CYTOCHEMICAL STUDY OF SUCCINIC DEHYDROGENASE ACTIVITY IN EXPLANTED CELLS

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We showed [3] that, in the early stages of cultivation of primary explanted kidney tissue, there was a pronounced fall in alkaline phosphatase activity. It was suggested that there might be a connection between this effect and the processes of adaptation of cells to new conditions of existence. In view of this, we undertook a study of other energetic enzymes (respiratory enzymes, phosphatases) in primary explanted and transplantable cultures. The presupposition of this investigation was that there might be differences in the level of activity and the dynamics of energetic enzymes in these two groups of tissue cultures.

The aim of the present work was a comparative cytochemical study of succinic dehydrogenase activity in epithelial cells of monkey kidney tissue and in a stable cell population HEp-2.

The number of cytochemical works devoted to the study of succinic dehydrogenase activity in tissue culture cells is small. It has been shown that the succinic dehydrogenase activity in primary explants of human malignant tumors and in the cultivation of tissue obtained from other centers of rapid cell multiplication is extremely high [6,7]. In an investigation of succinic dehydrogenase in HeLa cells [5], a high level of activity was found on the 1st-2nd day of growth of the culture.

A biochemical study of 7 to 10-day-old monolayer cultures of monkey kidney cells and cells of the transplantable stain HEp-2 [1,2] revealed a reduction in the rate of succinic dehydrogenase activity in comparison with cells taken immediately after trypsinization. However, these papers contained no data on the course of the changes in the enzyme activity during cultivation.

Experimental Method

As the primary explanted culture we used epithelial cells of the kidney cortical matter of rhesus monkeys. For preparing the tissue culture, the cortical substance was treated by Younger's method [9] and batches of 1.5 million cells (concentration 100,000 cells per 1 ml) were put into 100 ml Povitskaya flasks. The cells were grown for 20 days in a nutrient medium (Hanks' solution – 97.5%, lactalbumin hydrolyzate – 0.5%, bovine serum – 2%, antibiotics) at pH 7.5 and the medium was changed every 6-7 days.

As the transplantable strain we used HEp-2 cells derived from a human laryngeal carcinoma. A suspension of these cells was obtained by the method commonly adopted in virological practice. The cells were resuspended in medium No. 199 with 10% bovine serum and antibiotics. Fifteen-ml batches of the suspension (750,000 cells) were put into Povitskaya flasks. The cells were cultured for nine days without change of medium.

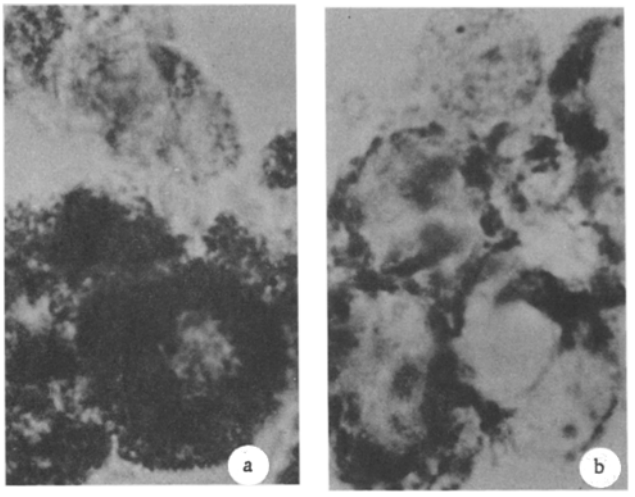

Fig. 1. Succinic dehydrogenase activity in tissue culture cells. Suspended cells before cultivation: a) cells of rhesus monkey kidney tissue; b) cells of HEp-2 strain. Photomicrograph. Magnification 2000 ×.

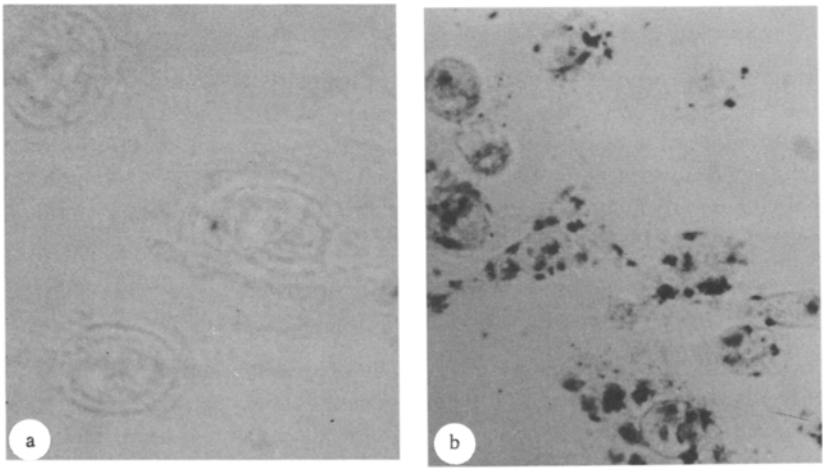

Fig. 2. Succinic dehydrogenase activity in tissue culture cells on first day of cultivation: a) cells of rhesus monkey kidney tissue. Magnification 2000 ×; b) cells of HEp-2 strain. Magnification 500 ×. Photomicrograph.

For the cytological and cytochemical investigation in both series of experiments, the flasks contained mica strips, on which the cells grew. The material was examined daily during the whole period of culturing. The succinic dehydrogenase activity was studied with the aid of neotetrazolium chloride by Seligman and Rutenburg's method [8] in Hirono's modification [4]. The incubation medium contained equal quantities of 1% neotetrazolium solution, 0.2 M sodium succinate solution, and 0.5 M phosphate buffer (pH 7.6). For a study of the endogenous dehydrogenase activity, the sodium succinate was replaced by a corresponding amount of physiological saline. The cells were incubated in freshly prepared substrate for two hours at 37°, fixed for 10 min in 10% neutral formalin, washed in two changes of flowing and distilled water, and, in certain cases, counterstained with methyl green and mounted in glycerine. Three series of experiments were made with each culture. The succinic dehydrogenase activity in the cells was determined from the precipitation of formazan deposit. We estimated the intensity of the reaction in nominal units (from + to ++++). For the morphological study, the material was fixed in Bouin's fluid and stained with Mayer's hematoxylin and eosin. The results obtained in all the series were of the same nature. We can conclude from this, despite some slight fluctuations, that the experimental results are reliable.

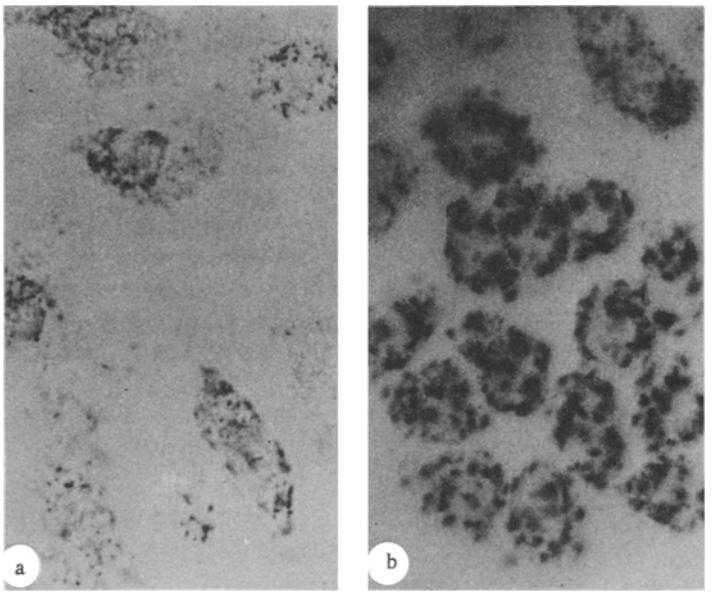

Fig. 3. Succinic dehydrogenase activity in tissue culture cells on sixth day of cultivation: a) cells of rhesus monkey kidney tissue; b) cells of HEp-2 strain.

We also used the same method to study frozen sections of kidney cortical matter and cells of suspensions of the two cultures before inoculation.

Experimental Results

Kidney tissue culture. On investigation of fresh frozen kidney slices, we found that the succinic dehydrogenase activity in the cells of the tubules was extremely high. The epithelium of the straight tubules contained a particularly large amount of deposit. It should be noted that the enzyme activity in the cells of some tubules was relatively low, this being probably due to their physiological state at the time of investigation. No diformazan deposit was found in the Malphigian corpuscle. The endogenous dehydrogenase activity was fairly high only in some of the straight tubules.

The suspension of cortical kidney matter before cultivation consisted of cells of rounded form with a compact, centrally placed nucleus. The overwhelming majority of the suspended cells contained a diformazan deposit, which filled the whole cytoplasm (Fig. 1a); this deposit consisted of granules of various sizes and acicular crystals. Some cells contained so much deposit that the outlines of the nucleus were difficult to detect, while in others the distribution of deposit was mainly perinuclear. In a few cells the reaction was very slight or absent altogether. A comparison of suspended cells incubated with and without the addition of sodium succinate revealed no essential differences in the nature and distribution of the deposit; this indicates a relatively high level of endogenous dehydrogenase activity.

In the early stages of growth of the kidney cell culture, there was a considerable decrease in their enzymatic activity: during the first 3 days of cultivation they were either completely devoid of deposit, or contained a very slight quantity of it (Fig. 2a). On the 3rd or 4th day we observed the appearance and subsequent increase of the diformazan content, which reached a maximum by the 5th or 6th day (Fig. 3a). After 10-11 days of cultivation, the intensity of the reaction fell and remained relatively low until the end of the experiment.

During the 3rd-7th days of cultivation, the endogenous dehydrogenase activity was very pronounced. However, by the 6th or 7th day the intensity of the reaction was reduced, so that, by the last days of the experiment, only a few cells contained diformazan deposit.

A thorough investigation of the culture showed that the cells, even those similar in morphological features, contained different amounts of deposit. More pronounced differences, affecting both the intracellular distribution of deposit and the degree of deposition were noted in cells of different morphological type [3]. For instance, in

"nidal" cells, the deposit was either absent altogether throughout the period of cultivation, or was found in extremely small quantity. In "flat" cells, the deposit granules were localized predominantly around the nucleus, whereas "fusiform" cells were characterized by a diffuse distribution of deposit. We noted a change in the nature of the precipitate during cultivation. While, at the early stages, it consisted of tiny homogeneous granules, from the 12th-14th day onward the deposit in certain cells consisted of coarse clots of irregular shape. Probably the change in the diformazan deposition with age was due to age-induced changes in the morphology of the cell mitochondria.

HEp-2 culture. The cytoplasm of the majority of cells of the suspension immediately before cultivation contained a considerable amount of diformazan deposit (see Fig. 1b) in the form of coarse aggregates of acicular crystals. In a few cells the intensity of the enzyme reaction was low, and in scattered cells it was absent altogether. On the elapse of the first day of cultivation, the amount of diformazan in the cells decreased a little (see Fig. 2b), but by the second day it reached a peak, and remained at this level with some fluctuations during the whole period of observation (see Fig. 3b). We noted that a feature peculiar to the cells of the growing culture was the extremely low endogenous dehydrogenase activity, whereas the cells of the suspension were characterized by a fairly high level of endogenous activity.

The diformazan deposits in the HEp-2 cells were in the form of coarse aggregates of acicular crystals, and in this they differed significantly from the deposit formed in the analogous reaction in kidney epithelium cells. We observed some fluctuations in the amount of deposit in individual cells: cells very rich in diformazan were accompanied by cells with low enzymatic activity. We did not notice any differences in the nature of the intracellular localization of the deposit.

Comparing the above data on the course of the changes in succinic dehydrogenase activity in cells of the kidney epithelium culture with the changes in alkaline phosphatase activity in the same culture [3], we see that the course of the changes in these enzymes is of exactly the same type. This is all the more interesting in that the compared enzymes differ considerably from one another both in the nature of their action and in the part they play in cell metabolism. We can surmise that the similar temporary reduction in the activity of the enzymes in the early stages of cultivation of the first generation of kidney cells is of a more general character and constitutes one of the manifestations of the response of cells to the new conditions of existence in the tissue culture. This view is supported also by the results obtained in this work in regard to the course of the changes in succinic dehydrogenase activity in the cells of the transplantable strain HEp-2. In these cells we observed no disappearance of enzymatic activity, and in this they differ markedly from the primary explanted culture. Similar results have been obtained for cells of another transplantable strain (HeLa strain) by Kaufman and Hill [5]. In our opinion, the high succinic dehydrogenase activity in cells of transplantable strains at precisely those times when it is completely absent in the primary explanted culture is associated with the complete adaptation of the cells to the conditions of cultivation. However, a confident assertion of this claim cannot be made until other enzymes and other tissue cultures have been investigated.

An interesting feature in connection with the problem under discussion was the observed low level of endogenous activity of dehydrogenase systems in cells of the transplantable strain in contrast to its high level in cells of kidney epithelium culture. We believe that this is related to the prolonged cultivation in vitro of cells of the transplantable strain, which utilize all their required nutrients and energy material directly from the medium. The conversion of the constituents of the medium into the form used by the cells is effected during metabolism to an extent not exceeding the minimum requirements of the cell. We can assume, in particular, that this applies also to the substrates of various enzyme systems. On cultivation of cells freshly extracted from an organism (primary explanted cultures), there is still no adaptation to the new conditions, and the cell continues, as before, to create reserves of required substances, which are only gradually used up in the process of metabolism.

SUMMARY

Succinic dehydrogenase activity was studied in the cells of primary cultures of monkey kidney and the HEp-2 strain. As distinct from the primary culture, the activity of the enzyme remains on the same level during the whole period of culturing in the cells of the transplantable strain. The data obtained are discussed in connection with the problem of cell adaptation to the conditions of culturing in vitro.

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