CYTOCHEMISTRY OF DEHYDROGENASES IN ISOLATED NUCLEI

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Dehydrogenase activity of isolated nuclei of the normal rat liver and of Zajdela's hepatoma was investigated. High NAD·H_2-dehydrogenase activity was found in the liver nuclei, mainly in the membrane; NAD·H_2-dehydrogenase activity in nuclei of Zajdela's hepatoma was 45% lower. No activity of succinate, glutamate, isocitrate, glucose-6-phosphate, or lactate dehydrogenase or of NAD-bound α-glycerophosphate dehydrogenase was found.

Detailed histochemical and biochemical data on the concentration and compartmentalization of the nuclear adenosinetriphosphatases are available in the literature [6, 7]. Information on dehydrogenase activity in the nuclei is mainly confined to biochemical data [2].

The writers have used cytochemical methods to study the following dehydrogenases in isolated nuclei of the albino rat liver and Zajdela's hepatoma: succinate, glutamate, isocitrate, glucose-6-phosphate, NAD, lactate, and NAD-bound α-glycerophosphate dehydrogenases.

EXPERIMENTAL METHOD

Dehydrogenase activity was studied in films made from suspensions of isolated nuclei in isotonic sucrose solution and in frozen sections. Nuclei of the normal rat liver and of the hepatoma were obtained by a method based on the Chauveau principle. Sections of the freshly frozen rat liver, 12 μ thick, were cut on a Slee (England) cryostat.

The cytochemical detection of dehydrogenase activity was carried out by the method of Nachlas et al. with slight modification [1]. Nitro-blue tetrazolium was used as hydrogen ion acceptor. To detect NAD·H_2-dehydrogenase, reduced NAD was added to the incubation medium in a concentration of 3 mg/ml. Slides with films of nuclei and sections were dried for 5-30 min in air at room temperature and then incubated for 20 min in the corresponding media at 37°C. The specimens were then dehydrated and mounted in balsam. Activity of the enzymes was judged from the number of diformazan granules precipitated during enzymic reduction of tetrazolium at the sites of localization of the corresponding enzymes. A parallel series of control tests was carried out without the addition of substrate to the incubation medium. In these cases no diformazan granules were formed.

EXPERIMENTAL RESULTS

A visual study of the specimens of the liver and hepatoma nuclei revealed no activity of succinate, glutamate, isocitrate, glucose-6-phosphate, lactate, or NAD-bound α-glycerophosphate dehydrogenases.

High activity of NAD·H_2-dehydrogenase was found in the nuclei of the normal rat liver, mainly in the nuclear membrane (Fig. 1a). The content of this enzyme in nuclei of Zajdela's hepatoma was considerably

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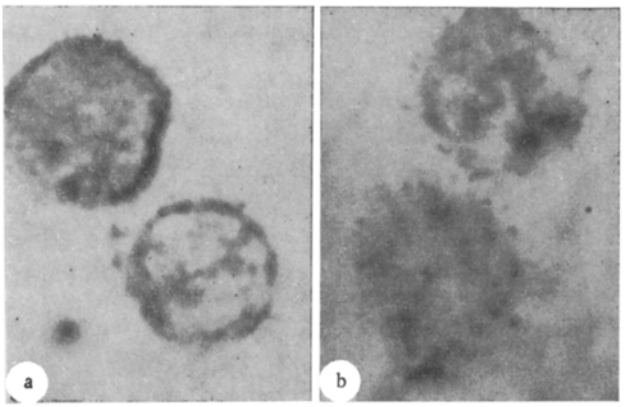

Fig. 1

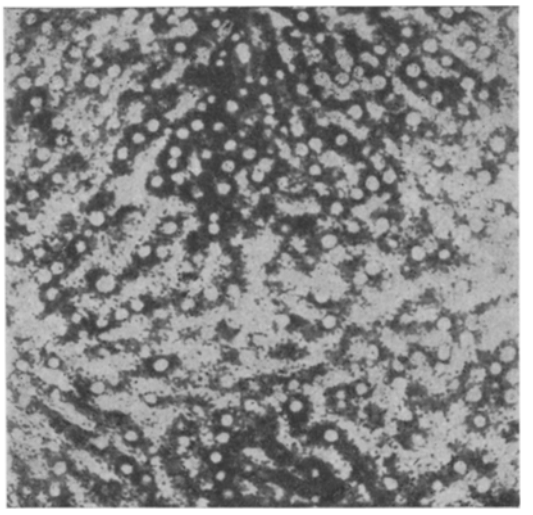

Fig. 2

Fig. 1. NAD·H_2-dehydrogenase activity in isolated nuclei of normal rat liver (a) and of Zajdela's hepatoma (b). Stained by Nachlas' method, 900 ×.

Fig. 2. Distribution of NAD·H_2-dehydrogenase in frozen sections of liver cells. Stained by Nachlas' method, 200 ×.

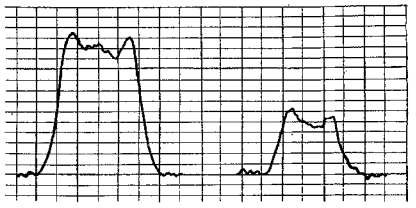

Fig. 3. Results of scanning isolated nuclei of liver (on left) and Zajdela's hepatoma (on right).

lower (Fig. 1b). The diformazan was distributed as tiny dust-like granules clearly outlining the nuclear membrane.

A study of frozen liver sections revealed high activity of all the enzymes tested in the cytoplasm; the contents of the nucleus remained unstained. It was impossible to localize the enzyme in the nuclear membrane by the study of NAD·H_2-dehydrogenase in frozen sections because its high activity in the cytoplasm adjacent to the nucleus masks the staining of the nucleus itself (Fig. 2).

Verification of the purity of the isolated nuclei under the electron microscope confirmed that NAD·H_2-dehydrogenase activity was in fact localized in the nuclear membrane and could not be due to cytoplasmic contamination.

The relative level of NAD·H_2-dehydrogenase activity in the nuclei of normal liver and of the hepatoma was also compared cytospectrophotometrically by scanning the preparations on a universal automatic-lighting MPS-50 microspectrophotometer (Shimadzu, Japan). The bean width was 2 μ and its wavelength 585 nm. In each specimen 30 nuclei were scanned. As an example, the results of scanning nuclei of the normal liver and hepatoma are shown in Fig. 3. The scanning line is trapezoidal in shape and frequently gives maxima at the beginning and end, corresponding to the edges of the nucleus. The mean height of the scanning curve was determined by averaging the areas of the component strips. The mean optical density of the nucleus corresponded to the mean height of the curve. The resulting optical density values (in conventional units) were subjected to statistical analysis in the ordinary way.

The mean density of the nuclei of the normal rat liver was 0.11 ±0.007 and of the Zajdela's hepatoma 0.06 ±0.006. The differences are statistically significant ($P < 0.001$). Activity of NAD·H_2-dehydrogenase in the nuclei of Zajdela's hepatoma was thus 45% lower than that in the normal liver nuclei.

NAD·H_2-dehydrogenase, located in the membranes, catalyzes the reaction of transhydrogenation between NAD·H_2 and NAD, and it thus takes part in the regulation of respiration of glycolysis [7, 4]. NAD·H_2 also takes part in the "shuttle mechanism" of NAD·H_2 oxidation, which has been demonstrated in many tissues and, in particular, in the brain and liver [6]. However, this mechanism does not function in tumor cells [7]. This may possibly be because of the low NAD·H_2-dehydrogenase activity in the membranes of tumor cells. Evidence in support of this view is given by the decrease in NAD·H_2-dehydrogenase activity

found in the nuclear membranes of Zajdela's hepatoma by comparison with its activity in normal liver nuclei. This fact requires further verification with respect to other tumors.

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