HISTOCHEMICAL INVESTIGATION OF ENZYMES IN DIPLOID AND POLYPLOID LIVER CELLS

(UDC 612.351.11.014.1)

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Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 59, No. 6, pp. 110-113, June, 1965
Original article submitted October 7, 1963

The tissues of various organs of animals and man contain diploid and polyploid cells. It has been concluded from several investigations [1, 4] that somatic polyploidy is of compensatory importance. In this connection the comparison of the properties of polyploid and diploid cells is of particular interest.

Polyploid cells usually differ from the corresponding diploid cells by the larger size of their nucleus and cytoplasm [14], with a corresponding increase in their dry weight (as shown by the results of histochemical investigation and ultraviolet miscoscopy of liver tissue), and by their higher content of RNA, polysaccharides, and lipids [2].

In the liver of the healthy adult rat there are many polyploid cells. About 70-80% of the mononuclear parenchymatous hepatic cells are tetraploid and 5-8% are octoploid. Moreover, many parenchymatous hepatic cells have two nuclei, containing altogether 4-8 sets of chromosomes, and sometimes many more—as many as 16-20 [2]. The size of the nucleus increases in proportion to the degree of ploidy.

The object of the present investigation was to study the enzymic activity of cells differing in their ploidy.

EXPERIMENTAL METHOD

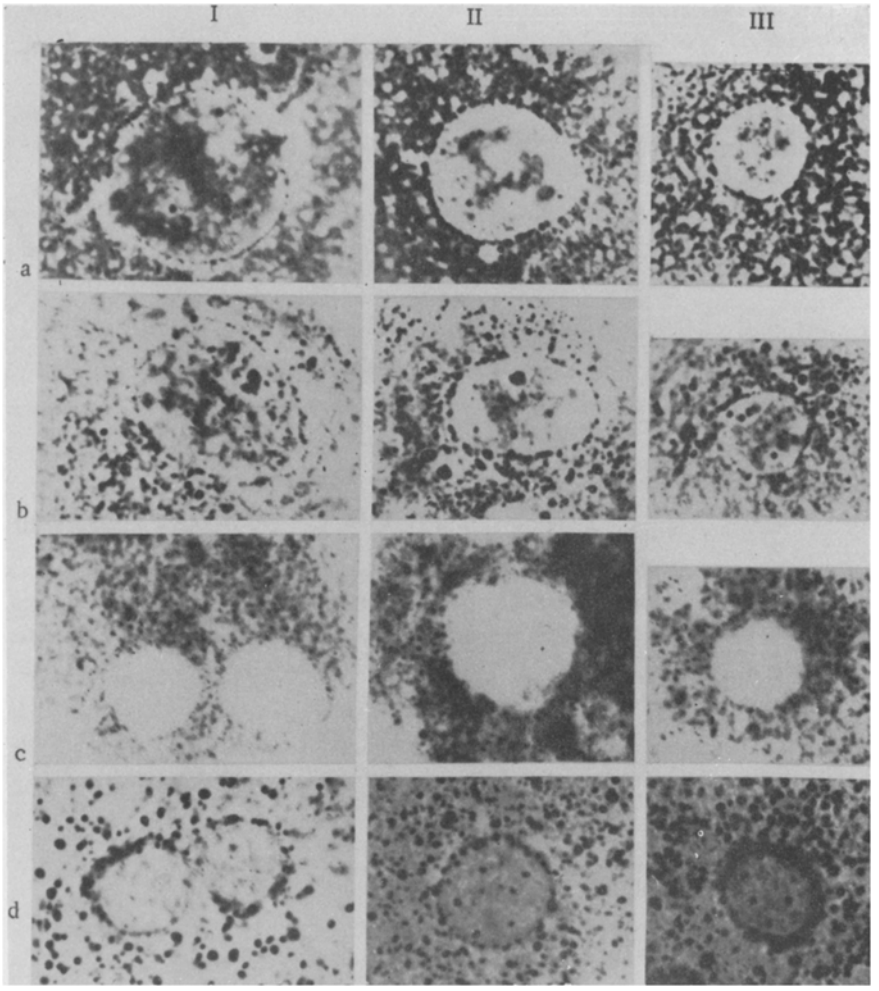
Adult albino rats were sacrificed by decapitation. The liver was extracted quickly and impressions taken from the recently cut surface.

By means of histochemical methods the following enzymes were studied in freshly prepared unfixed impressions of the liver: the oxidation-reduction enzymes cytochrome oxidase, succinate dehydrogenase, isocitrate dehydrogenase, malate dehydrogenase, glucose-6-phosphate dehydrogenase, glutamate dehydrogenase, α-glycerophosphate dehydrogenase, lactate dehydrogenase, and DPN- and TPN-diaphorase, and also certain hydrolytic enzymes: acid and alkaline phosphatase, adenosinetriphosphatase, glucose-6-phosphatase, esterase, and 5-nucleotidase. The enzymes studied are all concerned with the more important links in the chain of intracellular metabolism, so that they characterize the functional activity of the cells.

The dehydrogenases were detected by the method of Hess, Scarpelli, and Pearse [8], the diaphorases by the method of Nachlas and co-workers [10] and of Scarpelli and co-workers [13], and the succinate dehydrogenase by the method of Nachlas and co-workers [9]. In all cases the salt of nitro-BT was used. The cytochrome oxidase activity was investigated by Burstone's method [7].

The hydrolytic enzymes (phosphatase and esterase) were determined by Burstone's method [7], and 5-nucleotidase, adenosinetriphosphatase, and glucose-6-phosphatase by Pearse's method [12].

The ploidy of the nucleus was determined from its area, for in impressions of the liver this value is correlated



Demonstration of enzymic activity in diploid and polyploid liver cells. Reaction with nitro-BT. The number of diformazan granules in the cytoplasm of the diploid (2n), tetraploid (4n) and octoploid (8n) cells is comparatively unchanged. Objective 90 ×, ocular 7 ×. a) Glucose-6-phosphate dehydrogenase: I) 8n, II) 4n, III) 2n cells; b) DPN-diaphorase: I) 8n, II) 4n, III) 2n cells; c) cytochrome oxidase: I) binuclear cell, II) 4n, III) 2n; d) isocitrate dehydrogenase: I) binuclear cell, II) 4n, III) 2n.

with the DNA content, which is proportional to the number of chromosomes [1, 2]. The method used to measure the area of the cell nucleus is described in detail in the same cited papers. Cells with measurements approximately in the ratio 2 : 4 : 8 were compared, i.e., those which corresponded to the median values for each class of ploidy.

EXPERIMENTAL RESULTS

The study of the preparations (see figure) shows that the concentration of dye marking the localization of enzymic activity per unit area was the same in most cases in cells of different ploidy.

The oxidation-reduction enzymes were localized entirely in the cytoplasm.

The diformazan granules, which was the form in which areas of enzymic activity were revealed, filled a large part of the cell, and sometimes the whole cytoplasm, comparatively uniformly, reflecting the distribution of the mitochondria, the chief carriers of these enzymes. In many cases a cluster of granules was seen around the nucleus.

The activity of most of the investigated respiratory enzymes showed no difference in the diploid and polyploid cells.

The succinate dehydrogenase activity in the tetra- and octoploid cells was much higher than in the diploid.

The activity of the hydrolytic enzymes in the polyploid mononuclear liver cells was indistinguishable from that in the polyploid or was higher than the latter.

The almost equal concentrations of dye, corresponding to the enzyme activity, in the di- and polyploid cells did not imply almost identical properties of the cells.

Among certain cells of each class of ploidy, in individual cases low or, conversely, unusually intensive enzymic activity by comparison with the mean level was observed, most probably associated with differences in the functional state of the liver cells [11].

The binuclear cells, polyploid in relation to their total content of DNA, in some cases were distinguished from the mononuclear cells by their higher enzymic activity (cytochrome oxidase, diaphorase, succinate dehydrogenase).

The activity of the rest of the enzymes that we studied was no less marked in the binuclear cells than in the mononuclear, in agreement with data in the literature [6].

Hence, the activity of the enzymes of polyploid mononuclear and binuclear liver cells is not lower, and the content of the enzymes is probably higher in the polyploid cells than in the smaller diploid cells.

If these results are compared with the results of the histochemical investigation of RNA, polysaccharides, and lipids in polyploid cells [1], the view that these cells are capable of functioning normally is confirmed. In any case, this conclusion is valid for the tetraploid cells, most commonly found in the tissues of vertebrates.

The increase in the number of polyploid cells as a result of aging or of functional overloading, and the results of the study of their biochemical properties suggest that the polyploidization of somatic cells is one of the manifestations of the physiological compensatory regeneration of organs [1, 3-5].

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