ULTRASTRUCTURAL CHANGES IN RAT HEPATOCYTES AFTER PARTIAL HEPATECTOMY, AND COMPARISON WITH BIOCHEMICAL RESULTS

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SUMMARY

Ultrastructural changes in rat hepatocytes in the first 24 h following partial hepatectomy (p.h.), i.e. in the premitotic phase of liver regeneration, were studied using electron microscopic morphometry. Livers were investigated 1, 4, 8, 12, 20 and 24 h after p.h. and 1, 8, 20 and 24 h after a sham operation. Two effects appear to be associated specifically with the regeneration process: (1) an increase in the volume density of lysosomes to a peak between 4 and 8 h after p.h.; and (2) a doubling in the number of mitochondria per cell by 24 h without any associated increase in the total mitochondrial volume. Two further changes were observed only after p.h.: (1) a massive accumulation of lipid in the form of lipid droplets by 24 h; and (2) the appearance of 'protein droplets' (very large lysosomal-like structures) at various stages. Both these changes appear to be secondary effects associated with the stimulation for, but not necessary to, cell division. The loss of glycogen observed immediately after both p.h. and a sham operation is a non-specific effect probably resulting from operation-induced stress.

The results are discussed with reference to the changes observed in the biochemical composition of blood plasma. Glucagon appears to play an important role in the stimulation of some of the ultrastructural changes observed.

INTRODUCTION

Regenerating liver, after partial hepatectomy, is a model for the study of controlled growth in vivo and offers a useful comparative system for the study of the mechanisms underlying carcinogenesis or uncontrolled growth (Bresnick, 1971). Although regenerating liver has been studied widely (for a review see Bucher & Malt, 1971), the basic mechanisms underlying the initiation and the regulation of regeneration are not at present fully understood.

In an attempt to clarify the understanding of the processes leading to initiation of cell division after partial hepatectomy we have carried out parallel biochemical and ultrastructural investigations on rats in the first 24 h after partial hepatectomy (p.h.). This paper is primarily concerned with a quantitative description of the ultrastructural changes.

The only other report of a quantitative analysis of the ultrastructural changes that occur in the early stages after p.h. is that by Rohr, Strebel & Bianchi (1970). These

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experiments differed somewhat from those reported here. Animals were biopsied repeatedly, so that samples were taken at different times of day. Thus some of the changes described could have been partly the result of diurnal variation (Rohr, Hundstad, Bianchi & Eckert, 1970). The repeated stress could also have affected the values of certain ultrastructural components, e.g. glycogen. In addition, Rohr et al. did not report any changes earlier than 3 h after p.h., and between 12 h and 24 h after p.h., i.e. the period of DNA replication. Similarly, there are no strictly comparable published results for untreated, i.e. o h, controls. Other reports generally describe results from different strains of rat, rats of different age, rats fasted prior to investigation or material processed using different fixation schedules, any of which may affect the precise morphometric values obtained.

This report describes the quantitative ultrastructure of rat liver in the first 24 h after p.h., and after a sham operation. Results are given in terms of volume densities (directly comparable with biochemical concentrations) or, where significant, in terms of total amounts per cell (directly comparable with biochemical values given in terms of absolute amounts). An increase in the volume density of lysosomes 4-8 h after p.h. and a doubling in the number of mitochondria by 24 h after p.h. seem to be specific effects related to liver regeneration, whereas an increase in the volume of lipid droplets and the appearance of protein droplets are probably secondary effects. The massive reduction in glycogen deposits immediately after the operation is shown to be a non-specific effect probably resulting from operation-induced stress. The results are discussed with reference to the parallel biochemical changes that have been described (Strecker, Silz, Ruhenstroth-Bauer & Bottger, 1980; Strecker, Silz, Salem & Ruhenstroth-Bauer, 1980). Some of these results were reported in preliminary form by Murray, Strecker & Silz (1978).

MATERIALS AND METHODS

Animals

Female Wistar rats weighing 200-240 g (Gesellschaft für Strahlen- und Umweltforschung, München-Neuherberg) were used in all experiments. Animals were maintained at a room temperature of 22 °C and humidity of 50 ± 5 % and were fed food (Altromin 1324) and water ad libitum.

Partial hepatectomy

Partial hepatectomy was performed under ether anaesthesia according to the method of Higgins & Anderson (1931). The time for p.h. was chosen such that animals killed after the selected time-interval would be killed between 8.00 a.m. and 9.30 a.m. The proportion of liver removed was determined in 26 animals and found to be 65.5 ± 1.6 %. Control animals were subjected to a sham operation consisting of laparotomy, severing of the surrounding ligaments and soft manipulation of the liver.

Tissue preparation

Animals were killed at chosen intervals in the first 24 h after p.h. by bleeding from the aortal bifurcation while under ether anaesthesia. The blood was collected for further biochemical tests, the results of which have been reported elsewhere (Strecker et al. 1980a, b).
Ultrastructure of regenerating liver

Liver was immediately excised and a small piece of tissue was removed from the tip of the lobus caudatus, immersed in 3% glutaraldehyde in Hank's buffer and cut up into 1 mm³ blocks. The blocks were transferred to fresh fixative at 4°C for 2 h, washed in Hank's buffer followed by 0.1 M-sodium cacodylate buffer containing 2 mM-CaCl₂ and 4% sucrose, postfixed in 2% OsO₄ in cacodylate buffer for 1 h, dehydrated in alcohol and embedded in Spurr (1969) resin. Sections (1 μm and 0.5 μm thick) were cut and stained with toluidine blue for light microscopy. Silver-gold sections (approx. 80 nm) were cut from 2 blocks per animal on an LKB microtome with a diamond knife. Section thickness was kept as constant as possible and sections were expanded with xylene vapours to reduce compression artifacts before being picked up on 200-mesh copper grids and stained with uranyl acetate and lead citrate. Thin sections were cut from midlobular regions as identified in the light microscope (more than 3 cell diameters from a central vein or portal tract; Loud, 1968). Sections were examined in an Elmiskop 1A and random electron micrographs (selected as described by Weibel, Kistler & Scherle, 1966) were recorded on cut film. A diffraction grating replica 2160 lines/mm (Agar Aids) was recorded with each group of films for calibration.

Morphometry

The morphometric analysis was performed essentially as described by Weibel, Stäubli, Gnägi & Hess (1969). Nine electron micrographs per tissue block were taken at a primary magnification of ×5000 and printed at a final magnification of ×15000 together with a 9:1 double-lattice test system (Rohr & Riede, 1973) containing 1089 points, of which 121 were set off as heavy points, within a frame of known area (121 μm²). Fields containing less than 50% cytoplasm were discarded. A total of 36 micrographs per time-interval was evaluated: 9 micrographs from each of 2 blocks from each of 2 animals. The volume densities of hepatocyte cytoplasm, nuclei and mitochondria were estimated using heavy points only and those of lysosomes, peroxisomes, lipid vacuoles, glycogen and Golgi-rich areas (Schmucker, Mooney & Jones, 1978) using all points. The number of transected mitochondria within the frame was also counted.

The average ‘mononuclear’ hepatocyte volume at each of the selected times after p.h. was calculated from the change in total liver weight and average volume at 0 h (determined by light microscopic morphometry) as described in detail by Murray, Strecker & Silz (1980). The average ‘mononuclear’ hepatocyte volume of sham-operated controls was determined by light microscopic morphometry (Murray et al. 1980).

The volume densities as percentage of cytoplasm, and the total volume per mononuclear hepatocyte of mitochondria, lysosomes, peroxisomes, lipid vacuoles, glycogen and Golgi-rich areas, as well as the total number and single volume of mitochondria, were calculated according to standard methods (Weibel et al. 1969). The expression of stereological data in terms of the volume per mononuclear hepatocyte assumes that binucleate cells are approximately twice the size of mononucleate cells. This appears generally to be the case (Loud, 1968; Weibel et al. 1969). In this study the term hepatocyte will henceforth be taken to mean the cell portion related to one nucleus, or mononuclear hepatocyte.

No attempt was made to correct for errors arising as a result of section thickness, since all the organelles investigated were large in comparison with the section thickness (Weibel & Paumgartner, 1978). Other systematic errors were considered to be evenly distributed throughout the samples.

All results were subjected to a statistical analysis, which included calculation of the mean, standard deviation and standard error and an estimation of significance using Student's t-test (unless otherwise stated, significance taken as P < 0.05).

Results

Glycogen

A rapid decrease in the volume density of glycogen was observed shortly after operation in both p.h. and sham-operated animals (Fig. 1). By 30 min after operation the volume density of glycogen was reduced from 13% to 3–5% of the hepatocyte
cytoplasm and by 4 h after operation loss of glycogen was virtually complete (Figs. 2, 3 and 8). Reaccumulation of glycogen was apparent by 12 h, rising to 5-6% of the cell cytoplasm by 20 h. After 20 h a difference was observed between p.h. and sham-operated animals: glycogen continued to accumulate in the hepatocytes of sham-operated animals but was once more depleted, to 3.5% of the cytoplasm, by 24 h after p.h. This difference was still significant when the total volume of glycogen per cell was considered.

**Mitochondria**

The volume density and total volume per hepatocyte of mitochondria after p.h. and sham operation are shown in Fig. 4A,B. After sham operation the volume density remained constant whereas the total mitochondrial volume per cell was reduced to 68% of the initial value by ½ h after operation, reflecting the marked decrease in cell volume (Murray et al. 1980). The total mitochondrial volume per cell increased steadily thereafter, returning to the initial value by 24 h after operation. In contrast, after partial hepatectomy both the volume density and the total volume of mitochondria remained constant until 8 h after operation. The volume density then decreased gradually to 80% of the initial value by 24 h after p.h. whereas the total volume remained constant.

The total number of mitochondria per hepatocyte and the single volume are shown in Fig. 4C,D. After sham operation the total number remained constant whereas the single volume was reduced at ½ h after operation, returning to the initial value by 20 h. After p.h. an increase in the total number of mitochondria was observed by 8 h after operation, the total number doubling by 24 h after operation. This increase in total number was accompanied by a decrease in single volume to about half the initial value by 24 h after p.h. (see also Fig. 6).
Fig. 2. Zero hour control: typical section from untreated liver shows large areas of glycogen (gly), few lysosomes (ly) and little lipid (li). × 7200.

Fig. 3. One hour after p.h.: the amount of glycogen (gly) is greatly reduced, only a few scattered particles are visible. × 7200.
Lipid

The most striking feature observed after p.h. was an accumulation of lipid. The total volume of lipid droplets per hepatocyte is shown in Fig. 5. After sham operation the volume of lipid droplets per cell remained constant, lipid occupying 0.5–1% of the cytoplasm. After p.h. there was a steady accumulation of lipid, noticeable by 4 h after operation, resulting in a nearly 20-fold increase in total lipid volume by 24 h, at which time the lipid occupied over 7% of the cell cytoplasm. The lipid was very unevenly distributed within the liver and between animals. Even at 20 h after operation some animals showed little accumulation of lipid. The lipid droplets were often associated with newly accumulated glycogen (Fig. 6).

Lysosomes

For the sake of simplicity, dense bodies, autophagic vacuoles and multivesicular bodies were counted together as 'lysosomes' (Novikoff, 1973). Dense bodies formed the majority of identified lysosomes in all cases. The change in volume density of...
Ultrastructure of regenerating liver

Fig. 4. Mitochondria: A, volume density in cytoplasm; B, total volume per mononuclear hepatocyte; C, total number per mononuclear hepatocyte; D, average volume of single mitochondrion. After p.h.: A, •—•; B, •—•; C, •—•; D, *—•; or after sham operation A, O—O; B, △—△; C, △—△; D, *—*.

lysosomes is shown in Fig. 7. After sham operation the volume density remained constant, lysosomes occupying about 0.7% of the cell cytoplasm. After p.h. the volume density of lysosomes increased to a peak between 4 h and 8 h of approximately twice the initial value (Fig. 8). The increase at 4 h was highly significant ($P < 0.001$). The volume density returned to normal by 12 h after p.h., remaining constant thereafter.

An additional group of membrane-delimited bodies was observed much larger than lysosomes but with similar morphological features. These bodies have been called protein droplets and are probably lysosomal (Becker & Lane, 1965; Mori & Novikoff, 1977). Because of their extremely uneven distribution and disproportionate size in relation to other lysosomes they were excluded from the estimation of lysosome
Fig. 5. Lipid: total volume per mononuclear hepatocyte after p.h. (▲—▲) or sham operation (△—△).

Fig. 6. Twenty-four hours after p.h.: large accumulations of lipid are readily apparent in the form of lipid droplets (li) often associated with newly deposited glycogen (gly). The mitochondrial profiles appear on average smaller than those in control liver (see Fig. 2). ×7800.
Fig. 7. Lysosomes: volume density in cytoplasm after p.h. (●—●) or sham operation (○—○).

Fig. 8. Four hours after p.h.: a marked increase in the number of lysosomes (Ly) is apparent. There is no glycogen present. \( \times 7600 \).
Fig. 9. Protein droplets: three types of protein droplets are shown. A and B, dense protein droplets; C and D, less-dense protein droplets; E and F, pale vacuoles. A, B: 12 h after p.h.; C, E, F: 1 h after p.h.; D: ½ h after p.h. × 7400.
volume density. As a result of their uneven distribution meaningful morphometric measurements could not be performed on the group separately. However, a semi-quantitative estimate of their frequency and distribution was obtained by light microscopic observation of 0.5 μm thick toluidine-blue-stained sections. The protein droplets were divided into 3 categories on the basis of their morphology (Fig. 9): (1) the dense protein droplets, characterized by a round profile, a homogeneous ground substance similar in density to that of dense bodies, a 'halo' beneath the delimiting membrane and a diameter greater than 0.8 μm and occasionally as much as 8 μm; (2) the less-dense protein droplets, characterized by an appearance similar to that of the dense protein droplets but with a much less dense ground substance similar in density to that of peroxisomes or mitochondria; (3) pale vacuoles, characterized by a less regular profile and a very pale flocculated ground substance. All pale vacuoles observed were large (4 μm-12 μm in diameter).

No protein droplet of any type was observed in preparations from sham-operated control animals. Dense protein droplets were present in all preparations after p.h.: ½-1 h after p.h. mostly small dense protein droplets were present in over 50% of the cells and 4-8 h after p.h. in 25-50% of cells; at 12 h after p.h. mostly large dense protein droplets were observed in 50% of cells and by 16-24 h small dense protein droplets were seen in less than 25% of cells. Less-dense protein droplets were observed in 25% or less of cells ½-1 h after p.h. and occasionally thereafter up to 12 h after p.h. None was observed after 12 h. Pale vacuoles were observed occasionally ½ h after p.h., in approximately 25% of cells after 1 h, and occasionally 12 h after p.h. There was none present in any other preparation.

Peroxisomes and Golgi-rich areas

No significant changes were observed in the volume densities or total volume per cell of either the peroxisomes or Golgi-rich areas after p.h. or sham operation. Peroxisomes occupied approximately 1.5% of the cell cytoplasm (120 μm³) and Golgi-rich areas 0.2-0.3% (16-24 μm³).

**DISCUSSION**

**Glycogen**

The volume density of glycogen observed in hepatocytes of 0 h control animals (13% of cell cytoplasm) was considerably less than the value of nearly 20% reported by both Loud (1968) and Rohr et al. (1970b). However, in both reports the average cell volume was smaller and thus the total glycogen per cell was similar to that observed here. Glycogen is well known to be a labile substance, showing, for example, considerable diurnal variation in the extent of accumulation (Rohr et al. 1970a) and thus differences in animal maintenance might easily affect the precise values observed.

Glycogen depletion, reported by others (Rohr et al. 1970b; Marks, Markey, Dyer & Vaupel, 1975) by 3 h after operation and in mice by 1 h after operation (Verity, Travis & Brown, 1972), was observed as soon as ½ h after operation in both p.h. and
sham-operated animals and is clearly a non-specific operation-associated effect. It was accompanied by a marked drop in the concentration of insulin (a glycogen-storage promoter) in blood plasma (Strecker et al. 1980a) and a rise in concentration of plasma glucose (Strecker et al. 1980b). These observations are consistent with an explanation based on increased excretion of catecholamines as the result of operation stress followed by activation of hepatic glucan phosphorylase (the latter suggested by Verity et al. 1972). The reaccumulation of glycogen observed between 12 and 20 h is consistent with the increase in concentrations of plasma insulin (to the initial value in sham-operated animals and to two-thirds of the initial value in p.h. animals). The renewed depletion of glycogen 20-24 h after p.h. probably results from changes associated with the initial stages of cell division. Between 20 and 24 h after operation the concentration of plasma insulin continues to rise in sham-operated animals but remains constant after p.h. consistent with the observed differences in accumulation of glycogen.

**Lipid**

The volume density of lipid droplets in the hepatocytes of 0 h control rats was 0.5%, which is within the reported range of 0.3-1.1% (Loud, 1968; Rohr et al. 1970b; Schmucker et al. 1978). The massive accumulation of lipid by 24 h after p.h. has often been observed (e.g. see Stowell, 1948; Rohr et al. 1970b; Pieri, Zs.-Nagy, Giuli & Mazzufferi, 1975; Bartel, Orkisz & Kmiec, 1977) but the reason for it is not yet clear. Although the increase was observed as soon as 4 h after p.h. the main accumulation took place after 12 h.

The accumulation of lipid corresponded with a reduction in the serum concentration of cholesterol and triglycerides and an increase in the serum concentration of free fatty acids and glucagon. The glucagon concentration rose to a maximum at 12 h after p.h. whereas the increase in fatty acids was observed after 8 h rising to a maximum at 20 h after p.h. (Strecker et al. 1980a, b). Glucagon probably stimulates the rise in concentration of free fatty acid. As a result triglycerides may be incorporated into hepatocytes and appear in the form of lipid droplets.

The accumulation is very unevenly distributed within a single liver and between animals. It seems likely that accumulation of lipid is a secondary effect in liver regeneration and is not of direct importance.

**Mitochondria**

The value observed for the volume density of mitochondria in 0 h control rats (18.6% of cell cytoplasm) agrees with most reported values, which are of the order of 20% (Loud, 1968; Rohr et al. 1970b; Vergonet, Hommes & Molenaar, 1970; Pieri et al. 1975; Schmucker et al. 1978). However, the total number of mitochondria per cell (2640 ± 250) was higher, and the single mitochondrial volume (0.57 ± 0.07 μm³) generally lower, than the values reported by Rohr & Riede (1973) (1890 and 0.69 μm³), Weibel et al. (1969) (1140 and 0.66 μm³) and Pieri et al. (1975) (1939 and 0.53 μm³).

This difference may well result from the effects of starvation prior to death. In rats starved for 19 h prior to death, but with all other procedures identical to those
described here, we observed a total of 1790 mitochondria per cell and a single mitochondrial volume of 0.69 μm³.

The volume density of mitochondria was reduced by 24 h after partial hepatectomy. A similar decrease was observed by Rohr et al. (1970b). The total volume of mitochondria per cell, however, remained constant, showing that the decrease in volume density was simply the result of an increase in cell size without a concomitant increase in the total mitochondrial volume.

Two different effects were observed after p.h. and sham operation. Half an hour after sham operation both the total volume per cell and the single volume of mitochondria were reduced by nearly one-third, whereas the volume density and number of mitochondria were constant. This decrease directly parallels the decrease in cell volume and probably resulted from osmotic effects (Murray et al. 1980).

After p.h. the total volume of mitochondria per cell remained constant. The number of mitochondria per cell, however, doubled by 24 h, and this increase was accompanied by a halving of the single mitochondrial volume. (Rohr et al. (1970b) observed a similar decrease in single volume; his reported finding of the same number of mitochondria per unit volume presumably also corresponds to an approximate doubling in the total number per cell by 24 h after p.h., since the cell volume was nearly doubled by this time.) The most likely explanation is that between 8 and 24 h after p.h. each mitochondrion divides once, on average, with no accompanying increase in size. The increase in the number of mitochondria was already noticeable by 8 h after p.h., i.e. prior to the nuclear DNA replication phase, and may well be initiated by the same factors that promote cell division. The mechanisms stimulating DNA replication have been discussed elsewhere (Strecker et al. 1980a).

**Lysosomes**

The estimation of the volume of lysosomes depends on the criteria used to define 'lysosomes' as well as the ability to identify these bodies. This results in a considerable variation in reported values. The volume density observed here in hepatocytes of 0 h control animals (0.7%) lies within the reported range of 0.3–1.1% (Loud, 1968; Rohr et al. 1970b). There was a significant increase in the volume density (and total volume) of lysosomes 4–8 h after p.h. but not after sham operation. A similar increase was observed by Rohr et al. (1970b) and Marks et al. (1975).

The increase in the volume density of lysosomes was accompanied by an increase in the concentration of glucagon in blood plasma and cyclic AMP in the liver cell membrane (Strecker et al. 1980a). This is consistent with the observation of Deter & DeDuve (1967) that injection of glucagon causes an increase in lysosomal size by inducing cellular autophagy. This effect lasts only 3–4 h, i.e. about the same length of time as the peak in lysosomal volume density after p.h. The values for the concentration of glucagon and cyclic AMP in the membrane continued to rise after the peak in lysosomal volume density had been reached. Thus the initial increase in concentration of glucagon was probably responsible for the transient increase in lysosomal volume density. An increase in volume density and membrane fragility of lysosomes may be a precursor to cell replication (Barka, 1974).
The appearance of protein droplets after p.h. has been described in detail by Becker & Lane (1965) and Mori & Novikoff (1977), although in neither case do the bodies described appear identical to those observed here. Becker & Lane describe moderately dense or pale bodies with autophagic inclusions and the bodies described by Mori & Novikoff are generally not very electron-dense, with some resemblance to the pale vacuoles described here. In both cases, however, the size range of the bodies was similar to that observed here, and both authors report the presence of protein droplets as early as $\frac{1}{2}$-1 h after p.h. The protein droplets described here have not yet been characterized precisely and it remains to be seen whether they are variations of a single form or whether they represent 3 distinct types of body. The possibility that they represent progressive forms of the same organelle is suggested by the different times of maximum accumulation. The pale vacuoles have a maximum 1 h after p.h. and the dense protein droplets 12 h after p.h. However, all 3 types are present in varying amounts both $\frac{1}{2}$ h and 12 h after p.h. and the possibility that they represent progressive forms cannot be confirmed or denied. Similarly, it is not possible to designate the bodies as early or late varieties.

The protein droplets are not considered to be of fundamental importance in the regeneration process since they are not observed in animals starved prior to p.h. even though regeneration takes place (Mori & Novikoff, 1977). The bodies may arise as a result of the stimulation to cell division without themselves being necessary for subsequent mitosis. However, the bodies appear to form from pinocytotic structures (Mori & Novikoff, 1977) and thus indicate a massive increase in pinocytosis, which could enable the rapid transfer of essential plasma components into the cell. The changed conditions in hepatocytes of starved animals (reduced cell size, increased density of cell organelles) might obviate the necessity for this process or reduce the scale such that it was no longer readily observable.

**Endoplasmic reticulum**

Previous results published by Rohr *et al.* (1970b) indicate that p.h. and sham-operated animals show similar changes in the volume density of endoplasmic reticulum (ER) in the first 24 h after operation. Semi-quantitative inspection of the micrographs shown here indicated that no major changes took place in the distribution of the ER in the 24 h following p.h. or sham operation. After p.h. occasional dilation of the rough endoplasmic reticulum (RER) was evident with partial loss of ribosomes. This change was most marked at 8 h when a reduction in the amount of RER organized into large stacks of lamellae was also apparent. Twenty to 24 hours after p.h. the RER appeared normal, with possibly a slightly increased volume density. There was no massive loss of RER of the type reported by Marks *et al.* (1975). The only changes observed in smooth ER (SER) were a slight dilation, most apparent 8 h after p.h., and an increase in contrast mainly resulting from the loss of glycogen. No changes in the ER were observed after sham operation.

The detailed distribution of ER components was not investigated further since changes in the ER appeared not to play a major role after p.h. Minor changes are difficult to assess accurately because of the increased importance of systematic errors
in measurements of membrane components (Weibel & Paumgartner, 1978) and the
effect of differing cytoplasmic density on membrane contrast and recognition.

CONCLUSION

Of the various ultrastructural changes observed after p.h. two appear to be asso-
ciated specifically with the regeneration process: (1) the increase in volume density of
lysosomes; and (2) the doubling in number of mitochondria, probably in reaction to
the same stimuli that cause cell replication. The increase in lipid droplets and the
appearance of protein droplets are probably secondary effects associated with the
stimulation to cell division, whereas the decrease in glycogen between 20 and 24 h
after p.h. appears to be associated with cell division itself. The massive reduction in
glycogen deposits immediately after operation is a non-specific effect, probably
resulting from operation stress.

Immunoreactive glucagon appears to play an important role in the stimulation of
some of the ultrastructural changes observed. An increase in the concentration of
glucagon in the blood plasma apparently stimulates the increase in lysosomal volume
density, which may be a necessary precursor to cell division, as well as an increase in
free fatty acids and subsequent appearance of the lipid vacuoles. Even so, glucagon
is not the prime factor responsible for stimulation of liver regeneration. A hepatopoietic-factor-containing extract prepared by this group (Ruhenstroth-Bauer, Goldberg, Silz & Strecker, 1978; Silz & Ruhenstroth-Bauer, unpublished data) appears to
stimulate the regeneration directly.

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