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Changes in Lysosomal Enzyme Activities in Rat Liver Following Partial Hepatectomy

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Introduction

Recently, it has been proposed that lysosomes might participate in the cell division[1]. A remarkable fluctuation of lysosomes in the cells which enter into mitosis has been demonstrated morphologically[2~4]. In addition, the changes of lysosomal enzyme activities have been shown biochemically in the regenerating liver[5]. However, the role of lysosomes in the cell division seems to be still a matter of speculation.

On the other hand, no change of acid phosphatase activity has been seen in the regenerating liver[6,7]. It may be considered that the measurement of enzyme activity with a whole liver homogenate is not appropriate for the determination of lysosomal changes in the individual cells which constitute the liver tissue, because the lysosomal changes seem to occur independently in each type of cells after partial hepatectomy.

The present study has been undertaken to examine the changes in lysosomal enzyme activities with the liver tissue and isolated hepatocyte suspension during the regeneration and to elucidate whether lysosomes participate in the cell division. For this purpose, the activities of acid phosphatase, cathepsin, and acid ribonuclease were measured as the function of lysosomes and, in addition, the activity of glucose-6-phosphatase which is associated preferentially with the endoplasmic reticulum of hepatocytes[8,9] was also assayed.

Materials and Methods

Male Wistar albino rats weighing approximately 200 g were used. They were maintained on a standard laboratory diet and water *ad libitum*. Partial hepatectomy was performed by the method of Higgins and Anderson[10]. The animals were starved for 15 h before sacrifice. The operated animals were killed 6, 12, 24, 30, 36, 72 h, and 7 days after the treatment.

The isolation of hepatocytes from the liver tissue was made by the method of Jacob and Bhargava[11]. Both homogenates of the liver tissue (WLH) and hepatocytes (HH) were prepared by the method described elsewhere[12].

For the determination of RNA content and measurement of enzyme release, the nuclear and cytoplasmic fractions and the supernatant fluid were prepared by the method of Novikoff and Heus[13].

Glucose-6-phosphatase (G-6-Pase) activity was assayed by the method of Swanson[14]. The activity of acid phosphatase (acid Pase) was measured by the method of Wattiaux and De Duve[16] using β-glycerophosphate as a substrate. Cathepsin activity was determined according to the method of Gianetto and De Duve[16]. Acid ribonuclease (RNAase)

activity was estimated by the method of De Duve *et al.*[17]. To obtain the total activity of each lysosomal enzyme, Triton X-100 was added into each reaction medium. Other details concerning the assays of enzyme activities are given elsewhere[12].

The protein content was determined according to Lowry *et al.*[18] using bovine serum albumin as a standard.

According to the method of Schneider[19], RNA was extracted from the nuclear and cytoplasmic fractions, and the supernatant fluid, and then the RNA content was estimated. RNA value was expressed in terms of phosphorus content.

Results

Changes of Liver Weight and Enzyme Activities in the Whole Liver after Partial Hepatectomy

Table 1 *Increase in Weight and Enzyme Activities in the Liver after Partial Hepatectomy*

Time after partial hepatectomy (h)	Total liver weight (g)	Activity per g tissue (μmoles Pi/10 min)		Activity in total liver (μmoles Pi/10 min)	
		G-6-Pase	Acid Pase	G-6-Pase	Acid Pase
0	2.9±0.24 (30)	83.2± 8.0 (12)	53.6± 2.7 (12)	241	155
6	3.1±0.26 (6)	76.0± 7.2 (6)	53.2± 6.5 (6)	236	165
12	3.2±0.11 (6)	80.5± 5.5 (6)	54.0± 8.4 (8)	262	175
24	3.7±0.25 (7)	61.2±10.3 (7)	54.4±10.6 (7)	228	202
30	4.2±0.45 (8)	55.4± 4.9 (8)	37.6± 4.6 (7)	232	157
36	4.5±0.27 (9)	66.4±10.1 (6)	47.2± 5.6 (5)	299	213
72	6.6±0.88 (8)	65.6± 7.1 (8)	52.5± 9.2 (7)	434	347
168	8.8±0.32 (5)	67.1± 3.4 (4)	57.2±13.9 (4)	594	506

Results are given as mean values ± S.E.M.
Figures in parentheses indicate number of animals.
The weight of liver lobes remaining after partial hepatectomy was calculated as one-half the weight of removed liver lobes, and also corrected to the liver weight of a rat weighing 200 g.

Table 1 shows the recovery of liver weight and enzyme activities in the remaining liver. After removal of about 67% of liver, the weight of remaining liver lobes increased rapidly and the original weight was recovered almost completely by the 7th day.

G-6-Pase activity in the whole liver did not increase until 30 h after the operation in spite of an increase in liver weight and then increased gradually over 7 days at a similar rate as that of the recovery of liver weight. Acid Pase activity elevated 30% after 24 h, dropped at 30th h, and then increased like G-6-Pase activity.

Changes of Enzyme Activities per Protein of Liver

The enzyme activities in the liver tissue (WLH), expressed per mg protein of WLH, are shown in Table 2, G-6-Pase activity decreased 25% and about 10% after 24 and 36 h, respectively, and the latter activity was continued for 7 days. On the other hand, the

activities of lysosomal enzymes, all revealing similar tendency, rose within 24 h, declined by 30th h, and then increased again after 36 h.

Table 2 *Enzyme Activities in Liver Tissue Homogenate (WLH) after Partial Hepatectomy*

Time after partial hepatectomy (h)	Activity/mg protein /10 min			
	G-6-Pase a	Acid Pase a	Cathepsin b	RNAase c
0	397 ± 43.8 (7)	253 ± 25.3 (6)	30.8 ± 1.20 (6)	1.96 ± 0.49 (8)
12	393 ± 38.4 (6)	282 ± 29.3 (6)	39.9 ± 5.60 (6)	2.38 ± 0.63 (6)
24	304 ± 30.8 (6)	292 ± 28.7 (6)	42.3 ± 6.98 (6)	2.70 ± 0.40 (6)
30	318 ± 45.4 (6)	218 ± 28.5 (7)	36.6 ± 4.35 (8)	2.48 ± 0.74 (8)
36	362 ± 18.6 (4)	268 ± 34.2 (4)	45.2 ± 5.92 (4)	2.58 ± 0.68 (4)
72	359 ± 27.3 (4)	288 ± 35.0 (6)	35.4 ± 6.52 (6)	2.43 ± 0.37 (6)
168	325 ± 17.5 (4)	260 ± 27.7 (4)	32.4 ± 1.53 (4)	1.89 ± 0.19 (4)

Results are given as mean values ± S.E.M.
Figures in parentheses indicate number of animals.
a, mμmoles of Pi liberated; b, mμmoles of tyrosine liberated; c, absorbancy at 260 mμ.

Changes of Enzyme activities in Hepatocyte Suspension

Table 3 indicates the enzyme activities in hepatocyte homogenate (HH). Each activity is expressed per mg protein. G-6-Pase activity did not change for 30 h, elevated 20% over the normal level after 36 h, and then declined gradually.

In the premitotic stage before 30th h, lysosomal enzyme activities increased markedly and the activity peaks of acid Pase, cathepsin, and RNAase were at 24. 12, and 24 h, respectively. After 30 h, the second peaks of acid Pase activity and of cathepsin activity occured together at 36 h, while the activity of RNAase did not show the second elevation and declined from 24 h.

Table 3 *Enzyme Activities in Hepatocyte Homogenate (HH) after Partial Hepatectomy*

Time after partial hepatectomy (h)	Activity/mg protein/10 min			
	G-6-Pase a	Acid Pase a	Cathepsin b	RNAase c
0	623 ± 50.4 (6)	317 ± 45.8 (6)	38.6 ± 6.47 (6)	2.22 ± 0.54 (6)
12	639 ± 39.6 (6)	358 ± 57.0 (6)	48.8 ± 6.43 (6)	3.01 ± 0.89 (6)
24	627 ± 47.8 (6)	392 ± 71.4 (6)	44.7 ± 6.05 (6)	3.73 ± 0.28 (6)
30	636 ± 51.7 (6)	333 ± 53.7 (6)	54.3 ± 5.91 (6)	2.94 ± 0.36 (6)
36	737 ± 47.2 (4)	389 ± 36.5 (4)	56.6 ± 13.08 (4)	2.42 ± 0.36 (4)
72	682 ± 52.5 (4)	363 ± 30.8 (4)	53.2 ± 12.15 (4)	2.35 ± 0.58 (4)
168	573 ± 85.8 (4)	345 ± 46.7 (4)	49.6 ± 14.80 (4)	1.89 ± 0.97 (4)

Results are given as mean values ± S.E.M.
Figures in parentheses indicate number of animals.
a, mμmoles of Pi liberated; b, mμmoles of tyrosine liberated; c, absorbancy at 260 mμ.

Enzyme Activities and Protein Content per Number of Hepatocytes

Enzyme activities and protein content were estimated per 10^6 hepatocytes using the suspension of isolated hepatocytes as shown in Fig. 1. RNAase activity increased significantly, revealing two peaks at 12 and around 30 h, while the activities of other enzymes showed a small peak at 12 h and a marked increase after 24 h. The protein content in isolated hepatocytes decreased for 24 h and then increased up to 3 days. and again declined below normal at the 7th day.

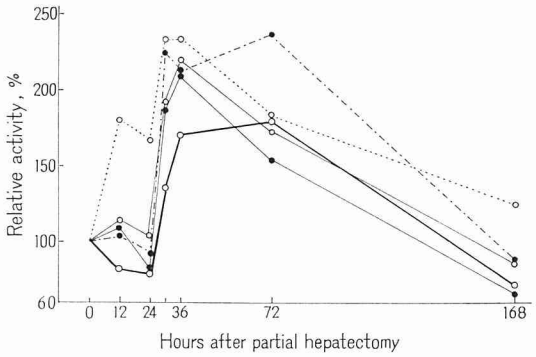

Fig. 1 Changes of enzyme activities and protein content in hepatocyte per cell number. Each point is expressed as percentage of normal value. Normal values per 10^6 cells were as follows. Protein, 2.61 mg; G-6-Pase, 1510 mμ moles Pi/10 min; acid Pase, 770 mμ moles Pi/10 min; cathepsin, 98 mμ moles tyrosine liberated /10 min; RNAase activity (absorbancy at 260 mμ/10 min), 4.5. All values represent the average of four animals. ●—●, G-6-Pase; ○—○, acid Pase; ●-•-•-●, cathepsin; ○- - -○, RNAase; ○—○, protein.

At 30, 36, and 72 h after hepatectomy, the increase in the enzyme activities of hepatocytes was not proportional to the increase in protein content. On the other hand, the hepatocytes obtained from the liver at 1 week after the operation contained less protein and less enzyme activities than those of the normal liver.

Release of Lysosomal Enzymes and Changes in RNA Content

The effect of hepatectomy on the release of lysosomal enzymes at various time after the operation is shown in Fig. 2, using the sham-operated rat liver as a control. In this study, the lysosomal enzyme activities in the supernatant solution from WLH were measured.

Acid Pase activity in the supernatant of hepatectomized rat liver increased slightly at 6th h, and after return to normal level at 12 h, it increased again by 18% at 24 th h. The releases of acid Pase of both the hepatectomized and sham-operated rat livers were almost of the same degree up to 12 h. The release of RNAase from the hepatectomized rats increased 17% and 27% at 6 and 12 h, respectively, and decreased gradually thereafter. As for the RNAase activity in the sham-operated rat liver, there was a slight rise of the supernatant activity at 12 h (Fig. 2).

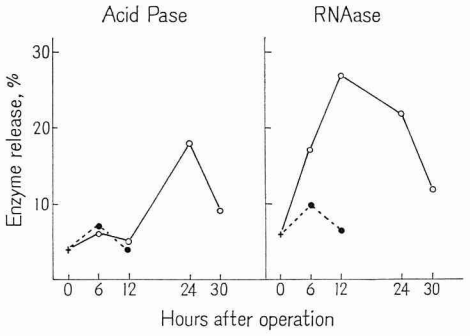

Fig. 2 Release of lysosomal enzymes into the cytoplasm of hepatectomized and sham-operated rat liver. Each point is expressed as follows: (the enzyme activity in supernatant fluid)/(the total enzyme activity in liver homogenate) × 100. All values represent the average of two animals.
+, normal; ○—○, hepatectomized; ●- - -●, sham-operated liver.

Total RNA content in the sham-operated rat liver was the same as that in the normal, whereas in the hepatectomized one it increased by 24th h and then recovered to the normal level at 30th h. RNA content in the nuclear fraction was not changed by sham-operation, while it increased slightly at 30 h after operation in the hepatectomized rat liver. On the other hand, the cytoplasmic RNA content in the hepatectomized rat liver decreased by 20% at 24 h, and returned toward normal at 30 h (Fig. 3).

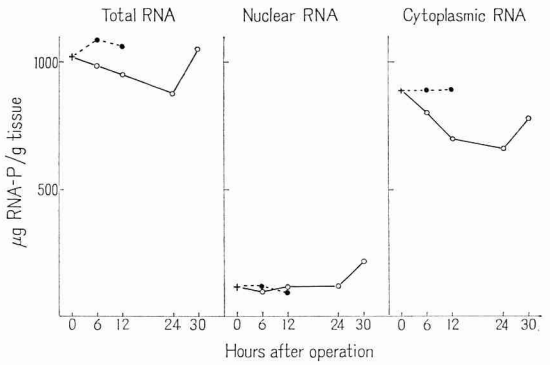

Fig. 3 RNA content in hepatectomized and sham-operated rat liver. RNA value is expressed as RNA-P μg/g tissue. The same samples as the experiments in Fig. 2 were used. +, normal; ○—○, hepatectomized; ●- - -●, sham-operated liver.

Discussion

The discussion below referes to the discrepancies between the changes in the enzyme activities and protein content in the liver tissue and isolated hepatocyte suspension.

Changes of Enzyme Activities in Premitotic Stage (12–24 h after Partial Hepatectomy)

Since no change in cell number and in cell population occurs in the premitotic

stage, it seems that an increase in liver weight is due to the enlargement of individual hepatocytes. Owing to the decrease in protein content of hepatocytes, the increase in cell size may be ascribed mainly to fatty accumulation[20]. During premitotic satge, G-6-Pase activity per hepatocyte showed almost no change, whereas the activity of lysosomal enzymes, especially RNAase activity, increased. As shown in Fig. 2 the lysosomal enzymes were released into cytoplasm from lysosomes. A marked release of RNAase seems to be related to the decrease of cytoplasmic RNA which consists of ribosomal RNA.

Changes of Enzyme Activities after Mitotic Stage

It has been known that a mitotic peak of parenchymal cells occurs around 28 h and the non-parenchymal cells enter into cell division on the second day after hepatectomy[21]. Therefore, the difference in fluctuation pattern of the enzyme activities in this stage between in WLH and HH may be explained by the consideration that the activities of enzymes in the hepatocytes fluctuate independently of those of the non-hepatocytes in the tissue.

The activity of G-6-Pase, which is localizd exclusively in the hepatocytes[9], decreased in the WLH, while it remained at a normal level for 30 h in the HH. This discrepancy may be explained by the assumption that the immature cells appearing after cell division possess a reduced G-6-Pase activity and/or are too fragile to be isolated from the liver by the method of Jacob and Bhargava[11] because of mechanical disruption. The hepatocyte suspension obtained one week after hepatectomy appears to consist mainly of small hepatocytes which are presumably in the process of maturation. Examination of Giemsa-stained specimen showed that the hepatocyte suspension at this stage contained many small-sized hepatocytes which mostly had a diploid nucleus.

Further experiments are necessary for the isolation of hepatocytes from the liver in regenerating process.

Lysosomes in Regenerating Process

It was observed that in the early stage after partial hepatectomy the lysosomal enzyme activities elevated and were released into the cytoplasm. If lysosomes participate in the cell division, it may be considered that the disturbed state in the cytoplasm resulting from the action of lysosomal enzymes would produce a favorable condition for mitosis. In the present study, acid RNAase appeared to be more activated than other lysosomal enzymes, although it has been found that alkaline RNAase decreases in the premitotic stage of the regenerating liver[22].

It has been suggested that the lysosomes are not responsible for the liver regeneration in the early stage after partial hepatectomy, from the fact that there is no change in enzyme activities[6,7]. The disagreement between these and our results seems to be due to the difference of the methods used for biochemical determination; the former had assayed enzyme activity only on the tissue (WLH) and the latter more on the isolated hepatocytes. The significance of the isolation of hepatocytes from the liver for the measurement of lysosomal enzyme activities in the regenerating parenchymal cells should be pointed out because there is a change in cell population and an activation of lysosomal enzymes occurs independently in hepatocytes and in non-hepatocytes at various times

after hepatectomy.

Many studies have been presented on the synthesis of DNA, RNA, protein, and other substances in the regenerating rat liver after partial hepatectomy as reviewed by Bucher[23]. As regards the synthesis of RNA and protein in the premitotic stage, an increase in the incorporation rate of isotopically labeled precursors into RNA was observed by 6th h and a net increase in RNA was seen by 24th h, whereas an increment of protein was not clearly detectable until 12 h and the most rapid rate of increase occurred thereafter until around 36 h. On the other hand, our observation showed RNAase activity to increase after 6 h, whereas a peak of cathepsin activity was around 12 h. It seems likely that the increase of RNAase activity precedes RNA synthesis and the increase of cathepsin activity precedes protein synthesis, and it is suggested that the increase in these activities would be responsible for the increase in concentration of precursors for RNA and protein synthesis. As for acid DNAase, Adams[5] has been proposed such a suggestion from the fact that DNAase activity rose before maximal DNA synthesis. Considering the substrate specificity, the increase of acid Pase activity may offer precursors available for the synthesis of nucleic acid, carbohydrate, or lipid. On the other hand, it has been indicated that the Kupffer cells are activated at around 48 h after hepatectomy and keep the activated state for a long time[24]. From this fact, it appears that the increase of cathepsin activity shown in WLH 2 days after hepatectomy is related to the activation of Kupffer cells in the liver.

In view of the above results, it is concluded that in hepatocytes the lysosomal enzymes respond first to a surgical stress for 6–12 h after hepatectomy and, in the next stage (12–24 h), the enzymes are activated to make a favorable condition for cell division, and after mitosis the enzymes are activated again during cell growth. On the other hand, it seems probable that in non-hepatocytes, the lysosomal enzymes respond to stress in the early stage as those in hepatocyte, and then are activated again in the premitotic stage of the Kupffer cells (2–3 days) as shown in cathepsin activity, which continues to such later stage as the function of Kupffer cells remains in an activated level. Accordingly, it would be conceivable that the lysosomes in both the cell types participate in the regeneration of liver and the activity of different lysosomal enzymes changes independently.

Summary

The changes in lysosomal enzyme activities in rat liver after partial hepatectomy were studied with the whole liver and isolated hepatocyte suspension.

The activity patterns of enzymes were different between the stages before and after mitosis, and also between the two cell types of hepatocytes and non-hepatocytes in the liver.

Increase of enzyme activities, especially of acid ribonuclease, in the premitotic stage suggests that the lysosomes may participate in the cell division during liver regeneration.

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