Effects of Ethanol Administration on Hepatocellular Ultrastructure of Regenerating Liver Induced by Partial Hepatectomy

JOSÉ A. MORALES-GONZÁLEZ, MD, LUIS F. JIMÉNEZ-GARCÍA, PhD,
JOSÉ GUTIÉRREZ-SALINAS, MD, JORGE SEPÚLVEDA, MD, ALFONSO LEIJA-SALAS PhD,
and ROLANDO HERNÁNDEZ-MUÑOZ, MD, PhD

Acute ethanol administration partially inhibits DNA and protein syntheses during liver regeneration (LR) induced by partial hepatectomy (PH) in rats. Previous findings that the magnitude of ethanol's deleterious effects on LR are related to the route and timing of its administration led us to perform studies at the ultrastructural level, comparing ethanol effects on PH-induced LR, as a consequence of its administration route. PH promoted alterations on the endoplasmic reticulum and mitochondria, accompanied by decreased glycogen and increased lipid content in cytoplasm. Structural nuclear and nucleolar activities were also evident. Intragastric ethanol administration practically abolished the adaptive changes found in PH-promoted regenerating hepatocytes, whereas its administration through the intraperitoneal route induced later ultrastructural modifications, indicating cellular proliferation. These results suggest that ethanol, under certain conditions, could stimulate liver proliferation triggered by PH. The mechanism underlying this surprising effect of ethanol on LR remains to be elucidated. However, it is suggested that an altered ethanol metabolism by rats subjected to PH could be involved.

KEY WORDS: alcohol; nuclear activity; nucleolus; cell proliferation; ethanol metabolism.

Liver regeneration (LR) after partial hepatectomy (PH) is an useful experimental model to investigate the regulatory mechanisms of cellular proliferation *in*

vivo. LR is a common mechanism which a patient recovers from liver injury due to trauma, infections, or hepatotoxins. This phenomenon has long been recognized (1) and is the subject of diverse biochemical, histochemical, and morphological studies (2–4).

The effects of ethanol on liver regeneration have been reported by several groups (5–8). We have demonstrated that ethanol can produce alterations in the PH-induced LR in the rat (3, 8). These alterations affect some physiological processes, such as levels of serum metabolites (glucose, triacylglycerols, albumin, and bilirubin); modifications in the serum activity of enzymes that reflect integrity of the liver (alanine and aspartate aminotrasferases, lactate dehydrogenase,

ornithine carbamyltransferase, and glutamate dehydrogenase) (3, 8). Furthermore, ethanol administration to rats with regenerating liver inhibits DNA synthesis and the specific activity of enzymes closely related with this process (eg, thymidine kinase), and it decreases the mitotic index. The magnitude of these alterations is related to the route and timing of ethanol administration. Results showed that the gastrointestinal route of ethanol administration elicited more damage than the intraperitoneal route. These alterations involve physiological and structural disturbances, evidenced by morphological changes produced by ethanol administration, which were noted along the overall process of LR.

Ultrastructural studies in liver parenchymal cells, at the time of active cellular proliferation, have revealed changes in nuclei and in the smooth endoplasmic reticulum (SER) (9, 10). However, there are no reports of the temporal changes in the ultrastructural organization of the regenerating hepatocyte after acute ethanol administration and the influence of the timing and route of its administration.

The aim of the present study was to evaluate the changes produced in some intracellular components (by electron microscopy) of the hepatocyte during LR, and those in the presence of a low dose of ethanol given by two routes: intraperitoneal and intragastric.

MATERIALS AND METHODS

Animals. Male Wistar rats (230–270 g) were housed under a 12-hr light–dark cycle and allowed standard rat pellet chow (Purina de México SA) and water *ad libitum*, before treatment. All manipulations were made according to our Institutional Research Guide Criteria for Animal Care (National University of Mexico). Partial hepatectomy (PH; 70% removal of liver mass) was performed in the morning, according to that reported by Higgins and Andersen (1), under light diethyl ether anesthesia. As controls, sham-operated rats were subjected to surgery without tissue removal.

After surgery, animals were grouped as follows: (A) Sham-operated rats receiving intragastric or intraperitoneal administration of saline solution (0.9% NaCl); (B) rats subjected to PH receiving saline solution by either administration route (control of PH); (C) PH animals receiving a single intragastric ethanol administration (1.5 g/kg body wt) and, (D) PH rats treated with the same dose of ethanol, but through the intraperitoneal route.

Ethanol was administered at 0, 12, 24, 36, 48, 72, and 96 hr after PH, and animals were killed 8 hr thereafter (when blood ethanol level was no longer detectable) (4), by a lethal dose of anesthesia with sodium pentobarbital.

Liver Histology. Hepatic samples from each group were used for electron microscopy. Samples were fixed in 3% glutaraldehyde in 0.1 mol/liter phosphate buffer (pH 7.4) for 2 hr at 4°C, and then washed overnight with 0.1 M phosphate buffer containing 0.25 mol/liter sucrose. The samples were fixed in 1% OsO_4 in 0.1 mol/liter phosphate buffer (pH 7.4) for 2 hr at 4°C. The fixed samples were washed eight times (15 min each) with 0.1 mol/liter phosphate buffer (pH 7.4), containing 0.25 mol/liter sucrose and dehydrated with absolute ethanol. The samples were transferred to propylen oxide (two changes; 15 min each), infiltrated for 24 hr with a 1:1 dilution of complete Epon 812 resin, as described by Luft (11), and then transferred to BEEM capsules containing fresh Epon 812 resin for polymerization at 60°C for 35 hr. Thin sections were recovered on 200 mesh copper grids, stained with uranyl acetate and lead citrate, and examined with a JEM 1200 EXII electron microscope operated at 60 kV. Photomicrograph material was taken from representative areas in all groups tested. This photomicrographic material was coded and read blindly by two independent observers without knowledge of the applied treatments.

RESULTS

Parenchymal liver cells in sham-operated animals exhibited no significant morphological abnormalities. Abundant organelles were present at the periphery of a central nucleus surrounded by a double-layered membrane and containing a single nucleolus. Numerous glycogen depots were evenly distributed throughout the cytoplasm, usually accompanied by a few fat droplets. No alterations were oberved in any cytoplasmic structure (Figure 1A). However, the normal structure of the hepatocytes was substantially modified at the onset of PH-induced LR, and after ethanol treatment in both sham-operated and PH-treated animals.

Sham-operated rats receiving ethanol by either the intragastric or intraperitoneal routes did not show any significant ultrastructural change, except for an evident fatty accumulation in their liver cytoplasm (data not shown).

Rats subjected to PH showed no significant changes in hepatocyte architecture during the first 12 hr after surgery (Figure 1B). However, in samples from regenerating liver taken after 12 to 24 hr of PH, we found enlarged mitochondria, a slight increase in fat droplets, and a predominance of SER. At this stage, nuclear organization was unchanged (Figure 1C).

Ethanol administration immediately after PH only promoted fatty liver, quite similar to that induced in sham-operated animals. This effect was independent of the administration route. However, we noted a higher increase of liver fatty accumulation and predominance of SER, when animals subjected to PH-induced LR were treated with ethanol 12 hr after
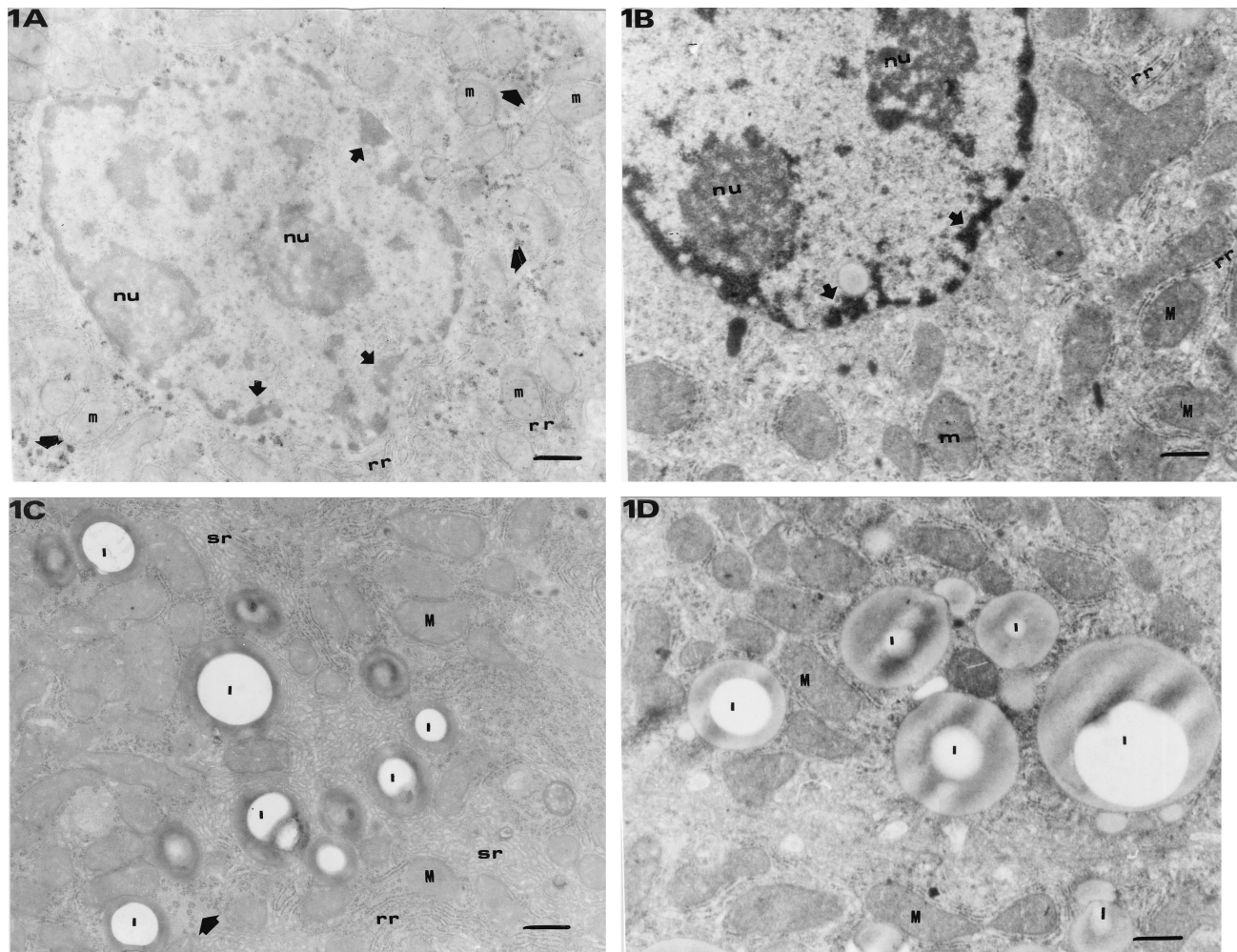
Fig 1. Electron micrographs of hepatocytes from rats at 12 hr of LR and treated with ethanol. (A) normal hepatocyte from sham-operated animals: nuclei with condensed chromatin (arrows); normal nucleolus (nu) without signs of activity. Glycogen deposits (arrowheads) were present in both mitochondria (m) and rough endoplasmic reticulum (rr). (B and C) Micrographs corresponding to proliferating hepatocytes after 12 hr of LR. (B) No nuclear activity was found, and only minor mitochondrial modifications are noted. (C) Lipid doplets (l) and a homogeneus distribution of both smooth endoplasmic reticulum (sr) and rough endoplasmic reticulum are shown. (D) Hepatocytes from rats 12 hr after PH and receiving intragastric ethanol administration. Here, lipid droplets were more abundant and mitochondria had reduced numbers and smaller cristae. No changes were recorded in the nuclei and nucleoli. Magnification: A, ×5000; B–D, ×7500. Bar represents 1 μm.

surgery. At this stage, the ethanol effects were more evident after intragastric administration (Figure 1D).

When livers were examined 24 to 32 hr after PH, many hepatic structural modifications were recorded, consequent to the regenerating process. Nuclei were enlarged and irregular in outline, displaying many pores in the double perinuclear envelope. Chromatin masses were prominent and occasional large clumps of chromosomal material were irregularly distributed in the nuclei. Nucleoli were also enlarged and increased in number; several were located near the inner perinuclear membrane (Figure 2A). In these hepatocytes, rough endoplasmic reticulum (RER)

was disorganized and reduced in amount. Most parallel tubules and cisternae of RER were broken into fragments and replaced by variably dilated vesicles containing electron-dense substance. Membranes of dilated RER were degranulated with partial or complete detachment of ribosomes, and a large number of free ribosomes was observed. Components of the Golgi complex were dilated and occasionally disorganized, and numerous SER vesicles were interspersed with groups of free ribosomes in the immediate vicinity of the Golgi complexes (Figure 2B).

At this time after PH, most mitochondria were swollen, rounded, or exhibited an irregular configu-
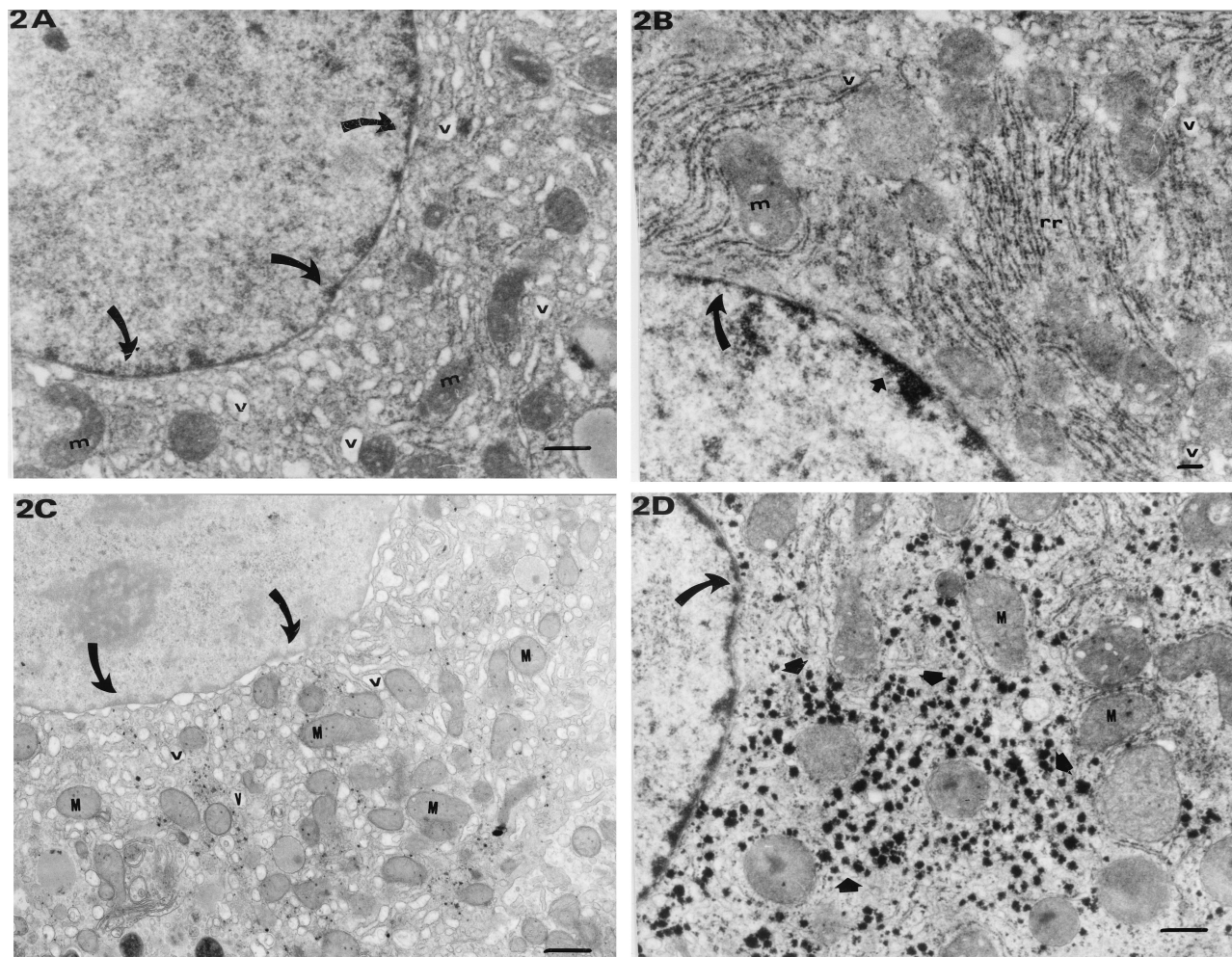
362

Fig 2. Electron micrographs of hepatocytes from rats at 24–36 hr of LR. (A) Nuclear condensed chromatin was largely diminished (large arrows) as compared with condensed chromatin (arrows). Cytosolic vesicles (v) of moderate density were observed. (B) Besides the diminution of nuclear condensed chromatin, the rough endoplasmic reticulum was more abundant and distributed in stratified layers. Micrograph C shows the integrity of the nuclear membrane and a normal nucleolus. Mitochondria were present in large numbers and were mostly enlarged. (D) There were abundant cytoplasmic glycogen deposits (arrowheads) in the cytoplasmic area. Magnification: A and D, ×7500; B, ×10,000; and C, ×5000. A, C, D: bar = 1 μm; B, bar = 500 nm.

ration. They seemed to be enlarged, while their cristae and intramitochondrial granules were reduced in number. Modifications of the hepatocellular plasma membrane were also noted (Figure 2C). In the cytoplasmic compartment, glycogen was almost entirely lost, and only a few scattered glycogen granules were observed among tubules and vesicles of the endoplasmic reticulum (ER). The hepatic cells also contained numerous lipid bodies (but less than at earlier surgical times) that did not appear to be membrane-limited. Lipid droplets were associated with myelin-like figures and closely related to mitochondria (Figure 2D).

The intragastric administration of ethanol to PH animals 24 hr after surgery induced a bigger fatty accumulation in the regenerating liver, whereas the amount of glycogen was practically unaffected by the hepatotoxin. Interestingly, the PH-induced changes on the distribution of the chromatin mass, as well as on the morphology and sizes of nuclei and nucleoli, were largely blocked by treatment with ethanol. In addition, the LR-promoted modifications of the relation between RER and SER were also strongly diminished by the hepatotoxin (Figure 3A). When ethanol was administered by the intraperitoneal route to these rats, the same effects were recorded but were of a lesser magnitude (Figure 3B).

During the 36- to 60-hr interval after PH, nuclei of the regenerating parenchymal cells continued displaying their characteristic alterations. Reorganization of
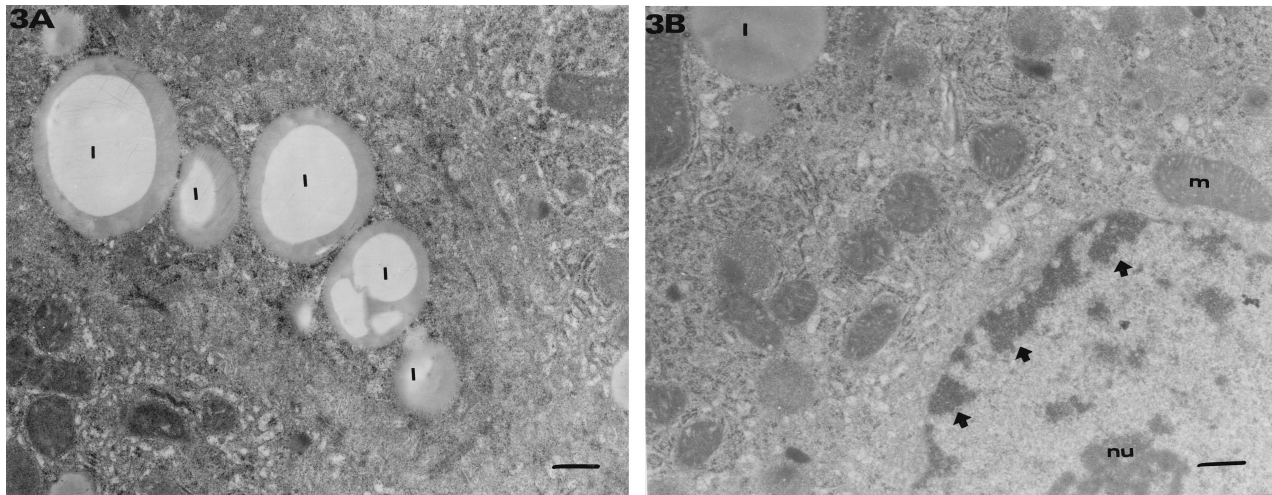
Fig 3. Electron micrographs of hepatocytes from rats at 24–36 hr of LR and treated with ethanol. (A) Regenerating hepatocytes after intragastric ethanol administration. Lipid droplets (l) were very abundant and larger. Rough and smooth endoplasmic reticulum and glycogen were absent, and no nuclear activity was detected. (B) proliferating hepatocytes after intraperitoneal ethanol administration. Again, no nuclear activity was denoted, as assessed by the presence of condensed chromatin (arrows) and the nucleolus was unchanged. Both electron microscopy patterns revealed no proliferative activity in the presence of ethanol administered by either intragastric or intraperitoneal routes. Magnification of micrographs: ×7500; bar = 1 μm.

the RER was noted around hypertrophic Golgi zones, and free ribosomes appeared to be largely confined to paranuclear locations, while SER was still scanty during this period. Mitochondria gradually returned to normal size and structure, and cytoplasmic glycogen deposits started to increase within the cell. In addition, lipid droplets were sparse, but larger in number when compared with control livers (Figure 4A).

At this stage of LR (36–60 hr), ethanol administration through the intragastric route promoted accentuated fatty accumulation, whereas glycogen was practically absent. Nuclear and chromatin modifications were not observed in PH animals receiving ethanol intragastrically, and RER appeared organized, with a predominance of SER, quite similar to that found in livers from sham-operated rats (Figure 4B). Intraperitoneal administration of the same ethanol dose to animals subjected to PH (36–60 hr after surgery), did not reproduce the inhibitory effect of intragastric ethanol on LR. Surprisingly, these rats did show diminution of fat droplets, an increase in granules of glycogen, and changes in nuclear activity. For instance, chromatin mass was less dense, starting to display prominent chromosomal clumps (mainly after 60 hr post-PH). RER began to fragment, with few free ribosomes around Golgi complexes. In addition, it was evident that mitochondria increased in number, which did not occur when PH animals were treated with the same dose of ethanol, but by the intragastric route (Figure 4C and D).

Beyond the first 72-hr after PH, morphological alterations gradually ceased in the regenerating liver. Cytoplasmic structure resumed its normal appearance and organelles were restored. The RER swelling and fragmentation was gradually reduced, since dilated vesicles became narrow and formed parallel rows. The number of glycogen granules increased in the membrane network, and the SER frequently increased in predominance; fat content became similar to that found in control livers. In addition, nuclear activity was clearly diminished, as reflected by chomatin condensation and by a reduction in nucleolus size (Figure 5A).

Animals subjected to PH (72–96 hr) and receiving ethanol by the intragastric route had abundant fat deposits in the cytoplasmic compartment, while no other modifications in the organelle's structure were recorded. Indeed, activities of nuclear and RER were completely absent (Figure 5B). When the same dose of ethanol was administered intraperitoneally to PH rats at this stage (72–96 hr), changes in chromatin were more evident, nucleoli were enlarged, and there was definitive RER activity predominating over the SER (Figure 5C and D).

The time course of ultrastructural changes in parenchymal cells, which followed at the onset of PH-induced LR, is summarized in Table 1. Also shown is the differential effect of ethanol on structural features characteristic of LR that were directly dependent on its administration route *in vivo*.
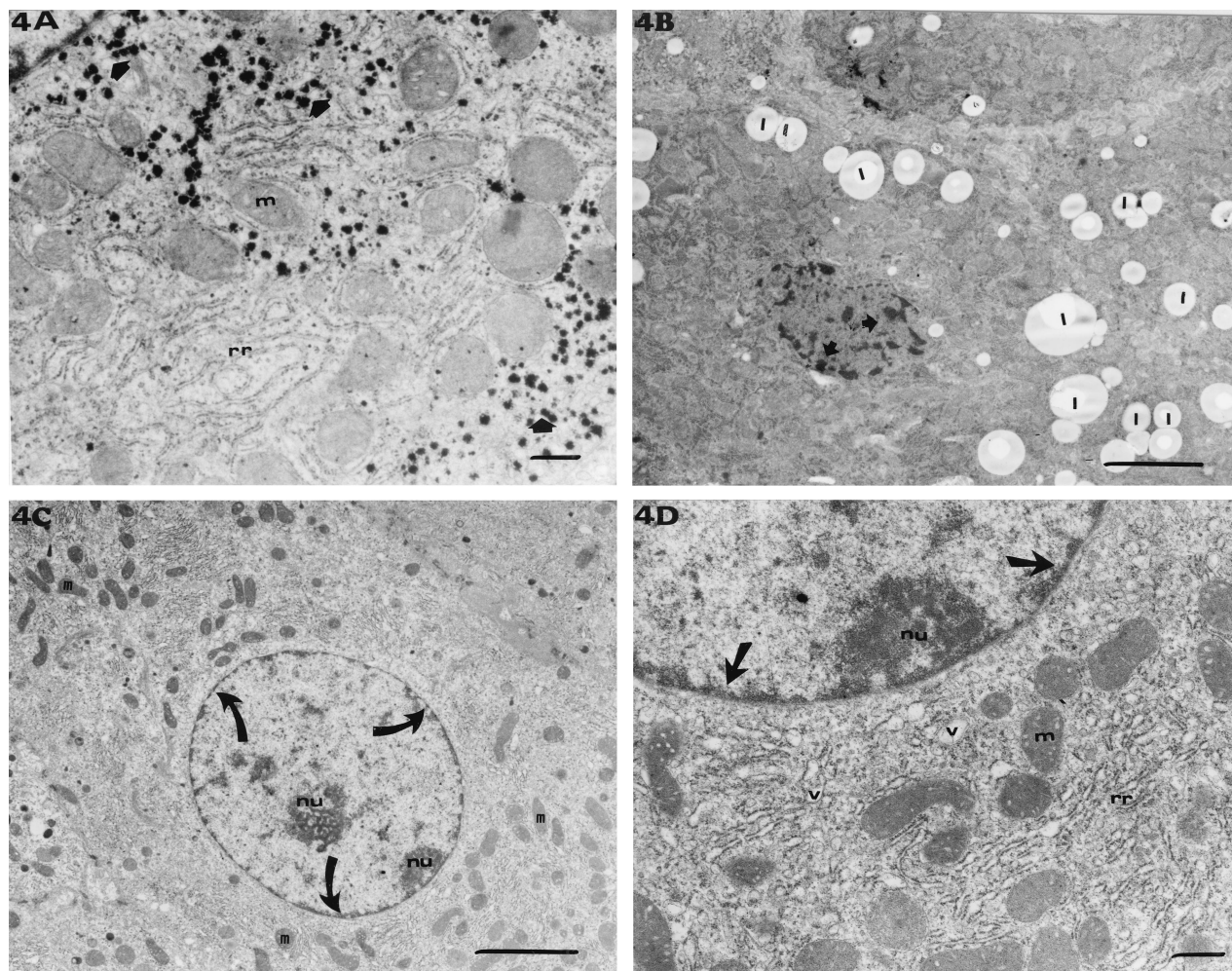
364

Fig 4. Electron micrographs of hepatocytes from rats at 36–60 hr of LR and treated with ethanol. (A) Regenerating hepatocytes show presence of glycogen deposits (arrowheads), as well as abundant rough endoplasmic reticulum, increased numbers of mitochondria were also noted. (B) Proliferating hepatocytes (36–60 hr after PH) from animals receiving intragastric ethanol administration are shown. There was striking fatty accumulation, and a clear absence of nuclear activity with condensed chromatin (arrows). (C) Hepatocytes from PH rats treated intraperitoneally with ethanol. Here, there was an increased number of mitochondria, as well as increased rough endoplasmic reticulum. Also evident were the enhanced size and number of nucleoli and the diminished amount of dense chromatin (large arrows). (D) Increased numbers of mitochondria and the presence of cytosolic vesicles of moderate density were evident. Changes in rough endoplasmic reticulum are in agreement with proliferative activity (compare with Figure 2A and B). Magnification of micrographs: A, ×7500; B and C, ×2500; and D, ×7500. Bar = 1 μm in A and D, 2 μm in B and C.

DISCUSSION

We showed recently that, in the PH-induced regenerating liver, ethanol metabolism is not directly implicated in the changes in the cellular redox state that occur during LR. These findings led us to propose that acute ethanol administration might minimize redox metabolic adjustments, probably leading to a decreased preparatory event culminating in the proliferative period that characterizes PH-induced liver regeneración (12).

We have also reported that a single low dose of ethanol produces a reliable and constant inhibition of the normal hepatic regenerative response induced by surgical removal of liver mass. This effect was strongly dependent on timing and the route of ethanol administration, and intragrastric administration of ethanol was more deleterious than the intraperitoneal route in inhibiting the regenerative process. However, ethanol administration to rats with regenerating liver did not induce hepatocellular necrosis, as evaluated by serum activities of hepatic marker enzymes (8).

These clearly established differential inhibitory effects of ethanol on the regenerating liver (dependent on its administration route), assessed by morpholog-
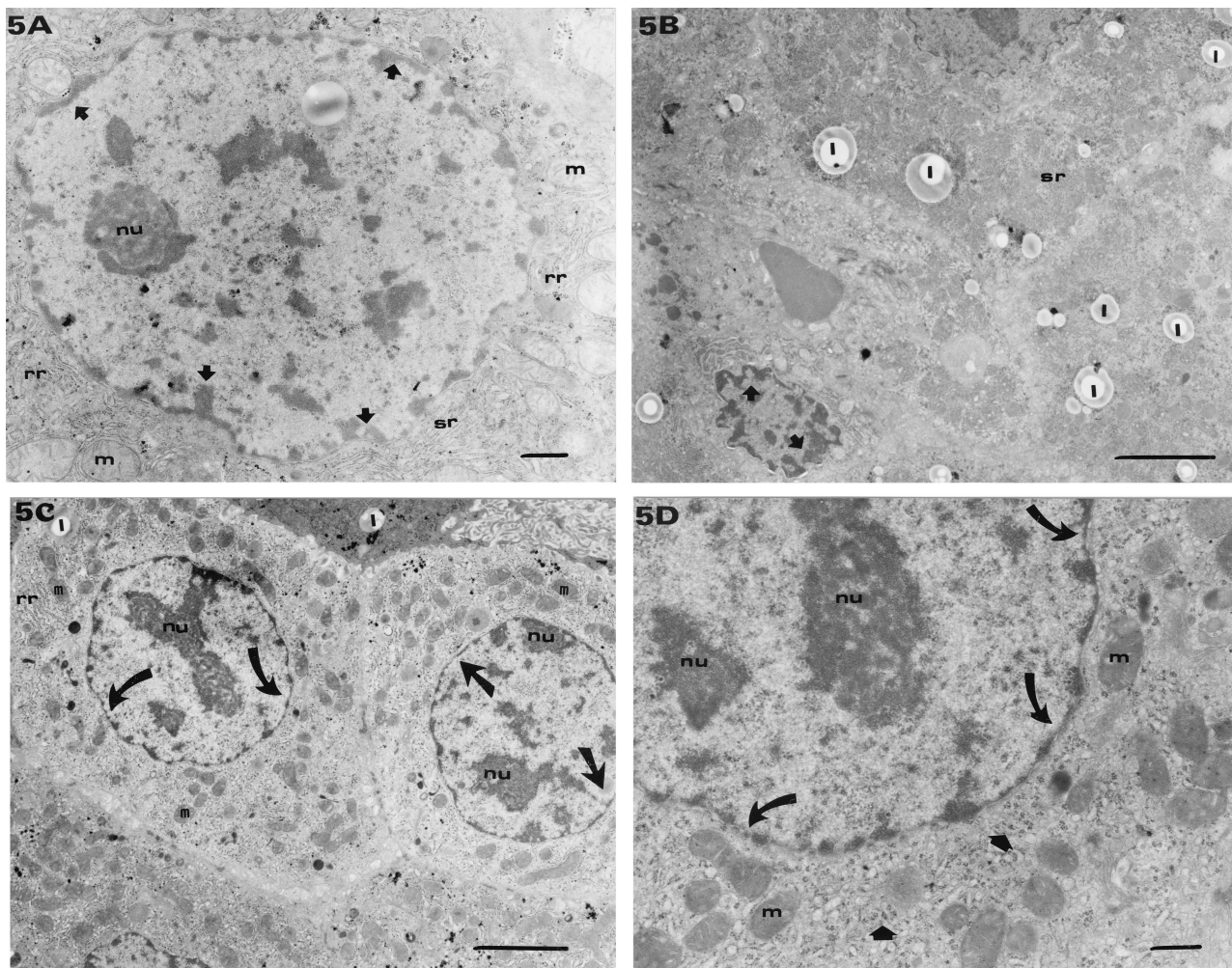
Fig 5. Electron micrographs of hepatocytes from rats at 72–96 hr of LR and treated with ethanol. (A) Hepatocytes at later LR stages, where restoration of the normal ultrastructural pattern is observed (compare Figure 1A). (B) Liver cells obtained from rats subjected to PH and receiving ethanol intragastrically showed essentially the same pattern observed along intragastric ethanol administration to rats subjected to PH (Figures 1D, 3A and 4B), mainly characterized by strong lipid accumulation and predominance of the smooth endoplasmic reticulum. (C) The intraperitoneal administration of ethanol resulted in increased size and number of nucleoli and of condensed chromatin (large arrows). Enhancement of mitochondria and rough endoplasmic reticulum was also evident, with restoration of glucogen deposits (arrowheads) (compare with Figure 4C). (D) Magnified picture of the latter groups shows the greater size of the nucleolus, and the diminution of dense chromatin. Magnification: A, ×5000; B and C, ×2500, and in D, ×7500. Bar = 1 μm (in A and D) and 2-μm in B and C.

ical studies (light microscopy) and by the liver's functional capacity (chemical determinations), were confirmed by the present findings extended to ultrastructural hepatocyte's organization studies.

It has been long recognized that morphological changes occur in the hepatic cells a few hours after PH. Indeed, there are excellent reports of the time-course of LR induced by PH at the ultrastructural level done through electron microscopy (9, 10). The main features include alterations on the ER and mitochondria, decreases in glycogen, and increases in lipid content. Mitochondrial and ER changes have been correlated with dramatic changes in their func-

tions, including alterations in specific enzyme activities (13, 14). Nonetheless, it can be considered that the LR process produced only minor ultrastructural differences in subcellular compartments, as well as in plasma membranes during the first 16 hr after PH. Therefore, our results agree with those previously reported (15, 16).

On the second postoperative day, cytoplasmic structural disorganization was followed by reparative and regenerative activities, and from the second to fourth days, most organellar changes returned to normal levels. Mitochondria were the organelles that took more time for normalization, correlating with

TABLE 1. SUMMARY OF LIVER ULTRASTRUCTURAL FINDINGS IN PARTIAL
HEPATECTOMIZED RATS AND TREATED WITH ETHANOL BY INTRAGRASTRIC (IG)
AND INTRAPERITONEAL (IP) ROUTES*

Treatment (hr after surgery)	Nuclear activity	RER activity	Lipid accumulation	Glycogen deposits
Controls				
0–96 hr	(−)	(−)	(−)/+	+++
PH rats + saline				
12 to 36 hr	++/+++	+++	++/+++	+
36 to 60 hr	+++	++/+++	+	+++
60 to 96 hr	+	+	+	++
PH rats + ethanol (IG)				
12 to 36 hr	(−)/+	+	++/+++	+
36 to 60 hr	(−)/+	(−)/+	+++	(−)
60 to 96 hr	(−)	(−)	++/+++	(−)/+
PH rats + ethanol (IP)				
12 to 36 hr	+	+	++/+++	+
36 to 60 hr	++	++	++	+/++
60 to 96 hr	++/+++	+++	+/++	++

*Parameters evaluated were: nuclear activity, (−) no change; (+) light; (++) moderate, or (+++) strong nuclear activity (enlarged nucleus, laxitude of chromatin mass and increased size and number of nucleoli); activity of the rough endoplasmic reticulum (RER): (−) no change, (+) light; (++) moderate, and (+++) complete RER activity (disorganization in fragments, dilatation, formation of vesicles, and appearance of free ribosomes in the Golgi vicinity); and fatty accumulation: (−) absent; (+) mild; (++) moderate, and (+++) severe. Presence of glycogen depots: (−) absent; (+) scanty; (++) mild, and (+++) abundant.

results obtained with radioactive labeling of proteins and membrane phospholipids, indicating that mitochondrial turnover lasts longer than other organelles in the regenerating rat liver (17).

Hence, the main manifestations of cellular proliferation in the normal regenerating liver were an increased perinuclear RER and a displacement of glycogen depots within the regenerating cells (mainly after 24 hr post-PH). These were accompanied by changes in the ultrastructure of the nucleus and nucleolus, which have been considered predictors of cell activity related to enhanced protein synthesis and proliferation (18), and they correlated well with a striking increase in the proportion of free ribosomes, indicating enhanced liver protein synthesis (19). Moreover, SER is the main source of drug-metabolizing enzymes and its predominance would suggest active metabolism of xenobiotics, whereas a predominant RER indicates that cells are subjected to an active protein synthesis (20). In any case, the hepatocytes seem to retain their functional activity and major structural attributes while proliferating, as reported previously (3, 8).

It is known that biochemical and physiological changes have to be accomplished in the regenerating hepatocyte, constituting the framework to drive mitosis and cell replication (21). Administration of ethanol to animals subjected to PH is able to inhibit LR, as evidenced by a diminution in DNA synthesis and in

enzyme activity, closely related to the duplicative process (thymidine kinase, thymidylate synthetase, and ornithine decarboxilase) (22, 23). Nonetheless, the underlying mechanism in the inhibitory action of ethanol is still poorly understood.

The present results showed that ethanol, instead of inducing a sort of hepatocellular damage to the regenerating liver, seemed to minimize the normal profile of changes that occurred in subcellular structures of the regenerating hepatocyte. Particularly, the intragastric route of ethanol administration blocked most PH-induced modifications in the liver ultrastructure, except for the more pronounced fatty liver in these animals. The changes in liver nuclear and nucleolar activities (eg, chromatin distribution) and that of RER were practically eliminated when rats subjected to PH received ethanol intragastrically. Indeed, livers from these animals did not show signs of cell proliferation. Furthermore, ER was predominantly smooth, indicating a lower (normal) rate of protein synthesis, as compared to non-ethanol-treated PH rats. This is noteworthy, considering that acute ethanol administration produces structural and biochemical changes in mammalian livers, including alterations in the porosity of the sinusoidal endothelium, in endothelial fenestrations, and in some subcellular organelles, such as mitochondria (3, 24).

In addition, it was clear that intragastric administration of ethanol induced bigger fat deposits at later

postsurgery times (60–96 hr) than at earlier times of LR. Normal animals developed fatty liver after ethanol ingestion, an effect closely related to a shifted cytoplasmic redox state towards a reduced condition (25). This ethanol-induced effect in fat accumulation largely depends on liver alcohol dehydrogenase (ADH) activity, as demonstrated by a strong diminution of fatty liver in animals receiving ethanol and treated with 4-methylpyrazole, a specific ADH inhibitor (26).

Therefore, the data suggest that liver ADH participation in ethanol oxidation is not the same at all LR stages after PH and that this enzyme contributes to a considerable ethanol catabolism at later stages of LR, as previously suggested in previous communications by our group (4, 12).

In addition, intraperitoneal ethanol administration to rats undergoing PH-induced LR promoted a lesser blockade of the PH-induced ultrastructural changes in the regenerating liver. More interestingly, a single low i.p. dose applied to PH rats at later post surgery times induced activation of hepatocyte proliferation instead of inhibiting this process. These data strongly agree with those found in studies of liver function and morphological appearance previously reported by us (8). Hence, we are demonstrating that, depending on the conditions, ethanol could stimulate LR rather than inhibit it.

The route-dependence of ethanol-induced changes in the ultrastructural organization of regenerating livers has not been previously described. The mechanisms involved in the blocking effect of ethanol on the progression of PH-induced LR is still unknown. Data would suggest that ethanol somehow promotes an 'uncoupling' of the remnant liver to respond to proliferation inducers, since the subcellular organization of the hepatocyte only depicted minor changes after PH and in the presence of ethanol. However, ethanol administration (mainly by the i.g. route) was also able to return liver cells to baseline (quiescent stage), when they are supposed to be already stimulated by the surgical loss of hepatic mass. It would seem that a single ethanol dose can turn cellular or molecular mechanism activated by PH (switch on) to the level normally present in the intact liver (switch off). The nature of such mechanism(s) is currently being studied in our laboratory.

In conclusion, lower doses of ethanol administered to rats subjected to PH elicited different modifications in the ultrastructural framework of the regenerating liver, largely dependent on its administration route. Whereas the i.g. administration practically abolished all adaptative changes of regenerating hepatocytes, its administration by the i.p. route promoted later ultrastructural modifications, indicative of cell proliferation. The underlying mechanism involved in this surprising effect of ethanol on LR remains to be elucidated.

REFERENCES

1. Higgins GM, Anderson RM: Experimental pathology of the liver: Restoration of the liver of the white rat following partial surgical removal. Arch Pathol 12:186–202, 1931

2. Bucher NLR: Regeneration of mammalian liver. Int Rev Cytol 15:245–300, 1963

3. Gutiérrez-Salinas J, Aranda-Fraustro A, Paredes-Díaz R, Hernández-Muñoz R: Sucrose administration to partially hepatectomized rats: A possible model to study ethanol-induced inhibition of liver regeneration. Int J Biochem Cell Biol 28:1007–1016, 1996

4. Morales-González JA, Gutiérrez-Salinas J, Hernández-Muñoz R: Pharmacokinetics of the ethanol bioavailability in the regenerating rat liver induced by partial hepatectomy. Alcohol: Clin Exp Res 22:1557–1563, 1998

5. Frank WO, Rayyes AN, Washington A, Holt PR: Effect of acute ethanol administration upon hepatic regeneration. J Lab Clin Med 93:402–413, 1979

6. Wands JR, Carter EA, Bucher NLR, Isselbacher KJ: Inhibition of hepatic regeneration in rats by acute and chronic ethanol Intoxication. Gastroenterology 77:528–531, 1977

7. Dugay L, Coutu D, Hetu C, Joly JG: Inhibition of liver regeneration by chronic alcohol administration. Gut 23:8–13, 1982

8. Morales-González JA, Gutiérrez-Salinas J, Yáñez L, Villagómez-Rico C, Badillo-Romero J, Hernández-Muñoz R: Morphological and biochemical effects of a low ethanol dose on rat liver regeneration. Role of route and timing of administration. *Dig Dis Sci* 44:1963–1974, 1999

9. Virágh S, Bartók I: An electron microscopic study of the regeneration of the liver following partial hepatectomy. Am J Pathol 49:825–839, 1966

10. Stenger RJ, Confer DB: Hepatocellular ultrastructure during liver regeneration after subtotal hepatectomy. Exp Mol Pathol 5:455–474, 1966

11. Luft H: Improvement in the epoxy resin embedding methods. J Biophys Biochem Cytol 9:409–411, 1961

12. Gutiérrez-Salinas J, Miranda-Garduño L, Trejo-Izquierdo E, Diaz-Muñoz M, Vidrio S, Morales-González JA, Hernández-Muñoz R: Redox state and energy metabolism during liver regeneration. Alterations produced by acute ethanol administration. Biochem Pharmacol 58:1831–1839, 1999

13. Pearson B, Grose F, Green R: Histochemical changes in liver succinic dehydrogenase during rapid growth following partial hepatectomy. Am J Pathol 35:139–151, 1959

14. Von der Decken A, Hultin T: The enzymatic composition of rat liver microsomes during liver regeneration. Exp Cell Res 19:591–604, 1960

15. Grisham JW, Tillman RL, Nägel AEH, Compagno N: Ultrastructure of the proliferating hepatocyte: sinusoidal surface and endoplasmic reticulum. *In* R Lesch, W Reutter (eds). Liver regeneration after experimental injury. New York, Stratton International, 1975, pp 6–23

16. Murray AB, Strecker W, Silz S: Ultrastructural changes in rat hepatocytes after partial hepatectomy. J Cell Sci 50:433–448, 1981

17. Díaz-Muñoz M, Cañedo-Merino R, Gutiérrez-Salinas J, Hernández-Muñoz R: Modifications of intracellular calcium release channels and calcium mobilization following 70% hepatectomy. Arch Biochem Biophys 349:105–112, 1998

18. Miller OL Jr: The nucleolus, chromosomes, and visualization of genetic activity. J Cell Biol 91 (Pt 2):15s–27s, 1966

19. Loeb JN, Yeung LL: Free and membrane bound ribosomes in regenerating rat liver. Biochim Biophys Acta 520:623–629, 1978

20. Phillips MJ, Latham PS, and Poucell-Hatton S: Electron microscopy of human liver diseases. *In* Diseases of the Liver. L Schiff, ER Schiff (eds). Philadelphia, JB Lippincott, 1993, pp 189–205

21. Michalopoulos GK: Liver regeneration: Molecular mechanism of growth control. FASEB J 4:176–186, 1990

22. Pösö AR, Pösö H: Inhibition of ornithine decarboxylase in regenerating rat liver by acute ethanol treatment. Biochim Biophys Acta 606:338–346, 1980

23. Yoshida Y, Komatsu M, Ozeki A, Nango R, Tsukamoto I: Ethanol represses thymidylate synthase and thymidine kinase at mRNA level in regenerating rat liver after partial hepatectomy. Biochim Biophys Acta 1336:180–186, 1997

24. Sarphie G, D'Souza NB, Van Thiel DH, Hill D, McClain CJ, Deaciuc IV: Dose- and time-dependent effects of ethanol on functional and structural aspects of the liver sinusoid in the mouse. Alcohol Clin Exp Res 21:1128–1136, 1997

25. Hernández-Muñoz R, Santamaría A, García-Sáinz JA, Piña E, Chagoya de Sánchez V: On the mechanism of ethanol-induced fatty liver and its reversibility by adenosine. Arch Biochem Biophys 190:155–162, 1978

26. García-Sáinz JA, Hernández-Muñoz R, Glender W, Piña E, Chagoya de Sánchez V: Effects of adenosine on ethanol-induced modifications of liver metabolism. Biochem Pharmacol 29:1709–1714, 1980