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ULTRASTRUCTURAL AND MORPHOMETRIC INVESTIGATION ON THE FETAL RAT LIVER

Michel Kraemer*, Jeannie Vassy*, Marie-Therese Chalumeau* and Albrecht Reith**

* Universite Paris XIII, UER Biomedicale, Laboratoire de Biologie, 74 rue Marcel Cachin, 93012 BOBIGNY CEDEX, FRANCE.

** Det Norske Radiumhospital, Laboratory for Electron Microscopy and Morphometry, Montebello, OSLO 3, NORWAY.

ABSTRACT

Qualitative and quantitative changes in liver tissue composition have been studied during prenatal development of the rat by electron microscopy and morphometric/stereologic methods. On the 12th day of gestation 26% of fetal liver volume consisted of hepatocytes, and the non hepatocytes cells amounted to 57%. On the 13th and 18th fetal days the volumetric density of hepatocytes occupied about 40% of the liver, and the non hepatocyte cells amounted respectively to 50% and 43%. By days 12 and 13, the rough endoplasmic reticulum and the Golgi complexes were well differentiated indicating that young fetal hepatocytes were able to synthesize and export plasma proteins. On days 12, 13 and 18 the mitochondrial compartment occupied 13% of the hepatocyte cytoplasm. On day 18, the mitochondria were larger and less numerous than on days 12 and 13, but without change in the shape. The ferrocyanide-reduced osmium post-fixation has been compared with conventional osmium post-fixation for the 18 days old fetuses. Ferrocyanide-reduced osmium post-fixation combined a good preservation and staining of glycogen with an increase density of membrane organelles.

Keywords: Development, electron microscopy, hepatocytes, morphometry, rat, stereology.

INTRODUCTION

Correlated ultrastructural and morphometric studies of adult and postnatal development of rat liver have been investigated by many authors, however few data are available on the prenatal development of rat hepatocytes. Most of these studies are restricted to some fetal ages or cell organelles (Daimon et al.,1982; Herzfeld et al.,1973; Rohr et al.,1971). The present work is a first step of a systematic investigation of the morphometric characteristics of fetal rat hepatocytes on the 12th, 13th and 18th day of gestation. The fetal rat livers were also investigated, from a qualitative point of view, at the electron microscopic level to identify and characterize the hepatic parenchyma.
A ferrocyanide-reduced osmium tetroxide post-fixation (Willingham and Rutherford, 1984) has been used to increase the membrane contrast of cells.

EXPERIMENTAL PROCEDURE

Preparation of Tissue

Livers were obtained on the 12th, 13th and 18th day of gestation. Liver tissue samples were immersion fixed for 2hrs in cold 0.1M phosphate-buffered 2.5% glutaraldehyde (pH=7.4; 560 mosm.) and then rinsed in 0.1M phosphate buffer + 0.1M saccharose (330 mosm.). Post-fixation was carried out either in a ferrocyanide-reduced osmium solution (1hr; 4oC) for the 12th, 13th and 18th day of gestation (exps.1, 2 and 3) or in OsO4 (1hr;4oC) for the 18th day of gestation (exp.4). After dehydration in graded alcohol solutions and embedding in epon, silver to grey thin sections were stained with lead citrate and examined.

Sampling and Stereologic Parameters

For each experiment 3 pregnant rats were sacrified and 5 fetuses per mother were studied. 75 electron micrographs were analyzed at each magnification step. At low magnification, electron micrographs were taken to give prints at x5000 magnification, which were used for recording the volume densities of the extracellular spaces (VVEX), non-hepatocyte cells (VVNHC) and hepatocytes (VVN). Electron micrographs at a final magnification of 15000 were studied for the determination of the volume densities of hepatocyte cytoplasm (VVCYT), nuclei (VVNH), mitochondria (VVMIT,CYT) and surface density of outer membrane of mitochondria (SVMO,CYT) and surface-to-volume density of hepatocyte chondriom (SVMO/VVM,CYT). For the estimation of volume densities of endoplasmic reticulum (VVER,CYT), Golgi apparatus (VVGOL,CYT), free ribosomes (VVRIBO,CYT), lipids (VVLIP,CYT),glycogen area (VVGLYCO,CYT) and surface densities of endoplasmic reticulum (SVER,CYT), Golgi apparatus (SVGOL,CYT), inner membrane of hepatocyte mitochondria (SVMI,CYT) the electron micrographs were printed at x 40000 magnification. Moreover, the nucleus-cytoplasmic ratio (VVNH/VVCYT) of hepatocytes and the mean profile area of mitochondria (am) were estimated. For the estimation of volume densities a lattice test system (spacing d=2 micrometers at a magnification of x 5000) was used. The test line lenght for the surface density estimation at a magnification of x 15000 was 210 micrometers. Analysis of variance and Student t-tests were used for statistical analysis. P values of 0.05 or less were regarded as being statistically significant.

QUALITATIVE RESULTS

On the 12th and 13th day of gestation the difference between the hepatocytes and the hemopoietic cells was done on the basis of mitochondria size, which was larger in hepatocytes than in other cell types, and the presence of lipid droplets in the hepatocyte cytoplasm. By day 12 (fig.1) the hepatocytes revealed numerous profiles of rough endoplasmic reticulum (RER) and the Golgi complexes (GA) showed saccules and vesicles. The mitochondria were few, rounded in section and small with fine cristae. On the 13th fetal day, the hepatocytes exhibited some intercellular adhesions assuming contact areas between cytoplasmic processes; the first bile canaliculi were noted. The hepatocyte population formed a cellular network invaded by hemopoietic cells. RER (fig.2) and GA profiles (fig.3) were observed. As for the 12th day of gestation, large area of free ribosomes were noted. On the 18th day of gestation, the hepatocytes were larger and more numerous than in previous ages. The mitochondria were larger but less rounded in section than in the 12th and

13th fetal days. The smooth endoplasmic reticulum (SER) was noticed in the glycogen areas which appeared. The glycogen was more easily demonstrasted in ferrocyanide- osmium post-fixed samples (fig.4) than in Os04 post-fixed specimens (fig.5). In osmium-potassium ferrocyanide samples the membrane contrast of the cytoplasmic organelles was increased and the mitochondria matrix contrast decreased, allowing a better visualization of fine delineated cristae.

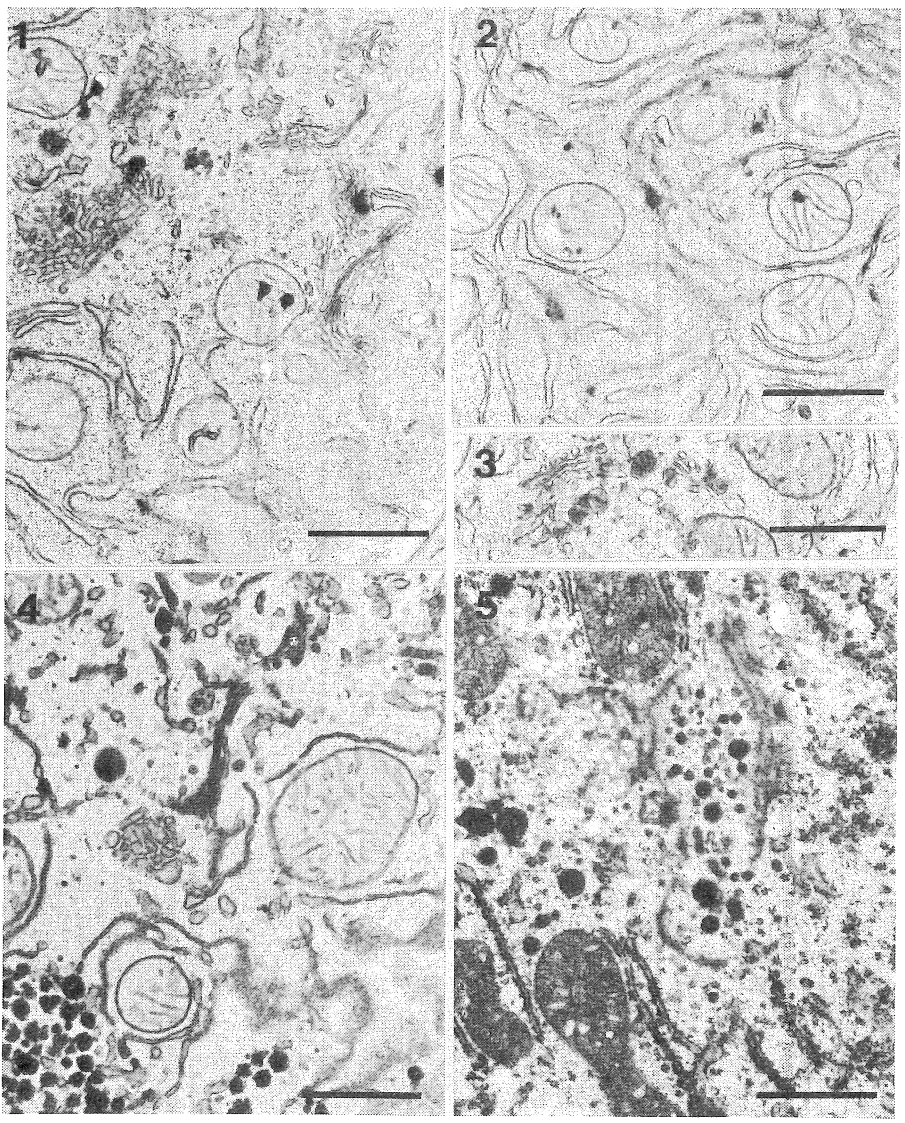

Fig.1. 12th day of gestation. Figs.2 and 3. 13th day of gestation. Fig.4. 18th day of gestation (ferrocyanide-osmium tetroxide post-fixation). Fig.5. 18th day of gestation (conventional osmium tetroxide post-fixation). Scale bar=1 micrometer.

QUANTITATIVE RESULTS

Composition of the Fetal Rat Liver

The composition of the fetal rat liver is summarized in Table 1 and Figure 6.

On the 12th day of gestation 26% of fetal liver volume consisted of hepatocytes and the extracellular spaces amounted to 15%. On the 13th fetal day, the volumetric density of hepatocytes increased significantly. At day 18 of gestation, the volumetric density of the hepatocytes increased significantly comparing with the 12th day but not with the 13th fetal day, and the hepatocytes occupied about 40% of the liver.

Table 1. Quantitative evaluation of developing rat liver. F. Ferrocyanide-reduced osmium tetroxide post-fixation. C. Conventional osmium tetroxide post-fixation. Significance $P<0.05$ for the pairs named, no significance (NS) for the pairs not named.

		AGE and EXPERIMENT				
PARAMETER	DIMENSION	12 days F. Exp. 1	13 days F. Exp. 2	18 days F. Exp. 3	18 days C. Exp. 4	SIGNIFICANCE
VVEX	cm^3/cm^3	0.154 ±0.075	0.126 ±0.050	0.163 ±0.057	0.082 ±0.044	1-4, 2-4, 3-4
VVNHC	cm^3/cm^3	0.574 ±0.077	0.501 ±0.088	0.434 ±0.091	0.487 ±0.133	1-3
VVH	cm^3/cm^3	0.264 ±0.078	0.373 ±0.092	0.403 ±0.128	0.434 ±0.135	1-2, 1-3, 1-4, 2-4
VVNH	cm^3/cm^3	0.068 ±0.028	0.096 ±0.030	0.073 ±0.035	0.079 ±0.026	NS
NVNH	10^6	13.83 ±5.20	14.35 ±6.26	20.74 ±5.09	17.70 ±5.18	1-3, 2-3
VVCYT	cm^3/cm^3	0.203 ±0.052	0.277 ±0.073	0.331 ±0.113	0.348 ±0.126	1-2, 1-3, 1-4, 2-4
VVMIT	cm^3/cm^3	0.129 ±0.034	0.127 ±0.035	0.131 ±0.031	0.141 ±0.047	NS
SVMO	m^2/cm^3	0.698 ±0.050	0.683 ±0.123	0.751 ±0.112	0.731 ±0.347	NS
SVMI	m^2/cm^3	1.883 ±0.465	2.556 ±0.696	2.061 ±0.903	1.851 ±0.431	1-2, 2-4
$\bar{a}_m$	μm^2	1.142 ±0.638	0.995 ±0.329	2.209 ±0.511	2.914 ±0.662	1-3, 1-4, 2-3, 2-4
VVER	cm^3/cm^3	0.207 ±0.044	0.200 ±0.074	0.319 ±0.077	0.226 ±0.065	1-3, 2-3, 3-4
SVER	m^2/cm^3	2.444 ±0.623	4.260 ±1.011	3.690 ±0.735	2.940 ±0.585	1-2, 2-3, 2-4
VVGOL	cm^3/cm^3	0.145 ±0.048	0.082 ±0.082	0.100 ±0.052	0.086 ±0.060	NS
SVGOL	m^2/cm^3	2.455 ±0.887	1.539 ±1.408	1.493 ±0.874	0.968 ±0.650	NS
VVRIBO	cm^3/cm^3	0.387 ±0.063	0.450 ±0.109	0.084 ±0.071	0.196 ±0.079	1-3, 1-4, 2-3, 2-4
VVLIP	cm^3/cm^3	0.045 ±0.027	0.066 ±0.033	0.007 ±0.008	0.009 ±0.012	1-3, 1-4, 2-3, 2-4
VVGLYCO	cm^3/cm^3			0.133 ±0.162	0.148 ±0.161	NS

Subdivision of the Hepatocytes

The results are summarized in Table 1 and Figures 6, 7 and 8.

The cytoplasm occupied respectively 77%, 74% and 82% of the hepatocytes on the 12th, 13th and 18th day of gestation. The volumetric density of the endoplasmic reticulum did not change between the 12th and 13th fetal day, while the surface density increased about 57%. The volume and surface densities of the Golgi apparatus did not change significantly between the 12th, 13th and 18th day of gestation. The volume and surface densities of mitochondria remained constant in the three ages. The surface density of inner membrane with cristae was 2.7 times larger than the SVMO on the 12th fetal day and showed a further increase of 3.7 times on the 13th fetal day. The surface-to-volume density of hepatocyte condriom did not vary significantly between the three ages. There was a significant increase of 1.9 time in the mean profile area between the 12th and 18th day of gestation, but no difference was noticed between the 12th and 13th fetal days. These results demonstrated that by day 18, the mitochondria were larger and less numerous than in the 12th and 13th day of gestation but without change in the shape. A significant decrease of 5.4 times in the volumetric fraction of free ribosomes was noticed between the 13th and 18th fetal days. The volumetric fraction of lipids decreased about 7 times, and the nucleocytoplasmic ratio of 1.5 time between the 12-13th days of gestation and the 18th fetal day.

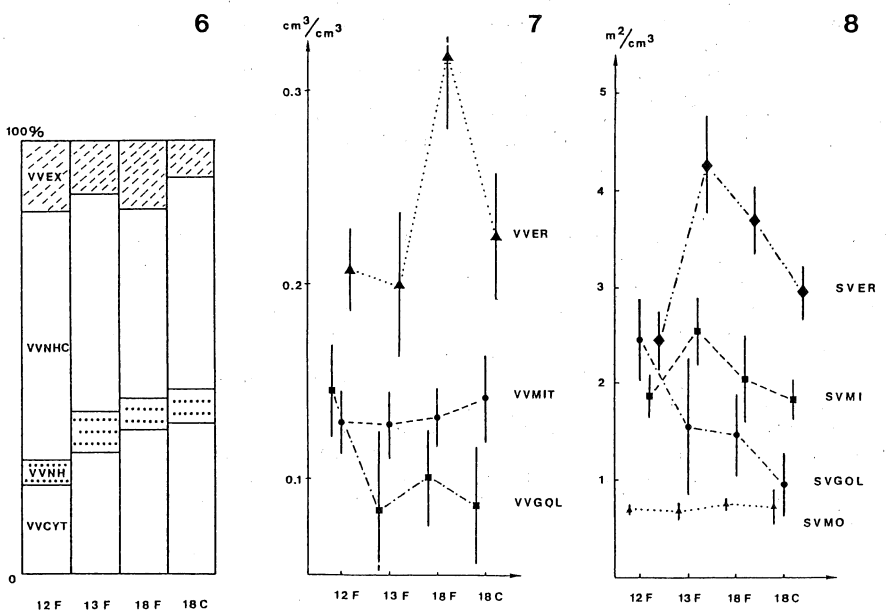

Fig.6. Volumetric subdivision of liver tissue. Fig 7. Volume densities in hepatocyte cytoplasm. Fig. 8. Surface densities in hepatocyte cytoplasm. F. Ferrocyanide-reduced osmium tetroxide post-fixation. C. Conventional osmium tetroxide post-fixation. (Brackets represent 1 standard error).

DISCUSSION

The most prominent changes during the prenatal development of the rat are the increase in number and volume of hepatocytes, correlated with a decrease of the hemopoietic cell compartment. The hepatocyte nucleus remains constant while the cytoplam increases. The volumetric fraction of the mitochondria does not change while the surface-to-volume ratio of the chondriom and the mean profile area indicate that three days before birth the mitochondria are larger and less numerous than on the 12th and 13th fetal days, the shape remaining the same.

For the 18th day of gestation, the differences noticed between other works (Daimon et al.,1982; Rohr et al.,1971) and our results for the volume and surface densities of the endoplasmic reticulum and Golgi apparatus, could be partly due to the different fixations used, as previously emphasized by Reith et al.(1984). On another hand, the differences noted between Daimon et al. (1982) and our data could also be related to a fractal problem as demonstrated by Paumgartner et al.(1981); the endoplasmic reticulum and Golgi apparatus data were obtained for Daimon's group at a magnification of 20000 and our results with a x40000 magnification.

The functional capacities of the hepatocytes can be estimated by the volumetric and surface densities of the RER and GA. These organelles are well developped as early as the 12th day of gestation, and the hepatocytes at this age seem to possess a high capacity of protein synthesis. These data are in agreement with previous immunocytochemical studies (Kraemer et al.,1981) demonstrating that fetal hepatocytes are able to synthesize and export plasma proteins.

As previously emphasized (Willingham and Rutherford, 1984) the use of a post-fixation in an osmium-potassium ferrocyanide mixture enhances membrane contrast and density of glycogen, extracts matrix proteins allowing a better visualization of mitochondrial cristae.

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