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A biochemical and stereological study of neonatal rat hepatocyte subpopulations

Effect of pre- and postnatal exposure to ethanol

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Summary. Hepatocytes from 12-day-old rats, pre- and post-natally exposed to alcohol, together with those from pair-fed controls, were isolated and subfractionated in six cell subpopulations on Percoll density gradients. These cells were characterized using a combination of biochemical and stereological methods. The low density cells (F_2) mainly showed biochemical and stereological features of perivenous hepatocytes, whereas the heavier cells (F_6) were primarily periportal hepatocytes. The alcohol-metabolizing enzymes, alcohol dehydrogenase and aldehyde dehydrogenase (high and low Km) showed more activity in the F_2 fraction. Alcohol-altered mitochondria and Golgi apparatus occurred mainly in F_2 cells, whereas the endoplasmic reticulum and lysosomes appeared to be more altered in the F_6 hepatocytes. Alcohol also induced the appearance of some small hepatocytes, with a well-developed rough endoplasmic reticulum and an increased number of mitochondria. Biochemical data indicated that glutamate dehydrogenase and alanine aminotransferase were more affected in F_2 cells from alcohol-treated rats, and that the activity of the ethanol-metabolizing enzymes was also reduced in these hepatocytes. Our results indicate that alcohol exposure during zonal development in the liver could have a selective effect on specific cell components depending on the acinar zone, and that the perivenous hepatocytes appear to be more damaged under these conditions.

Key words: Alcohol exposure – Hepatocyte subpopulations – Zonal enzyme markers – Stereology – Neonatal rats

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Introduction

Several authors have demonstrated functional, biochemical and ultrastructural heterogeneity of hepatocytes within the liver acinus (for reviews see, Gumucio and Miller 1981; Jungermann and Sasse 1978; Ma and Biempica 1971). From these studies it has been proposed that the liver acinus is divided in three different areas, defined by Rappaport et al. (1954) as zone 1 or periportal (PP), zone 2 or intermediate, and zone 3 or perivenous (PV). These zones have been shown to have specific hepatic functions (Gumucio and Miller 1981; Jungermann and Sasse 1978).

Although it has been extensively demonstrated that chronic exposure to ethanol induces severe biochemical and ultrastructural alterations in hepatocytes (Lieber 1983; Rubin and Lieber 1967), only a few studies have considered the heterogeneity of adult liver parenchymal cells. From these studies it appears that in adult liver, the early alcohol-induced alterations occur in the PV region (Popper and Lieber 1980). Work in our laboratory has shown that prenatal exposure to alcohol causes severe ultrastructural alterations as well as changes in the cytochemical activity of several phosphatases in the liver of newborn rats (Renau-Piqueras et al. 1985a, b, 1987). However, it is not yet known if these alterations show a preferential localization within the liver acinus.

The different types of hepatocytes have been clearly identified "in situ" using quantitative electron microscopy (Loud 1968; Meihuizen and Blansjaar 1980), but there are technical problems in showing biochemical and functional heterogeneity of hepatocytes in the tissue. Therefore, during the last few years several methods have been devel-

oped to study this heterogeneity in adult animals (Gumucio and Miller 1982), including isolation and subfractionation of hepatocytes on density gradients after collagenase perfusion of liver (Drochmans et al. 1975; Tonda et al. 1983). However, due to the difficulty in perfusing small livers, new procedures have recently been developed to purify fetal rat hepatocytes (Radford and Bhathal 1985).

The aim of the present work has been to analyze the effect of pre- and post-natal exposure to ethanol on rat hepatocyte subpopulations. Using the methods for fetal liver mentioned above, we have isolated and subfractionated hepatocytes from 12-day-old control and alcohol-exposed rats. The subpopulations of cells obtained were analyzed using qualitative and quantitative biochemical and ultrastructural procedures.

Materials and methods

Animal treatment. Female Wistar rats weighing 150–200 g were used. All rats were maintained under controlled conditions of light and dark (12/12 h), temperature (23° C) and humidity (60%). They were fed the Lieber-DeCarli liquid diet containing either 5% (w/v) ethanol or isocalorically balanced with dextrin-maltose for pair-fed controls (Lieber and DeCarli 1976). Female rats received liquid diet (ethanol or control) for a minimum of 40 days prior to exposure to male rats. After mating, the rats were placed in separate cages on the ethanol or control liquid diet, during gestation. The day of parturition was considered day 0. Since metabolic zonation of the liver acinus does not occur until the 2nd week of life (Jungermann and Sasse 1978; Katz et al. 1976), the litters were culled at birth to 8–10 pups per dam and maintained with their corresponding mothers for the next 12 days. During lactation mothers were fed with ethanol or control liquid diet, as above. All the animals were sacrificed by decapitation at the same time of day (8:30 a.m.) to avoid circadian variations in the hepatocyte ultrastructure (Gumucio and Miller 1981).

Isolation and fractionation of hepatocytes. The method described by Radford and Bhathal (1985), and Tonda et al. (1983) was followed with some modifications. Briefly, livers from control and treated rats were finely minced, incubated in Ca^{++}-free Krebs-Henseleit bicarbonate buffer containing 0.5 mM EGTA for 30 min at 37° C. After centrifugation, the liver fragments were resuspended in the same buffer containing Ca^{++} and collagenase (0.5 mg/ml) for 60 min at 37° C. After washing with Ca^{++}-free buffer the cells were passed through several nylon meshes (500, 90, 60 and 30 μm pore size) and resuspended for 15 min in the same buffer containing 1% BSA. Then 2.2 ml of the cell suspension (4×10^6 viable cells/ml) were layered on a discontinuous Percoll isosmotic gradient (Table 1) and centrifuged for 20 min at $600 \times g$ at 12° C. During all the steps of this process the cells were gassed with carbogen. After centrifugation seven distinct layers were obtained. Hepatocytes from the same fractions were pooled and washed three times. The cells were resuspended in Ca^{++}-free buffer containing 0.5 mM EGTA and 1% BSA. Aliquots were used for cell viability, biochemical assays, and electron microscopy.

To check the reliability of the subfractionation method, rebanding experiments were carried out in some cases. Hepatocytes from one fraction were layered on top of a new discontinuous Percoll gradient. After centrifugation ($600 \times g$, 20 min, 12° C), the cells appeared located in the same position as in the original experiment.

Biochemical methods. The cell pellets were resuspended in 10 mM HEPES-buffer with 1% Triton X-100 and frozen at −80° C. The next day the hepatocytes were disrupted by freezing and thawing three times and homogenized with a super Dispox Tissumizer at full speed for 50 s. Biochemical analyses were made on aliquots of this homogenate. Alanine aminotransferase was determined according to Segal and Matsuzawa (1970); alcohol and aldehyde dehydrogenases were assayed as described by Koivula and Koivusalo (1975), differentiating between the high and low Km forms of the latter by using 5 mmol/liter and 0.05 mmol/liter acetaldehyde substrate, respectively. Glutamate dehydrogenase activity was measured by the procedure of Salinas et al. (1974). Protein concentrations were determined by the method of Lowry et al. (1951).

Criteria of cell preservation and cell size. Viability was determined in the whole hepatocyte population and in each fraction by the trypan blue exclusion test. The preservation of hepatocytes was assessed by both phase-contrast microscopy and in semithin sections (2 μm).

Electron microscopy. The hepatocytes of each fraction (1.5×10^6 cells/fraction) were washed three times in buffer containing 1% BSA and centrifuged at $90 \times g$ for 15 min. The pellets were fixed at 4° C in 1.0% glutaraldehyde-0.7% formaldehyde in 0.05 M cacodylate buffer (pH 7.4 at 4° C) containing 2 mM $CaCl_2$. The effective osmolarity of the fixative solution was 300 mOsm. After 15 min, the pellets were detached, fixed in fresh fixative for 15 additional min, and cut into 30 cubes. From each pool of blocks, eight were chosen at random and fixed in the aldehyde-based solution for 30 min at 4° C, washed for 120 min at 4° C in 0.1 M cacodylate buffer (300 mOsm, 2 mM $CaCl_2$, pH 7.4 at 4° C), postfixed for 3 h in the dark at room temperature in 0.2% OsO_4 containing 0.8% potassium ferrocyanide, washed in cacodylate buffer, stained in block for 45 min at room temperature in aqueous 0.5% uranyl acetate, dehydrated in a graded series of acetones and embedded in Vestopal. Samples of liver tissue from control and alcohol-treated rats were also processed for electron microscopy using the same procedure.

Stereology. The sampling method was carried out according to Cruz-Orive and Weibel (1981) and Weibel (1979). Hepatocytes from the different fractions from each of the four cell separation experiments were fixed as described above. After cutting the pellets into 30 cubes, a sample of eight blocks were selected at random by the lottery method and processed for electron microscopy as described. Then, three blocks per fraction and experiment were randomly chosen, and from each block four ultrathin sections (interference colour, silver) were cut at equally spaced intervals, not less than 200 μm. The sections were mounted on carbon-coated 200-mesh copper grids and double stained with uranyl acetate and lead citrate in a LKB 2168 Ultrostainer. Micrographs of hepatocytes were taken at two levels of magnification. At level I (×9576), the cell, cytoplasm and nucleus were assessed. At level II (×20520), mitochondria, lysosomes, glycogen, rER, sER, Golgi apparatus, lipid droplets and peroxisomes were estimated. Micrographs were always taken at both levels following the systematic quadrats (aligned) subsampling method (Cruz-Orive and Wei-

bel 1981; Weibel 1979). Level II electron micrographs were taken within the fields covered by level I (Weibel 1979). Stereological analysis of micrographs were carried out using point counting procedures and standard stereological formulas (Weibel 1979; Renau-Piqueras et al. 1985b).

The minimum sample size (MSS) for each parameter was determined by the progressive mean technique (Williams 1977; confidence limit ±5%). Progressive averages of the different parameters were plotted against the test area analyzed, and the test area required to achieve parameter constancy limited within ±5% of the overall average was regarded as MSS for a particular parameter. In all cases the number of micrographs analyzed was twice that determined by the MSS analysis.

Since no statistical differences (analysis of variance, $p \leqq 0.05$) were found between particular fractions within each group (control and alcohol), the results are expressed as the mean ±SD of each group.

Statistical comparison of biochemical and stereological data was made by the Student's *t*-test ($p \leqq 0.05$).

Results

Viability and recovery of subfractionated hepatocytes

The isolated hepatocytes, after separation by discontinuous gradients, appeared distributed in all experiments in a similar seven-layer pattern. The first, at the top of the tube, was composed of non-sedimented material, mainly cell debris, dead cells, and small clumps of hepatocytes. Therefore, the second layer was considered as F_1. The buoyant density, viability and yield of each fraction are summarized in Table 1. Although the highest recovery was obtained in the F_1 fraction, biochemical studies, phase-contrast microscopy and semithin sections revealed that this fraction, in both control and treated samples, also contained many doublets and damaged cells. This was also confirmed by electron microscopy. It is noteworthy that viability in all the fractions was higher than 90% (Table 1).

Activity of enzymes in hepatocyte density populations

Activities of two enzymes were measured as markers to identify the perivenous or periportal origin of cells in fractions. The specific activity of alanine aminotransferase (ALAT), considered a periportal marker enzyme (Morrison et al. 1965; Shank et al. 1959; Welsh 1972), increased from F_2 to F_6 (F_2, F_6; $p \leqq 0.05$). In contrast, glutamate dehydrogenase (GDH), used as a perivenous marker enzyme (Morrison et al. 1965; Shank et al. 1959), showed the highest activity in F_2 (F_2, F_6; $p \leqq 0.05$), (Fig. 1). The same distribution pattern of ALAT and GDH activities was obtained for cells from alcohol-treated rats. However, a lower GDH specific activity was found in all fractions (Fig. 1).

As shown in Fig. 2, the distribution of the alcohol-metabolizing enzymes alcohol dehydrogenase (ADH) and high and low Km aldehyde dehydrogenase (ALDH) was similar to that of GDH (F_2, F_6; $p \leqq 0.01$). In alcohol-treated cells the distribution was similar, but with a decrease in specific activity of ADH in all fractions and some small variations in that of ALDH (Fig. 2).

Electron microscopy

Qualitative results. Hepatocytes of the different fractions, of both control and alcohol-treated rats, showed a good ultrastructural perservation except for F_1 where some cells showed marked alterations. F_2 control hepatocytes were characterized

Table 1. Characteristics of hepatocyte density populations

Hepatocyte fractions	Buoyant density	Viability		Yield[b]	
		Control	Ethanol	Control	Ethanol
	g/cm³[a]	%		%	
OS	–	90	90		
F_1	1.086	>90	90	36.3±2.3	40.5±10
F_2	1.086–1.089	>90	>90	16.6±2.1	12.5± 3.2
F_3	1.089–1.095	>90	>90	11.6±2.3	9.2± 2.3
F_4	1.095–1.101	>90	>90	17.8±3.0	14.3± 4.3
F_5	1.101–1.107	>90	>90	11.2±3.1	15.3± 5.1
F_6	1.107–1.112	>90	>90	6.4±1.4	7.98±3.1

Mean values ±SD of 5–6 experiments are given; OS = original suspension of hepatocytes

[a] Nine volumes of Percoll (Pharmacia), one volume of 1.5 M NaCl and BSA (1% final concentration) were mixed

[b] The yield was calculated as percentage of the total number of cells in the different layers, excluding the top layer (clumps and damaged cells)

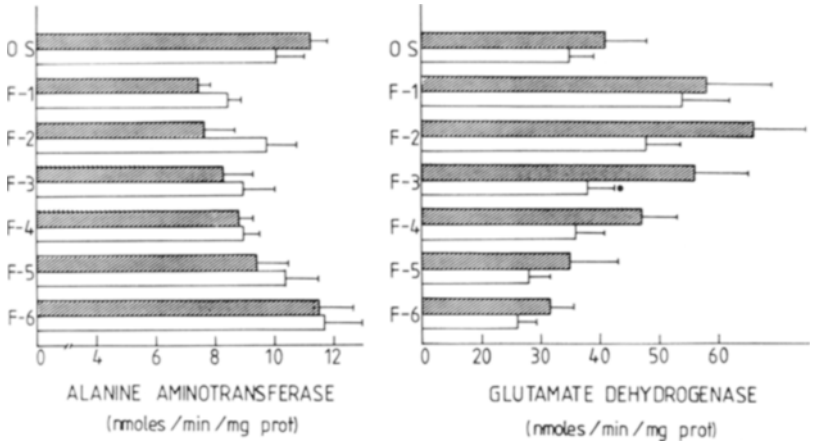

Fig. 1. Alanine aminotransferase and glutamate dehydrogenase activities of the original hepatocyte suspension (OS) and hepatocyte subpopulations (F_1–F_6) isolated from pair-fed control (▨) and pre+postnatally exposed to alcohol (□) 12-day-old rats. Values are mean ± SE from 5–6 different experiments. Significant differences from control group ($*p \leqq 0.05$)

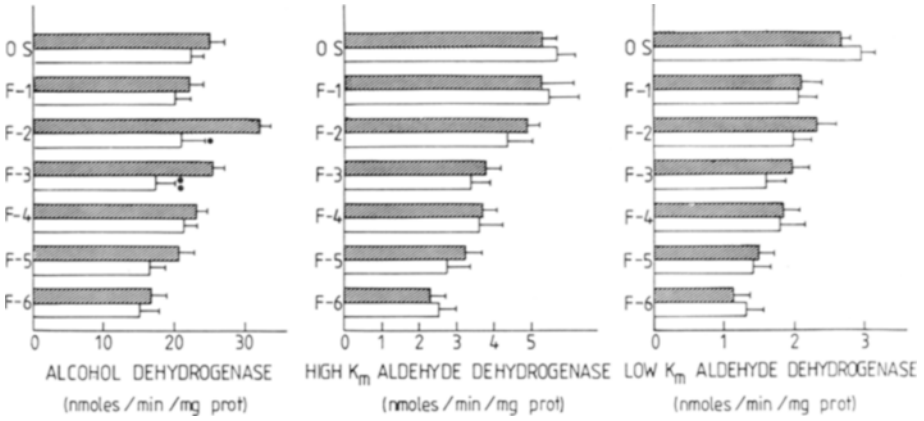

Fig. 2. Alcohol dehydrogenase and aldehyde dehydrogenase (high and low Km) activities, of hepatocyte subpopulations. Symbols as in Fig. 1. Values are mean ±SE from 5–6 different experiments. Significant differences from control group ($*p \leqq 0.01$; $**p \leqq 0.05$)

by a relative abundance of sER, large cytoplasmic areas, small mitochondria, scarcity of free ribosomes and few glycogen particles. The latter, when present, appeared as isolated α-particles. In contrast, glycogen was present in F_6 hepatocytes in large, compact areas. Other ultrastructural features of these cells were a prominent rER, free ribosomes and a poorly developed sER. Golgi apparatus in these hepatocytes appeared more prominent than in F_2 cells (Figs. 3, 4). The cells of F_3, F_4, and F_5 fractions showed a morphology intermediate between that of F_2 and F_6 cells.

The ultrastructural features of the hepatocytes from alcohol-treated rats were similar to those of control cells. However, as previously reported (Renau-Piqueras et al. 1985a, 1987), about 30% of cells showed the Golgi apparatus lacking cisternae and composed, mainly of small vesicles. In F_3, F_4, F_5 and F_6 fractions of alcohol-treated rats, there was a variable proportion of hepatocytes with a morphology clearly different from that of the rest of the cell population. The cytoplasm of these cells, which we have called "dense cells", was almost completely filled by mitochondria, free ribosomes and rER. Mitochondria in these hepatocytes showed a clear matrix and were larger than those of the rest of the cells. The morphology of the nucleus was also altered, mainly by condensed chromatin. These cells were found as well in ultrathin sections of liver tissue of alcohol-treated rats.

Some of the main ultrastructural characteristics of F_2 and F_6 hepatocytes, as well as those of "dense cells", are illustrated in Figs. 3–6.

Quantitative results. Absolute and relative stereological data obtained in the analysis of the hepatocytes of the different fractions are summarized in Figs. 7–10. A progressive increase in the mean absolute cell volume was observed from F_2 to F_6 fractions, with the largest cells in the F_6 fraction. The same pattern was found in the fractions from alcohol-treated rats (total cell population) although there was a significant reduction in the hepatocyte volume in all fractions (Fig. 7). This distribution was confirmed by cell sorting analysis.

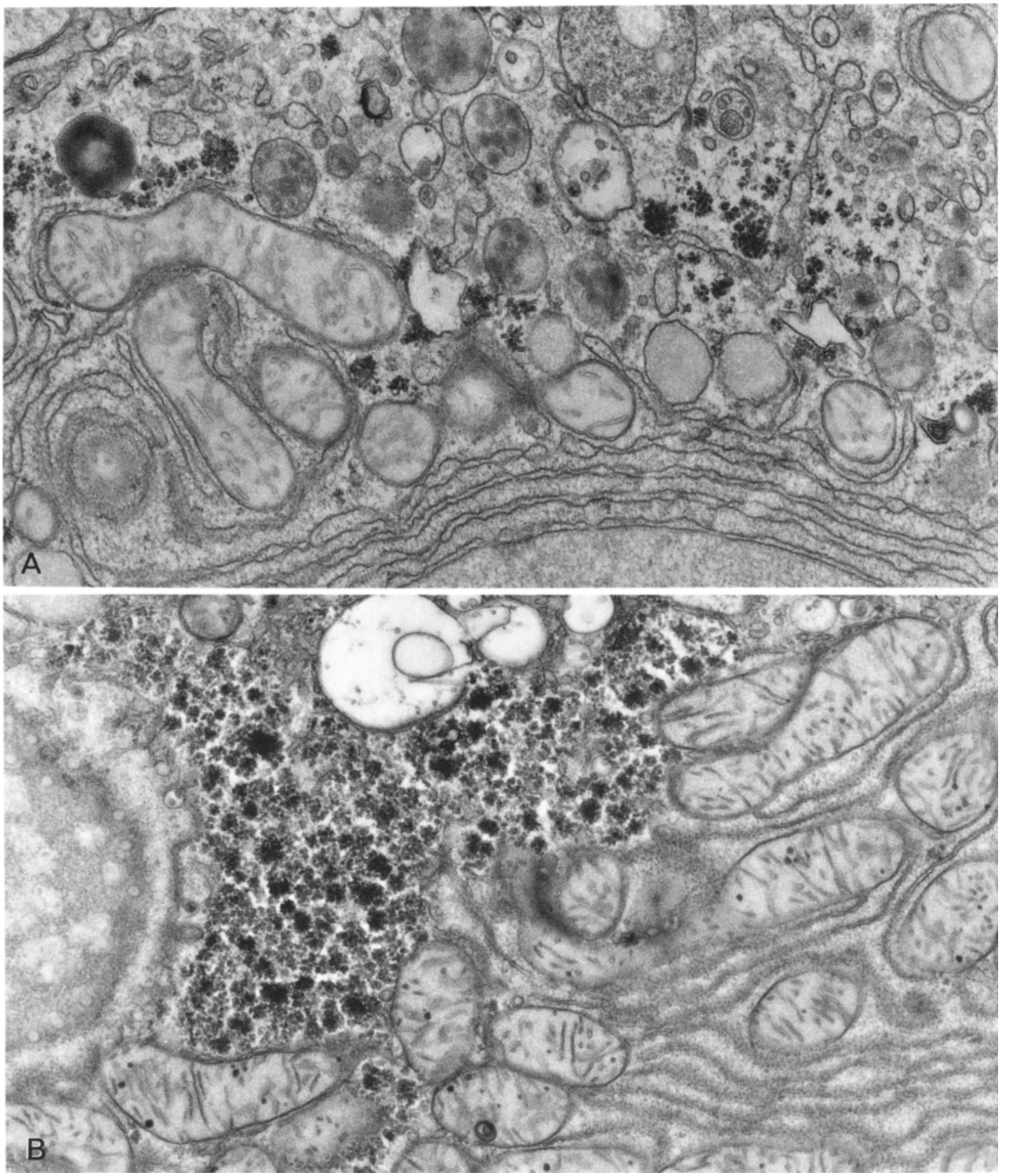

Fig. 3A, B. Micrographs showing the glycogen distribution in a F_2 (**A**) and a F_6 (**B**) control hepatocyte. In F_2 cells, glycogen is dispersed in small, isolated rosettes near the sER. In F_6 cells glycogen appears as large aggregates (**A**, ×31350; **B** ×25080)

When "dense cell" were considered separately, it was seen that they were smaller than the rest of the hepatocytes.

Regarding the cytoplasmic components of control hepatocytes (Figs. 8–10), it was found that the volume density of mitochondria, Golgi apparatus, glycogen and rER was higher in F_6 than in F_2 hepatocytes. Also, mitochondria of F_6 hepatocytes were 2.2 times larger than those of F_2 cells, although the total number of these organelles per

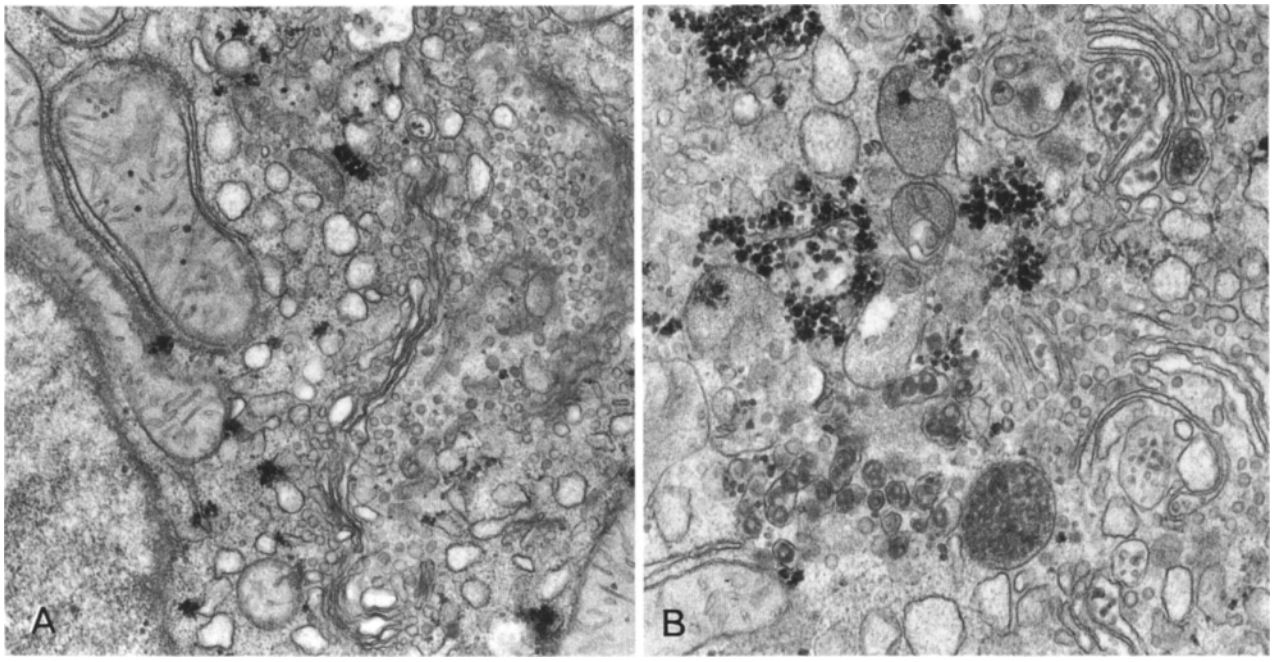

Fig. 4A, B. Details of control hepatocytes of F_2 (**A**) and F_6 (**B**) fractions. In (**A**) a Golgi area, several sER profiles and mitochondria are shown. **B** Electron micrograph illustrating a portion of a F_6 hepatocyte; a well developed Golgi apparatus and several vesicles containing very low density lipoprotein particles are present. Notice the well-preserved ultrastructure of the different cell components (**A**, ×22800; **B**, ×28700)

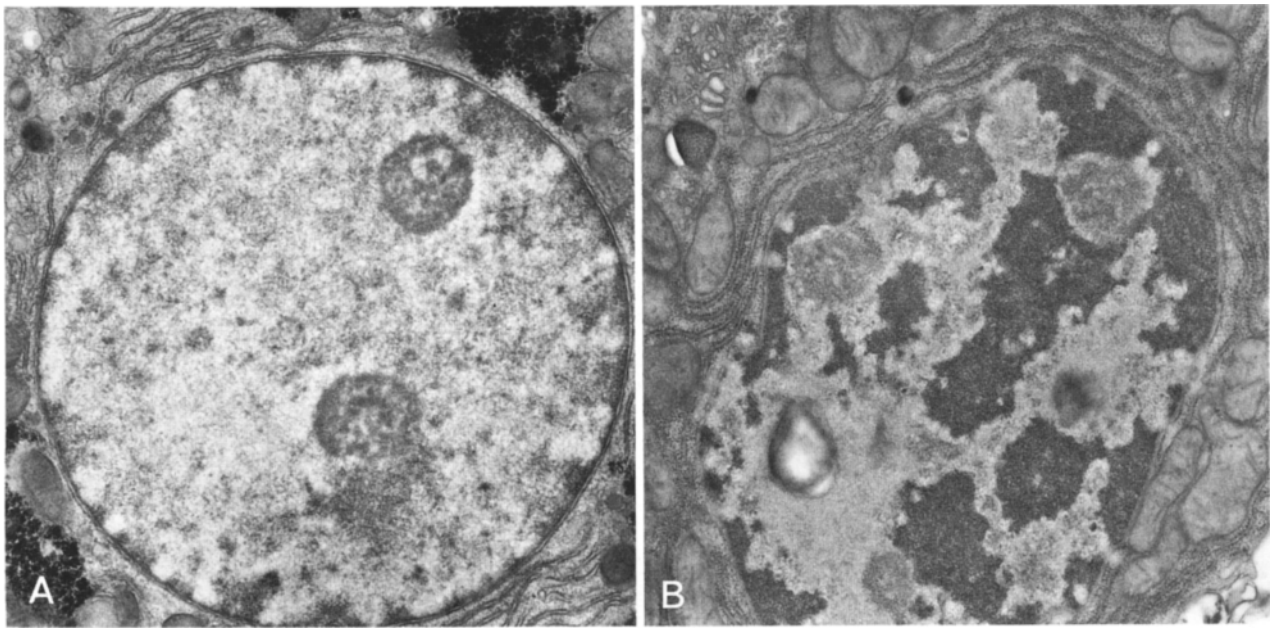

Fig. 5A, B. Micrographs showing the ultrastructural features of the nucleus of a control isolated hepatocyte **A** and a dense hepatocyte **B**. Note the difference in condensed chromatin patterns in the two cell types (**A**, ×12300; **B**, ×15300)

cell was similar in both cell populations. The numerical density of mitochondria and lysosomes and the volume density of sER and lysosomes were greater in F_2 than in F_6 cells. No significant differences were found in the stereological variables concerning peroxisomes.

The stereological analysis of subfractionated hepatocytes derived from alcohol-treated rats was carried out on the two morphologically different types of cells, "dense cells" and the normal (non-dense) cells. The proportion of "dense hepatocytes" in the different fractions was: 0% (F_2),

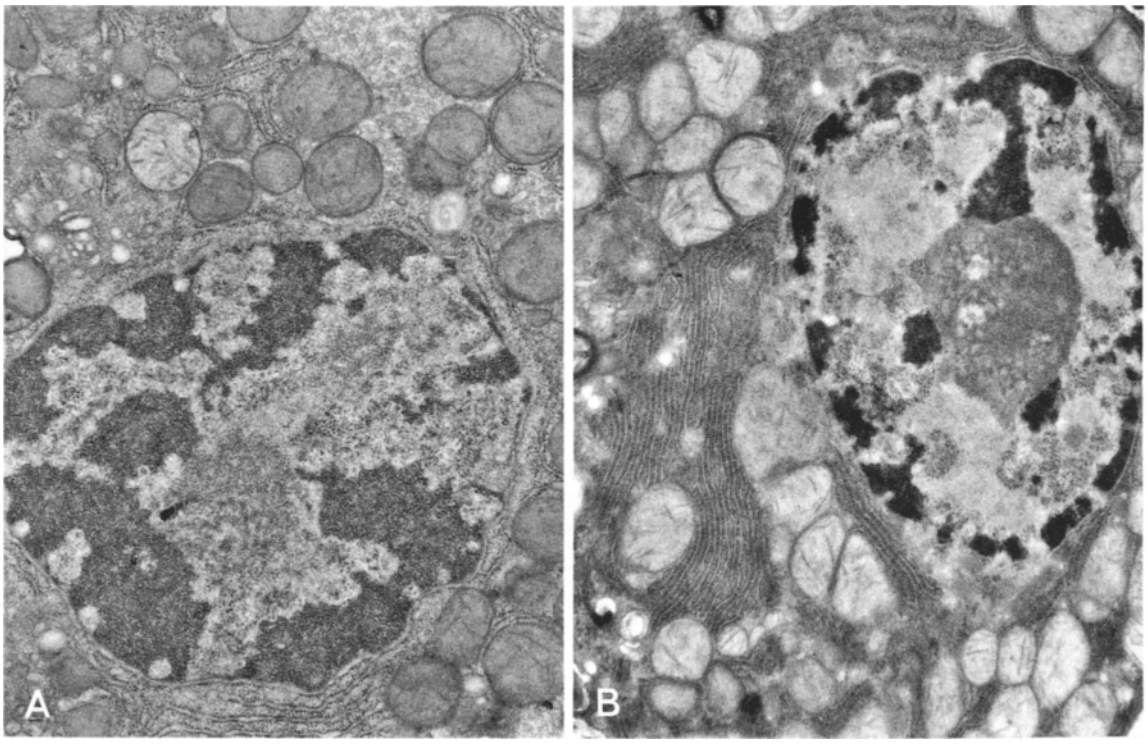

Fig. 6A, B. Micrographs showing some ultrastructural characteristics of hepatocytes isolated from alcohol-treated rats. (**A**) Hepatocyte showing a nucleus with features intermediate between normal and dense hepatocytes. In **B** a dense hepatocyte showing the cytoplasm almost completely filled by rER and mitochondria. The nucleus of this cell appears pycnotic. (**A** ×12413; **B**, ×12413)

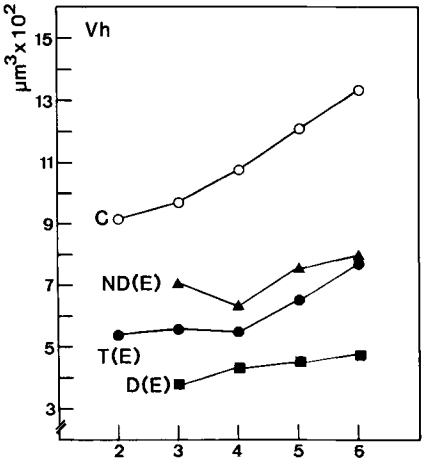

Fig. 7. The absolute volume of isolated and subfractionated hepatocytes is plotted as a function of their location in the different fractions. (o) Control hepatocytes (C). (•) Total ethanol-treated hepatocytes (T(*E*)). (■) Dense hepatocytes (ethanol) (D(*E*)) and (▲) non-dense hepatocytes (*ethanol*) (ND(*E*))

45.5% (F_3), 36% (F_4), 28% (F_5) and 9% (F_6). These cells, as noted above, were smaller but contained more rER and larger mitochondria than normal cells (Fig. 6B). The comparison between the two types of hepatocytes is summarized in Figs. 7–10. On the other hand, the variation in the different stereological parameters for the five fractions of alcohol-treated hepatocytes was similar to that described for control cells.

Comparison between control and treated hepatocytes showed that the volume density of mitochondria, rER and sER, the numerical density of mitochondria, and the total number of mitochondria per cell were greater, in all fractions, in alcohol-treated cells than in control (Figs. 8, 9). In contrast, the mean volume of a single mitochondria and the volume density of the Golgi apparatus and glycogen appeared greater in all the fractions of control hepatocytes (Figs. 8, 10).

Exposure to ethanol altered equally the size of F_2 and F_6 hepatocytes. However, mitochondrion and Golgi apparatus appeared to be more altered in F_2 cells, whereas glycogen, rER and probably sER were more affected in F_6 cells (Figs. 8–10, Table 2).

Discussion

Isolated hepatocytes constitute a model system with the "in situ" biochemical, ultrastructural and functional properties of hepatic parenchymal cells,

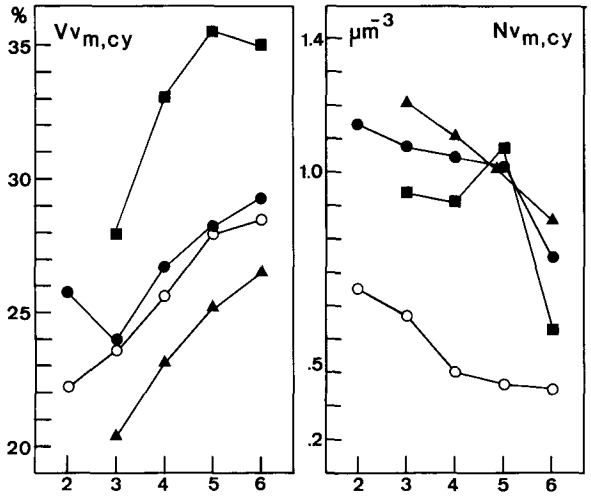

Fig. 8. Mitochondrial stereological parameters of isolated and subfractionated control and ethanol-treated hepatocytes. $Vv_{m,cy}$: volume density of mitochondria with respect to cytoplasm; $Nv_{m,cy}$: volume density of mitochondria per volume unit of cytoplasm; Nt_m: total number of mitochondria; $V_{s,m}$: mean absolute volume of a single mitochondrion. Symbols as in Fig. 7. *Abscissa*: Fraction number

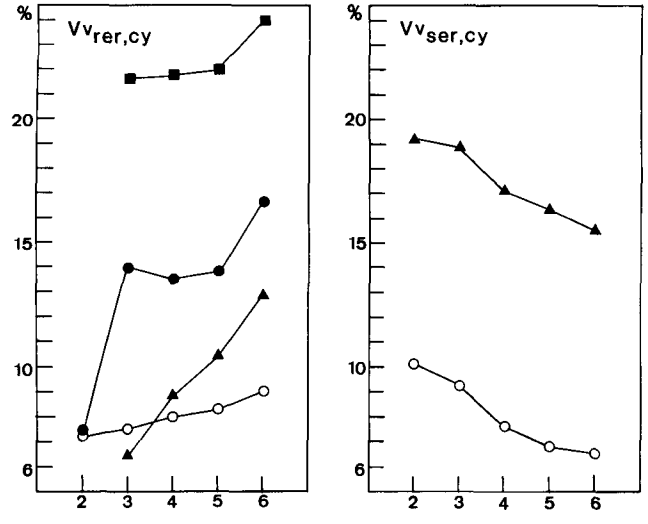

Fig. 9. Volume density of rER and sER of isolated and subfractionated control and ethanol-treated hepatocytes. Symbols as in Fig. 7. *Abscissa*: Fraction number

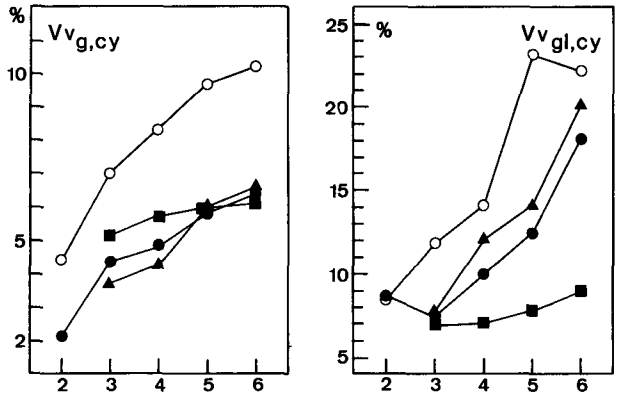

Fig. 10. Volume density of Golgi apparatus and glycogen of isolated and subfractionated control and ethanol-treated hepatocytes. Symbols as in Fig. 7. *Abscissa*: Fraction number

and have therefore been extensively used for the study of general as well as drug metabolism (Bernaert et al. 1979; Seglen 1976). Since the intraacinar heterogeneity is conserved after dispersion of the liver into cells, several authors have tried to separate the different populations of hepatocytes, according to different procedures. The advantages and problems of these have been discussed widely (Lindros et al. 1986). Isolation and subfractionation of hepatocytes in density gradients has been used in the present work mainly because it permits using biochemical, electron microscopy and flow fluorocytometric techniques in studying cells from the same sample.

Our biochemical and stereological results show significant differences between F_2 and F_6 cells subfractionated from control rats, suggesting that F_2 and F_6 cells do in fact constitute two different populations of hepatocytes. Since few data are available on the stereological and biochemical characteristics of neonatal PV and PP rat hepatocytes "in situ", we compare our "in vitro" results with those obtained in adult rats (Loud 1968). Although age-related changes in the quantitative ultrastructure of rat hepatocytes have been reported, it seems that the general differences between PV and PP cells are maintained almost during the first year of life (Kanamura et al. 1985; Meihuizen and Blansjaar 1980; Schmucker et al. 1977; Schmucker et al. 1978; Sturgess and de la Iglesia 1972).

Several studies have indicated a gradient of enzyme activities rather than exclusive localization of enzymes along the acinus. Thus, ALAT activity has been shown to be mainly located in PP adult hepatocytes and GDH had the highest activity in PV cells (Morrison et al. 1965; Shank et al. 1959; Welsh 1972). Our results are in agreement with these findings since we found that the highest

Table 2. Stereological parameter ratio between F_6 and F_2 hepatocytes and between control and treated hepatocytes

Component	Parameter	F_6/F_2		F_2/F_2	F_6/F_6
		Control	/ Ethanol	Control/Ethanol	Control/Ethanol
Hepatocyte	Vh	1.46*	1.44*	1.70*	1.74*
Nucleus	$Vv_{n,h}$	0.92	0.97	0.89	0.92
	V_n	1.41*	1.31*	2.41*	2.61*
Mitochondria	$Vv_{m,cy}$	1.28*	1.21*	0.86*	0.97
	$Sv_{m,cy}$	1.09	1.07	0.93	0.95
	$Nv_{m,cy}$	0.54*	0.66*	0.57*	0.47*
	Nt_m	1.05	0.73	0.66*	0.95
	Vs.m	2.21*	2.10*	1.58*	1.66*
Lysosomes	$Vv_{l,cy}$	0.69*	0.83	0.90	0.75*
	$Sv_{l,cy}$	0.58*	0.79	0.91	0.65*
	$Nv_{l,cy}$	0.25*	0.73	0.76*	0.26*
	Nt_l	1.05	1.21	0.87*	0.42*
rER	$Vv_{rer,cy}$	1.25*	2.21*	1.04	0.54*
	$Sv_{rer,cy}$	1.62*	2.30*	1.10	0.77*
sER	$Vv_{ser,cy}$	0.64*	0.81	0.53*	0.42*
	$Sv_{ser,cy}$	0.61*	0.78	0.90	0.71*
Golgi	$Vv_{g,cy}$	2.08*	3.00*	2.08*	1.44*
Glycogen	$Vv_{gl,cy}$	2.65*	2.08*	0.98	1.17
Lipid droplets	$Vv_{ld,cy}$	1.07	1.08	1.35*	1.35*
	$Sv_{ld,cy}$	1.13	1.08	3.97*	3.05*
	$Nv_{ld,cy}$	1.13	0.98	1.25*	1.45*
	$Nt_{ld,cy}$	2.10*	1.53*	1.71*	2.28*
Peroxisomes	$Vv_{p,cy}$	0.93*	1.29*	0.86*	0.62*
	$Sv_{p,cy}$	1.14	1.12	0.96	0.98
	$Nv_{p,cy}$	0.86	1.10	0.94	0.74*
	$Nt_{p,cy}$	0.77	1.77*	2.54*	1.10

Vv, volume density; Sv, surface density; Nv, numerical density; cy, cytoplasm; h, hepatocyte. The first subindex corresponds to cell component and the second subindex corresponds to reference volume.
* Significant differences (Student's t-test, $p \leqq 0.05$). Alcohol-treated hepatocytes: total cell population

ALAT activity was in F_6 whereas that of GDH was in F_2. At the same time, our stereological results agree with data obtained "in situ" in adult animals (Loud 1968). Thus, we have found that F_6 hepatocytes have more rER, glycogen and peroxisomes than F_2 cells. Moreover, the Golgi apparatus in the former cells is more developed than in the latter. Mitochondria in F_6 hepatocytes are twice as large as those of F_2 cells, in agreement with a recent stereological "in situ" analysis indicating that in developing mouse liver PP mitochondria are larger than those of PV (Kanamura et al. 1985). Finally, whereas glycogen in F_6 cells appears distributed in large aggregates, in F_2 cells it is dispersed as isolated rosettes. F_2 cells, on the other hand, contain more sER than do F_6 hepatocytes. The volume density of lysosomes in these cells is also greater than in F_6 cells. Although we have not considered the different types of lysosomes in our analysis, this study is in progress. From our results we can suggest that F_2 fraction is enriched in PV hepatocytes whereas F_6 fraction is composed mainly of PP hepatocytes. F_3, F_4 and F_5 contain mainly hepatocytes located in zone 2. Moreover, our data on the cell components and enzyme distribution in the subfractionated hepatocytes are in agreement with the specific functions assigned to PP and PV cells (Gumucio and Miller 1981; Jungermann and Sasse 1978).

Although previous studies on the acinar distribution of ADH are contradictory, drug bio-transformation enzymes are preferentially located in the PV zone (Loud 1968; Wanson et al. 1974). We have found ADH was predominantly in the F_2 cells in 12-day-old rats, which agrees with the results of Morrison and Brock (1967) for adult human and rat liver.

The few reports on the acinar distribution of low and high Km ALDH activities in adult livers have also been controversial (Bengtsson et al. 1981; Väänänen et al. 1984), but in our 12-day-old rats both ALDH activities were mainly in the F_2 hepatocytes, as was ADH, in agreement with Lindahl et al. (1983). Since the PV zone is apparently

the main site for drug detoxification, the enzymes of this zone may be developed earlier to take care of detoxification in the developing liver.

One of the major disadvantages of subfractionation methods for hepatocytes, including centrifugation in density gradients, is that when animals have been exposed to a drug there is no guarantee that cells which separate at a certain density correspond to hepatocytes that separate at the same density from normal animals (Gumucio and Miller 1982; Lindros et al. 1986).

Indeed, the altered characteristics of the "dense cells" we observed from alcohol-treated rats, does not permit, on the basis of their density, correlating them with the hepatocytes from control rats which sediment at the same density. However "dense cells" constitute a minor population, mainly in F_2 and F_6 fractions. On the other hand, the enzymatic and stereological characteristics of the normal, i.e. non-dense, cells from alcohol-treated rats were distributed in the fractions in a pattern similar to that of hepatocytes from control animals, although there were some morphological and stereological differences between F_2 and F_6 hepatocytes from alcohol-treated and control rats.

Analysis of stereological data showed that some cell components such as mitochondria and Golgi apparatus are more affected by prenatal alcohol exposure in F_2 cells than in F_6. In contrast, lysosomes and ER appear more altered in F_6 hepatocytes (Table 2). This indicates that prenatal exposure to alcohol could have a selective effect on specific cell components depending on the acinar zone.

The biochemical data show that the activities of both marker enzymes GDH and ALAT were altered after alcohol treatment, mainly in F_2 hepatocytes. Whereas ALAT activity showed an increase, GDH activity decreased. It has been suggested that ethanol produces a perivenous hypoxia due to an increase in oxygen uptake (Ji et al. 1983; Lemasters et al. 1983; Thurman et al. 1986); the results we observed on ALAT and GDH activities in treated animals would seem to confirm this hypothesis. The increase in ALAT activity may reflect an adaptive response to the hypoxia. On the other hand, the decrease in GDH in all fractions probably reflects mitochondrial damage, which may be enhanced in the PV zone. In fact we have demonstrated here that prenatal exposure to alcohol induces injury in the mitochondrial ultrastructure of F_2 cells and have shown previously similar mitochondrial alterations in the liver (Renau-Piqueras et al. 1985a). A 45% decrease in GDH activity in chronic alcoholic rats has also been reported (Idéo et al. 1972). The increase or decrease in the GDH and ALAT ratios we observed between F_6 and F_2 fractions from alcohol-treated animals vs. controls, could be due to some contamination of these fractions by "dense cells" which are biochemically indistinguishable from the "normal" (non-dense) cells.

When the ethanol-metabolizing enzymes, ADH and high and low Km ALDH, of hepatocyte subpopulations of control and alcohol-treated animals were compared, ADH activity was found to be decreased in the F_2 hepatocytes, with less significant differences in the other fractions. A small decrease in ADH activity in liver homogenates, after alcohol exposure, was reported from our laboratory (Guerri and Grisolía 1982), and another group also found a moderately lowered ADH activity in hepatocytes from chronic alcoholic rats, although they found no differences in ADH activity across the acinus (Väänänen et al. 1984). In the work indicated above (Guerri and Grisolía 1982), alcohol induced a small increase in ALDH activity; however no significant differences between ALDH activities of control and alcohol-treated rats were found in this study. In chronic alcoholic animals, changes in ALDH activities have not been consistent (Guerri et al. 1978; Koivula and Lindros 1975). It is possible that these discrepancies may be related to loss of mitochondrial integrity on prolonged exposure to ethanol.

We have shown in previous studies (Guerri and Grisolía 1982) that prenatal and pre + postnatal exposure to alcohol induced a significant reduction in the body and liver weights of rats. We have observed similar decreases in rats exposed to alcohol in this study (data not shown). Although nutritional deficiencies may have played a role due to the interference of ethanol with lactation (Sanchis and Guerri 1986), we have recently demonstrated the presence of alcohol and acetaldehyde in the milk (Guerri and Sanchis 1986), both of which could have deleterious effects on growth and on the development of the liver.

In summary, the results presented here show that ethanol exposure during zonal development in rat liver could have a selective effect on specific cell organelles depending on the acinar zone. It appears, in addition, that the perivenous hepatocytes are more susceptible to ethanol injury on the basis of the biochemcial and stereological results.

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