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CHANGES IN THE LIVER OF THE RAT AFTER PARTIAL HEPATECTOMY

By R. D. HARKNESS

From the Department of Physiology, University College, London

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It is well known that after partial hepatectomy in rats the residual part of the liver grows rapidly and reaches the size of a normal liver in 2–3 weeks (Higgins & Anderson, 1931). In the 24 hr or so after operation the hepatic cells increase only in size, not in number. After this latent period they begin to divide rapidly (Brues, Drury & Brues, 1936). Many of the chemical changes which accompany this growth have been investigated. The object of the present investigation was to confirm some previous observations, and to make additional observations, particularly in the latent period before the commencement of cell division. A preliminary account of this work has already been published (Harkness, 1949).

METHODS

Such rats as were available from the local stock were used, namely male albinos, and black and white hooded rats of both sexes. The median and left lateral lobes of the liver were removed aseptically under ether anaesthesia (Higgins & Anderson, 1931). The animals were taken in groups of the same sex and colour, from which individuals were chosen by reference to a table of random numbers, to serve as normals or to be killed 10, 24, 48 or 72 hr after operation. They were kept in individual cages in a thermostatically controlled enclosure at 26–27° C from a week before the operation until death. They received food and water *ad lib.* all the time. The earlier groups had the Rowett Institute diet, the later ones the M.R.C. diet No. 41 (Parkes, 1946).

When an animal was to be killed it was given sodium pentobarbital, about 5 mg/100 g body weight in about ¼ ml. of distilled water, intraperitoneally. When it was anaesthetized the abdomen was opened rapidly, and a small sample of liver for glycogen estimation was taken, and placed at once in 30% KOH in a weighed tube. A sample of liver for estimation of blood was taken next. After this a sample of blood was taken, usually from the aorta, but on occasion from the carotid. The liver was then removed, and cut up into pieces which were rapidly blotted, weighed and used for the other estimations.

Changes in the composition of the liver

Water. By drying to constant weight in an oven at 100° C.

Total lipid. By the saponification procedure of Leathes & Raper (1925) adapted for use on a small scale.

Glycogen. By the method of Good, Kramer & Somogyi (1933).

Total nitrogen. By the micro-Kjeldahl procedure on powdered dried liver.

Blood. By the method of Klein (1945). This method was found to give practically nothing which estimated as blood when done on normal liver which had been perfused with isotonic saline to

remove blood. The samples of liver used for estimation of blood were blotted quickly on filter-paper before being weighed. The blood which is left is mainly that in the smaller vessels.

Chloride. The Carius-Van Slyke method (Van Slyke & Sendroy, 1923), with the addition of removal of the precipitated silver chloride before sodium thiocyanate titration, was used at first on powdered dried liver. Later the same method was used on wet liver broken up in KOH (Sunderman & Williams, 1933). The first procedure gave lower results than the second. However, a direct comparison of the two showed that the ratio was consistent. In twelve comparisons the first gave a mean figure 82·3% (standard error 1·2%) of the second. Figures obtained by the first procedure were multiplied by 100/82·3 to bring them into line with figures obtained by the second. Though this may introduce a slight absolute error, it does not affect comparison between rats killed at different times.

Extravascular space was calculated from data on chloride and blood in liver, and chloride in blood plasma. Total blood chloride was estimated by the same method as total chloride, plasma chloride by the method of Sendroy (1937). Data on blood and plasma chloride were obtained from three normal animals, and from three animals at each of the times at which animals were killed after operation. The mean values in normals, and at the different times after operation, did not show significant variation judged by Snedecor's F test (variance ratio). The mean value for all the animals was therefore used for calculating extravascular space for animals on which data on blood and plasma chloride were not obtained.

Extravascular space was calculated from the following formula, which is based on the assumption that protein concentration is the same in plasma as extravascular fluid, and that there is no need therefore to make allowance for a Donnan membrane equilibrium effect; the known high concentration of protein in liver lymph makes it clear that such an effect will be very small or absent in liver.

$$\text{Extravascular space (ml./g liver)} = \frac{\text{Cl/g of liver} - \text{Cl in blood in 1 g liver}}{\text{Cl/ml. of plasma}}.$$

Changes in the total quantity of these various constituents of the liver were also followed. For this purpose the weight of the whole liver preoperatively, and of the remnant left at operation were estimated from the weight of the liver removed at operation (Brues *et al.* 1936). This latter is a relatively constant proportion of the whole for a given strain of rats. Unfortunately, however, it was found to differ significantly in the two types of rat used in these experiments, viz. albino, and black and white hooded rats, both from the local stock. Among the albinos the removed portion of the liver averaged $67{\cdot}0 \pm 1{\cdot}0$% of the whole, among the black and white rats $60{\cdot}3 \pm 1{\cdot}9$%. The figure for black and whites on a larger sample, including six more not used in these experiments, was $62{\cdot}4 \pm 0{\cdot}9$%.

Histological changes

Counts and measurements of diameter of hepatic parenchymal cell nuclei were made on sections cut at 6 μ setting of the microtome. The samples of liver used for this purpose were obtained both from regenerating liver and, for comparison, from the liver removed at operation. They were fixed in Zenker-acetic fluid, embedded in paraffin, and cut in the usual way. Sections were stained with Ehrlich's haematoxylin after incubation for 15 min in 0·8 N-HCl at 56° C, as is done in the Feulgen method for staining. This method gives very clear nuclear staining with little background in the cytoplasm. It facilitates nuclear counts and measurements on parenchymal cells, which are easily distinguishable from others. In the comparison of the counts in removed and regenerating liver no allowance was made for differences in contraction in size of the sample during fixation and embedding. It was found that there was no significant systematic difference in the contraction of normal and regenerating liver. In twenty-four comparisons of removed liver and regenerating liver taken 10–72 hr after operation, the correction for this contraction was on average $2{\cdot}5 \pm 1{\cdot}5$% greater for regenerating than removed liver.

The counts were made on twenty randomly chosen areas of 192 μ square under $\frac{1}{12}$ in. objective. The original counts were corrected for the error introduced by the fact that all nuclei visible do not have their centres within the section, by multiplying by section thickness/section thickness

plus mean nuclear diameter (Brues *et al.* 1936; Abercrombie, 1946). The nuclear diameter was obtained by measuring 30–50 nuclei per rat in five randomly chosen fields of section. The diameter of each nucleus was measured under $\frac{1}{12}$ in. objective, once only, in whatever axis happened to run along the scale of a graticule in the eyepiece of the microscope. The mean nuclear diameter obtained in this way is somewhat less than the true mean because measurements on fragments of nuclei, whose centres are outside the section, are included. A correction was made for this effect to obtain the true diameter (Abercrombie, 1946). This amounts on average to about 1 μ. Mean nuclear volume was calculated from the corrected diameter on the assumption that the nuclei were spherical, as they seem to be. Morgulis (1911) gives a figure of 0·92 as the ratio between the mean of a number of measurements of shortest and of longest diameter of nuclei in the liver of a rat. The error from the assumption of spherical shape must be small.

Counts of numbers of cells in meta-, ana- and telophase of mitosis were also made. No distinction was made between mitoses in hepatic parenchymal cells, and cells lining sinusoids (Abercrombie & Harkness, 1951). The majority were in parenchymal cells.

RESULTS

Changes in composition of liver

General information is given in Tables 1 and 2, and the combined results for all animals are given in Table 3. To check the methods and the assumption often made that the lobes removed at operation and those left behind have the same composition, the composition of these two parts of the liver was compared in a number of normal rats. No significant differences were found

TABLE 1. Numbers of rats, their body weights at the time of operation and the change in body weight post-operatively till death

		Experimental rats Time after operation (hr)			
	Normal rats	10	24	48	70
Number of rats:					
Albino ♂	6	4	5	3	4
Black and white ♂	2	2	1	1	1
♀	1	1	1	0	0
Total	9	7	7	4	5
Body weight (g):					
Preoperatively	252±16	258±14	255±20	236±24	218±25
Post-operative loss	—	10±3	10±6	7±4	2±2

The estimates of variation are in all cases the standard errors of the means.

(Table 4), except perhaps with glycogen, which is on the borderline at the 5 % level of probability. Differences in glycogen concentration in different parts of the liver have been described before (e.g. Gomori & Goldner, 1947). The difference is small enough to be immaterial to the present investigation and has been ignored. The main findings in regenerating liver are summarized below.

Blood and extravascular space. The extravascular space showed a small fall from 21·2 ml./100 g liver to a minimum of 16·8 ml./100 g at 24 hr and rose thereafter to nearly normal at 48 hr, slightly above at 72 hr. Blood space fell

TABLE 2. Weight of liver lobes removed at operation, estimated total at time of operation, and the liver weight at death. All weights are in g. The estimates of variation are in all cases the standard errors of the means. The figure for total liver weight in operated animals was calculated from the weight of removed liver. The exact times of death were 10·0±0·2, 23·7±0·5, 48·0±0·7 and 70·4±0·2 hr. The description of parts of liver refers to the experimental animals, while the data on normal animals refer to the corresponding lobes

		Experimental rats Time after operation (hr)			
	Normal rats	10	24	48	70
Albino rats:					
Lobes removed at operation	6·36±0·43	5·78±0·34	6·43±0·49	5·81±0·37	5·21±0·60
Estimated total at time of operation	9·51±0·64	8·65±0·50	9·65±0·69	8·72±0·57	7·82±0·90
Liver weight at death	3·15±0·24	3·19±0·25	4·64±0·38	5·23±0·54	5·63±0·34
Black and white rats:					
Lobes removed at operation	4·80±0·47	5·90±0·72	4·79±0·18	4·76	4·20
Estimated total at time of operation	7·98±0·75	9·44±1·16	7·67±0·30	7·62	6·72
Liver weight at death	3·18±0·31	3·33±0·49	3·95±0·30	4·38	5·89

TABLE 3. Changes in composition of the liver

		Experimental rats Time after operation (hr)			
	Normal rats	10	24	48	70
Blood (ml./100 g)	6·7±0·3	6·3±0·6	5·4±0·4	3·8±0·2	4·2±0·2
Extravascular space (ml./100 g)	21·2±1·1	19·3±1·9	16·8±1·6	19·8±1·4	22·7±0·5
Extracellular space (ml./100 g)	27·9	25·6	22·2	24·6	26·9
Water (g/100 g)	71·0±0·3	70·8±0·7	72·4±0·7	73·2±0·3	73·9±0·3
Lipid (g/100 g)	3·3±0·1	7·6±0·8	7·4±0·7	5·8±0·7	4·5±0·8
Glycogen (g/100 g)	3·8±0·5	0·4±0·1	1·7±0·4	1·1±0·5	1·3±0·4
Residual solid (g/100 g)	22·0±0·6	21·2±0·5	18·6±0·5	19·9±0·3	20·4±0·5
Nitrogen (g/100 g)	3·3±0·1	2·9±0·1	2·7±0·1	2·9±0·1	3·1±0·1

The estimate of variation is in each case the standard error of the mean. The units are g or ml./100 g fresh liver.

TABLE 4. Comparison between lobes normally removed at operation (median and left) and residual lobes (right and caudate) in normal rats

	Residual	Difference	Removed	No. of rats
Water (g/100 g)	71·0	0·2±0·2	70·8	7
Lipid (g/100 g)	3·3	0·0±0·2	3·3	5
Glycogen (g/100 g)	3·4	0·4±0·2	3·8	7
Nitrogen (g/100 g)	3·4	0·0±0·2	3·4	7
Residual solid (g/100 g)	22·6	0·2±0·3	22·4	7
Blood space (ml./100 g)	6·6	0·1±0·4	6·7	6
Extravascular space (ml./100 g)	21·2	0·1±1·1	21·1	5
Nuclear count per field (92 × 92 μ)	8·7	0·1±0·3	8·8	6
Parenchymal cell nuclear diameter (μ)	7·8	0·1±0·2	7·9	6

The estimate of variation is the standard error of the mean.

from 6·7 ml./100 g to a minimum of 3·8 ml./100 g later, at 48 hr, and was still well below normal at 72 hr.

It is to be noted that the interpretation of these changes is dependent on certain assumptions. In the case of extravascular space it is assumed that the distribution of chloride between cells and extravascular fluid is the same in normal and regenerating liver. There is no evidence whether this is so or not. It is unlikely, however, that the distribution is grossly abnormal, since there is no necrosis in the liver which is also functioning well enough to keep the animal in a reasonably normal condition. In the case of blood space it is assumed that the blood in the liver has the same haemoglobin content as in the aorta. The extent of the error will depend on the relation between blood flow and lymph formation. The changes in blood space agree with expectation from the naked eye appearance of the liver. Up to 48 hr it is usually much paler than normal but to a less extent at 72 hr. Infiltration with fat, however, undoubtedly contributes greatly to this pallor. A decrease in 'vascular tissues' in regenerating rat liver has been reported by Stowell (1948), who made measurements on sections of liver by Chalkley's method (1943). He found that 'vascular tissues' normally occupy 28 % of liver substance, a figure which agrees well with the present one for blood + extravascular space in normal rats (27·9 ml./100 g). In regenerating liver 6–48 hr post-operatively the percentages of 'vascular tissues' were 31–47 % of the normal, a greater drop than was found for extravascular and blood space in the present experiments.

One further point of interest arose out of the determinations of chloride in liver. In earlier experiments analyses were made of portions of the liver removed at operation. These turned out, in a comparison of thirteen against five normals, to have an average chloride content 17 % above normal. This difference was just significant ($P = 0{\cdot}05$ by 'Student's' t test). It was attributed to oedema arising during the handling involved in removal. This might be expected to upset the calculation of original weight of liver from the weight of removed lobes, but apparently the effect is small, since calculation from the weights of animals and relation of this to liver weight gave an almost identical average result for the original weight of liver. Possibly a slight error introduced by oedema is compensated by the slight error in opposite sense produced by the small fragment, 100 mg or so of liver, left in the animal from the removed lobes.

Water. There was a rise from 71·0 g/100 g of liver to 73·9/100 g at 70 hr. Calculation from the data on extracellular space shows a rise in the ratio of water to solid in the cells. The results of other workers on the water content of regenerating liver are conflicting. In the first 24 hr Higgins & Anderson (1931) and Szego & Roberts (1949) found a considerable drop. Gurd, Vars & Ravdin (1948), on the other hand, obtained results very much like those reported here. It may be significant that only the latter workers appear to

have kept their animals at a temperature near to that used in the present experiments (26–27° C). The present experiments showed a significant rise in water content of the liver at 72 hr, though Drabkin (1947) and Davidson & Waymouth (1944) found no such rise about this time. The discrepancy may again be explained by difference in experimental technique, since these workers starved their animals for 24 hr before death.

Lipid. The main point of interest is that the concentration of lipid was already maximal at 10 hr after operation. Other figures for times earlier than 24 hr are those of Bogetti & Mazzocco (1939), and Szego & Roberts (1949), who found a significant rise at 10 hr and even earlier, but not yet to maximum. The figures for later times agree with those of Ludewig, Minor & Hortenstine (1939), and of Gurd *et al.* (1948).

Glycogen. There was a drop to almost zero at 10 hr with an irregular rise subsequently. The 72 hr value was still only about one-third of normal, but is a little higher than that found by Stone (1935) in an investigation which did not include earlier times. The results are in agreement with those of Bogetti & Mazzocco (1941), Novikoff & Potter (1948) and Gurd *et al.* (1948), who found low values over the same range of time after operation.

Residual solid and nitrogen. The term residual solid is used for the solid left after subtracting the weight of glycogen and lipid from the total solid. The greater part of it is protein. Residual solid fell progressively from 22·0 g/100 g wet liver to a minimum of 18·6 g/100 g at 24 hr. Nitrogen followed a very similar course. These results are in agreement with those of Brues *et al.* (1936), Gurd *et al.* (1948), and Szego & Roberts (1949).

Nuclear and cell volume

As the necessary figures were available, it seemed worth while to attempt for comparison to compute the volume of the parenchymal cell as a whole; or more strictly the volume of the intracellular material associated with one nucleus, the presence of binucleate cells being ignored. In order to obtain this, it was necessary to know the volume of the part of the section on which the nuclear count was made. Information on the actual thickness of the section as it lay on the slide was obtained as follows.

Serial sections were cut through five embedded blocks of liver, the volumes of which had been measured previously by weighing in air and water, the method used by Brues *et al.* (1936). Every tenth section was mounted on a slide and its area measured with a planimeter by tracing its image, projected at known magnification on to paper. Area of section was plotted against distance through the block, the latter measured by number of sections cut. The area under this curve is equal to the volume of the block. Since this was already known, the correct scale for the axis representing distance through the block could be determined and hence the section thickness. This averaged $7{\cdot}3 \pm 0{\cdot}2\,\mu$ for sections cut at $6\,\mu$. It is to be noted that this method is based on the assumption that there is no change in the volume of the section during cutting, but only a distortion, lateral compression being associated with a corresponding increase in the thickness of the section. That this is so is borne out by measurements of thickness of sections immediately after

cutting, before they have been floated out on water, which gave a still greater value, $8{\cdot}4 \pm 0{\cdot}2\,\mu$, for five sections cut at $6\,\mu$. These measurements were made by melting sections on to the top of a block of wax by radiant heat and then cutting them again at right angles to the original direction, so that their thickness could be measured directly when they had been mounted on a slide (Marengo, 1944).

The average cell volume was computed by dividing the total cell volume by the corrected count of number of hepatic cell nuclei. The total hepatic cell volume was computed from the total volume in the area of measurement on the assumption that extracellular space was proportionately the same as that obtained by chloride and blood measurements. That this assumption is valid is indicated by the fact that Truax (1939) found agreement between extracellular space measured from chloride estimations and visually on sections. It was also assumed that 5% of the total volume was occupied by the cells other than parenchymal cells. This is a rough figure, obtained by measurements on sections from a small number of animals by Chalkley's method (1943).

It will be seen from Table 5 that both nuclear and cell volume rose in regenerating liver, the latter lagging behind the nuclear volume and falling earlier. That both volumes rise agrees with the results of Stowell (1948). He, however, found that cytoplasmic volume rose earlier than nuclear volume and fell earlier too, the opposite of the present findings. He also reported a rapid fall in nuclear volume after 24 hr. The present results for nuclear volume agree excellently with those of Bucher & Glinos (1950).

TABLE 5. Parenchymal cell and nuclear volume in fixed material

	Time after operation (hr)			
	10	24	48	70
Cell volume	98 ± 6	163 ± 7	145 ± 7	140 ± 15
Nuclear volume	117 ± 10	179 ± 11	203 ± 22	202 ± 29

Cell and nuclear volume are expressed as a percentage of the value found in the liver removed at operation. The mean cell volume for all removed livers was $6190 \pm 240\,\mu^3$ and the mean nuclear volume was $330 \pm 14\,\mu^3$. No correction has been made for shrinkage during fixation and embedding. The estimate of variation is in each case the standard error of the mean.

Mitotic activity was negligible in normal and 10 hr rats, and very low in the 24 hr rats (mean number of meta-, ana- and telophase mitoses $0{\cdot}2 \pm 0{\cdot}1$ per 1000 parenchymal nuclei). It was evident at 48 and 70 hr ($7{\cdot}8 \pm 2{\cdot}4$ and $7{\cdot}2 \pm 2{\cdot}1$ per 1000 parenchymal nuclei).

Increase in total quantity of different constituents of liver

It has already been mentioned that the quantity of liver left initially in the black and white rats was significantly greater than in the albinos. This fact makes it difficult to combine the data of the two different types. As there were only a small number of black and white rats, and the changes in them were very little different in general trend from those found in the albino rats, except for a slight fall instead of a rise in liver weight at 10 hr, only the results for the albinos are actually given (Fig. 1). The total quantities of the different components of course change relatively to one another in accordance with the changes in concentration given in Table 2. The most rapid change is in the

lipid. It is interesting to find that this returned to a total very close to the amount present in the liver preoperatively. The figures given by Gurd *et al.* (1948) and Szego & Roberts (1949) show the same trend. This return to a total so close to normal seems to suggest a homeostatic mechanism controlling the total lipid in the liver, independently of the control of other components. This idea is complicated, however, by the findings of Ludewig *et al.* (1939), who have reported that the proportions of the different components of the total lipid change in regeneration.

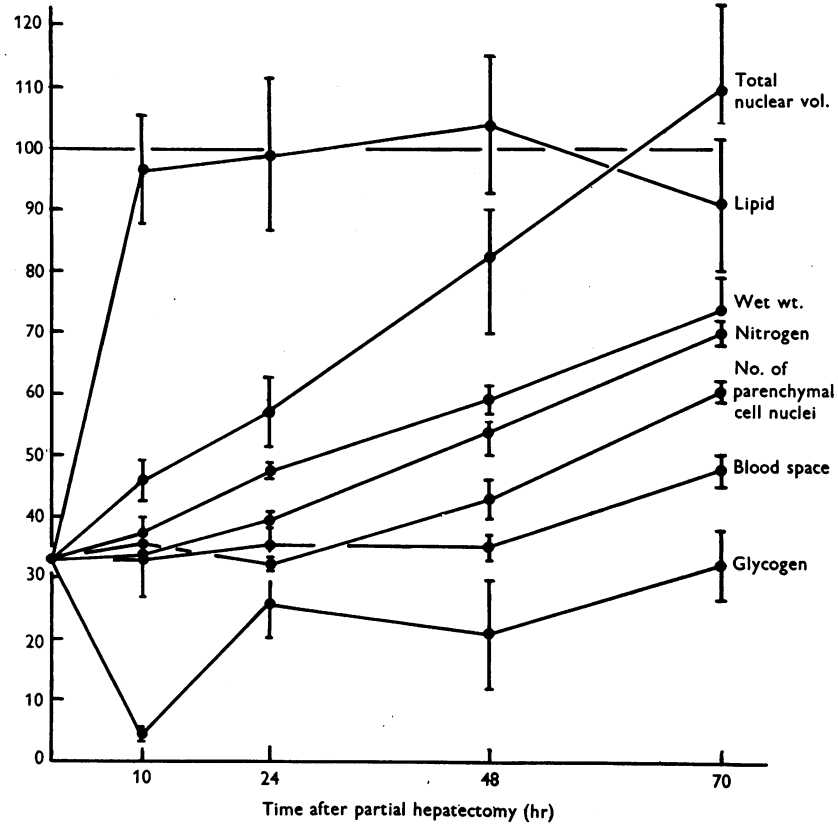

Fig. 1. Total quantities of different components of liver as a percentage of the total in the liver preoperatively. The vertical lines about the points go from + to − the standard error of the mean.

The component which rose next most rapidly after lipid, among those studied, was parenchymal cell nuclear volume, that is, the product of mean nuclear volume and total number of nuclei. This reached normal between 2 and 3 days post-operatively, but appeared to be going on above it.

Next in order of rapidity of rise came the wet weight and its major components, residual solid, and water. Lagging about 24 hr behind the increase

in weight came the increase in number of parenchymal cell nuclei, as found by Brues *et al.* (1936). Blood volume lagged still further behind and had not increased significantly till 72 hr. Glycogen lagged most of all.

DISCUSSION

The present results on the whole confirm earlier comparable work. Nevertheless, several discrepancies have been noted. These might of course be due to any of a number of possible causes, such as slight difference in the operative technique, and handling of the animals, or the strain of rat, etc. However, two of these causes, food intake and temperature at which the animals are housed, seem especially to merit some short discussion since they can be standardized relatively easily. The latter is particularly easy to control and it appears to the author important to do this. For one thing the animals eat little at first, and it is known that surrounding temperature has a considerable effect on changes in the liver of animals under these conditions (e.g. Chevillard, Hamon & Mayer, 1937). The temperature used in these experiments (26–27° C), and in those of Gurd *et al.* (1948) (28° C), is near the temperature of minimum heat production for the rat (Herrington, 1940), and the effects of small variations in temperature are minimized. It is high enough to be maintained easily at all times of the year, at any rate in England.

Another possible cause of discrepancy lies in variations in the food intake of the animals. Some workers have starved their animals in an attempt to overcome this. This procedure seems undesirable, particularly in the first few days after partial hepatectomy. The food intake of the animals is normally small in the first 24 hr after operation; and if, for example, they are starved preoperatively and killed 48 hr post-operatively after a further 24 hr fast, they may, in effect, have been made to fast for nearly 3 days. It seems too that the interpolation of an arbitrary period of fasting is likely to make any theoretical treatment of the changes after partial hepatectomy more difficult, and on the balance that the disadvantage in not allowing the animals free access to food outweighs the advantage. However that may be, it is relevant to plead that this point deserves more consideration than it has had in the past. In view of increasing interest in regenerating liver it seems desirable that methods should be standardized as much as possible to facilitate comparison between the results of different workers. The gain from standardization is likely to outweigh some loss of generality of conclusions associated with variability of method.

It is clear from the investigations of many workers that the different components of regenerating liver grow at different rates. The main interest in the chemical changes observed seems to be in their relation to the outburst of cell division which starts in the first day after operation. It appears that almost every chemical component of liver, except glycogen, increases more

rapidly than the number of parenchymal cell nuclei. The increase of protein in liver runs most nearly parallel to the number of parenchymal cell nuclei; there seems to be little doubt, however, that it precedes the latter.

Though it appears that increase of protein as a whole precedes increase in nuclear population, it is possible that individual components of the protein may follow the latter more closely. The early rapid increase of phospholipid (Ludewig *et al.* 1939) and ribonucleic acid (Novikoff & Potter, 1948) suggests an early increase in the mitochondria and submicroscopic particles, which together account for a great part of these components of liver (see Claude, 1946; Price, Miller & Miller, 1948). There is evidence, however, that the mitochondria do not increase early since succinoxidase activity (Novikoff & Potter, 1948), which is almost confined to mitochondria (Hogeboom, Schneider & Pallade, 1947), is less than normal per unit weight of tissue, though probably increased slightly in total at 24 hr after operation. It seems probable, therefore, that the early increase in phospholipid and ribonucleic acid is an indication of an early increase in the submicroscopic particles. If these increase more rapidly than the protein as a whole, then there must be other components of protein which increase less rapidly. Such rough calculations as can be made from published data indicate that the hypothesis that the increase of these other components runs closely parallel to the increase in number of parenchymal cell nuclei, is not an unreasonable one.

The last point for discussion is the change in extravascular and blood space. We know very little about how the volumes of these are controlled normally, but it seems that they must increase with the rest of the liver during regeneration, since the final regenerated liver is apparently very like the normal in structure and function. The curious divergence between the changes in extravascular and intravascular space, the latter expanding before the former, is difficult to understand. The delay in the increase of vascular space seems to indicate that the growth and division of hepatic cells is a separate process which precedes in time the reorganization of the vascular channel of the liver. This reorganization is likely to involve movement rather than division of the cells concerned. Investigation of the outwandering activity of cells of regenerating liver in tissue culture (Abercrombie & Harkness, 1951) has shown very little change from normal 1 day after operation, but a clear change at 2 days. Unfortunately, parenchymal cells did not give sufficient growth, with the technique used, for quantitative data to be obtained. For this and other reasons the results only showed that considerable change in migratory activity did occur without giving sufficient information for correlation with the present results. The problem of the organization of the new tissue and of the growth and organization of supporting tissue in regenerating liver is clearly one of great interest, and has received very little attention in the past.

SUMMARY

1. Changes in extravascular and blood space (chloride and haemoglobin estimations) in water, lipid, glycogen, nitrogen, and number and nuclear volume of parenchymal cells have been investigated concurrently in adult rats 10, 24, 48 and 70 hr after removal of two-thirds of the liver.

2. Extravascular space per unit weight of liver fell to a minimum about 20 % below normal at 24 hr but was nearly normal by 48 hr and slightly above by 70 hr.

3. Blood space per unit weight of liver fell to a minimum about half normal at 48 hr and had risen only slightly above that towards normal by 70 hr.

4. Changes in other constituents of liver were on the whole similar to those found by other workers.

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