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Qualitative and quantitative ultrastructural alterations in hepatocytes of rats prenatally exposed to ethanol with special reference to mitochondria, golgi apparatus and peroxisomes

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Summary. To assess the effect of ethanol on the liver of the offspring of alcohol-fed rats, the hepatocytes of newborn rats whose mothers were fed: a) a liquid diet containing alcohol, b) the same diet isocalorically balanced, or c) a chow diet, were analyzed using both quantitative and qualitative electron microscopy as well as cytophotometric and biochemical methods.

Hepatocytes of chow-fed and pair-fed controls showed differences in the amounts of glycogen and lipids as well as in several stereologically measured variables including mitochondria, Golgi apparatus and smooth endoplasmic reticulum. These differences are probably due to the composition of the diet.

Rats prenatally exposed to alcohol showed increases in the hepatocyte and mitochondria volumes and in the number of peroxisomes. Moreover, the Golgi apparatus of these cells appeared disorganized and composed exclusively of small size vesicles, suggesting an impairment of their function.

Key words: Ethanol – Prenatal exposure – Stereology – Cytophotometry

Studies of the fetal alcohol syndrome in humans have established a serious risk of developmental and growth abnormalities in the offspring of mothers consuming ethanol during pregnancy (Abel 1982; Colangelo and Jones 1982; Streissguth et al. 1980). This teratogenic effect of ethanol has also been clearly established in laboratory experimental animals (Chernoff 1977; 1980; Kronik 1976; Randall and Taylor 1979; Streissguth et al. 1980). Thus, several reports indicate that heavy ethanol consumption by experimental animals during pregnancy causes a reduction in fetal and postnatal growth, fetal or embryo death, alterations in neuro-epithelial cells and important

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biochemical alterations in brain and liver (Banningan 1984; Chernoff 1977, 1980; Guerri and Grisolia 1982; Guerri et al. 1984; Kronik 1976; Randall and Taylor 1979).

Despite the large amount of work in this field (Abel 1982; Colangelo and Jones 1982; Streissguth et al. 1980) very few reports have been focused on the study of ultrastructural effects of ethanol on the tissue of humans and experimental animals prenatally exposed to this compound (Banningan 1984; Beskid et al. 1975; Lefkowitch et al. 1983; Rømert and Matthiessen 1983), and although the effect of alcohol on liver is well known, little information is available on ultrastructural alterations in the liver in these cases (Beskid et al. 1975; Lefkowitch et al. 1983; Rømert and Matthiessen 1983). Therefore, we have investigated, using qualitative and quantitative electron microscopy, the alterations at birth in hepatocyte ultrastructure of rats prenatally exposed to alcohol (PEA). We have used newborn rats whose mothers received a liquid diet containing ethanol or, for control purposes, the same liquid diet lacking alcohol but isocalorically balanced with maltose-dextrin (Lieber and DeCarli 1976 and 1982). In addition, the ultrastructure of hepatocytes of these control rats was compared with that of newborns from rats receiving standard solid food and water "ad libitum".

Material and methods

Treatment of rats. Female wistar rats weighing between 200 and 250 g were treated as follows (Guerri and Grisolia 1982): all rats were maintained on a 12-h light day cycle in stainless steel cages. They received the Lieber-DeCarli liquid diet (Lieber and DeCarli 1976, 1982) either containing 5% (v/v) ethanol (treated rats) or isocalorically balanced with maltose-dextrin for pair-fed controls (control-1). Another group of rats was given rat chow and tap water "ad libitum" (control-2). Female rats were maintained on the ethanol liquid diet for a minimum of 30 days prior to exposure to male rats. Pairs of a male and a female rat were housed in separate cages, the male animals being removed once pregnancy was confirmed. Immediately after birth three newborns per group were randomly chosen and sacrificed between 8:30 and 9:00 a.m.

Electron microscopy. Tissue samples were taken and processed for electron microscopy as described previously with some modifications (O'Connor et al. 1984). The left lateral lobes of the livers of both control and treated newborn rats were fixed by immersion (David and Uerling 1983) in 1% glutaraldehyde – 0.7% formaldehyde in 0.05 M cacodylate buffer (pH 7.4 at 4° C) containing 2 mM $CaCl_2$. Effective osmolarity of the fixative solution (300 mOsm) was calculated as described (Mathieu et al. 1978; Renau-Piqueras et al. 1980). After 30 min, a thin slice from the central region of the lobe of each treated and control rat was cut. These slices were cut into some 30 cubes, and from these blocks, 12 were chosen randomly. After 120 min at 4° C in fresh fixative solution, the blocks were 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 2% OsO_4 containing 1.8% potassium ferrocyanide (White et al. 1979), washed in cacodylate buffer, dehydrated in a graded series of acetones and embedded in Vestopal. Of the 12 blocks per rat, five were chosen randomly and ultrathin sections (1 section per block; interference color, silver) were stained with uranyl acetate and lead citrate.

Stereology. From each ultrathin section five micrographs were made at two levels (Weibel 1979; Weibel et al. 1969). At level I (×8,938) the cell (hepatocyte), nucleus, nucleolus, mitochondria, peroxisomes, and lipid droplets were assessed. At level II (×47,880), the rough endoplasmic reticulum (RER), smooth endoplasmic reticulum (SER), and Golgi apparatus were estimated. Stereological analysis of the micrographs was made by planimetry using a

Leitz ASM system for semi-automatic image analysis (O'Connor et al. 1984; Renau-Piqueras and Cervera 1983; Renau-Piqueras et al. 1983) which permits the direct determination of the volume and surface densities (Vv_i and Sv_i, respectively). The other parameters used in this study (see Tables 1 and 2) were obtained using a H.P. 2100 computer and a stereological program developed by us (QUAN VIII). For this, the following formulas were used (Weibel 1979; Weibel and Bolender 1973; Williams 1977):

Absolute nuclear volume (Vn):

$$Vn = Vn_n / Nv_n \tag{I}$$

where Vv_n is the nuclear volume density per unit volume of tissue and Nv_n, the nuclear numerical density per unit volume of tissue. Nv_n was calculated according to:

$$Nv_n = \frac{k}{\beta} \cdot \frac{Na_n^{1.5}}{Vv_n^{0.5}} \tag{II}$$

where Na_n is the numerical profile density, $k = 1.05$ and $\beta = 1.38$ (Weibel et al. 1969).

Absolute hepatocyte volume (Vh):

$$V_h = Vn \cdot Vv_h / Vv_n \tag{III}$$

where Vv_h is the hepatocyte volume density per unit volume of tissue and Vv_n the nuclear volume density per unit volume of tissue.

Formula (II) was also used for determination of the numerical density of mitochondria (Nv_m), peroxidsomes (Nv_p) and lipid droplets (Nv_{ld}); k was calculated by the expresion:

$$k = (M_3/M_1)^{1.5} \tag{IV}$$

where

$$M_1 = (d_1 + d_2 + d_3 + \ldots + d_n)/n \tag{V}$$

and

$$M_3 = [(d_1^3 + d_2^3 + d_3^3 + \ldots + d_n^3)/n]^{1/3} \tag{VI}$$

d_1, d_2, d_3, ... and d_n are the mean diameter of each profile. The coefficient β was calculated after determining the mean axial ratio of the same profile population by using a normogram for ellipsoids and cylinders (Weibel and Bolender 1973).

Finally, the absolute volume of a single mitochondrion, a single peroxisome, or a single lipid droplet was calculated by the formula:

$$Vi = Vv_i \cdot V_{cy} / Nt_i \tag{VII}$$

where V_{cy} is the absolute volume of cytoplasm and Nt_i the total number of elements per cell ($Nt_i = Nv_i \cdot V_{cy}$).

The minimal sample size for each variable was determined by the progressive mean technique (Williams 1977) (confidence limit, $\pm 5\%$). Since no statistical differences (analysis of variance, $P \leqq 0.05$) were found between animals within each group, the results are expressed as the mean $\pm$s.d. of each group. Finally, statistical comparison of the stereological data was by the Student's *t*-test ($P \leqq 0.05$).

Morphometric no-stereologic parameters. As a measure of the shape of cells, nuclei and mitochondria, their coefficient of form (CF) was determined. For circular or elliptical profiles, as nuclei or mitochondria, we have used the expression $CF = 4\pi \text{ Area/Perimeter}^2$ (Renau-Piqueras and Cervera 1983; Renau-Piqueras et al. 1983) (The CF for a circle is 1.0 and for an ellipse of $a = 1$ and $b = 0.5$, $CF = 0.8$). In the case of hepatocytes, and assuming a hexagonal shape, we have tested several formulas to determine the CF of: a) a series of 13 concentric regular hexagons, and b) a series of 13 concentric irregular hexagons. The formulas were: a) $CF_1 = \text{Area/Perimeter}^2$; $CF_2 = 4\pi \text{Area/Perimeter}^2$, and $CF_3 = \text{Surface/Volume}$ (Williams 1977). For the regular hexagons we obtained: $CF_1 = 0.0685 \pm 0.00059$ (mean $\pm$s.d.); $CF_2 = 0.8618 \pm 0.0078$, and $CF_3 = 0.1005 \pm 0.0754$. Similar variation coefficients were obtained for the irregular hexagons and the three CF values were smaller than those for regular hexagons.

Since the CF_2 is the same for circular or elliptical profiles and considering the variation coefficients, we have also used this CF as a measure of the hepatocyte shape.

To assess the area distribution curve of mitochondrial profiles a total of 600 mitochondria per case was measured.

Cytophotometric and Biochemical determinations. Lipid content in the three types of rat livers was determined cytophotometrically. Vestopal sections (1 μm) were incubated in a 1% alcoholic solution of Sudan black B for 4 min at room temperature (Barka 1967). Absorption between 400 and 800 nm was then determined in a Leitz MPV-2 cytophotometer. As reference, the absorption of a 1% Sudan black B solution was measured in a Perkin-Elmer spectrophotometer. Cytophotometric determination of glycogen was carried out by the PAS technique (Barka 1967), and absorption between 400 and 800 nm was also determined.

Biochemical determination of liver glycogen was made in Control-1 and PEA rats according to Hassid and Abraham (1957).

Results

Qualitative observations. When ultrathin sections of the periphery of blocks were considered, lipid droplets, extracellular spaces, lipoprotein particles, and glycogen showed a prominent electron-dense staining (Fig. 1), probably due to the OsO_4-ferrocyanide fixation. Subjective analysis of electron micrographs of Control-1 and Control-2 hepatocytes revealed several differences between cells from the two sources. Thus, Control-2 cells showed more lipid droplets than Control-1 hepatocytes. Moreover, while very low density

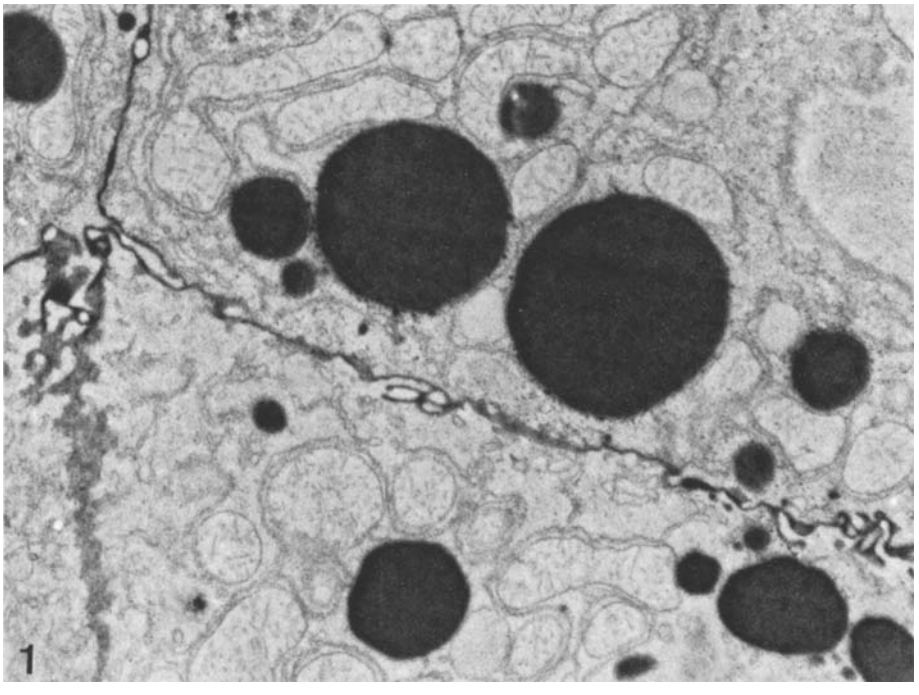

Fig. 1. Detail of Control-2 (chow-fed) rat hepatocytes showing lipid droplets and extracellular spaces with a prominent electron-dense staining. No counterstain. (× 12,155)

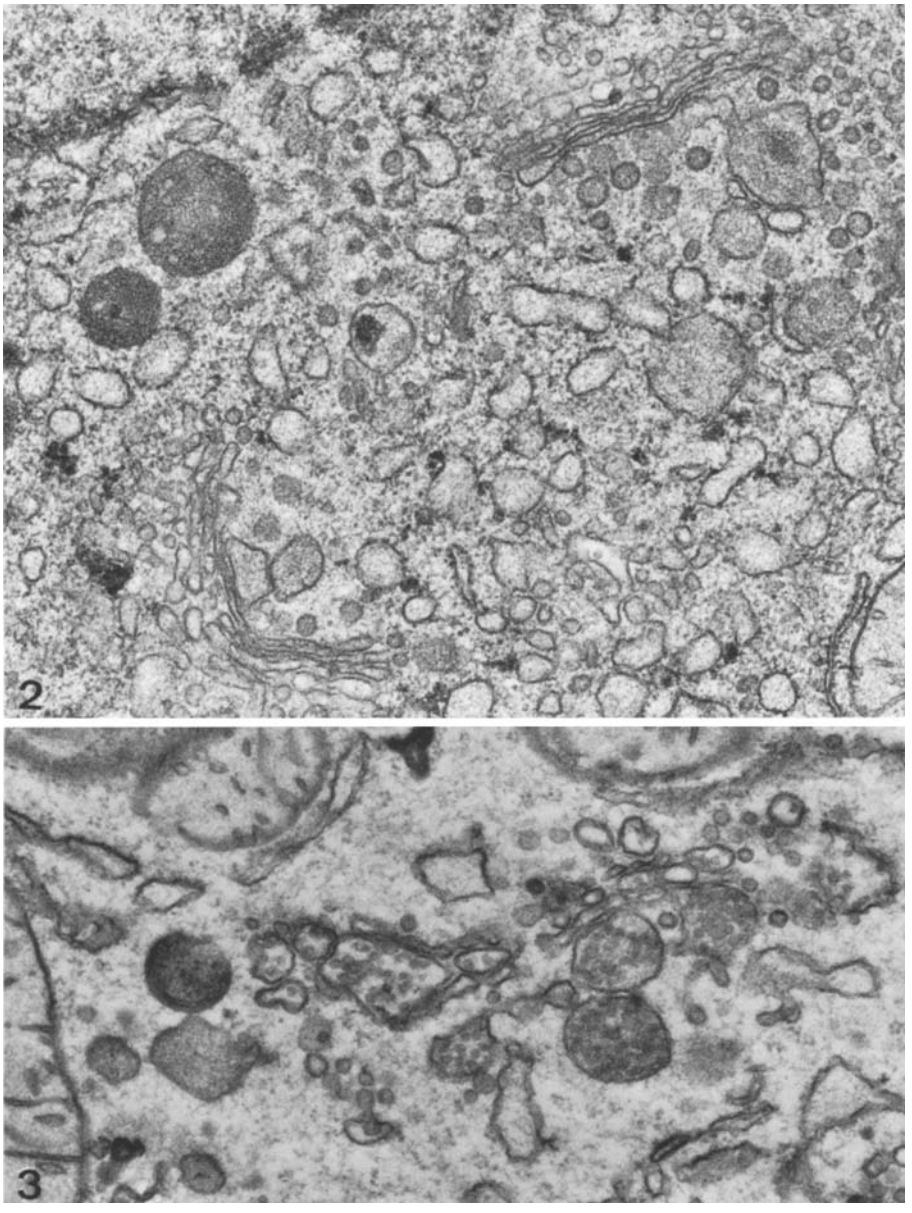

Fig. 2. Golgi apparatus of a Control-1 (pair-fed) rat hepatocyte. VLDL particles are absent. Smooth endoplasmic reticulum appeared organized in vesicles. Compare the morphology of Golgi apparatus in these cells with that of PEA rats illustrated in Fig. 5 (× 35,910)

Fig. 3. Detail of the Golgi apparatus of a Control-2 (chow-fed) rat hepatocyte. Note the presence of abundant VLDL particles in the cisternae (× 36,936)

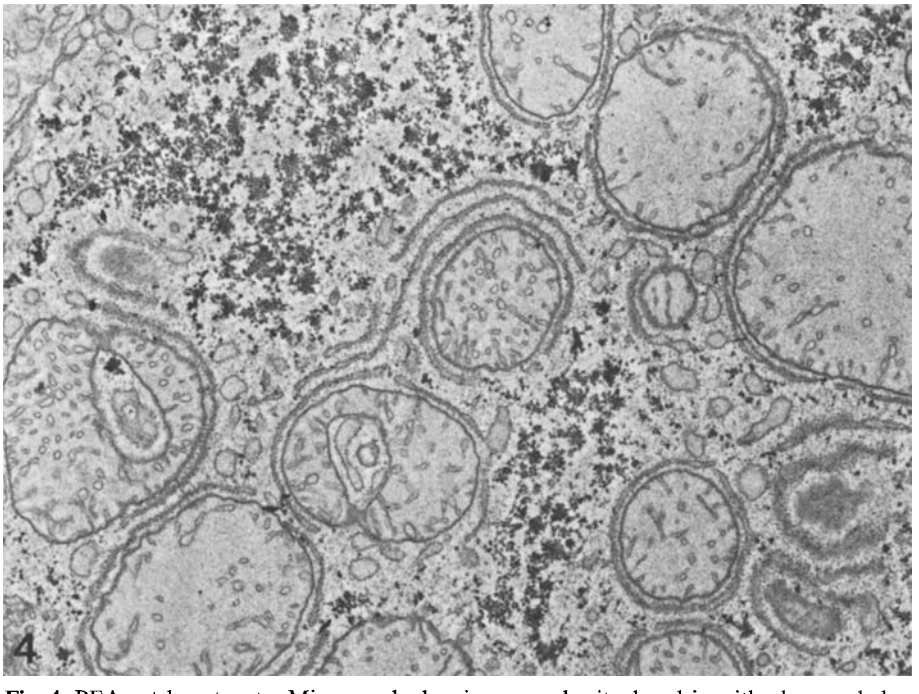

Fig. 4. PEA rat hepatocyte. Micrograph showing several mitochondria with abnormal shape and size. Mitochondria in PEA rat hepatocytes are 1.53 times larger (absolute volume) than those of pair-fed controls (× 17,000)

lipoprotein particles (VLDL) were frequently seen in the Golgi stacks of Control-2 hepatocytes, they were scarce in most of the Control-1 cells examined (Figs. 2 and 3). Cells also differed in the morphology of SER. Thus, whereas Control-2 hepatocytes showed a typical, tubular SER, this organelle in the pair-fed cells was composed mainly of vesicles of different sizes (Fig 2). Moreover, the cisternae of RER of Control-1 cells were more dilated than those of Control-2 hepatocytes. Finally, Control-1 cells showed more glycogen than Control-2 hepatocytes.

Comparison between Control-1 and alcohol treated cells indicated some ultrastructural differences, particularly in the mitochondria and Golgi apparatus. Mitochondria of treated cells were larger than those of Control-1 hepatocytes (Fig. 4), and also showed abnormal shapes (Fig. 4). The Golgi apparatus of these cells appeared to be composed mainly of vesicles. Cisternae and VLDL particles were rarely found (Fig. 5). Glycogen was not uniformly distributed among the cells in the liver of PEA rats. Thus, in the same area, cells containing large amounts of glycogen could be seen close to others in which it was lacking. In addition, the pattern of glycogen distribution was diffuse. Although lysosomes were scarce in both cell types, no qualitative differences were observed. Finally, in some cells, myelinic figures indicating residues of membrane lipid metabolism were found (Fig. 6).

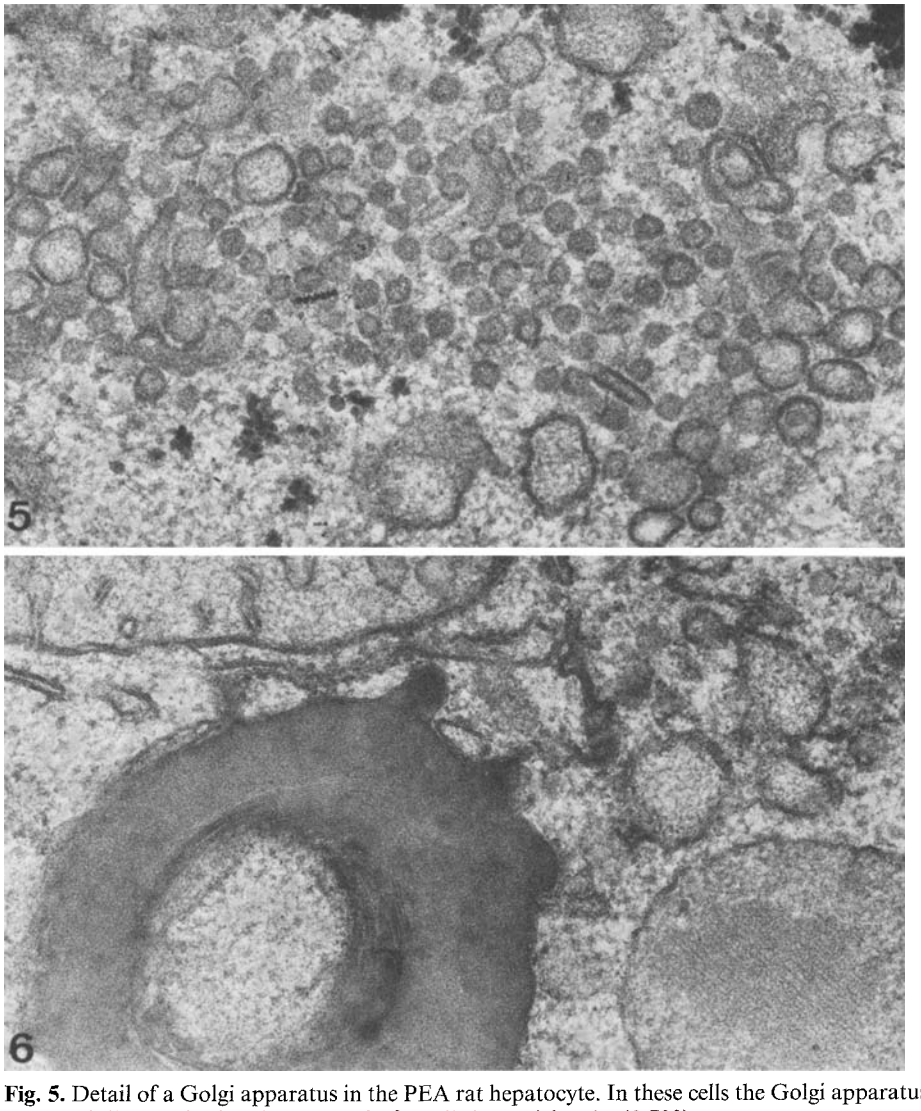

Fig. 5. Detail of a Golgi apparatus in the PEA rat hepatocyte. In these cells the Golgi apparatus appeared disorganized and composed of small size vesicles. (× 49,783)

Fig. 6. Micrograph showing a cytoplasmic myelinic figure in a PEA rat hepatocyte. (× 62,000)

Stereological and morphometric results. Absolute and relative stereological data obtained in the study of the three types of hepatocytes considered are summarized in Tables 1 and 2. These data indicate that Control-1 and Control-2 are significantly different in several variables including the absolute nuclear volume, the absolute volume of a single mitochondrion, the volume density of nucleus, Golgi apparatus and SER, and the surface density of nucleus and SER, which are greater in pair-fed than in Control-2

Table 1. Relative stereological data (mean ± s.d.) of hepatocytes from rats prenatally exposed to alcohol (PEA), Control-1 (pair-fed) and Control-2 (chow diet)

Component	Parameter	PEA	Control-1	Control-2	Units
Nucleus	$Vv_{n,h}$	18.97 – 4.07	20.25 – 4.16	15.52 – 4.37[b]	μm^0
	$Sv_{n,h}$	0.15 – 0.03	0.22 – 0.07[a]	0.14 – 0.03[b]	μm^{-1}
Nucleolus	$Vv_{nu,n}$	8.24 – 2.11	10.22 – 2.23	9.03 – 2.14	μm^0
	$Sv_{nu,n}$	0.22 – 0.07	0.25 – 0.09	0.23 – 0.09	μm^{-1}
Mitochondria	$Vv_{m,cy}$	22.08 – 5.55	16.44 – 3.97[a]	18.26 – 5.00	μm^0
	$Sv_{m,cy}$	0.85 – 0.22	0.72 – 0.18[a]	1.09 – 0.24[b]	μm^{-1}
	$Nv_{m,cy}$	0.09 – 0.03	0.10 – 0.03	0.23 – 0.09[b]	μm^{-3}
Lipids	$Vv_{l,cy}$	2.11 – 0.42	1.48 – 0.23	12.19 – 2.72[b]	μm^0
	$Sv_{l,cy}$	0.14 – 0.09	0.08 – 0.02	0.68 – 0.15[b]	μm^{-1}
	$Nv_{l,cy}$	0.08 – 0.04	0.06 – 0.04	0.35 – 0.12[b]	μm^{-3}
Golgi	$Vv_{g,cy}$	3.71 – 1.74	5.97 – 3.19	1.84 – 0.33[b]	μm^0
	$Sv_{g,cy}$	0.17 – 0.07	0.22 – 0.09	0.24 – 0.02	μm^{-1}
RER	$Vv_{r,cy}$	3.14 – 1.17	3.12 – 1.03	4.15 – 0.92[b]	μm^0
	$Sv_{r,cy}$	0.46 – 0.19	0.74 – 0.29[a]	0.72 – 0.06	μm^{-1}
SER	$Vv_{sr,cy}$	4.28 – 1.41	4.74 – 1.74	0.48 – 0.08[b]	μm^0
	$Sv_{sr,cy}$	0.98 – 0.24	1.27 – 0.43	0.22 – 0.02[b]	μm^{-1}
Peroxisomes	$Vv_{p,cy}$	0.79 – 0.22	0.94 – 0.29	0.83 – 0.27	μm^0
	$Sv_{p,cy}$	0.19 – 0.04	0.09 – 0.03[a]	0.07 – 0.02	μm^{-1}
	$Nv_{p,cy}$	0.13 – 0.05	0.09 – 0.02[a]	0.08 – 0.03	μm^{-3}

Vv, volume density (in percentage); Sv, surface density; Nv, numerical density; The first subindex corresponds to cell component and the second subindex corresponds to reference volume

[a] Significant differences between PEA and Control-1 hepatocytes ($P \leqq 0.05$)
[b] Significant differences between Control-1 and Control-2 hepatocytes ($P \leqq 0.05$)

Table 2. Absolute stereological data (mean ± s.d.) of hepatocytes from rats prenatally exposed to alcohol (PEA), Control-1 (pair-fed) and Control-2 (chow diet)

Component	Parameter	PEA	Control-1	Control-2	Units
Hepatocyte	V_h	1,357.20 – 364.80	984.37 – 200.37[a]	1,031.86 – 253.64	μm^3
Nucleus	V_n	243.08 – 55.06	201.22 – 45.97[a]	157.06 – 58.57[b]	μm^3
Cytoplasm	V_{cy}	1,108.40 – 328.91	783.65 – 108.88[a]	877.73 – 209.83	μm^3
Mitochondria	Nt_m	104.00 – 52.00	82.00 – 33.00	198.00 – 99.00[b]	μm^0
	$V_{s \cdot m}$	2.55 – 0.80	1.67 – 0.44[a]	0.94 – 0.33[b]	μm^3
Lipids	Nt_l	129.00 – 96.00	52.00 – 22.00[a]	413.04 – 52.00[b]	μm^0
	$V_{s \cdot l}$	0.40 – 0.03	0.41 – 0.21	0.48 – 0.19	μm^3
Peroxisomes	Nt_p	157.00 – 81.00	64.00 – 31.00[a]	69.00 – 15.00	μm^0
	$V_{s \cdot p}$	0.06 – 0.01	0.12 – 0.03[a]	0.11 – 0.02	μm^3

V, absolute volume; Nt, total number of elements; $V_{s \cdot i}$, volume of a single element

[a] Significant differences between PEA and Control-1 hepatocytes ($P \leqq 0.05$)
[b] Significant differences between Control-1 and Control-2 cells ($P \leqq 0.05$)

Table 3. Coefficient of form (CF) values of hepatocytes, nuclei and mitochondria from rats prenatally exposed to alcohol (PEA), Control-1 (pair-fed) and Control-2 (chow diet)

Component	Parameter	PEA	Control-1	Control-2	Units
Hepatocytes	CF_h	0.76–0.09	0.73–0.10	0.75–0.10	μm^0
Nucleus	CF_n	0.92–0.01	0.90–0.02	0.85–0.12	μm^0
Mitochondria	CF_m	0.83–0.15	0.81–0.14	0.79–0.16	μm^0

Values os mean ± s.d.

[a] Significant differences between PEA and Control-1 hepatocytes ($P \leqq 0.05$). No significant differences were found between Control-1 and Control-2 cells

Table 4. Stereology of Golgi apparatus vesicles (mean ± s.d.) of PEA hepatocytes

Vesicle parameter	$\bar{x} \pm$ s.d.	Units
$Vv_{v,g}$	14.67– 2.51	μm^0
$Sv_{v,g}$	12.68– 1.96	μm^{-1}
$\bar{D}$	6.29– 0.29	$\mu m \times 10^{-2}$
$Nv_{v,g}$	783.15–117.68	μm^{-3}
$V_{s,v}$	1.88– 0.23	$\mu m^3 \times 10^{-4}$

$Vv_{v,g}$, vesicle volume density per unit volume of Golgi apparatus (in %); $Sv_{v,g}$, vesicle surface density per unit volume of Golgi; $\bar{D}$, mean caliper diameter of vesicles ($\bar{D} = 6\ Vv/Sv$) (Weibel and Bolender, 1973); $Nv_{v,g}$, numerical density of vesicles per unit volume of Golgi apparatus (see equation II in Material and Methods) ($k = 1.05$ and $\beta = 1.382$); $V_{s,v}$, absolute volume of a single vesicle ($V = Vv/Nv$). Similar values for $\bar{D}$, Nv and $V_{s,v}$ were obtained when other methods were used (see Abe et al. 1983, for calculation of $\bar{D}$ and $V_{s,v}$, and DeHoff and Rhines 1961, for determination of Nv)

cells. On the other hand, the absolute number of mitochondria and lipid droplets, the volume density of lipid droplets, and the surface and numerical density of mitochondria and lipid droplets appeared increased in Control-2 hepatocytes. In the morphometric data of Table 3 Control-1 and Control-2 cells differ in the nuclear CF and in the mean mitocondrial area ($0.874 \pm 0.518\ \mu m^2$ and $0.610 \pm 0.336\ \mu m^2$, respectively), although the mitochondrial area curve distribution was similar in both cases.

Analysis of results of PEA and pair-fed hepatocytes reveals that they differ in the absolute volume of whole cell, nucleus, cytoplasm, and single mitochondrion, the volume and surface density of mitochondria and surface density of peroxisomes, the total number of peroxisomes and lipid droplets, and in the numerical density of peroxisomes, which are greater in alcohol treated than in Control-1 cells. However, other stereological parameters such as the surface density of nucleus and SER, and the absolute volume of a single peroxisome, are smaller in these cells than in pair-fed hepatocytes. Finally, these cells also differ in the mean mitochondrial area (Control-1: $0.610 \pm 0.336\ \mu m^2$; PEA: $1.385 \pm 0.719\ \mu m^2$) as well as in their respective mitochondrial area distribution curves.

Stereological analysis of the Golgi complex in treated cells (Table 4) shows this cell component to be composed of vesicles with a mean diameter

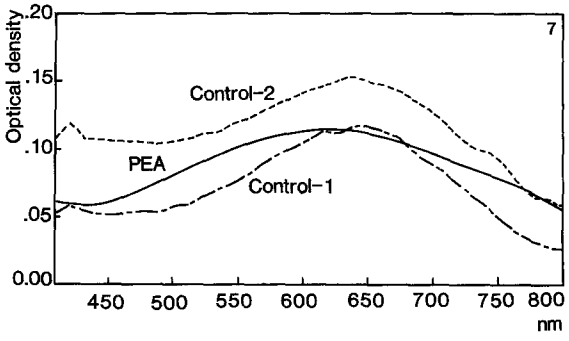

Fig. 7. Absorption curves for lipids (Sudan black B) of PEA, Control-1 and Control-2 rat hepatocyte

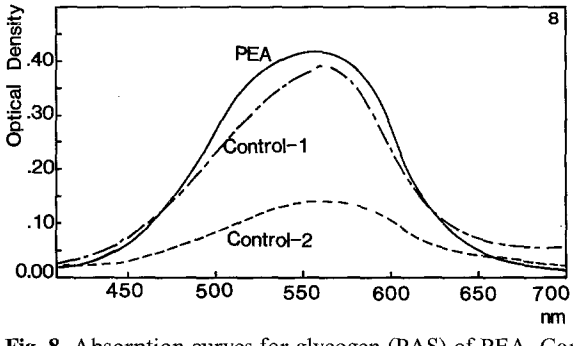

Fig. 8. Absorption curves for glycogen (PAS) of PEA, Control-1 and Control-2 hepatocytes

of 6.29×10^{-2} μm and a mean individual volume of 1.88×10^{-4} μm^3. They represent 14.67% of total Golgi apparatus volume and their number per μm^3 of Golgi is 783. Finally the mean intervesicle distance (r_o) was estimated as 0.136 ± 0.013 μm ($r_o = 1/\sqrt{\rho}$; $\rho = N_a$ = Number of vesicles/Area of Golgi apparatus (Markovics et al. 1974; Renau-Piqueras and Cervera 1982)).

Cytophotometric and biochemical results. Cytophotometric analyses of lipid composition of the three types of hepatocytes are summarized in Fig. 7. There were no observable differences in the lipid content of PEA and pair-fed hepatocytes. However, there was an increased lipid content in Control-2 cells.

Glycogen content, as determined by cytophotometric and biochemical assays, is similar in pair-fed and treated cells, but significantly less (cytophotometric data only) in Control-2 hepatocytes (Fig. 8). (The data from biochemical analysis are: PEA rats, 33.87 ± 8.80 mg/g tissue; pair-fed rats, 42.46 ± 2.35 mg/g tissue).

Discussion

It is known that diet composition is an important factor affecting the biochemical and ultrastructural characteristics of liver (Novikoff 1982; Zern

et al. 1982). In fact, different mitochondrial characteristics have been reported in studies on rats receiving the same chow and liquid diets used for our controls (Gordon 1984). However, no comparison has been made of the ultrastructure of cells from pair-fed and chow-fed controls. In addition, the majority of morphological studies on newborn rats have been made on chow-fed rats (David 1979, 1980a and b). It therefore appeared advisable, before attempting to analyze the ultrastructure of hepatocytes of PEA rats, to delimit the changes induced by the diet. Our results indicate qualitative and quantitative differences in hepatocytes from the two types of controls, confirming the effect of diet composition on the liver ultrastructure and the necessity for adequate controls in studies which evaluate the effects of drugs on liver. Thus, some differences observed between the control hepatocytes, mainly those concerning lipid droplets, glycogen and VLDL particles can be directly related to the differences in the nutritional composition of the diets. The remaining differences are probably the morphological expression of changes in metabolism induced by the varied components of the diets.

Among the alterations observed in the comparison of ethanol treated rats with pair-fed animals, those concerning cell size, mitochondria, Golgi apparatus and peroxisomes are notable. Since little information is available on the changes in liver morphology in either PEA humans or experimental animals, we will consider the changes observed following acute and chronic alcholism in humans as well as in amimals.

The significant increase of hepatocyte volume described in chronic alcoholism has been postulated to be due to an accumulation of K^+ in the cell and therefore an increase in intracellular water (Israel and Orrego 1981; Israel et al. 1982). The increment in size has been determined to be 1.5 times that of the control cells (Israel et al. 1982) and is similar to the cell volume increase (1.4 times) observed in our PEA rats.

Several ultrastructural qualitative and quantitative studies have clearly demonstrated that chronic and acute exposure to alcohol of adult rats and humans induces changes in both size and shape of mitochondria of hepatocytes (Dobbins et al. 1972; Oudea et al. 1973a and b; Petersen 1977). Qualitative electron microscopy has shown that exposure to ethanol also causes similar mitochondrial changes in hepatocytes of half-term mini-pig fetuses and pups and in neuroepithelial cells of mouse embryos (Banninghan 1984; Baskid et al. 1975; Rømert and Matthiessen 1983). Our work confirms and extends these latter reports with data showing that the variation in the mitochondrial volume density in PEA rats is due to an increase in the size of these organelles and not to an increment in their number. The mechanism of ethanol-induced swelling of mitochondria remains controversial (Gordon 1984; Rottenberg et al. 1984), with two main theses. In the first, the effect has been attributed to an alteration in the structure of mitochondrial membranes induced either by ethanol or its metabolite, acetaldehyde, which would lead to changes in the function of mitochondria (Rottenberg 1984; Rubin and Rottenberg 1982). The second suggests that the increase in the NADH:NAD ratio induced by ethanol has several metabolic conse-

quences including a slowing of the rate of oxidative phosphorilation (Zern et al. 1982). This decreased capacity to oxidize substrates is known to cause mitochondrial swelling (Harman and Feigelson 1952). Although the alteration in the oxidative ability is more apparent in acute alcoholism (Zern et al. 1982), it has been reasoned that the decreased reducing equivalent formed via other pathways of ethanol metabolism yielding acetaldehyde could also be responsible for most of the mitochondrial swelling in chronic alcoholism (Gordon 1984). Moreover, it has been claimed that this would be independent of mitochondrial membrane lipid alterations (Gordon 1984). Whatever the cause, it is likely that alteration in the fine structure of mitochondria of PEA rats is related to other marked cellular changes which occur in both acute and chronic alcoholism.

One of the most striking qualitative morphological changes observed in PEA rats is the anatomic disorganization of the Golgi complex, suggesting an alteration in its function. Although to a lesser extent, morphological changes in the Golgi complex have been reported in both chronic and acute alcoholism, including increase in the number and size of cisternae, vacuoles and vesicles as well as an accumulation of VLDL particles (Ehrenreich et al. 1973; Matsuda et al. 1979; Stein and Stein 1967). This together with biochemical evidence would suggest that glycosylation and transport of several secretory proteins are inhibited by ethanol (Baraona and Lieber 1982; Lieber 1980; Marinari et al. 1978; Volentine et al. 1984; Zern 1982). A similar effect on the morphology and function of the Golgi apparatus has been described after using other drugs, including monensin (Oda et al. 1983; Tartakoff 1980 and 1983). This carboxilic ionophore which binds Na^+, K^+ and protons, produces a hyperpolarization of cell membranes due to an increase in the K^+ efflux, Na^+ influx and, therefore, in the (Na^+, $-K^+$) ATPase. Monensin produces a dramatic slowing of intracellular transport of newly synthesized secretory proteins, and failure of some proteins to aquire terminal sugars has also been reported. Morphologically, monensin treatment induces a swelling of cisternae of Golgi apparatus which appears composed by of different sized vacuoles. This swelling might be due to a passive influx of water induced by the presence in the cisternae of an impermeant weak acid whose protons exchange for Na^+ (Tartakoff 1980 and 1983).

It is interesting to note the similarity between the alterations induced by monensin to those induced by ethanol. Thus, in addition to the morphological alterations of the Golgi complex described by others (Ehrenreich et al. 1973; Matsuda et al. 1979; Stein and Stein 1967) and by us, and to the inhibition of glycosylation and transport of several proteins as mentioned above, ethanol increases the levels of intracellular K^+ and water as well as the (Na^+, $-K^+$) ATPase (Guerri and Grisolía 1982; Israel and Orrego 1981; Israel et al. 1982). This parallelism in effects on the Golgi apparatus could indicate a similar mechanism of action for ethanol and monensin; additional experiments are in progress to clarify this point.

Finally, an increase in the peroxisome compartment, as described here,

has also been reported both in humans and rats consuming ethanol (Porta et al. 1965; Rubin and Lieber 1967). Moreover, an increment in ethanol peroxidation in rats chronically exposed to alcohol (Khanna and Israel 1980) has also been described. However, the volume density of peroxisomes of adult rats receiving chow and 25% ethanol in water for 35 days showed no differences (Dobbins et al. 1972). In our results, the volume density of peroxisomes of PEA and pair-fed hepatocytes was essentially the same. However, there was a significant variation in the number and size of peroxisomes of PEA hepatocytes indicating a proliferation of these organelles after ethanol exposure. Peroxidatic oxidation of ethanol by catalase might therefore also be an alternative mechanism for alcohol clearance in PEA rats as it is in chronic ethanol-fed rats (Lazarow 1982).

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