LETTER TO THE EDITOR

DIFFERENCE IN RESISTANCE TO GLUTARALDEHYDE OR FORMALDEHYDE FIXATION BETWEEN MOUSE AND RAT HEPATIC GLUCOSE 6-PHOSPHATASE

Glucose 6-phosphatase (G-6-Pase) activity has been known to be easily inactivated during glutaraldehyde or formaldehyde fixation. In fact, the enzyme activity of rat hepatocytes was rapidly abolished both in sections of aldehyde fixed tissue (Chiquoine, J. Histochem. Cytochem. 1 : 429, 1953; Tice and Barnett, J. Histochem. Cytochem. 10 : 754, 1962; Sabatini et al., J. Cell Biol. 17 : 19, 1963) and in fresh frozen sections followed by aldehyde fixation (Ericsson, J. Histochem. Cytochem. 14 : 361, 1966; Manns, J. Histochem. Cytochem. 16 : 819, 1968). My chance observation that the enzyme activity survived longer in mouse liver cryostat sections fixed with glutaraldehyde led to a closer investigation of the effect of aldehyde fixation on G-6-Pase activity of mouse and rat liver. The present report deals with the difference in the resistance to glutaraldehyde or formaldehyde fixation between mouse and rat hepatic G-6-Pase activity.

Male adult DDD mice (20~29 g) and Wistar rats (180~230 g) fed the same kind of standard pellet (CE-2, Nippon Clea Co., Ltd. Tokyo) were used. The nonfasted animals were killed at 10 : 30 to 11 : 30 A.M. Rats fasted for 48 hr from 9.00 A.M. were also used. Blocks of tissue from livers were removed rapidly and frozen on dry ice. For the histochemical study, 12 μ cryostat sections of liver were cut and mounted on slides. The sections were immersed for various periods of time; at intervals of 5 min up to 2 hr; in 4% glutaraldehyde or; at intervals of 10 or 15 min up to 7 hr; in 4% formaldehyde buffered to pH 7.0 with 0.03 M cacodylate at 3.5~4°C, washed in 0.25 M sucrose at 4°C for 15 min, incubated in Wachstein-Meisel medium for 15 min at room temperature (Wachstein and Meisel, J. Histochem. Cytochem. 4 : 592, 1956), washed in water, then briefly immersed in dilute yellow ammonium sulfide and washed in water. For the biochemical assay, fresh liver homogenates were prepared according to the method described by Nordlie (Nordlie et al., J. Biol. Chem. 240 : 3479, 1965). G-6-Pase activity (Glucose-6-phosphate hydrolysis) was assayed by the method of Nordlie (Methods in Enzymology Vol. IX, 619p. 1966). The released inorganic phosphate was estimated by the method of Allen (Allen, Biochem. J. 34 : 858, 1940). Enzyme activity is expressed as μ moles of inorganic phosphate released in 10 min at 30°C by 1 mg of liver homogenate protein. The protein content of the liver homogenates were assayed by the method of Lowry et al. (Lowry et al., J. Biol. Chem. 193 : 265, 1951).

Glutaraldehyde fixation caused marked reduction in rat enzyme activity even after immersion for 10 min, and the reaction was abolished after immersion for 25 or 30 min. In mice, the reaction was only moderately reduced after immersion for 10 min, markedly reduced after immersion for 30 min. A weak reaction was still visible after immersion for 60 min, and abolished after immersion for 70, 75 or 80 min. In fasted rats, the reaction was markedly reduced after immersion for

Fig. 1. Mouse liver. Fresh frozen section fixed in 4% glutaraldehyde for 30 min.

Fig. 2. Mouse liver. Fresh frozen section fixed in 4% formaldehyde for 2 hr.

These sections were incubated for 15 min in Wachstein-Meisel medium for the demonstration of G-6-Pase. The reaction is stronger in periportal than in centrilobular areas but is weaker than in unfixed section. No reaction could be seen in sections of rat liver treated by the same method. $\times 40$

30 min, and abolished after immersion for 55, 65, 70 min. Formaldehyde fixation also caused reduction of rat enzyme activity after immersion for 1 hr, and inactivation after 2 or $2\frac{1}{6}$ hr. In mice, the reaction was only moderately reduced even after immersion for 2 hr and abolished after $4\frac{1}{2}$, 5, $5\frac{1}{4}$, $5\frac{1}{2}$ or $5\frac{3}{4}$ hr. In fasted rats, the reaction was markedly reduced after immersion for 2 hr, and abolished after immersion for $3\frac{1}{2}$ or 4 hr.

TABLE 1
G-6-Pase activity of mouse, rat and fasted rat liver

Experimental animal	G-6-Pase activity of liver
Mouse	0.64 ± 0.06
Rat	0.50 ± 0.06
Rat fasted for 48 hr	0.89 ± 0.07

Experimental details and definition of unit of activity are given in the text. The data presented are the results from thirteen mice, thirteen rats and six rats fasted for 48 hr. The data are given as mean values $\pm$ standard deviations of means. Difference between mean values of the enzyme activity of rat and mouse liver, or fasted rat and mouse liver is statistically significant ($P < 0.001$).

The substrate specificity of the enzyme stained in fixed sections was ascertained by substituting a number of other sugar phosphates for glucose-6-phosphate in the Wachstein-Meisel medium. No reaction was found with glucose-1-phosphate, fructose-1,6-diphosphate or β -glycerophosphate, while slight or no staining occurred with ribose-5-phosphate and fructose-6-phosphate.

To characterize the enzyme further the effects of heat, various metal ions and other substances were examined. Total loss of enzyme activity followed treatment with hot water at 90°C for 5 min; 0.1 M acetate buffer, pH 5.0 at 37°C for 15 min; acetone or ethanol at 4°C for 5 min; the preincubation in 0.25 M sucrose containing Cu^{++} (1 mM), Zn^{++} (10 mM), Ca^{++} (1.0 M), or Mg^{++} (1.0 M) and the addition of these inhibitors to the incubation medium. Fluoride (10 mM) caused partial inhibition while iodoacetate (50 mM) or preincubation in arsenite (50 mM) had no effect. Results were comparable on both fixed and unfixed sections and are in agreement with the known reactions of G-6-Pase.

The results presented here indicate that there was an apparent difference in resistance to glutaraldehyde or formaldehyde fixation between mouse and rat hepatic G-6-Pase and the enzyme activity survived in mice twice to three times longer than in rats during fixation in glutaraldehyde or formaldehyde. Biochemical assays showed (Table 1) that mouse liver showed only a slightly higher, although significant, level of the enzyme activity than rat liver. Further, although fasted rat liver showed a significantly higher level of the enzyme activity than mouse liver, the histochemical reaction in fasted rat liver was abolished by fixation for roughly equal or shorter periods of time than in mouse liver. These results suggest that the difference in resistance to glutaraldehyde or formaldehyde fixation between mouse and rat hepatic G-6-Pase activity is not due to any difference in the level of the enzyme activity between mouse and rat liver, but to the difference in the properties of the enzyme itself between mouse and rat liver.

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