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Haemodynamic and ultrastructural observations on the rat liver after two-thirds partial hepatectomy

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ABSTRACT

Rat liver ultrastructure was investigated after partial hepatectomy (PH), by scanning and transmission electron microscopy. Portal pressure was monitored before and after PH and, after killing performed at 6, 12, 24, 48 h and 10 d, regenerating livers were fixed by portal vein perfusion under haemodynamic conditions identical to those existing in vivo. An early and persistent increase in portal pressure after PH was found ($P < 0.01$ for normal vs sham-operated controls). Ultrastructural study showed sinusoid dilatation and disappearance of the sieve-plate arrangement of small endothelial pores, thus leaving the parenchymal liver cell surface directly exposed to portal blood. Widening of sinusoids, endothelial fenestrations, intercellular spaces and spaces of Disse, was accompanied by dilatation of bile canaliculi. At 10 d, liver ultrastructure had returned to normal. Our observations suggest that a rise in portal pressure, as a consequence of PH, may be related to the observed ultrastructural changes in the liver.

Key words: Liver regeneration; portal pressure.

INTRODUCTION

Historically, surgeons have primarily approached the elucidation of liver regeneration mechanisms by elaborating the concept of haemodynamic factors according to the original blood flow theory (Mann, 1940; Grindlay & Bollman, 1952; Fisher et al. 1954). After partial hepatectomy (PH), portal pressure is elevated in experimental animals (Rabinovici & Wiener, 1963; Oliver & Sutton, 1966; Banerjee & Aikat, 1968; Ohlsson et al. 1969; Mizumoto et al. 1970; Rice et al. 1977; Fogli et al. 1990), as well as in man (Stone et al. 1969; Sirinek & Thomford, 1974; Stone, 1975; Nagasue et al. 1983; Kanematsu et al. 1985) and the volume of blood passing through the liver remnant is increased during the initial phase of the regenerative response (Benacerraf et al. 1957; Rabinovici & Wiener, 1963; Aronsen et al. 1969; Rice et al. 1977; Kahn et al. 1982). The demonstration that liver regeneration could occur after reduction or absence of portal blood flow (Wienbren, 1956; Thompson & Clarke, 1965) downgraded the importance of haemodynamic factors to a solely 'permissive role' (Brauer, 1963; Bucher, 1963). The concept that specific hepatotrophic factors, blood and/or liver-borne, exert the basic control over liver regeneration and that the quality rather than the quantity of blood reaching the liver is important for regeneration after PH, is fully accepted today. However, the blood flow theory must be completely rejected only with caution, since the volume of blood passing through the liver is definitely important with respect to all liver functions, including regeneration.

The aims of this work were to study the consequence of two-thirds PH on portal pressure in the rat and, by means of scanning electron microscopy (SEM) and transmission electron microscopy (TEM), to investigate the ultrastructural modifications during compensatory liver growth under the effects of such haemodynamic changes.

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MATERIALS AND METHODS

Animals

Male Sprague-Dawley rats averaging 358 ± 25 g (Charles River, Italy) were used. The animals were maintained in plastic cages, with no more than 3 rats for each cage, at room temperature. They were fed a standard laboratory rat food (MIL, S. Morini, Italy) and water ad libitum, and were exposed to a 12 h darkness-light daily cycle. All experiments were performed according to national Research Council criteria for the care and use of laboratory animals.

Operative procedures

Under ether anaesthesia and via a midline laparotomy, 2 23-gauge needles were introduced into the portal vein and into the infrarenal inferior vena cava of the rat. The needles were connected to 2 central venous pressure monitoring sets (Medifix, B. Braun, Germany) filled with physiological saline. Zero pressure was based on the anatomical levels of the portal vein and inferior vena cava at the midportion of the rat. Pressures were read in cm saline from the height of the fluid columns after having reached a steady level. These values were considered to be the basal portal pressure and the basal caval pressure. The needles were removed and haemostasis was obtained by gentle sponge compression or by microfibrillar collagen haemostat (Avitene, Alcon Laboratories, USA). Rats were randomly allocated to the following groups: (1) partially hepatectomised rats (PHR: n = 25); (2) sham-operated rats (SOR; n = 20); and (3) normal control rats (NCR; n = 18). PHR were submitted to a standard two-thirds PH (Higgins & Anderson, 1931). SOR underwent a sham operation consisting of soft manipulation of the liver, severing of the surrounding ligaments and positioning of an untied ligature temporarily placed around the lobes. In all animals, a second measurement of the portal and caval pressures was performed before closure of the abdominal wall. These pressures were considered the posthepatectomy portal pressure and the post-hepatectomy caval pressure. In PHR, the left and median liver lobes were lightly blotted with tissue paper, and weighed immediately after PH, while the volume of the resected lobes was evaluated as saline displacement in ml at 25 °C. The total liver weight and volume were previously estimated in 6 intact controls averaging 335 ± 29 g body weight, and after PH, the resected liver represented 68.1 ± 1.4% of the total rat liver weight. All operations, as well as portal and caval pressure measurements, were performed between 9 and 11 a.m.

Autopsy

Rats were killed within 6, 12, 24, 48 h and 10 d after surgery. The portal and caval pressures were measured as described above, and these values were taken as the terminal portal pressures. The needle in the portal vein was replaced by a 18-gauge teflon cannula (Abbocath-T, Abbott Ltd, Ireland). Around the cannulae, both the portal vein and the hepatic artery were ligated with a double ligature, while the inferior vena cava was clamped. Heparin (1000 i.u. in 1 ml saline) was slowly injected into the liver via the portal vein and perfusion was begun with saline at 37 °C, after having opened the chest and cannulated the thoracic inferior vena cava with a polyethylene catheter (o.d. 2.5 mm) to drain the effluent from the liver. A perfusion flow ranging from 10 to 12 ml/min (1.5–1.8 ml/g of liver tissue/min) was maintained by gravity and by adjusting the resistance in the tube leading to the portal vein by the insertion of a flow control extension set (Dial-A-Flo, Abbott Ltd, Ireland). During perfusion, the upper level of the perfusate in the drip chamber above the animal was maintained at the height corresponding to the previously recorded portal pressure. In this way, each animal was perfused at the appropriate portal pressure, while the flow through the liver was monitored by measuring the volume of the perfusate exiting the liver from the thoracic inferior vena cava. When the blood was cleared from the liver, perfusion was continued with 1.5% glutaraldehyde solution in 0.1 M cacodylate buffer (pH 7.4, 440 mOsmol), adjusted with 2% sucrose and 2 mM $CaCl_2$, at room temperature for 10 min (McCuskey et al. 1983). Livers were excised from the abdominal cavity, cleaned of ligaments, vessels and of stumps in PHR. Weight and volume of the regenerating livers and those of the corresponding right lateral and caudate lobes in SOR and NCR, were measured as described above. Liver regeneration was estimated as the weight and percentage volume of regenerated liver to residual liver (% RW, % RV), previously calculated by the values of the resected lobes, and as percentage weight and volume increase in relation to the original liver weight and volume (% W, % V).

Microscopic evaluation

An average of 10–12 small blocks of fixed tissue for each animal, 1–2 mm in width, were cut with a

surgical blade from different regions of the caudate and right lateral lobes. The specimens were immersed in buffered 2% glutaraldehyde at 4 °C. For SEM, the liver specimens were left in 2% glutaraldehyde at 4 °C for 2 wk (Gores et al. 1986). Subsequently, the pieces were dehydrated in a graded series of ethanol, cracked with jewellers' forceps and subjected to critical-point drying. After mounting the dried tissue on aluminium stubs, it was sputter coated with gold and then examined in a Cambridge Instruments-360 SEM. For TEM, the specimens were postfixed in 0.1 mol/l sodium cacodylate-buffered 1% OsO_4, dehydrated through a graded acetone series and embedded in Eponate (Ted Pella Inc., USA). Sections (80 nm) were contrasted with lead citrate and uranyl acetate and examined with a JEOL 100B TEM. All data were expressed as mean ± standard deviation and the nonparametric Wilcoxon's test was used for statistical analysis with a 5% level of statistical significance.

RESULTS

In PHR, a statistically significant percentage volume increase, in comparison with the percentage weight increase, was recorded at 6 h (% RV vs % RW $P < 0.01$, and % V vs % W $P < 0.01$), 12 h and 24 h (% RV vs % RW $P < 0.01$, and % V vs % W $P < 0.05$). The percentage weight and volume increase in relation to residual liver weight and volume, and the percentage weight and volume increase in relation to original liver weight and volume in PHR, are summarised in Figure 1.

In PHR, the portal pressure significantly increased (PHR vs SOR and PHR vs NCR, $P < 0.01$), and the caval pressure significantly decreased (PHR vs SOR and PHR vs NCR, $P < 0.05$). Portal hypertension significantly persisted in all the PHR until killing at 6, 12, 24, 48 h and 10 d, when no significant modifications of the portal pressure were recorded in NCR and SOR. In PHR, the terminal caval pressure had returned to the pre-PH values, while in NCR and SOR, the terminal caval pressure did not show any statistically significant modification (Table).

On SEM, examination of liver specimens in PHR at 6 and 12 h showed an organisational pattern of the liver lobules not different from that of control animals. Sinusoids and hepatocyte plates maintained a normal radiating disposition from the portal tracts at the periphery to the central vein. A difference in sinusoid diameter, which was recorded between periportal acinar zone 1 and pericentral acinar zone 3 in control animals, was not present at 6 h after PH, because of dilatation of the sinusoid lumen in the periportal area,

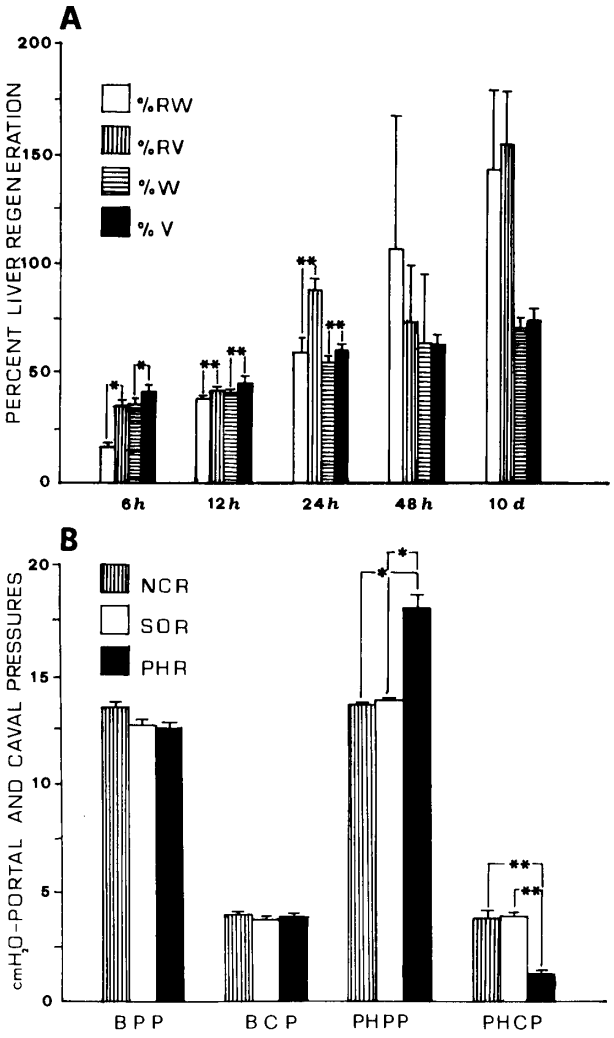

Fig. 1. (*A*) Liver regeneration in partially hepatectomised rats at 6, 12, 24, 48 h and 10 d, expressed as percentage weight increase (% RW) and percentage volume increase (% RV) in relation to residual liver weight and volume, and as percentage weight increase (% W) and percentage volume increase (% V) in relation to original liver weight and volume. (*B*) Portal and caval pressures, expressed in cm H_2O, in normal control rats (NCR), sham-operated rats (SOR) and partially hepatectomised rats (PHR) before operation (basal portal pressure: BPP; basal caval pressure: BCP), and immediately after operation (post-partial hepatectomy portal pressure: PHPP; post-partial hepatectomy caval pressure: PHCP). Values are given as means ± standard deviation. * $P < 0.01$, ** $P < 0.05$.

while in zone 3 sinusoid diameter did not increase. At 6 h, the fenestrations in zone 1 sinusoid endothelium appeared enlarged, and the disposition of the small pores in clusters conforming to a sieve-plate arrangement disappeared (Fig. 2). Widening of the intercellular spaces and spaces of Disse was observed at 6 h, hepatocytes were mostly rounded in shape but not significantly increased in volume, and their surfaces appeared covered by microvilli (Fig. 3). The above mentioned ultrastructural modifications were more prominent in zone 1 at 6 h, but became evident throughout the entire liver lobule at 12 h. At this time,

Table 1. *Portal pressures at killing* (*SPP*) *and caval pressures at killing* (*SCP*), *expressed in cm* H_2O, *in normal control rats* (*NCR*), *in sham-operated rats* (*SOR*) *and in partially hepatectomised rats* (*PHR*), *at 6*, *12*, *24*, *48 h and 10 d after operation**

Group	n	Time	SPP	SCP
NCR	3	6 h	13.00±0.10*	3.80±0.75
NCR	4	12 h	13.29±0.52*	3.75±0.50
NCR	4	24 h	13.43±0.30*	3.91±0.90
NCR	3	48 h	12.73±0.37*	3.88±0.75
NCR	4	10 d	13.10±0.90**	4.00±0.10
SOR	4	6 h	12.90±0.09*	3.50±0.40
SOR	3	12 h	13.33±0.46*	4.01±0.20
SOR	3	24 h	13.40±0.45*	3.85±1.11
SOR	5	48 h	12.56±0.60*	3.97±0.81
SOR	5	10 d	13.10±0.90**	3.99±0.42
PHR	6	6 h	18.30±1.47*	3.86±1.05
PHR	4	12 h	17.76±1.36*	3.80±1.20
PHR	4	24 h	17.29±1.94*	3.96±0.41
PHR	5	48 h	16.70±0.65*	3.87±0.90
PHR	6	10 d	15.70±0.91**	3.78±0.55

* Values are given as mean±standard deviation.
* PHR vs NCR at 6, 12, 24, 48 h, $P < 0.01$; ** PHR vs NCR at 10 d, $P < 0.05$; * PHR vs SOR at 6, 12, 24, 48 h, $P < 0.01$; ** PHR vs SOR at 10 d, $P < 0.05$.

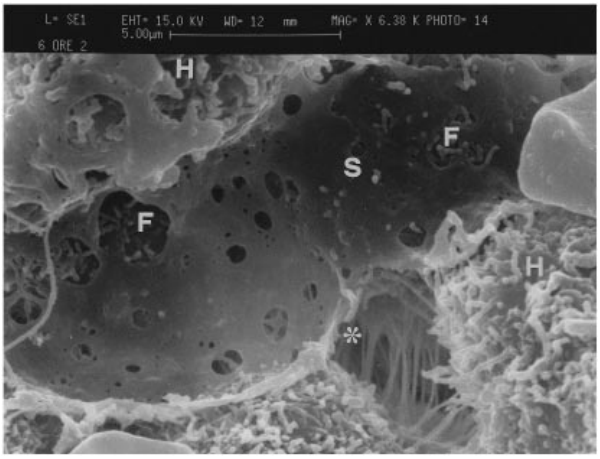

Fig. 2. Scanning electron micrograph of rat liver at 6 h after partial hepatectomy. The sinusoid endothelium (S) shows large fenestrations (F) through which microvilli on the surfaces of hepatocytes (H) are evident. Enlarged intercellular spaces (*) are present between neighbouring hepatocytes. ×3150.

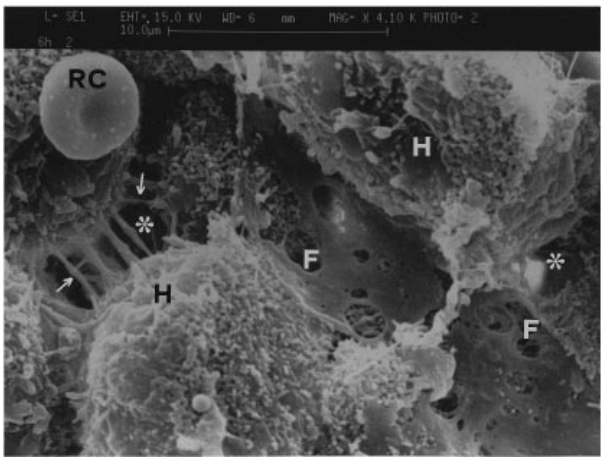

Fig. 3. Scanning electron micrograph of rat liver at 6 h after partial hepatectomy. Hepatocyte (H) surfaces are covered by microvilli and enlarged intercellular spaces are evident (*). Neighbouring hepatocytes are anchored to each other by pseudopods (arrows), which bridge between the cells across the enlarged intercellular space. RC, red cell; F, fenestration in sinusoid endothelium. ×2000.

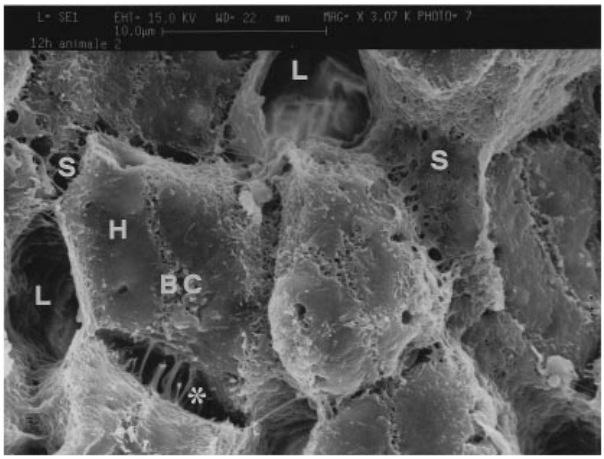

Fig. 4. Scanning electron micrograph of rat liver at 12 h after partial hepatectomy. The sinusoid endothelium lining the hepatocyte surfaces (H) is recognisable as a widely opened membrane (S). L, sinusoid lumen. Dilated bile canaliculi (BC) are seen in the midportion of the hepatocyte surfaces. Enlarged intercellular spaces (*) are evident. ×1500.

a decrease in the density of microvilli on hepatocyte surfaces was observed and because of fenestration enlargement, the sinusoid endothelium was often recognisable as a widely open membrane interposed between the cell plates (Fig. 4). In PHR, bile canaliculi running in the centre of the hepatocellular surfaces appeared dilated and in communication with the intercellular spaces (Fig. 4). The intercellular spaces, which in controls were recognisable as virtual spaces interposed between the smooth surface of neighbouring hepatocytes, at 6 and 12 h appeared markedly enlarged. However, in spite of marked intercellular spaces enlargement, hepatocytes appeared connected to each other by elongated cytoplasmic bridges that were easily recognisable within the enlarged intercellular spaces (Figs 2–4).

At 24 h, widening of the intercellular spaces was more evident throughout the entire lobule and the liver cells still appeared anchored one to each other by intercellular bridges in spite of the almost complete loss of the normal radiating pattern of cell plates and

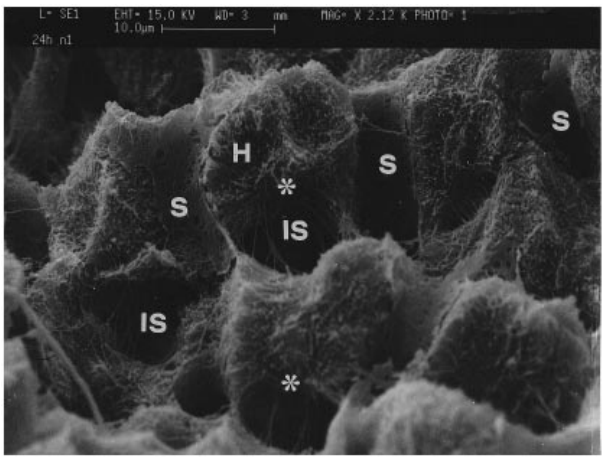

Fig. 5. Scanning electron micrograph of rat liver at 24 h after partial hepatectomy. A normal radiating pattern between hepatocyte plates (H) and sinusoids (S) is not recognisable because of widening of the intercellular spaces (IS). Bile canalicular lumina (*) appear to communicate with the enlarged intercellular spaces. Liver cells are loosely attached to each by cytoplasmic bridges. $\times 1000$.

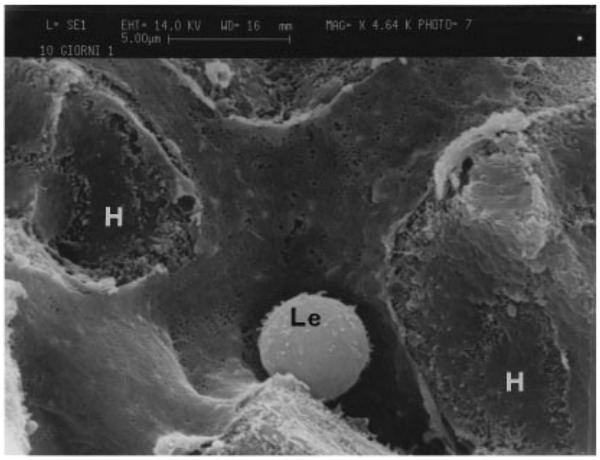

Fig. 7. Scanning electron micrograph of rat liver at 10 d after partial hepatectomy. The sinusoid endothelium overlying the hepatocytes (H) show small pores, which are mostly arranged in a sieve-plate arrangement. Le, leucocyte. $\times 2300$.

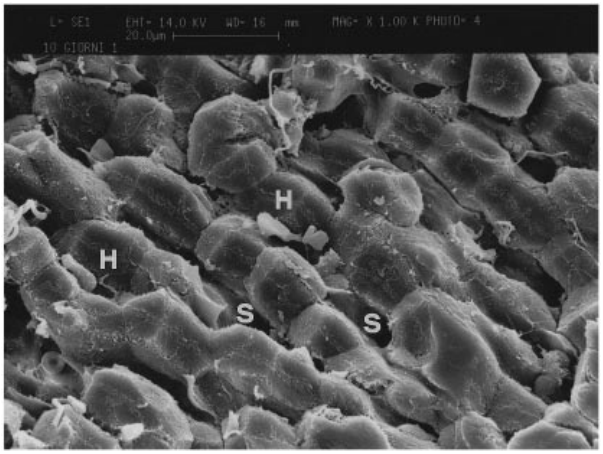

Fig. 6. Scanning electron micrograph of rat liver at 10 d after partial hepatectomy. The cell plates have returned to 1 cell thickness and hepatocytes (H) appeared well attached one to another. S, sinusoids. $\times 500$.

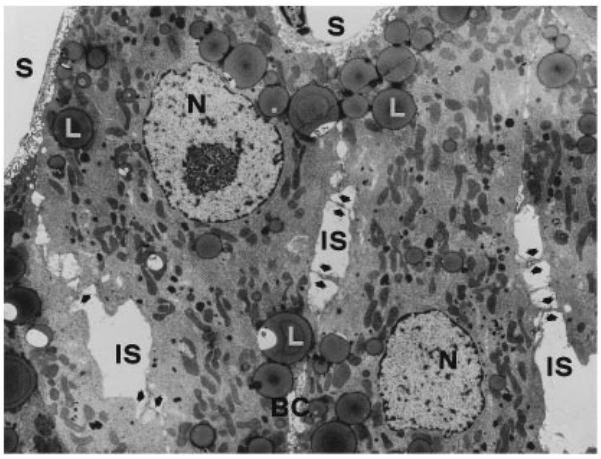

Fig. 8. Transmission electron micrograph of rat liver at 24 h after partial hepatectomy. Enlargement of the intercellular spaces (IS) is present. Cytoplasmic prolongations, which contain junctional complexes (arrows), cross the intercellular spaces. Intracellular accumulation of lipids, in the form of electrondense droplets (L), is evident. S, sinusoidal lumen; N, hepatocyte nuclei; BC, bile canaliculus. $\times 2200$.

sinusoids. Sinusoid dilatation and enlargement of fenestrations were still evident and the bile canalicular lumina became markedly dilated and indistinguishable on the liver cell surfaces (Fig. 5).

At 48 h, several cell plates were more than a single cell in width and many sinusoids did not show a normal radiating pattern because the lumen was narrowed by replicating hepatocytes. Bile canaliculi, fenestrations in the sinusoidal endothelium and intercellular spaces showed a wide range of diameters. At 10 d, the multiple-cell thick plates were still present in some instances, but most of the cell plates had returned to a cell thickness (Fig. 6). At this time, the features of hepatocyte surfaces, i.e. the number and density of microvilli in the lateral sinusoidal and bile canalicular lumina, had completely returned to normal and hepatocytes appeared well attached to one another. Large fenestrations in the sinusoid endothelial wall were only occasionally seen and small pores were mostly arranged in sieve plates (Fig. 7).

On TEM, sinusoids from PHR showed a marked dilatation, starting at 6 h in zone 1, and becoming more evident throughout the entire liver lobule at 12 h. Intercellular spaces were enlarged at 6, 12 and 24 h, and hepatocytes appeared to be in communication by cytoplasmic bridges (Fig. 8) which appeared

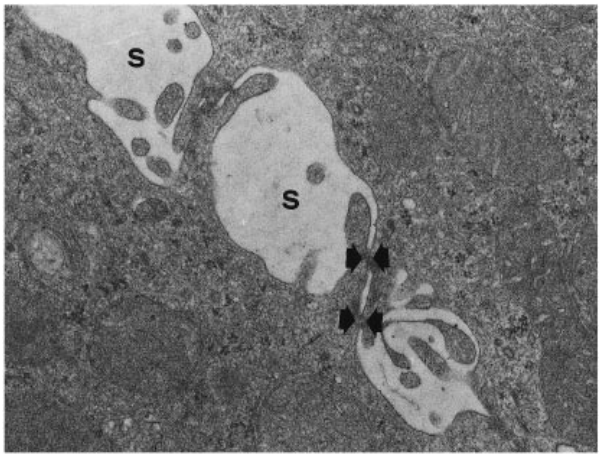

Fig. 9. Transmission electron micrograph of rat liver at 24 h after partial hepatectomy. At higher magnification (see Fig. 8) the cytoplasmic prolongations, which cross the dilated intercellular spaces (S), show the presence of desmosomes (arrow). ×9500.

to possess desmosomes (Fig. 9). Hepatocytes from PHR showed a typical intracellular lipid accumulation in the form of electrondense droplets (Fig. 8).

DISCUSSION

Portal pressure monitoring both in morphological and functional studies of isolated perfused rat liver is of undoubted importance. Control of perfusion pressure and flow are required to approximate to physiological conditions, since perfusion at a lower pressure may result in reduction of liver size, caused by nonhomogeneous distribution of perfusate into the liver, and perfusion at a higher pressure may cause an increase of the intrahepatic vascular bed with consequent barotrauma to the liver parenchyma (Brauer et al. 1959; Ogawa et al. 1979). In addition, liver perfusion at an appropriate physiological portal pressure is needed after PH, since the removal of two thirds of the hepatic parenchyma resulted in a prompt and stable increase of portal pressure in the rat.

Conflicting results have been reported on the occurrence and duration of portal hypertension after PH; this appears mostly to be due to different experimental conditions and study methods (Rabinovici & Wiener, 1963; Oliver & Sutton, 1966; Banerjee & Aikat, 1968; Rice et al. 1977; Fogli et al. 1990). A significant rise in portal pressure after two-thirds PH is universally accepted in the rat, and post-PH portal hypertension can be explained by the acute reduction in the portal vascular bed, while the splanchnic venous flow remains unchanged (Brauer, 1963). The persistence of portal hypertension, even during the late phase of liver regeneration, could be explained by narrowing of the intrahepatic vascular bed by growing hepatocytes, distorting the normal lobular pattern (Brauer, 1963; Bucher, 1963; Banerjee & Aikat, 1968).

The use of SEM for evaluating morphological changes during liver regeneration in the rat has been limited (Brooks & Haggis, 1973; Ogawa et al. 1979; Meyer et al. 1981; Tomoyori et al. 1983), in spite of the relatively simple method of manual fracture of perfusion-fixed liver and critical-point drying (Fahimi, 1967). To our knowledge, none of the aforementioned studies took into account the post-PH portal pressure increase during liver perfusion. The need to perform liver perfusion through the portal vein at an appropriate portal pressure and controlled flow during liver perfusion-fixation for SEM study, is just as important as the use of buffered fixative solutions that are isotonic with the blood plasma to minimise cell shrinkage (Arborgh et al. 1976; Grisliani et al. 1976; McCuskey et al. 1983). By means of SEM, it was possible to recognize a variety of aspects concerning the surface structure of hepatic cells and their supracellular organisation. In particular, we focused on the sinusoids, hepatocellular plates, bile canaliculi, sinusoid endothelium, spaces of Disse, and intercellular spaces. In NCR and SOR, we found an ultrastructural pattern similar to that previously reported (Fahimi, 1967; Miller et al. 1979). Sinusoids in zone 1 showed a narrower diameter and a more tortuous pathway than those in zone 3 (Motta & Porter, 1974). Fenestrations in sinusoid endothelium differed in size, and the smaller ones were mostly arranged in clusters, conforming to a sieve-plate arrangement (Wisse, 1970; Motta & Porter, 1974; Motta, 1975; Montesano & Nicolescu, 1978). At 6 h, we did not detect any difference in sinusoid diameters between zones 1 and 3 because of marked dilatation of sinusoids in the periportal areas. One of the most evident ultrastructural changes observed during the early phase of liver regeneration was enlargement of fenestrations in the sinusoid endothelium and the complete disappearance of the sieve-plate arrangement of small pores, as previously reported (Morsiani et al. 1995).

A morphometric TEM study in the rat showed that 8 h after PH there was a significant widening of the sinusoidal bed, whereas hepatocyte cytoplasm diminished (Vizzotto et al. 1989). Sinusoid dilatation after experimental passive congestion of the rat liver has also been reported (Brauer et al. 1959; Nopanitaya et al. 1976), as well as an increase in size of sinusoid endothelium fenestrations (Fraser et al. 1989; Greenway & Lautt, 1970). On SEM, we observed all the

above mentioned ultrastructural changes at 6 h, i.e. before the onset of cell division. At 6, 12 and 24 h after PH, we found a statistically significant increase in liver volume in comparison with the increase in liver weight. This could be related, at least in part, to the observed sinusoid dilatation and individual extracellular spaces. An early transient volume increase after experimental passive liver congestion was described as an effect of transsinusoidal filtration of fluid in the liver, depending only on the increased sinusoid hydrostatic pressure (Brauer et al. 1959; Greenway & Lautt, 1970). This increase in liver volume when the sinusoidal pressure is raised was found to be accompanied by dilatation of sinusoids, oedema of the portal area, and separation of the sinusoid endothelium from the underlying parenchymal cells with dilatation of the spaces of Disse (Brauer et al. 1959; Greenway & Lautt, 1970). Ultrastructurally this effect was characterised by enlargement of sinusoid fenestrations, allowing large particles, for instance chylomicrons, access to the spaces of Disse from which they are normally excluded (Greenway & Lautt, 1970; Fraser et al. 1989).

Widening of the intercellular spaces after PH has been extensively described in the rat as being accompanied by a dramatic decrease in junctional components, i.e. in the number and size of gap junctions and desmosomes (Yee & Revel, 1978; Meyer et al. 1981). On SEM, we confirmed the observation of an increase in intercellular space during the early phase of the liver response to PH. However, we found that hepatocytes did not completely lose cell-to-cell contacts, remaining anchored to each other by cytoplasmic bridges even after complete disappearance of the normal pattern of cell-to-cell contacts in the hepatocyte plates. These ultrastructural features were already present at 6 h and were maintained throughout the entire liver regenerative response. This observation was confirmed on TEM, allowing us to exclude an artifact due to specimen preparation. Together with widening of sinusoid fenestrations, intercellular spaces and spaces of Disse, a progressive dilatation of bile canalicular lumina was evident from the 12th to the 24th hours and, during this period, bile canaliculi appeared to communicate widely both with intercellular spaces and spaces of Disse. Similar ultrastructural changes were analysed both after SEM observations and TEM morphometric studies (Pfeifer & Reus, 1980; Tomoyori et al. 1983); it was suggested that growth of bile canaliculi proceeds at a higher rate than the overall growth of hepatocytes and that widening of intercellular spaces, spaces of Disse and dilatation of bile canalicular lumina may be related to the increased sinusoid pressure after PH (Tomoyori et al. 1983).

Since pressure in the periportal spaces of Disse is grossly equivalent to that in zone 1 sinusoids due to the presence of numerous endothelial fenestrations (Brauer, 1963) and since these fenestrations appear enlarged in the early phase of the liver regenerative response after PH, it is conceivable that an increase in portal pressure may have contributed to the widening of spaces of Disse and intercellular spaces in the periportal area. Disappearance of the barrier interposed between the bile canaliculus and the intercellular space, and the consequent dilatation of the bile canalicular lumen, may also be related to the increased portal pressure. To the classic objection that a certain degree of liver regeneration occurs even after reduction or absence of portal blood flow, the reply could be made that the existence of a humoral stimulus to regeneration does not prove that haemodynamic factors have no causative role in liver regeneration. In fact, after a portacaval shunt in the rat, a certain degree of hepatic blood flow is maintained after 24 h (Chauvaud et al. 1973), as well as after 3–5 mo, because of increased compensatory flow from the hepatic arterioles into the periportal sinusoids (McCuskey et al. 1983). When portal flow to an area of liver parenchyma is reduced or abolished, this area will receive a significantly increased arterial flow (Brauer, 1963; Hanna & Maheshwari, 1986; Doi et al. 1988; Lautt et al. 1990). The observation that early after PH in the rat there was a significant increase in liver volume, which corresponded ultrastructurally to widening of all extracellular spaces without a significant increase in cell volume (Vizzotto et al. 1989), suggests a lowering in cell density, i.e. the number of cells per tissue volume in the liver remnant before the onset of cell division. Interestingly, this finding may correspond to the density-dependent mechanism of hepatocyte proliferation in vitro, in which mature parenchymal liver cells become fully competent to respond to insulin and epidermal growth factor, and start DNA synthesis about 20 h after plating, but only when cultured at low density (Michalopoulos et al. 1982; Nakamura et al. 1983).

In conclusion, we hypothesise that the 'permissive role' of haemodynamic factors in liver regeneration after PH in the rat should not merely be considered as an increase in the amount of any hepatotrophic factor transported to the liver by the blood stream (Bucher, 1963). Our ultrastructural observations suggest that haemodynamic changes after PH could play a role in liver regeneration, primarily by increasing sinusoid endothelial permeability to any circulating hepato-

trophic substances. Raised sinusoidal pressure may further decrease cell-to-cell contacts by mechanically enlarging the intercellular spaces and contributing in this way to extracellular matrix degradation (Liu et al. 1994; Mars et al. 1995) and, ultimately, by inducing hepatocytes in the periportal area to become competent and to respond to circulating and liver-borne growth factors.

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