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BINUCLEATED HEPATOCYTE SUBPOPULATIONS IN MALE AND FEMALE RATS FED WITH OLIVE AND SUNFLOWER OILS

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ABSTRACT

The possible differences due to sex and diet on the percentage and cytomorphological features of the binucleated cells across the hepatic acinus are analized in this study. Stereological quantitative determinations were performed on males and females of two groups of rats fed either with olive or sunflower oil. The results indicate that binucleated hepatocytes only represent a scarce cell subpopulation within the hepatic acinus, being their mean values in males higher than in females in both groups of diet. In addition, these binucleated hepatocytes follow a characteristic pattern of distribution across the acinus, with a progressive decrease in number from the portal zone to central vein. The average cellular and nuclear areas are higher in binucleated hepatocytes than in mononucleated for both males and females in the two diet groups. According to the diet, binucleated hepatocytes show greater cellular and nuclear areas in the olive oil group than in the sunflower oil group. When these parameters are studied across the acinus, it is observed that the size of the cells is linked to the fat stored in the cytoplasm. No significant differences were found in the nuclear areas across the hepatic acinus.

Keywords: binucleated hepatocytes, quantitative techniques, olive, sunflower

INTRODUCTION

The liver is the main organ involved in the regulation of lipid metabolism and specifically in metabolic pathways related to massive intestinal fat absortion (Brisson, 1981). The funtional unit of the liver based on its microvascular architecture is the hepatic acinus (Elias, 1949; Rappaport et al., 1959). There is a metabolic zonation in the acinus that is reflected in the heterogeneity of the hepatocytes from portal to the central zone. This heterogeneity is the consequence of the blood flow, which produces a gradual reduction of available subtrate and oxigen (Weibel et al., 1969; Schumuker et al., 1978; Matsumura et al., 1986; Gumucio and Chianale 1988).

The hepatocytes are the main funtional cells of the liver. There are about 250 billion hepatocytes in the normal adult human liver (Elias, 1949) About 30-80% of this number are poliploidic cells, and near to 25% are binucleated cells; this characteristic is probably caused by an endomitotic process (Gonzalez Fernandez, 1967; Carriere, 1969). Thus, the rate hepatocytes division must exert some influence on this process. However, the turnover that hepatocytes are subjected to, if the liver is not injured, is relatively slow (Leffert et al., 1988). In addition, different works suggest that factors such as ageing (Dice, and Goff, 1988), hormones (Carriere,1969; Van Thiel, 1988) and some drugs (Bolender R.P. and Gittes F,1988), are involved in the poliploidic process and development of binucleated hepatocytes. Nevertheless, the role which might exert on this hepatocyte poliploidic process, factors such as nutrition and particularly daily fat diet, has not been extensively studied; moreover if the implications of the liver in lipid metabolism is considered (Brisson, 1982).

The purpose of the present study was to analyze the influence of two different diets, either containing olive oil or sunflower oil, on the number, acinar distribution and cytomorphometric features of the binucleated hepatocytes in male and female rats. These parameters will be compared to the total hepatocyte population. In addition, the relations between binucleated hepatocytes and fat storage in their cytoplasms was also studied .

EXPERIMENTAL PROCEDURE

Twenty Wistar albino rats, that had just been weaned, weighing 65±5 gr. at the time of the experiments, were divided into two different groups. Each group, with 5 males and 5 females, was fed "ad libitum" either with a diet enrriched with olive oil or sunflower oil. The total diet composition is shown in table 1.

Table 1.-Composition of the olive/sunflower fat diet.

Starch	28,8%	Sugar	28,5%
Fat (Olive/Sunflower)	14%	Casein	12%
Fibre (celulose)	8%	Vitamin corrector	5%
Mineral corrector	4%		

After 30 days on these diets all the rats were sacrificed and their livers were quickly removed, weighed and rinsed with a saline buffered solution.

From each liver, and in the same location, eight (4+4) tissue blocks were obtained. These blocks were fixed with a 10% formalin-saline solution during 48h. Four of these blocks were embedded in parafin to obtain sections of 6µ thickness. The sections were stained following the Hematoxilin-Eosin technique. The other four blocks were cut on a cryostat to obtain frozen sections of 20µ thickness. These sections were stained with Sudan III to reveal the hepatocyte lipid stores as well as their cytoplasms and nuclei.

All the data in this study refer to the hepatic acinus as an anatomic and funtional unit of the liver; this unit, based on their microvascular architecture (Rappaport et al., 1959), is divided in three different zones, named "portal" "midzonal" and "central", following the direction of the blood flow.

Simple counts were carried out in stained H-E sections, to calculate the volume density not only for the mononucleated hepatocyte subpopulation but also for the subpopulation of the binucleated hepatocytes. The procedure used was based on the utilization of a calibrate eye piece graticula and 100X immersion objetive (Haug, 1967). The nucleus has been taken as a marker for each cell, considering the cells with two nuclei as a unique binucleated cell. A total number of 600 data were analysed.

Cytomorphometric determinations were performed, from 20 µ thick stained Sudan III sections, by quantitative image analysis (Elias et al., 1949; Weibel et al., 1969) using a semiautomatic analiser Mop-10 Kontron.

The cytomorphological mean values of the cells have been estimated from a sample of 3000 hepatocytes, whose cellular, nuclear and fat drop profiles were measured. In binucleated hepatocytes, both nuclear profiles were assumed as a unique measure. Samples were obtained homogeniously, in the three acinus zones, from these 3000 sampled hepatocytes, being 726 from "olive" males, 732 from "olive" females, 766 from "sumflower" males and 766 from "sunflower" females.

The statistical analysis was performed using a paired Student's "t" test. When the three acinus zones were compared the analysis of variance was used.

RESULTS

The binucleated hepatocyte percentage, are shown in table 2. The number of mononucleated and binucleated hepatocytes per $10^6\mu^3$ can also be seen.

There is a greater number of binucleated hepatocytes in males of both groups (olive and sunflower) than in females. These hepatocytes also present a decreasing gradient in their number from portal to central zone, particulary in rats fed with olive oil.

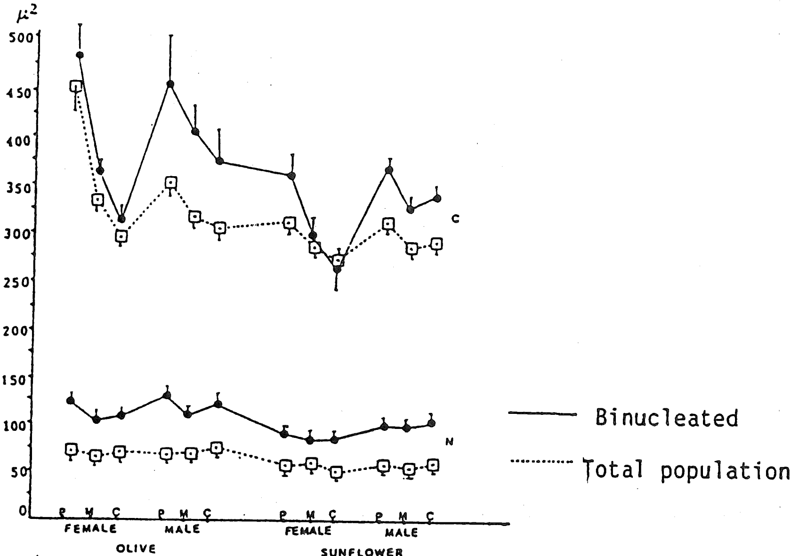
Table 2.- Number (mean ± smd; n=150) of mononucleated and binucleated hepatocytes per each liver tissue volume of $10^6\mu^3$ The percentage of binucleated hepatocytes is also shown in the whole of the hepatic acinus and in their three zones (P=portal; M=midzonal; C=central) for both sexes (males and females) of each diet group (olive and sunflower). These percentages referring to all the population of hepatocytes.

		Mononucl.	Binucl.	Binucl. percentages			
				Acinus	P	M	C
OLIVE	females	686 ± 13	35 ± 9	4,85 %	6,80%	4,26%	3,47%
	males	676 ± 10	44 ± 8	6,11%	8,62%	5,14%	4,56%
SUNFLOWER	females	666 ± 14	31 ± 7	4,45%	5,61%	3,99%	3,75%
	males	663 ± 9	48 ± 9	6,75%	7,91%	6,10%	6,24%

The mean values for cellular and nuclear areas, are shown in Graph 1, not only for binucleated hepatocytes but also for all the hepatocyte population (both mononucleated and binucleated subpopulations) in the three acinus zones for all the experimetal groups. In binucleated hepatocytes, the nuclear area corresponds to the sum of the two nuclei.

Table 3.-Percentages of hepatocytes with fat in the three acinus zones (P=portal; M=midzonal; C=central) for males and females of both diet groups (olive and sunflower). Theses percentages referring to the total hepatocyte population (mononucleated and binucleated) and to the binucleated hepatocyte subpopulations respectively.

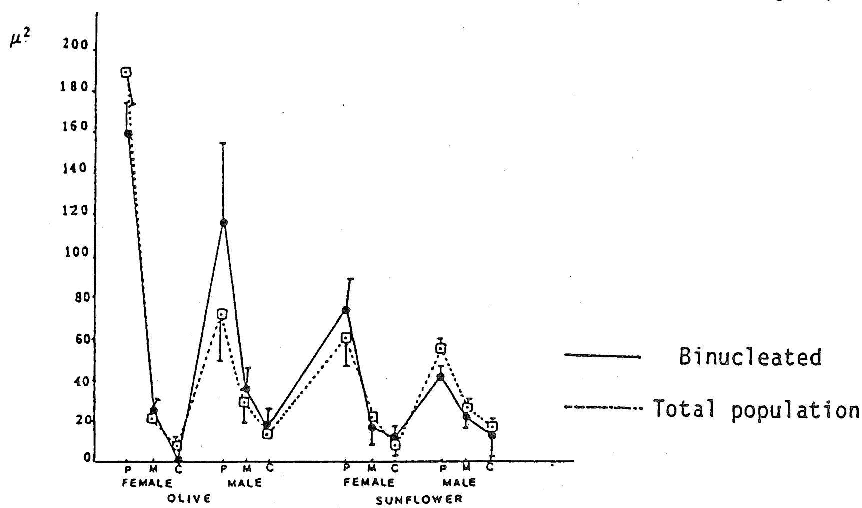
	Olive						Sunflower					
	Female			Male			Female			Male		
	P	M	C	P	M	C	P	M	C	P	M	C
Total hepatocytes	80%	33%	5%	78%	70%	60%	96%	92%	65%	98%	95%	80%
Binucleated	81%	67%	-	100%	100%	85%	100%	100%	66%	100%	100%	85%



Graph 1.- Values (mean ± smd), expressed in square microns, of both cellular and nuclear areas of the binucleated hepatocytes in relation to these same parameters in all the hepatocyte population. These data correspond to the three acinus zones (Portal, Midzonal and Central) in both sexes (males and females) of each diet (olive and sunflower)

The percentage of the binucleated hepatocytes that have fat in their cytoplasms are shown in table 3. These percentages are in relation to all the hepatocyte population (Including mononucleated and binucleated hepatocytes).

Finally, graph 2, shows the mean fat drop areas in those hepatocytes which contain them. These parameters are also shown in relation to the total hepatocytes population in the three acinus zones of each experimental group.



Graph 2.-Areas (mean± smd), expressed in square microns, for the fat drops contained in the hepatocytes with fat (binucleated and total population) in the three acinus areas (portal, midzonal and central) of both sexes (male and female) in each diet groups (olive and sunflower).

DISCUSSION

The obtained percentages of the binucleated hepatocytes in males and females of each diet group (olive and sunflower) mean that the frequency formation of two nuclei in hepatic cells, show greater relation with sex than with diet. In fact, the females of both groups showed a lesser number of these hepatocytes with two nuclei than their corresponding males. Thus, several work performed in males and females (Carriere, 1969; Van Thiel, 1988) point out that the endocrine system and particularly the steroidic hormones, play an important role over the hepatic metabolism and could also contribute to the genetic ploydie mechanism of these cells.

Cellular and nuclear profiles of the binucleated hepatocytes in the two diet groups show greater values than the mononucleated one. In addition, the greater differences between cellular sizes of both binucleated and mononucleated hepatocytes, were found in portal zone of the males fed on olive oil which reached an average of 30%. Generally, greater differences were found in the males of both groups than in the corresponding females. In the latter, the average size differences between the binucleated and the total hepatocyte population, fluctuated from a minimum of 4% to a maximum of 12%.

Referring to the nuclear cell area, we can observe less variability across the hepatic acinus and among different experimental groups. In fact, the total sum of the areas of both nuclei of the binucleated hepatocytes was homogeniously about 40% to 50% bigger than in all hepatocytes population.

However, both cellular and nuclear areas, in all hepatocytes population (binucleateted and mononucleated cells) were bigger in the olive oil group than in the sunflower. Consequently, the size of both parameters must be influenced by diet and particularly by the type of fatty acids contained in the olive or sunflower oils (Peinado et al., 1987).

It is appropiate to underline that the binucleated as well as the monunucleated hepatocytes, display a similar size distribution pattern across the hepatic acinus. Thus, the hepatocytes showed cellular sizes higher in portal zones than in the other acinus zones. Though the highest differences were shown by females. As can be seen in table 3 and graph 2, this distribution pattern is correlated not only with the number of cells with fat but also with the amount of fat drops contained in their cytoplasm. This particular distribution of the hepatocytes with fat across the acinus must be the consequence of their microvascular architecture (Peinado al., 1987). In fact, hepatocyte heterogeneity due to this vascular organization has been extensively described (Weibel et al., 1969; Schmuker et al., 1978; Peinado et al., 1987; Lamers et al., 1987; Gumucio and Chianale, 1988) and it is also known that it is the cause of a different hepatocyte turnover across the acinus. Thus, hepatocytes in portal zone have a faster grade of division than the hepatocytes located in the other acinus zones (Leffert et al., 1988); this feature could also explain the high number of binucleated hepatocytes found in this zone.

Referring to the nuclei sizes for the total hepatocyte population, both binucleated and mononucleated hepatocytes hardly ever showed heterogeneity across the liver acinus. This feature has already been observed referring to phenobarbital treatment (Stuabli et al., 1969) and in quantitative studies about ageing hepatocytes (David, 1983)

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