ULTRACYTOCHEMICAL CHANGES OF GLUTOSE-6-PHOSPHATASE ACTIVITY IN DEGENERATIVE HEPATOCYTES IN CHRONIC HEPATITIS—APPLICATION OF GLUTOSE-6-PHOSPHATASE ULTRACYTOCHEMISTRY TO HUMAN LIVER BIOPSY SPECIMENS—

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Using glucose-6-phosphatase (G6Pase) as a marker enzyme, ultracytochemistry was performed on human degenerative hepatocytes in liver biopsy specimens from 7 cases of chronic aggressive hepatitis and 3 cases of chronic persistent hepatitis. Some modifications of prefixation, incubation time and postwashing time were made to apply the ultracytochemical method for this enzyme originally designed for animals to biopsied human liver. Normal G6Pase activity was found in the smooth endoplasmic reticulum (SER), the rough endoplasmic reticulum (RER) and the nuclear envelope (NE) of nearly normal hepatocytes in chronic persistent hepatitis. In degenerative hepatocytes of chronic aggressive hepatitis, lead phosphate deposition was reduced in the dilated cisternae and observed only on the inner surface of the cisternal membrane. SER was vesiculated and contained no G6Pase activity. Occasionally, in markedly dense and shrunken hepatocytes which were detached from neighboring cells, G6Pase activity had mostly disappeared in SER and RER but it was well preserved in NE.

It is most interesting to determine the correlation between ultrastructural changes of hepatocytes and their function. As a marker of hepatocyte function, G6Pase was chosen because it is one of the liver specific enzymes (3, 5), though it is also observed in other organs (6, 11, 12, 13, 16, 19), and because it is well known that the enzyme activity decreases histochemically and biochemically during various liver injuries (1, 22).

In previous experiments using rats and mice, G6Pase activity was ultracytochemically demonstrated in hepatocytes (4, 7, 8, 17, 23). However, in human materials little G6Pase ultracytochemistry has been studied except for the authors' short communication on liver biopsy specimens (21) and a report on human kidney tissues (25).

This study was undertaken to determine the ultracytochemical distribution and changes of G6Pase in degenerative hepatocytes in chronic hepatitis, especially in chronic aggressive hepatitis.
MATERIALS AND METHODS

Liver biopsy specimens were obtained under peritoneoscopy from 7 cases of chronic aggressive hepatitis and 3 cases of chronic persistent hepatitis. The specimens were cut into slices less than 1 mm thick with a razor blade, fixed with 2.0% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.2) for 30 min at 0-4°C, washed in 0.1 M cacodylate buffer (pH 7.2) for 2 hours at 0-4°C, then cut into 30 μm thick sections using a cryostat, incubated in Wachstein-Meisel’s medium (24) for 15 min, 30 min or 60 min at 37°C, washed briefly in 0.1 M cacodylate buffer (pH 7.2), postfixed with 1% osmium tetroxide, dehydrated in a graded series of ethanol and embedded in Epon. Ultrathin sections were cut with glass knives on a Ultramicrotome, stained with uranyl acetate and lead citrate and examined with a HS-8 electron microscope.

As control, the tissue sections were incubated in the medium without glucose-6-phosphate (G6P) and in the medium with 20 mM of β-glycerophosphate substituted for G6P. For histochemical observation on the microscopical level, after incubation in Wachstein-Meisel’s medium the sections were immersed in ammonium sulfide solution.

RESULTS

The fixation time of 30 min gave better results than 15 min or 60 min as far as enzyme activity and ultrastructural preservation. In the present study, postfixation washing time of 2 hours well preserved the enzyme activity, but the activity was barely found after overnight washing.

The incubation time for 30 min was more adequate than that for 15 min or 60 min for the preservation of the maximum amount of reaction product with decreased artificial product. Only slightly affected areas of liver biopsy specimens from chronic persistent hepatitis were chosen as controls. There were no recognizable ultrastructural changes in the human hepatocytes of these areas.

Ultracytochemically, deposits of lead phosphate showing G6Pase activity were observed in SER, RER and NE of the hepatocytes. But no activity was observed in the nucleus, mitochondria, Golgi apparatus, bile canaliculus and plasma membranes (Fig. 1).

Histologically, characteristic findings in chronic aggressive hepatitis were necrosis and degeneration of hepatocytes accompanied with mononuclear cell infiltration around the portal tracts (Fig. 2).

Histochromically G6Pase activity was well preserved in the non-degenerative hepatocytes, but was reduced in the degenerative area and barely observed in the necrotic area (Fig. 3). When the hepatocytes which had histochemically normal enzyme activity were studied ultracytochemically, the enzyme activity was well preserved in SER, RER and NE. But in the degenerative hepatocytes which had decreased enzyme activity, the lead phosphate deposits showing G6Pase activity appeared sparse and localized on the inner surfaces of the membranes where dilatation and deformity of the cisternae of RER were observed (Fig. 4). In the hepatocytes of necrotic area, enzyme activity of SER and RER was not observed where numerous vesicles of SER were noted in the cytoplasm of hepatocytes with a
Fig. 1. Ultracytochemical demonstration of G6Pase in hepatocyte from a case of chronic persistent hepatitis. Enzyme activity is positive in the endoplasmic reticulum (ER) and the nuclear envelope (NE), but negative in mitochondria (M), the bile canaliculus (B) and the Golgi apparatus (G). Note that ER are not dilated. Minute deposits in the plasma membrane (P) are possibly reaction products of non-specific phosphatases. ×26,000
Fig. 2. Periportal area of liver lobule from a case of chronic aggressive hepatitis. Note degeneration and necrosis of hepatocytes which are marked around the portal tracts, accompanied with mononuclear cell infiltration. HE stain. × 300

Fig. 3. G6Pase histochemistry in the same case as Fig. 2. G6Pase activity is marked in some hepatocytes (H) but decreased in the degenerative area (D) and lost in the necrotic area (N). × 350
Fig. 4. Degenerative hepatocyte of chronic aggressive hepatitis. Lead phosphate deposits are decreased, localized on the inner surface of the dilated RER and not found in the vesiculated SER. But the enzyme activity is well preserved in NE. × 23,000
Fig. 5. A hepatocyte of necrotic area in chronic aggressive hepatitis. Note SER vesicles without G6Pase activity and NE with positive activity. Collagen fiber (F). ×18,000
Fig. 6. Severely degenerated hepatocyte probably undergoing coagulation necrosis. The enzyme activity of NE is well preserved. Bile canaliculus (B). ×14,000
decreased amount of RER (Fig. 5).

In some hepatocytes which were markedly dense, shrunken and separated from neighbouring hepatocytes, G6Pase activity was barely found in endoplasmic reticulum (ER) (Fig. 6). However, even in these hepatocytes of degenerative or necrotic areas the enzyme activity was well preserved in NE (Figs. 4, 5, 6). In sections incubated in the medium from which G6P was omitted and in the medium in which G6P was replaced by 20 mM of β-glycerophosphate, the enzyme activity was not observed (Figs. 7, 8).

The enzyme activity was observed in hepatocytes constituting canalicular-ductular junction, but no activity was found in the biliary epithelial cells. Epithelial cells of bile ductules also showed no activity. The intermediate form between parenchymal cells and ductural cells was not observed in the present study (14).

DISCUSSION

In ultracytochemical localization of only partially degenerative areas of chronic persistent hepatitis, reaction products showing G6Pase activity were localized in RER, SER and NE of hepatocytes and the enzyme activity was not observed in other intracellular organelles. This intracellular distribution pattern is the same as that observed in the hepatocytes of rat (4, 17, 23). These findings are in agree-
ment with the results of biochemical studies (3, 5, 20).

In hepatocytes from the degenerative area to the necrotic area in chronic aggressive hepatitis, swelling, vacuolization, eosinophilic degeneration and necrosis of hepatocytes were observed and decreased or nonexistent activity in these hepatocytes was proven histochemically. This also agrees with a recent report (1).

In the degenerative hepatocytes, dilatation and distortion of RER were observed and lead phosphate deposits appeared to be sparsely localized on the inner surface of cisternae of RER. In these hepatocytes vesiculated SER was often observed and there the enzyme activity was barely observed. In hepatocytes which appeared to be more degenerative or necrotic, more vesiculated SER was observed with a decreased amount of RER and the enzyme activity was not observed in SER. These findings seem to indicate that the enzyme activity decreases proportionately with the degeneration of hepatocytes on the ultracytochemical level, too.

In rats treated with phenobarbital, prominent proliferation of SER with normal ultrastructure and enzyme activity has been observed (2, 15, 17). However, during the course of carcinogenesis induced by 3'-methyl-4-dimethyl-aminoazobenzene (3'-MeDAB), as soon as SER degenerates, the enzyme activity is lost (18). The ultrastructural degeneration in ER seems to resemble the disappearance of G6Pase activity in chronic aggressive hepatitis in this study. It has been known that G6Pase activity decreases biochemically in hepatitis (1, 22) and histochemically in the hepatocytes of degenerative and necrotic areas (1). The decrease of the enzyme activity of vesiculated SER appears before that in dilated RER in liver with chronic aggressive hepatitis. However, even in hepatocytes of these or more severely degenerative areas, the enzyme activity in NE was well preserved. The precise reason for this finding is not known, but it might be concerned with the different functions of ER and NE.

Some modifications of the ultracytochemical method for the demonstration of G6Pase activity were made for biopsy specimens from human liver. In these experiments, the fixation of the biopsy specimens cut into slices of less than 1 mm thickness in 2% glutaraldehyde at 0-4°C for 30 min was adequate for the preservation of ultrastructure and enzyme activity. According to Kanamura, G6Pase cannot be considered aldehyde-sensitive during a short fixation time, such as 30 min (10). Also in this study, even when longer fixation time such as 40 min or 45 min was applied, the enzyme activity was not so diminished.

As for the postfixation wash, it is said that a much shorter washing time should be used for the ultrastructural demonstration of G6Pase activity than is generally used (9). Ultracytochemically in biopsy specimens of human liver, the enzyme activity gradually decreased during the prolongation of postwashing time, such as 6 hrs or 10 hrs. Moreover, the enzyme activity was scarcely found in overnight washing. Therefore, especially in small tissues such as biopsy specimens of human liver, a much shorter postwashing time or about 2 hrs seems to better.

Incubation time of 30 min was adequate because the enzyme activity in sections incubated for 30 min was higher than in those incubated for 15 min or 60 min and the artifacts were not observed. The modified methods described by the author seem to be useful in investigating ultracytochemically the biopsy specimens of the human liver.
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REFERENCES


