ELECTRON-HISTOCHEMICAL AND BIOCHEMICAL INVESTIGATION OF THE GLUCOSE-6-PHOSPHATASE AND β-GLYCEROPHOSPHATASE ACTIVITIES IN NUCLEI OF THE NORMAL LIVER AND HEPATOMA-27 OF RATS

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The presence of glucose-6-phosphatase activity was demonstrated electron-histochemically and biochemically in rat liver cell nuclei, and the histochemical reaction product was localized mainly in the perinuclear space. The activity of this enzyme in hepatoma-27 nuclei was much lower than in nuclei isolated from normal liver. A low level of β-glycerophosphatase activity was detected histochemically in liver nuclei incubated at pH 6.4. No activity of this enzyme was found in hepatoma-27 nuclei. Biochemical tests revealed traces of activity in both liver and hepatoma-27 nuclei.

KEY WORDS: glucose-6-phosphatase; β-glycerophosphatase; liver nuclei; hepatoma-27.

The opinion has been expressed without any justification that many phosphatases are localized exclusively in cytoplasmic structures. Attempts have been made to explain the positive reaction for nonspecific phosphatase in the nuclei during histochemical tests by diffusion of the enzyme or of the histochemical reaction product [2, 12]. Nevertheless, there is electron-histochemical evidence of acid phosphatase activity in the chromatin of the cell nuclei of certain hepatomas [3], and in the chromatin, chromosomal discs, and nuclear membrane of corn and pea root-cap cells [13]. Bukhvalov and Unger [1] found the product of the histochemical reaction for nonspecific phosphatase with pH-optimum at 6.4 in the perinuclear space in mouse liver cells. The presence of the enzyme in the perinculear space has also been demonstrated in the Turbellaria [6] and in certain other species [5].

The question of the localization of glucose-6-phosphatase (G6Pase) in the nucleus is no less contradictory. In some biochemical laboratories the absence of activity of this enzyme in the nuclei is used as evidence of the purity of the nuclear fraction. Meanwhile, there is definite histochemical evidence of the localization of G6Pase not only in the endoplasmic reticulum but also in the perinuclear space [7, 9]. In addition, relatively high specific activity of G6Pase has been found by biochemical methods in Franke's laboratory [8] in the nuclear membranes of rat liver, in an amount that increased on purification, indicating that the enzyme is located in the nuclear membrane.

The investigation described below was carried out to study the ultrastructural localization of nonspecific phosphatase and G6Pase in nuclei of normal rat liver and of hepatoma-27.

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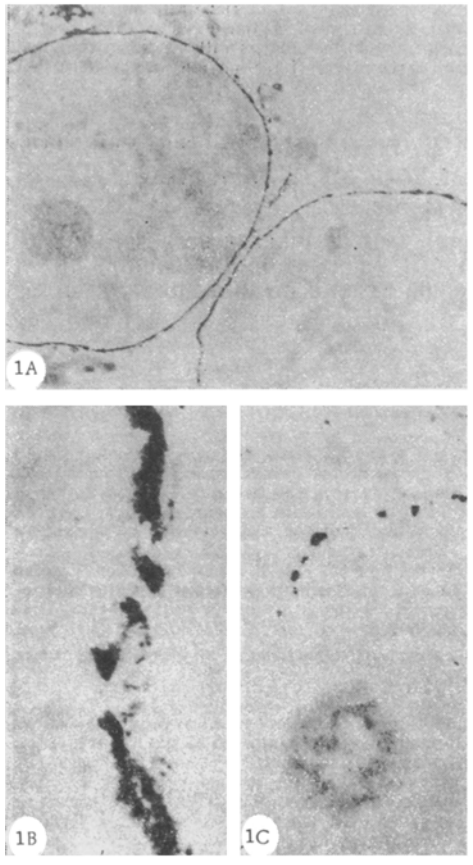

Fig. 1

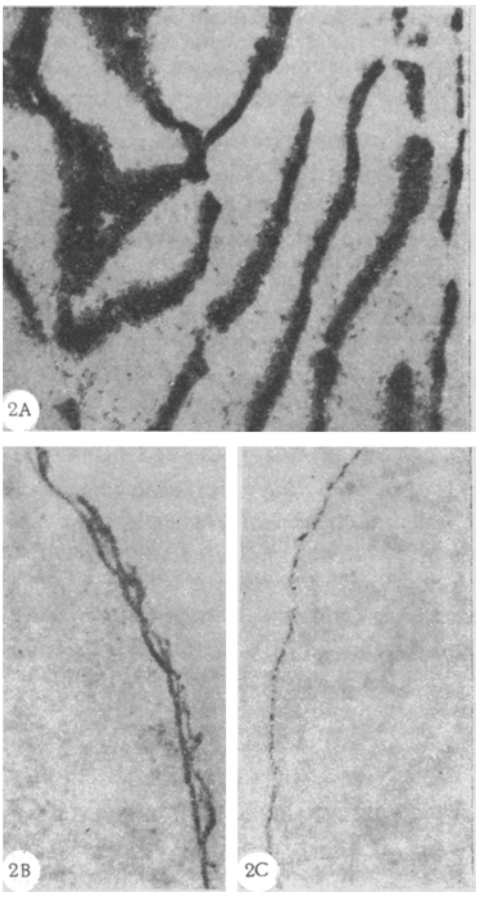

Fig. 2

Fig. 1. G6Pase activity in isolated rat liver nuclei: A) 7200 ×; B) 140,000 ×; C) control with addition of 0.01 M p-chloromercuribenzoate to the medium, 25,400 ×.

Fig. 2. G6Pase activity in hepatoma-27: A) cisterns of endoplasmic reticulum (92,300 ×); B and C) isolated hepatoma-27 nuclei (14,600 × and 16,400 ×, respectively).

EXPERIMENTAL

Nuclei from the rat liver and hepatoma-27 were isolated by Chauveau's method with some modifications and prefixed in glutaraldehyde [1].

For the electron–histochemical detection of β-glycerophosphatase activity the nuclei were incubated in a medium of the following composition: 5 ml 0.25 M tris-maleate buffer, 2 ml 2.3 M sucrose, 2.5 ml 1.25% sodium β-glycerophosphate, and 0.75 ml 2% $Pb(CH_3COO)_2$. Incubation was carried out at 30°C for 15 min at pH 5.6, 6.4, and 8.8.

For the detection of G6Pase activity the nuclei were incubated for 15 min at 30°C in a medium of the following composition: 5 ml 0.25 M tris-maleate buffer (pH 6.7), 2 ml 2.3 M sucrose, 2.5 ml 1.25% G6Pase, and 0.75 ml 2% lead acetate.

The subsequent treatment of the preparations for electron microscopy was as described previously [5].

For the biochemical determination of β-glycerophosphatase activity the nuclei were incubated at 30°C for 30 min in the same medium as for electron-histochemical study, but without lead acetate. G6Pase activity was determined biochemically by the method of Duke and Pears [4, 12]. The protein concentration was estimated by Lowry's method [10].

RESULTS

No β-glycerophosphatase could be detected electron-histochemically in the nuclei of normal liver and hepatoma-27 at pH 5.6 and 8.8. At pH 6.4 the product of the reaction catalyzed by this enzyme was found in the perinuclear space of some nuclei, but its activity was much lower than in mouse liver nuclei [1]; no activity could be detected in hepatoma-27 nuclei even at pH 6.4. Traces of β-glycerophosphatase activity were detected biochemically in normal liver and hepatoma-27 nuclei at pH 6.4 and 5.6. The determination could not be carried out at pH 8.8 because of agglutination of the nuclei.

The product of the histochemical reaction for G6Pase was found in the perinuclear space of the normal rat liver nuclei (Fig. 1A). In some cases a clear space could be seen in the zone of the perinuclear space filled with the histochemical reaction product (Fig. 1B), although there was no evidence that the enzyme was located chiefly along the inner surface of the nuclear membranes, as described by Kartenbeck et al. [8]. Nor was the enzyme seen to be associated primarily with the membranes in the cisterns of the endoplasmic reticulum (Fig. 2A).

The G6Pase activity in the perinuclear space of the nuclei isolated from the hepatoma was considerably lower than normal (Fig. 2B) and in most cases the reaction product was confined to the perinuclear space itself; only very rarely could it be seen on the nuclear membranes also (Fig. 1B). This possibly confirms results obtained in Franke's laboratory [8] to show the localization of G6Pase along the inner surface of the nuclear membranes, although the compromise conclusion cannot be ruled out that G6Pase is located both in the perinuclear space or the cavity of the endoplasmic cisterns and also in their membranes. Kartenbeck et al. [8] consider that the histochemical reaction product is formed through the activity of enzyme located along the inner surface of both nuclear membranes and that it diffuses into the perinuclear space; however, another possibility is that the product formed in the space may be adsorbed on the membranes (Fig. 2C).

As a control of the specificity of the histochemical reaction for G6Pase, nuclei were incubated in the absence of the exogenous substrate or with the addition of 0.01 M p-chloromercuribenzoate to the medium. No color developed whatsoever in the control without the substrate, and in the control with the inhibitor the intensity of the reaction was substantially less (Fig. 1C).

The biochemical determination of G6Pase activity revealed a sharp decrease in hepatoma-27 (1.2 ± 0.08 μmole P/min/g protein) compared with normal (37 ± 0.45 μmoles P/min/g protein). This result reflects the partial loss of specialization of the hepatoma cells as a result of their structural and functional changes, for one of the specific functions of the hepatocytes is to synthesize glycogen, and G6Pase is a key enzyme of gluconeogenesis.

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