Rapid Increase in Nuclear Hydrolase Following Partial Hepatectomy in the Rat (37086)

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Increased lysosomal labilization is often followed by increased mitotic activity and cell division. Phytohemagglutin (1), streptolysin A (2), Vitamin A (3), specific antigens (4), chemical carcinogens (5, 6) and partial hepatectomy (7–12) increase lysosome fragility and lead to cell mitosis. Investigators have shown that cytoplasmic hydrolase activity increases during liver regeneration prior to cell division (7, 9). Similar increases in free acid hydrolase activity have been observed during chemical carcinogenesis (13, 14). Hormones may also activate lysosomes in target tissues. Szego (15) has demonstrated that as early as 15 min after estradiol treatment, uterine endometrium lysosomes appear to attach to the nucleus. At 30 min there is a strong positive staining for acid hydrolases (acid phosphatase) within the nucleus. A similar migration and attachment of lysosomes to the nuclei has been observed after lymphocytes were treated with phytohemagglutinin (5).

This report describes a rapid increase in cytoplasmic-free hydrolase and a subsequent increase in nuclear hydrolase activity following 70% hepatectomy in the rat.

Materials and Methods. Male Cox/Holtzman rats weighing 200–240 g were 70% hepatectomized according to the technique of Higgins and Andersson (16) with the animals under anesthesia. Control animals were either sham operated (livers not removed) or 30% hepatectomized. Livers were removed at various times following operation and then weighed. The tissue was minced and homogenized with a glass homogenizer with a Teflon pestle and made up to a 10% final suspension in 0.32 *M* sucrose buffered with potassium phosphate, pH 7.4 and containing 2 m*M* $MgCl_2$. The suspension was filtered through a cheese cloth and then sedimented at 600*g* for 10 min in a refrigerated centrifuge. The nuclei (Fraction I) were resuspended in 4 ml of solution A and then mixed with 22 ml of 2.2 *M* sucrose, containing 2 m*M* $MgCl_2$. The final sucrose concentration was 1.9 *M*. The nuclear suspension was sedimented at 41,000*g* for 1 hr. Before the pellets were resuspended small samples were removed, stained with .005% acridine orange and examined microscopically. The pellets prepared by the method described were free from contaminating whole cells, mitochondria, or lysosomes. The nuclear pellets were resuspended in 10 ml of distilled water and then subjected 3 times to freezing and thawing to liberate hydrolase activity. Enzyme assays were also run in the presence of 0.15% Triton X-100. The post-nuclear supernatant from the first spin was resedimented at 12,000*g* for 20 min. The supernatant from this spin (Fraction II) and the pellet (Fraction III) were used to determine the free and bound activity in the cytoplasm. These fractions were also subjected to freeze–thaw before enzyme assays were run. Results for the cytoplasmic enzymes are reported as a ratio of free-to-bound activity. The results for nuclear hydrolase activity are reported as relative specific activity (RSA) based on weight of DNA. DNA was determined by the method of Giles (17).

β-Glucuronidase was assayed by the method of Fishman *et al.* (18). One milliliter of freeze–thaw lysate was incubated with 0.25 ml of 5 m*M* phenolphthalein-β-glu-

curonide sodium in 0.5 *M* acetate buffer, pH 4.8. Incubations were for 20 min at 37°. The reaction was stopped with 2 ml of 0.4 *M* glycine buffer pH 10.7 containing 0.25% sodium dodecyl sulfate (SDS).

Acid phosphatase was assayed using the method described by Torriani (19). The substrate was *p*-nitrophenol phosphate, disodium tetrahydrate (Sigma). Neutral esterase activity was assayed with the substrate *N*-CBZ-L-tyrosine *p*-nitrophenyl ester. One hundred μl of 5 m*M* substrate in acetonitrile was added to 1 ml of lysate. Samples were incubated for 20 min at 37°. The reactions were stopped by the addition of 2.0 ml of 0.25% SDS buffered at pH 7.5 with 0.02 *M* tris buffer. Color was read at 410 nm.

Acid ribonuclease was assayed by the method of McDonald (20). Cytoplasmic (free and lysosomal) lysates were incubated with the substrate (yeast RNA) for 1 hr. Nuclear levels of the enzyme were sufficiently low to require a 2-hr incubation. Acid-soluble nucleotides were determined spectrophotometrically at 260 nm.

Results. Ratios of free to bound acid hydrolase were compared in the post nuclear supernatants from rats following partial

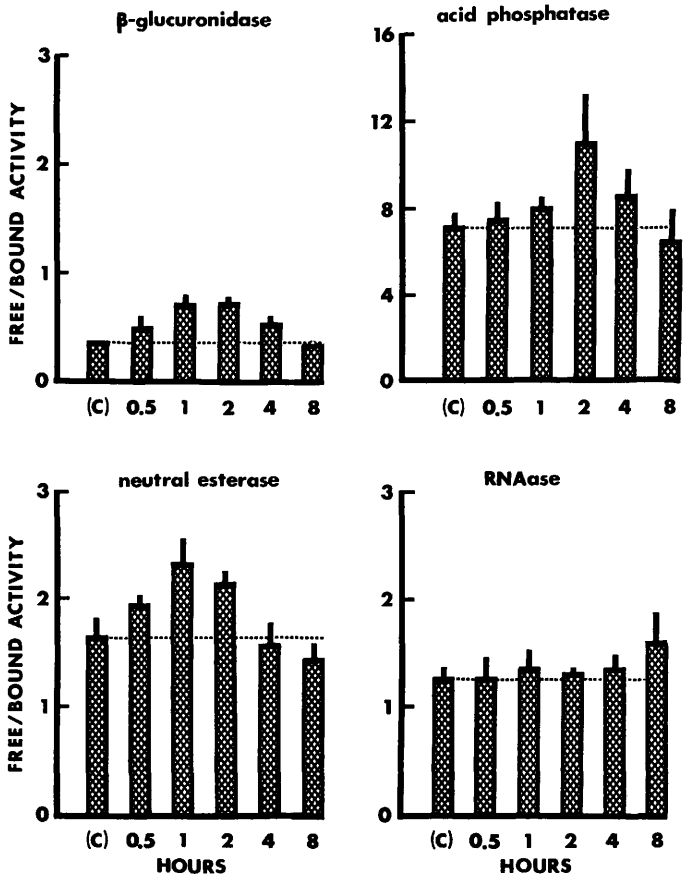

FIG. 1. The ratio of hydrolase activity in cytoplasmic fractions II and III following 70% hepatectomy. One milliliter of lysed fraction was used for each enzyme assay. Total activity (Fractions II + III) for a typical control were as follows: (in μg/equivalents of substrate hydrolyzed/10 min) RNAase, 122; β-glucuronidase, 190; acid phosphatase, 135; neutral esterase, 210. Blanks for RNAase were 30–40% of O.D. For the other enzymes blanks were less than 5%. C = control. Dashed line = 30% hepatectomized. S.E.M. are presented.

hepatectomy (70%, 30%) and from sham-operated controls. The results for various times from 30 min to 8 hr are shown in Fig. 1. An increase is seen for all free hydrolases except RNAase as early as 30 min following 70% hepatectomy but not in the 30% hepatectomized or sham-operated animals. The greatest increases in free activity were in β-glucuronidase and in neutral esterase. A significant increase in acid phosphatase activity was observed at 2 hr. The activities that increased to a peak by 1 or 2 hr had returned to a normal ratio of free/bound by 4 hr. RNAase showed only a slight increase by 2 hr but a significant increase at 8 hr. The total cytoplasmic hydrolase activity decreased less than 10% ($p < 0.1$) and must not be considered significant. Thus any increase in free activity is reflected in a decrease in bound activity.

The relative specific activity (RSA) of acid nuclear hydrolase was based on the weight of nuclear DNA. Figure 2 shows a slight but significant ($p < 0.05$) increase of all the hydrolases at 30 min following a 70% hepatectomy except RNAase. Those levels were significantly increased by 1 hr. β-Glucuronidase showed the greatest increase (3.4 $\times$ control levels) at 2 hr. Nuclear β-glucuronidase was significantly below control level at 8 hr. RNAase and acid phosphatase were still slightly elevated. The other hydrolases were at the same level in the hepatectomized and sham-operated controls at 8

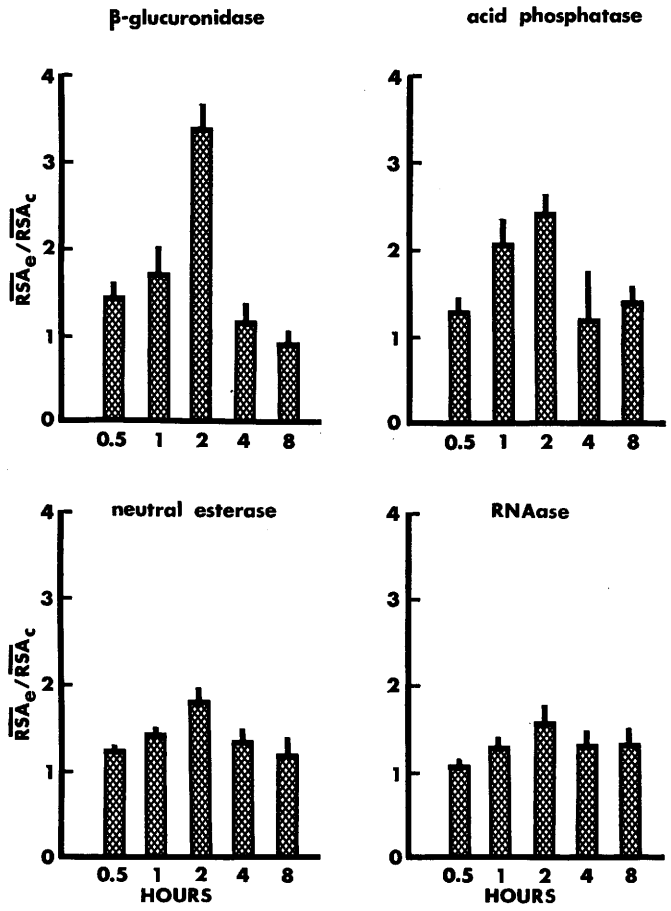

FIG. 2. The ratio of relative specific activity of hydrolases in the nucleus of 70% hepatectomized (RSA_e) and control (RSA_c) rats. The RSA was calculated on a DNA weight basis. One milliliter of nuclear lysate (total = 10 ml) used for each assay. DNA per milliliter lysate = 80–100 μg. Hydrolase activities were between 3.5–6.5% of the total cellular activity (Fractions I + II + III).

hr. The association of lysosomes to nuclei from hepatectomized and sham operated controls could not be demonstrated microscopically with acridine orange. Organelles loosely bound to the surface of the nucleus would have been removed by hydrodynamic shear during the sedimentation through dense sucrose at 41,000*g*.

Discussion. This study has shown that 70% hepatectomy leads to a rapid increase in the free hydrolase activity in the cytoplasm followed by a greater increase in the hydrolases associated with the nucleus. The increase in free cytoplasmic activity must be associated with a proportional decrease in the bound activity since no significant decrease was demonstrated for the combined Fractions I + II. The free cytoplasmic activity reaches a peak in approximately 1 hr. The nuclear hydrolases peak in 2 hr. Other investigators have shown that hydrolase activity increases in the cytoplasm prior to DNA synthesis (7, 12, 14). Szego (13) has shown that estrogen treatment produces changes in the lysosomes of the uterus as early as 2.5 min and that by 15 min lysosomes appear fused to the nuclear envelope. Similar observations were made by Allison and Mallucci (5) with lymphocytes treated with phytohemagglutinin. In the same study they showed that there was a disappearance of normal lysosomes from the cytoplasm of regenerating liver at 12 hr following partial hepatectomy. The increase in free cytoplasmic hydrolases followed by a decrease is in agreement with previous findings that these organelles disappear following partial hepatectomy (5). The reduction of activity both in the cytoplasm and in the nucleus may be the result of the inactivation or the sequestering of the enzymes. Klockars and Wigelius (12) demonstrated an increase in free cytoplasmic hydrolase activity prior to DNA synthesis and mitosis but found that the increases in specific enzyme activity did not parallel that of other lysosomal hydrolases. These authors concluded that the lysosome organelle does not itself play a specific role in the process of cell division.

Investigators have postulated various roles for lysosomes in the process of cell division. Allison and Paton (21) have shown that uv irradiation of cells leads to lysosomal damage and subsequent chromosome breakage. The hydrolases may activate the priming reactions (8) possibly by causing degradation of repressor molecules (22) or by increasing the amount of precursors for RNA and DNA synthesis (23, 24). DeTerra (25) has suggested that hydrolytic activity may increase nuclear membrane permeability to enhance the nuclear–cytoplasm interaction. This interaction could result in an increased transport of precursors into the nucleus (26) or increased migration of nuclear RNA into the cytoplasm (27). Increased nuclear hydrolytic activity may result in the dispersion of the dense heterochromatin into an active diffuse template. The amount of diffuse chromatin has been shown to increase twofold during liver regeneration (28). In a recent review, Filov *et al.* (29) have proposed that the site of acid hydrolase action is not directed against the nuclear chromatin but against mitochondrial DNA.

Summary. β-Glucuronidase, acid phosphatase, RNAase, and neutral esterase were measured in the cytoplasm and nuclei following partial (30% and 70%) hepatectomy in rats. A significant increase in the free to bound hydrolase level in the cytoplasm was shown for β-glucuronidase, acid phosphatase, and neutral esterase by 2 hr in the 70% hepatectomized but not in 30% hepatectomized or sham operated controls. RNAase increased significantly at 8 hr. In the nucleus, all 4 enzymes increased significantly within one hour and peaked at 2 hr following 70% hepatectomy.

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