Endocytosis of Serum Albumin in Regenerating Rat Liver (44126)

HO YUL RYOO, MASAKI TAGA, TOSHIHIRO SASSA, TATSUZO OKA, AND YASUO NATORI[1]

Department of Nutritional Chemistry, School of Medicine, University of Tokushima, Tokushima 770, Japan

Abstract. **Lysosomes, isolated from rat liver after 70% partial hepatectomy (PHX), were found, by Western blotting, to contain a considerable amount of serum albumin. The level of intralysosomal serum albumin after PHX showed biphasic patterns: it increased immediately after PHX, peaked at 30 min, rapidly declined within a few hours, rose again with a peak at 15 hr, and gradually declined thereafter. At 15 hr after PHX, the content of lysosomal proteins in the liver increased to twice the level of unoperated control, and the electron-microscopic observation of the isolated lysosomes revealed numerous large membrane-delimited structures with ground substances of variable electron opacities. The increase in the intralysosomal serum albumin at 30 min and 15 hr was accompanied by changes in the buoyant densities of endosomes in Percoll density gradients. At both time points, the density profiles of endosomes isolated from hepatectomized rats shifted to the denser direction, suggesting that PHX activates fusion and/or maturation of endosomes. Formaldehyde-treated bovine serum albumin is known to be taken up by the liver by receptor-mediated endocytosis. The uptake of the modified heterologous albumin was shown to be activated as early as 30 min after PHX. Both the uptake of serum albumin into lysosomes and the shift of buoyant density profile of endosomes after PHX were inhibited by the administration of adrenergic receptor antagonists, particularly by the α_1-antagonist prazosin. Further, the concentration of catecholamines in rat serum, particularly that of norepinephrine, was found to increase immediately after PHX, relative to that in serum from sham-operated rats. These results suggest that the elevation of serum norepinephrine levels after PHX activates endocytosis and facilitates delivery of endocytosed serum albumin to lysosomes, where albumin is digested to yield amino acids for possible use in protein synthesis during liver regeneration.** [P.S.E.B.M. 1997, Vol 215]

The regrowth of the liver after 70% partial hepatectomy (PHX) in the rat is remarkably rapid, attaining its original size by 7–10 days. The molecular mechanism of liver regeneration has been the subject of intensive studies for many years, and most of the recent studies have been concerned with the role of various growth factors in the regulation of hepatic growth (1, 2). The liver regeneration, in terms of protein metabolism, represents an increase in the protein mass in the liver. However, few studies have been concerned with the problem of the source of amino acids for protein synthesis during the hepatic growth. The results of the present study suggest that, during regeneration, serum albumin is actively taken up into liver cells by endocytosis and is delivered to the lysosomal compartment where albumin is digested to yield amino acids for possible use in protein synthesis. We also present evidence that norepinephrine may play a role in the enhancement of endocytosis after PHX.

Materials and Methods

Animals. Male Wistar rats, weighing 210–250 g, were fasted for 24 hr and subjected to two-thirds PHX under light ether anesthesia according to the method of Higgins and Anderson (3). Sham operations consisted of identical abdominal incisions and gentle manipulation of the liver lobes before closure. Food was restored after surgery. For adrenergic blockade experiments, rats were injected ip with prazosin or propranolol (Sigma Chemical Co., St. Louis, MO) (1.5 mg/kg body wt) and PHX was performed 1 hr later. For the endocytosis experiment with modified serum

[1] To whom requests for reprints should be addressed at Department of Nutritional Chemistry, School of Medicine, University of Tokushima, Kuramoto, Tokushima 770, Japan.

Received September 3, 1996. [P.S.E.B.M. 1997, Vol 215]
Accepted December 30, 1996.

0037-9727/97/2152-0179$10.50/0

albumin, formaldehyde-treated albumin was prepared from bovine serum albumin by the method of Horiuchi *et al.* (4). The modified albumin (80 mg/kg body wt) was injected 5 min before PHX, and lysosomes were isolated from the liver 30 min after PHX.

Isolation of Lysosomes. Highly purified lysosomes were isolated from the livers by the method developed in our laboratory (5). The isolated lysosomes were not contaminated with mitochondria, as evidenced by the absence of activity of a mitochondrial marker enzyme (succinic-INT reductase).

Isolation of Endosomes. Endosomes were isolated from a low-density fraction of liver homogenates by modifying the method described by Belcher *et al.* (6). Briefly, the livers were homogenized in three volumes of ice-cold SI buffer (250 m*M* sucrose and 3 m*M* imidazole, pH 7.4). Homogenates were centrifuged at 1000*g* for 10 min, and supernatants were centrifuged again at 10000*g* for 20 min. Four-milliliter portions of the supernatants were mixed with 24 ml of 31.5% Percoll (Pharmacia Biotech AB, Uppsala, Sweden) in SI buffer to give a final concentration of 27% Percoll, and centrifuged in a Hitachi RP50T rotor for 2 hr at 25000*g*. Density profiles of the Percoll gradients were determined by density calibration beads in the range of 1.035–1.149. After the centrifugation, 2-ml fractions were collected from the bottom by pumping. After turbidity determination at 600 nm, each fraction was mixed with two volumes of ice-cold 0.15 *M* NaCl, and the fractions were layered onto 2 ml of 2.5 *M* sucrose. The tubes were centrifuged at 17800*g* in a Hitachi RPR18B rotor for 1 hr. The white endosome bands were removed from the sucrose cushions in a volume of 1.5 ml/tube and diluted with five volumes of ice-cold 0.15 *M* NaCl. The suspensions were centrifuged to pellet the endosomes.

Estimation of Lysosomal Protein Content. The lysosomal protein content in the liver after PHX was estimated by measuring the specific activities of arylsulfatase in the liver homogenates and isolated lysosomes as described previously (7).

Western Blot Analysis. Electrophoresis of the lysosomal and endosomal proteins and Western blot analysis were carried out as described previously (7) using antiserum against rat serum albumin or bovine serum albumin.

Catecholamine Assay. Blood samples were withdrawn from orbital sinus of the operated rats under light ether anesthesia and the concentrations of epinephrine and norepinephrine in the serum were assayed by high-pressure liquid chromatography using electrochemical detection as described (8).

Electron Microscopy. Electron-microscopic examination of the isolated lysosomes was performed as described previously (5).

Results

Content of Lysosomal Proteins in the Liver and Concentration of Serum Albumin in Lysosomes. Inasmuch as lysosomes are considered to be the main site of intracellular protein degradation (9, 10), we first checked if PHX would change the protein content in the lysosomal compartment in the remaining liver cells. The lysosomal protein contents were estimated by assaying the specific activities of arylsulfatase, a lysosomal marker enzyme, in the liver homogenate and isolated lysosomes (7). As shown in Table 1, the lysosomal protein content began to increase as early as 30 min after PHX and almost doubled by 15 hr after PHX.

We next examined if the increase in the lysosomal protein content is, at least in part, ascribable to engulfment of serum albumin into lysosomes. Western blot assays of the lysosomal proteins with the use of the antiserum against rat serum albumin showed that a remarkable amount of serum albumin was found in the lysosomes as early as 30 min after PHX. However, the level of serum albumin in the lysosomes was rapidly decreased during the few hours subsequent (Fig. 1A). Serum albumin content in the lysosomes began to rise again several hours after PHX, attaining the maximal value at 15 hr, and gradually declined during the next several days (Fig. 1B).

Morphological Change of Lysosomes after Partial Hepatectomy. In view of the changes in the lysosomal protein contents in the liver of hepatectomized rats, we isolated lysosomes from the liver at various times after

Table I. Estimation of the Lysosomal Protein Contents in the Control and Partially Hepatectomized Livers

	Specific activity of arylsulfatase (units[a]/g protein)		Relative specific activity (lysosome/homogenate)	Lysosomal protein content[b] (mg/g liver protein)
	Homogenate	Lysosome		
Control	44.3 ± 6.7	3520 ± 755	79.3 ± 10	12.3 ± 2.4
PHX (0.5 hr)	51.6 ± 7.4	2830 ± 680	55.1 ± 11[c]	18.8 ± 3.9[c]
PHX (15 hr)	52.0 ± 8.1	2660 ± 1320	49.1 ± 20[d]	24.0 ± 10.8[c]

Note. Values are mean ± SD of eight animals.
[a] Units of enzyme activity are defined as the amount of enzyme causing the transformation of 1 μmol of substrate per min under the condition of the assay.
[b] mg Lysosomal Protein/g Liver Protein = Enzyme Units/g Liver Homogenate Protein/Enzyme Units/mg Lysosomal Protein.
[c] $P < 0.01$.
[d] $P < 0.001$ versus control.

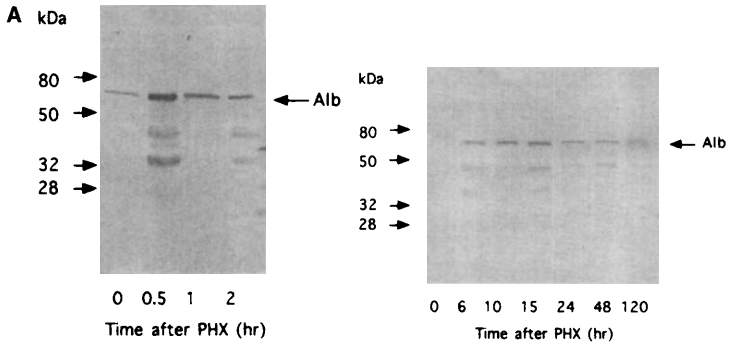

Figure 1. Western blot analysis of intralysosomal serum albumin in the livers of partially hepatectomized rats. Lysosomal proteins (36 μg) from the livers of rats 0–2 hr (A) or 0–120 hr (B) after PHX, were subjected to SDS-polyacrylamide gel electrophoresis and immunoblotted with antiserum against rat serum albumin. Numbers on the left represent the positions of molecular weight markers in kilodaltons. Alb, the position of authentic rat serum albumin.

PHX for electron microscopic examination. As seen in Figure 2, the general features of lysosomes did not change within 30 min after PHX. However, PHX induced profound changes in the morphology of lysosomes at 15 hr after the operation. Compared with unoperated control, the lysosomes from the liver at 15 hr after PHX contained numerous large membrane-delimited structures with ground substances of variable electron opacities.

Effect of Adrenergic Blockers on Accumulation of Serum Albumin in Lysosomes. Since adrenergic hormones have been implicated in the regulation of liver regenerative growth following PHX (for a review see Ref. 11), the effect of adrenoreceptor blockade on the uptake of serum albumin into lysosomes was investigated. Rats, injected ip with prazosin (α_1-adrenergic receptor antagonist) or propranolol (β-adrenergic receptor antagonist) 1 hr before PHX, showed a marked reduction in the serum albumin uptake into lysosomes during 1 hr after PHX. The effect of prazosin was considerably greater than that of propranolol (Fig. 3).

Serum Catecholamine Levels after Partial Hepatectomy. The observation of the inhibitory effect of adrenergic blockers on the accumulation of serum albumin in lysosomes has prompted us to measure the level of catecholamines in sera from animals subjected to either PHX or sham operations. Both epinephrine and norepinephrine levels began to increase immediately after surgery; the elevation could be observed within 1 min after PHX. However, the time courses of the elevation of epinephrine and norepinephrine levels were somewhat different. During the initial 15 min after PHX, the rise in epinephrine levels in the hepatectomized and sham-operated rats essentially followed the same course. By contrast, the level of norepinephrine in the serum from hepatectomized rats was clearly higher than that from the sham-operated rats at all times measured (Fig. 4).

Change in Buoyant Density of Endosomes after Partial Hepatectomy: Effect of Adrenergic Blocker. Endosomes are generated when segments of hepatic cell membrane invaginate, enclosing a minute volume of serum and its contents. Most endosomes fuse with primary lysosomes to form secondary lysosomes. Since PHX appears to enhance the delivery of serum albumin to lysosomes by way of endocytosis, we determined the buoyant density of endosomes isolated from the liver of hepatectomized rats on 27% Percoll gradients. The buoyant density profiles of endosomal fractions, obtained from the liver at 30 min post-PHX, are shown in Figure 5A. The endosomal fraction from the liver at 30 min after PHX showed a small but reproducible increase in the buoyant density, compared with that from the sham-operated rats. Furthermore, the treatment of rats with prazosin 1 hr before PHX prevented the increase in the densities. Lysosomes, contaminated in the endosomal fraction, were localized in the high-density fractions (fractions 1–3 in Fig. 5A, with the density around 1.100) on the basis of the activity of the lysosomal marker enzyme, β-galactosidase (data not shown). The effect of PHX on the buoyant density profiles of endosomes from the liver at 15 hr after PHX is shown in Figure 5B. PHX induced a marked shift toward higher density compared with the sham-operated control. Prazosin administration again clearly prevented the shift. One may notice the difference in

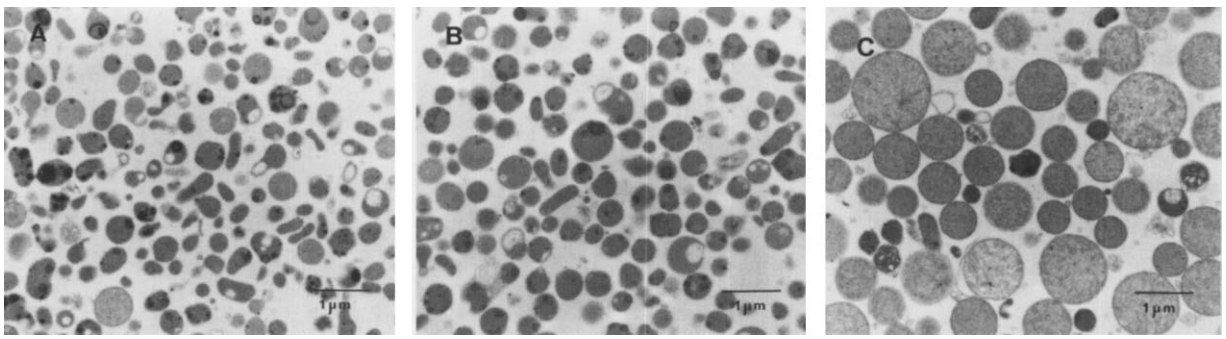

Figure 2. Electron micrographs of lysosomes isolated from the livers of rats at 0 (A), 30 min (B), or 15 hr (C) after PHX. Bar, 1.0 μm.

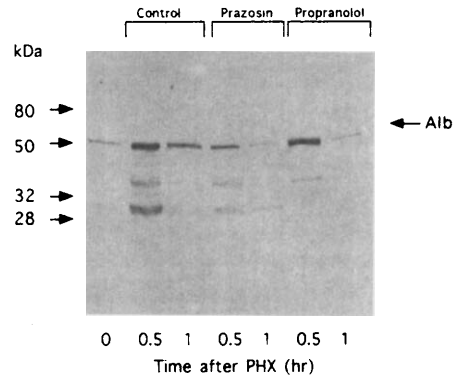

Figure 3. Effect of prazosin and propranolol on the intralysosomal uptake of serum albumin in the livers of partially hepatectomized rats. Experimental conditions were the same as in Figure 1.

the buoyant density profiles between the samples from 30 min (Fig. 5A) and 15 hr (Fig. 5B) after PHX. This is because of the difference in the food intake of the two groups of rats; the 30-min group had been fasted for 24 hr at the time of endosome preparation, while the 15-hr group had access to food after PHX. The food intake influences the buoyant density profiles of endosomes from rat liver (Ryoo HY, Natori Y, unpublished observation).

Contents of Serum Albumin in Endosomes.

Since PHX affects the buoyant densities of endosomes, we examined if the protein composition of endosomes might have changed after PHX. Western blot assays of endosomal proteins with the use of the antiserum against rat serum albumin indicated that the proportion of serum albumin in the total endosomal proteins was essentially constant over a wide density range of endosomes and the relative content of serum albumin was not changed at 30 min post-PHX

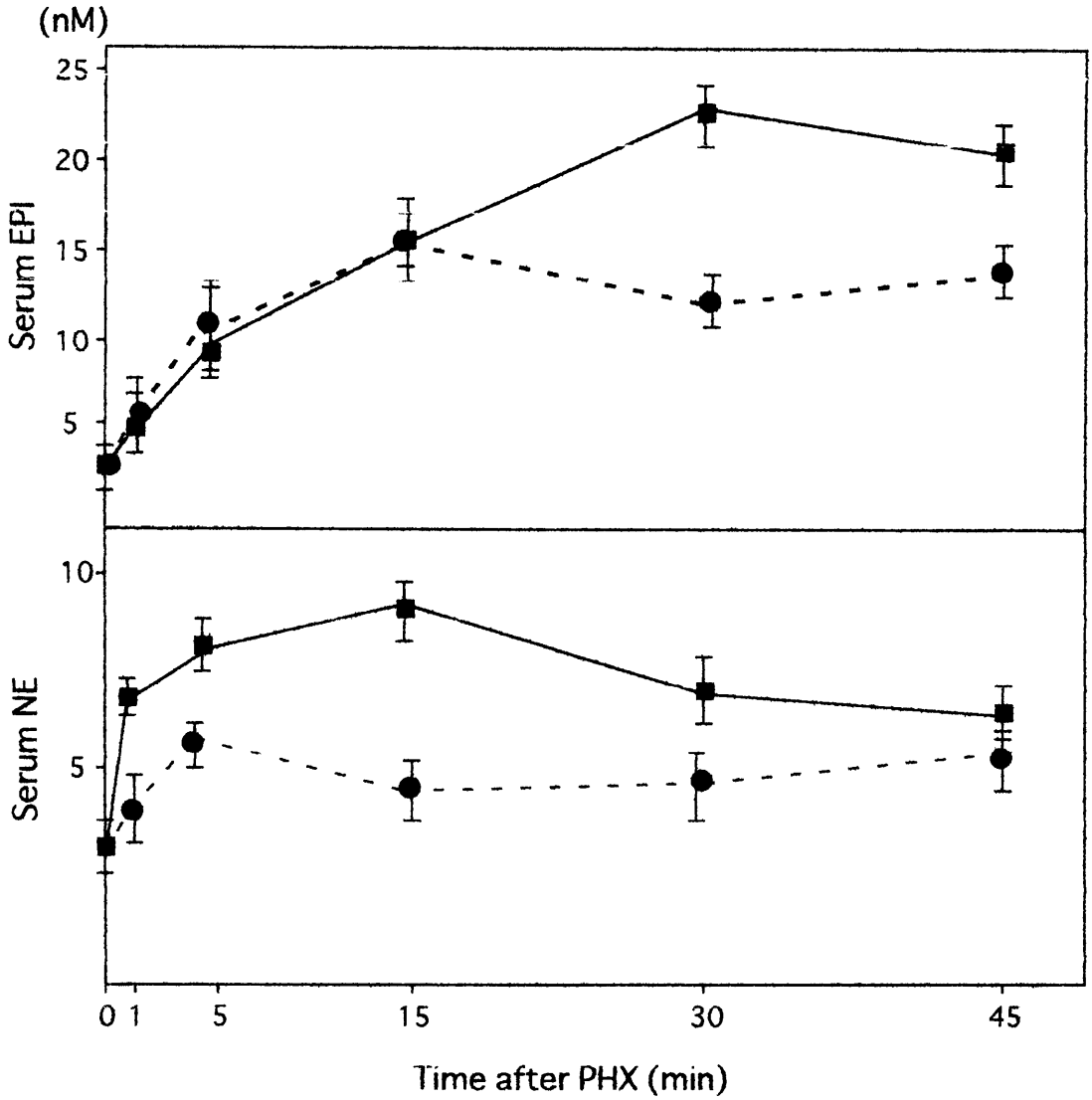

Figure 4. Serum catecholamine levels after PHX. Levels of epinephrine (EPI) (upper panel) or norepinephrine (NE) (lower panel) at various times in sera from rats subjected to PHX (—) or sham operation (----). Values represent means ± SD for five animals in each group.

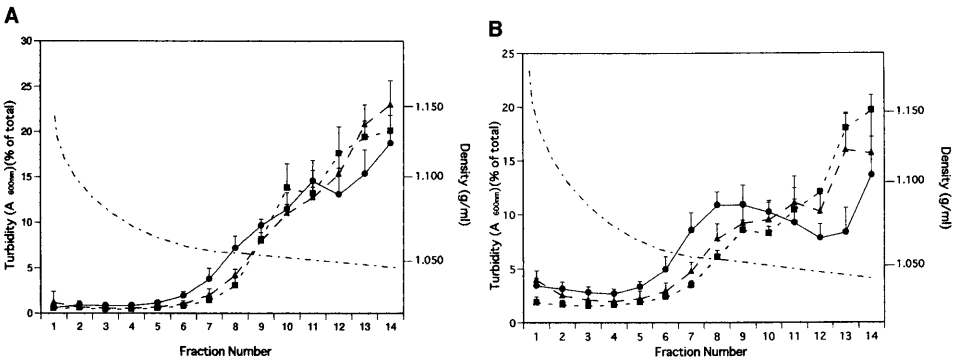

Figure 5. Change in buoyant density of endosomes isolated from the livers of rats at 30 min (A) or 15 hr (B) after PHX. Post-mitochondrial supernatants were prepared and fractionated on 27% Percoll gradients as described in Materials and Methods. To determine the density profiles of endosomes, turbidity at 600 nm was measured for each fraction. Endosomes from the livers of sham-operated (■), partially hepatectomized (●), and prazosin-pretreated/partially hepatectomized rats (▲), respectively. The density of the gradient, determined by density calibration beads, is indicated on the right. Values represent means ± SD for five animals in each group.

(Fig. 6A). A similar picture was obtained for the endosomal proteins at 15 hr post-PHX (Fig. 6B). It was further shown that prazosin treatment did not alter the relative content of serum albumin in the total endosomal proteins (Fig. 6B).

Endocytosis of Formaldehyde-treated Bovine Serum Albumin. Formaldehyde-treated serum albumin is known to be taken up by the liver by receptor-mediated endocytosis (4, 12). We prepared formaldehyde-treated bovine serum albumin, injected it 5 min before PHX, and lysosomes were isolated from the liver 30 min after PHX. Western blotting of the lysosomal proteins with the use of antiserum against bovine serum albumin showed an increased lysosomal uptake of the modified albumin by the hepatectomized rats compared with the sham-operated controls (Fig. 7). This result indicates that receptor-mediated endocytosis is activated by PHX.

Discussion

It has long been known that protein droplets, 20–30 μm in diameter, occur in the hepatocytes of rat liver after PHX (13). The droplets have been shown to develop initially by addition to smaller pinocytotic structures and later by fusion with lysosomes (14). Rat hepatocytes are normally not pinocytotic cells, but they become pinocytotic after PHX. The previous studies on this problem have been mostly morphological, and the biochemical basis for the enhancement of pinocytosis has remained unknown.

In the present study, the lysosomes isolated from the liver at 15 hr after PHX contained numerous large membrane-delimited structures with ground substances of variable electron opacities (Fig. 2). These structures should represent previously described protein droplets. The increase in the lysosomal protein content in the liver at this time point is also compatible with the previous morphological findings. At 30 min post-PHX, the isolated lysosomes appeared essentially the same as the unoperated control although the lysosomal protein content already began to increase (Table I and Fig. 2).

The liver synthesizes and secretes albumin but does not usually take up albumin for degradation. A striking observation in the present study was an abrupt appearance of serum albumin in the lysosomes as early as 30 min after

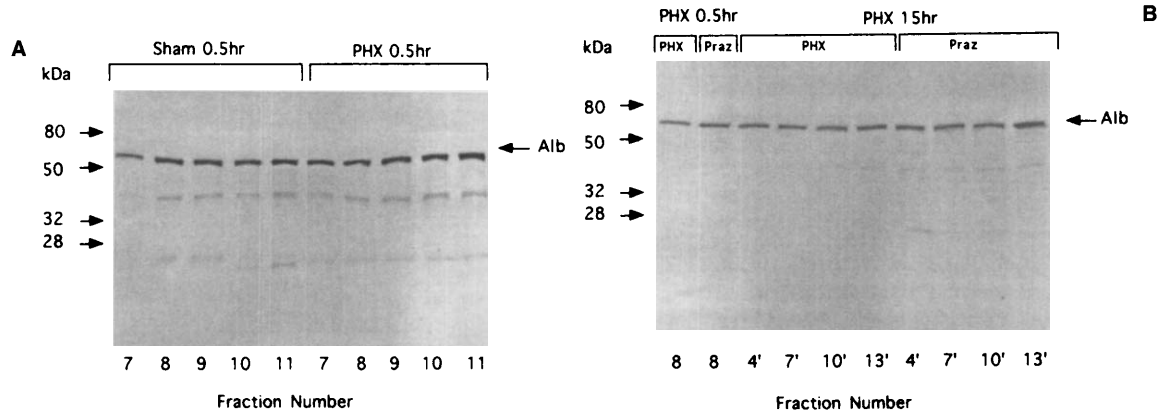

Figure 6. Western blot analysis of intra-endosomal serum albumin in the livers of partially hepatectomized rats. Endosomal proteins (6 μg) from the livers of rats, at 30 min (A) or 15 hr (B) after PHX, were subjected to Western blot analysis as in Figure 1. Fraction numbers without an apostrophe correspond to those in Figure 5A, while numbers with an apostrophe correspond to those in Figure 5B. Prazosin (Praz) was administered as described in Materials and Methods.

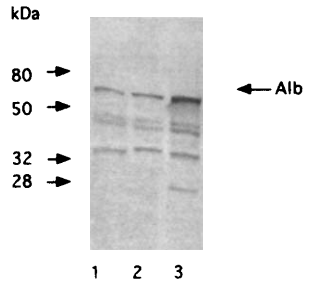

Figure 7. Endocytotic uptake of formaldehyde-treated bovine serum albumin in the livers of partially hepatectomized rats. Formaldehyde-treated bovine serum albumin was prepared and injected into rats as described in Materials and Methods. Lysosomes were isolated from the livers of nonoperated control (Lane 1), the livers from rats pretreated with prazosin and 30 min after PHX (Lane 2), or the livers at 30 min after PHX (Lane 3). The lysosomal proteins were subjected to Western blot analysis as in Figure 1 except that antiserum against bovine serum albumin, instead of antiserum against rat serum albumin, was used.

PHX. The uptake of serum albumin by the liver appears biphasic in the course of liver regeneration: initially (immediate-early phase), the albumin content peaks at 30 min and rapidly declines within a few hours, then several hr later (delayed-early phase) it rises again, peaks at 15 hr, and gradually declines (Fig. 1). A similar biphasic time course has been observed in the expression of a number of cell cycle–regulated genes after PHX (15). The expression of albumin gene, by the way, is transiently induced after PHX, peaking at 6 hr (15).

In the absence of an appropriate marker enzyme for endosomes, we cannot quantitate the amount of endosomes in the liver. However, the increase in the intralysosomal serum albumin levels at 30 min and 15 hr post-PHX was accompanied by changes in the buoyant densities of endosomes in Percoll density gradients (Fig. 5). At both time points, the density profiles of endosomes isolated from hepatectomized rats shifted in the denser direction. It has been proposed that fusion of light vesicles with preexisting dense primary lysosomes mediates the delivery of endocytosed material to dense hydrolytic compartments (16). Recently, the possibility has been raised that the appearance of endocytosed material in dense compartments may involve a maturation event (17). We may suggest that PHX activates fusion and/or maturation of endosomes so that the delivery of endocytosed serum albumin to lysosomes is facilitated. One should note, however, that the PHX-activated endocytosis is not selective for serum albumin. The amount of serum albumin, relative to the total endosomal proteins, was virtually the same for sham-operated and hepatectomized rats (Fig. 6). It thus appears that PHX does not induce selective uptake of serum albumin but rather activates invagination of total serum proteins for delivery to the lysosomal compartment. Although several lines of tumor cells have been reported to bind not only heterologous but also isologous serum albumin on their surface with the aid of the albumin binding protein (18), the presence of isologous albumin-specific binding protein on the surface of liver cells has not been demonstrated.

Direct evidence for the activation of endocytosis immediately after PHX came from the endocytosis of formaldehyde-treated bovine serum albumin (Fig. 7). The modified albumin is known to be taken up by the liver by receptor-mediated endocytosis (4, 12), and the uptake of the modified heterologous albumin was found to be activated as early as 30 min after PHX.

The present demonstration of the activation of endocytosis after PHX is consistent with the report of Chawdhury *et al.* (19), who showed that receptor-mediated endocytosis of DNA complexed with asialoglycoprotein was enhanced following PHX. Kamimoto *et al.* (20) also reported that the endocytotic uptake of formaldehyde-treated bovine serum albumin into the liver was somewhat enhanced after PHX. These studies have dealt with the delayed-early phase (several hr) to growth phase (several days) of liver regeneration, while the present study is mainly concerned with the immediate-early phase (30 min).

Macromolecules presenting to the liver may be internalized by hepatocytes or phagocytic cells such as Kupffer cells. It has been reported that formaldehyde-treated bovine serum albumin is endocytosed *via* scavenger receptors on the endothelial cells (21), while asialofetuin is mainly taken up by hepatocytes (22). Although the activated endocytosis of formaldehyde-treated serum albumin, in the present study, suggests the involvement of endothelial cells, the involvement of hepatocytes cannot be excluded in view of the enhanced uptake of asialoglycoprotein conjugates after PHX (19). This question may be settled when the two cell types are separated and lysosome/endosome fractions are examined separately.

The work of several investigators has suggested a role for adrenergic hormones in the regulation of liver regenerative growth following PHX (for a review see Ref. 11). We found a dramatic increase in serum catecholamines immediately after PHX (Fig. 4). Both epinephrine and norepinephrine levels were increased, but the time courses of the elevation of the two hormones were different. Although the epinephrine levels in the hepatectomized and sham-operated rats followed the same course during the initial 15 min after PHX, the level of norepinephrine in the serum from hepatectomized rats was clearly higher than that from the sham-operated rats. This result suggests that norepinephrine, rather than epinephrine, may play a role in the activation of endocytosis observed at 30 min after PHX. This contention is supported by the present finding that both the uptake of serum albumin into lysosomes and the shift of buoyant density profile of endosomes are inhibited by the administration of adrenergic receptor antagonists, particularly by the α_1-antagonist prazosin (Fig. 3). Cruise *et al.* (23) previously measured catecholamine concentrations in the blood following PHX and found that the levels of both epinephrine and norepinephrine in the hepatectomized rats were consistently higher than those in the sham-operated rats. However, the

shortest time point of their measurement was 2 hr after PHX, and the differential kinetics of elevation of two hormones during the initial 15 min after PHX must have escaped their attention.

The α_1-adrenergic receptor has been reported to mediate the stimulative effect of norepinephrine on regenerative DNA synthesis by an alteration in the binding of epidermal growth factor to regenerating liver (23). The molecular mechanism of activation of endocytosis by norepinephrine will be the subject of future investigation.

1. Michalopoulos GK. Liver regeneration: Molecular mechanism of growth control. FASEB J **4:**176–187, 1990.
2. Taub R. Transcriptional control of liver regeneration. FASEB J **10:**413–427, 1996.
3. Higgins GM, Anderson RM. Experimental pathology of the liver. I. Restoration of the liver of white rat following partial surgical removal. Arch Pathol **12:**186–202, 1933.
4. Horiuchi S, Takata K, Morino Y. Characterization of a membrane-associated receptor from rat sinusoidal liver cells that binds formaldehyde-treated serum albumin. J Biol Chem **260:**475–481, 1985.
5. Yamada H, Hayashi H, Natori Y. A simple procedure for the isolation of highly purified lysosomes from normal rat liver. J Biochem **95:**1155–1160, 1984.
6. Belcher JD, Hamilton RL, Brady SE, Hornick CA, Jaeckle S, Schneider WJ, Havel RJ. Isolation and characterization of three endosomal fractions from the liver of estradiol-treated rats. Proc Natl Acad Sci USA **84:**6785–6789, 1987.
7. Sato A, Nagai M, Tagami A, Natori Y, Okada M. Effect of pyridoxine-deficiency on degradation of cytosolic aspartate aminotransferase in rat liver lysosomes. J Nutr Sci Vitaminol **37:**419–424, 1991.
8. Kilts CD, Gooch MD, Knopes KD. Quantitation of plasma catecholamines by on-line trace enrichment high performance liquid chromatography with electrochemical detection. J Neurosci Methods **11:**257–273, 1984.
9. Holtzman E. Lysosomes. New York: Plenum Press, 1989.
10. Natori Y, Chiku K, Oka T, Sato A. Role of lysosomes in hepatic protein degradation. In: Katunuma N, Kominami E, Eds. Intracellular Proteolysis: Mechanism and Regulations. Tokyo: Japan Scientific Societies Press, pp 445–451, 1989.
11. Morley CGD, Royse VL. Adrenergic agents as possible regulators of liver regeneration. Int J Biochem **13:**969–973, 1981.
12. Nilsson M, Berg T. Uptake and degradation of formaldehyde-treated ^{125}I-labelled human serum albumin in rat liver cells in vivo and in vitro. Biochim Biophys Acta **497:**171–182, 1977.
13. Becker FF, Lane BP. Regeneration of the mammalian liver. I. Autophagocytosis during dedifferentiation of the liver cell in preparation for cell division. Am J Pathol **47:**783–801, 1965.
14. Mori M, Novikoff AB. Induction of pinocytosis in rat hepatocytes by partial hepatectomy. J Cell Biol **72:**695–706, 1977.
15. Haber BA, Mohn KL, Diamond RH, Taub R. Induction patterns of 70 genes during nine days after hepatectomy define the temporal course of liver regeneration. J Clin Invest **91:**1319–1326, 1993.
16. Mellman I, Fuchs R, Helenius A. Acidification of the endocytic and exocytic pathways. Annu Rev Biochem **55:**663–700, 1986.
17. Roederer M, Bowser R, Murphy RF. Kinetics and temperature dependence of exposure of endocytosed material to proteolytic enzymes and low pH: Evidence for a maturation model for the formation of lysosomes. J Cell Physiol **131:**200–209, 1987.
18. Wang J, Ueno H, Masuko T, Hashimoto Y. Binding of serum albumin on tumor cells and characterization of the albumin binding protein. J Biochem **115:**898–903, 1994.
19. Chowdhury NR, Wu CH, Wu GY, Yerneni PC, Bommineni VR, Chowdhury JR. Fate of DNA targeted to the liver by asialoglycoprotein receptor-mediated endocytosis in vivo. J Biol Chem **268:**11265–11271, 1993.
20. Kamimoto Y, Tanabe D, Tashiro S, Hiraoka T, Miyauchi Y. Changes in receptor-mediated endocytosis in liver sinusoidal cells after partial hepatectomy. Liver **14:**141–147, 1994.
21. Blomhoff R, Eskild W, Berg T. Endocytosis of formaldehyde-treated serum albumin via scavenger pathway in liver endothelial cells. Biochem J **218:**81–86, 1984.
22. Tolleshaug H, Berg T, Nilsson M. Uptake and degradation of ^{125}I-labelled asialo-fetuin by isolated hepatocytes. Biochim Biophys Acta **499:**73–84, 1977.
23. Cruise JL, Knechtle SJ, Bollinger RR, Kuhn C, Michalopoulos G. α_1-Adrenergic effects and liver regeneration. Hepatology **7:**1189–1194, 1987.