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THE LIVER CELLS IN ACUTE STARVATION AND REFEEDING

AN EXPERIMENTAL HISTOQUANTITATIVE STUDY ON MICE

By

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OKSANEN, AILI: *The liver cells in acute starvation and refeeding. An experimental histoquantitative study on mice.* Acta vet. scand. 1972, *13*, 218—227. — A series of 52 young male white laboratory mice were starved for a maximum of five days, and 45 normally fed animals were used as controls. A group of experimental animals and a corresponding control group were sacrificed after two, three, and four days' starvation and after six, 12 and 17 days' refeeding. Even after two days the animals had lost weight, and the liver cells had diminished highly significantly. A return to the initial values was noted after 12 days' refeeding. In the experimental animals a strong negative correlation was noted between the cell count per field and body weight ($r = -0.712$, $b = -7.520$), while no such correlation was observed for the controls. The volume of the liver cells was reduced by 61.7 %, the volume of their nuclei by 16.2 % after four days' starvation. After two to three days' starvation the liver cells often showed a slight accumulation of fat. Mitoses were frequent in the liver cells during the stage of regeneration, when the cells had attained the same size as in the controls.

starvation; liver; mitosis; refeeding.

During acute starvation the weight of the liver is reduced by 36—63 % (*Grande* 1964). The reduction is due to loss of water glycogen, protein, esterified fatty acids and phospholipid phosphorus from the cytoplasm of the liver cells, while the nuclei do not change in size or composition (*Harrison* 1963, *Herrera & Freinkel* 1968).

During starvation the mitotic activity of the liver cells of growing mice ceases, only to rise transiently over the normal level in connection with refeeding (*Leduc* 1949). Similarly, starvation depresses the abundant mitotic activity of the regenerating liver after partial hepatectomy (*Vilchetz et al.* 1968).

Since on scanning of the literature no qualitative or quantitative histological reports on changes of the liver due to starvation were found, it seemed useful to study the liver cells by histoquantitative methods during acute starvation and refeeding.

MATERIAL AND METHODS

White, about 6-wecks-old male laboratory mice with a mean weight of 28.1 g at the beginning of the experiment were divided into two groups as similar as possible in regard to weight. One group was completely starved for a maximum of five days though with free access to water. The other group serving as controls was given commercial mouse feed ad libitum. Otherwise the two groups were kept under similar external conditions. All animals who died during starvation and all those showing any symptoms of disease were omitted from the series. Some of the samples had to be omitted because of evaporation of formalin from the flasks during storing. One group of starved animals and a corresponding control group were sacrificed after two, three and four days. After starvation the experimental animals were given the same feed ad libitum as the controls, and an experimental and a corresponding control group were sacrificed six, 12 and 17 days after the beginning of refeeding. The number of animals in the various subgroups may be seen in Table 1. The animals

Table 1. Occurrence of fat in the liver cells.

Group	Number of animals	Fat content in the scale 0—3			
		1	2	3	0
2 days' starvation	10	1	—	4	5
controls	10	—	—	—	10
3 days' starvation	10	1	1	4	4
controls	5	1	1	—	3
4 days' starvation	7	3	1	—	3
controls	8	2	—	—	6
6 days' refeeding	8	1	1	—	6
controls	9	2	2	—	5
12 days' refeeding	8	2	1	2	3
controls	8	1	2	—	5
18 days' refeeding	9	—	1	—	8
controls	5	—	1	—	4
total controls	45	6	6	—	33

were killed with town gas at 9 a. m. Immediately after sacrifice they were weighed and the abdominal aorta was cut so that the liver could be collected as blood-free as possible. Histological specimens were immediately placed in 10 % neutral formalin.

Specimens 10 μm in thickness, fixed in formalin, were stained with Scharlach red and Sudan black. From sections mounted in paraffin, specimens 5 μm in thickness were stained with haematoxylin and eosin.

Using a chequered measuring ocular the liver cell nuclei were counted in all experimental animals and controls in two fields measuring 320 μm × 320 μm. One field was chosen so as to be in contact with the central vein, the other from the periphery of a lobulus. The results (cell count per field) were pooled for statistical analysis.

To allow comparison of the areas of the cells visible in the specimens, the inverse values of the cell counts per field were calculated. For comparison of the cell volumes, the third power of the square root of the cell areas was calculated.

The nuclear areas were measured in the group starved for four days and its corresponding control group. Using oil immersion one hundred nuclei were drawn in perpendicular fields. By the aid of a mirror applied to the ocular tube the outlines of the nuclei were drawn at 187.5. The areas were measured with a planimeter adjusted at x 3.31. The nuclear volume was calculated on the basis of the third power of the square root of the nuclear area. The areas of cells and nuclei were determined using different methods and different, empirically chosen magnifications. Hence, the results were comparable within the two categories, but these could not be compared with each other owing to the lack of accuracy of the magnifications and the possibly different rate of shrinkage of the nucleus and cytoplasm on fixation. For the same reasons no attempt was made to calculate the exact volume in μm^3 of either the cells or the nuclei.

The means (m.) and standard deviation (s) for the nuclear areas, the liver cell counts per field and the body weight of the animals were calculated for the different experimental and control groups. Student's t-test was used on comparing these parameters in the various experimental groups to the corresponding values in the respective control groups. The cell counts per field and the body weight of the animals were compared by correlation regression analysis in the experimental and control groups.

The lipid content of the specimens stained with Scharlach red was estimated using the following scale 0—3: no fat = 0, traces of fat = 1, an appreciable amount of fat = 2, the heaviest content of fat seen (see Fig. 5) = 3.

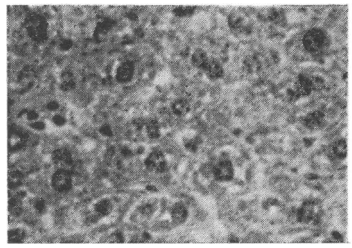

Figure 1. Liver of a control animal. Scharlach red × 350.

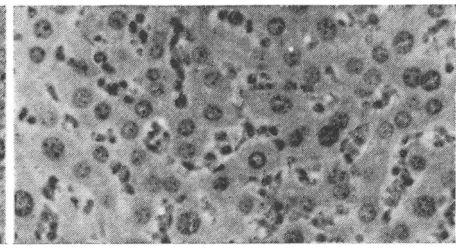

Figure 2. Liver of a mouse starved for four days. Scharlach red × 350.

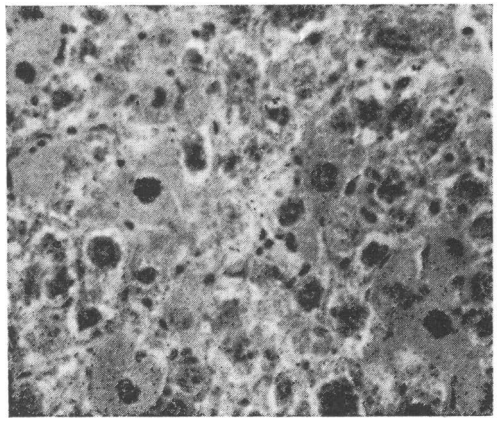

Figure 3. Liver after 12 days' refeeding. Seven mitotic nuclei are seen. Scharlach red × 350.

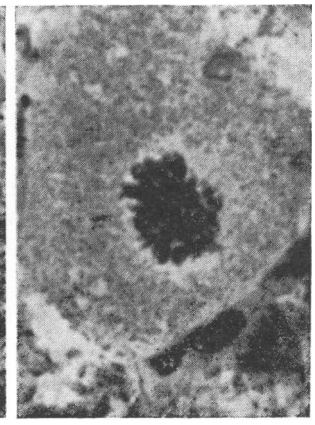

Figure 4. Same as Figure 3. Scharlach red × 825.

RESULTS

Histological examination

In all groups the liver cell nuclei were of various sizes in the same specimen. In the controls, the liver cells were ordinary, displaying a slightly eosinophilic cytoplasm with slight granulation (Fig. 1). In connection with continued starvation the liver cells diminished. The cytoplasm became uniformly eosinophilic (Fig. 2). No mitoses were seen in the controls nor in the experi-

mental animals during starvation, but during regeneration mitoses were frequent, in particular after 12 days' refeeding. The highest frequency of mitoses was 23 per 1000 cells. The cytoplasm of the mitotic cells was larger and more homogeneous than that of the remainder of cells in the same specimen (Figs. 3 and 4). In livers showing an appreciable amount of mitoses the mean cell count per field was 120.1.

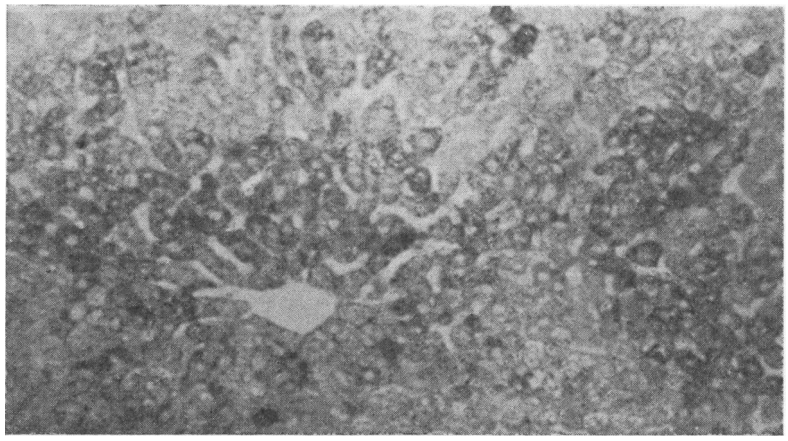

Figure 5. Liver of a mouse starved for three days. Fat content estimated at 3 in the scale 0—3. Sudan black × 150.

In all groups traces of fat were found in the liver cells of some individuals. Fat in greater amount (Fig. 5) was seen as fine droplets in the animals sacrificed after starvation for two or three days and in those killed after refeeding for seven days (Table 2).

Histoquantitative investigations and statistical results

The m. and s for the cell counts per field and the body weight of the animals in the various groups are shown in Fig. 6 and Table 2, in which the differences between the starvation and control groups as estimated by t-test also appear. In the control groups the liver cell counts per field remained very much the same throughout the experiment. In the experimental animals the cell count per field rose markedly during continued starvation (Table 2). After four days' starvation the mean cell count per field was 91.3 % higher than in the controls. As calculated by area the reduction of the cells was 48.3 % and by volume 61.7 %.

Table 2. Means and standard deviations for the liver cells per field in the different groups, differences in % between the means in the experimental and control groups, the corresponding differences as estimated by the Student's t-test, and weight of the animals in the different groups.

Group	Cells per field m. ± s	Change of mean value %	t	P	Body weight in g m. ± s
2 days' starvation	171.3±17.9	+ 42.2	5.413	0.001	24.9±1.45
controls	123.5±21.5				28.5±3.27
3 days' starvation	180.9±33.2	+ 50.1	4.103	0.001	24.6±2.39
controls	124.4±19.9				27.5±3.49
4 days' starvation	230.0±32.6	+ 91.3	7.846	0.001	20.3±2.43
controls	120.0±18.9				28.9±2.23
6 days' refeeding	158.8±19.8	+ 31.9	4.537	0.001	24.0±2.78
controls	122.1±13.3				28.4±3.96
12 days' refeeding	108.3±12.2	— 10.1	1.87	not signif.	30.0±2.90
controls	122.1±16.9				30.6±1.41
18 days' refeeding	119.4±19.7	— 0.9	1.61	not signif.	30.2±3.24
controls	105.6±12.3				30.2±3.85
total controls	120.5±17.3				

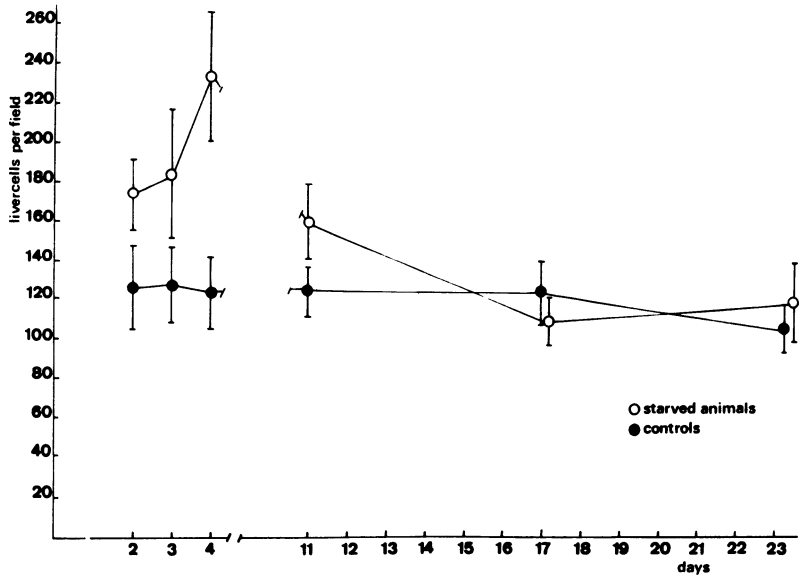

Figure 6. Mean values and standard deviation for cell count per field in the different groups. Days of starvation are indicated on the abscissa.

The mean value for the nuclear area was 5.7 after four days' starvation against 6.4 in the corresponding controls. The mean value in question was 11.3 % smaller in the experimental animals. As estimated by Student's t-test the difference in nuclear area was highly significant ($t = 6.760$, $P = 0.001$). The reduction of the nuclear volume was 16.2 %. During continued starvation the body weight steadily dropped (after two days $t = 3.025$, $P = 0.001$ and after four days $t = 6.615$, $P = 0.001$).

After six days' refeeding the differences in body weight ($t = 2.511$, $P = 0.05$) and in liver cell count per field ($t = 4.53$, $P = 0.001$) were significant, only to disappear after 12 days' refeeding.

On regression analysis a strong negative correlation between body weight and cell count per field was found for the experimental animals ($r = -0.712$, $b = -7.520$) (Fig. 7). No corresponding observation was made for the controls ($r = 0.048$).

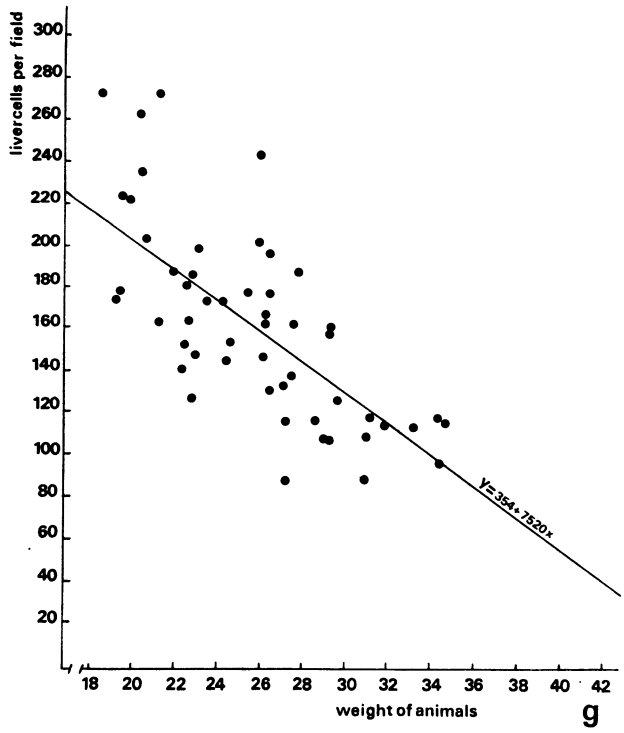

Figure 7. Correlation between body weight (x) and cells per field (y), $r = -0.712$, $b = -7.520$, $P = 0.001^{***}$.

DISCUSSION

In the present study the increase of the liver cell count per field reflected a marked and rapid diminution of these cells during starvation. In the control animals the liver cell count per field and body weight showed no correlation. In the experimental animals on the other hand there was a strong negative correlation between these two parameters. This was due to the simultaneous reduction in weight of the animals and size of the liver cells caused by starvation. The calculated reduction in volume of the liver cells (after four days' starvation), i.e. 61.7 %, corresponds to the upper limit of the rate of reduction of the liver weight reported by *Grande* (1964). Since the calculated reduction of the nuclear volume during the same period was only 16.2 %, most of the diminution of the liver cells was due to a decrease of the cytoplasm, obviously implying loss of the substances mentioned by *Herrera & Freinkel* (1968): water, glycogen, protein, phospholipid phosphorus and esterified fatty acids. The decrease in volume of the nuclei observed in this study after four days' starvation is in contrast to the results with chemical methods reported by *Harrison* (1953) and *Herrera & Freinkel.* However, the diminution of the liver cell nuclei observed in this study lends support to the conclusion drawn by *Vilchetz et al.* (1968), although it was not possible on the basis of the histological picture to say which part of the nuclei that was reduced.

No spontaneous mitoses were seen in any liver in the control groups. This is in agreement with previous observations (*Leduc* 1949). The abundance of mitoses during refeeding reflects the regeneration of the liver. This finding corroborates *Leduc*'s results and those of *Vilchetz et al.*, who reported that starvation had a depressing effect on cell division after partial hepatectomy. It may be assumed that starvation also inhibits the normal mitotic activity of the adult liver cells, by *Brues & Marble* (1937) estimated at one per 10,000—20,000 cells. The large number of mitoses observed in the present study in connection with refeeding may be considered as compensating the inhibition of cell division caused by starvation. The greatest frequency of mitoses was observed after 12 days' refeeding. This is markedly later than in *Leduc*'s study. The difference may be due to the longer starvation period in the present investigation. In this study mitoses appeared considerably later than one or two days after partial hepatectomy (*Brues & Marble*). It seems possible that the

liver cells increase in size before they begin to increase in number. This assumption is corroborated by the correspondence between the mean cell count per field in the animals showing mitoses and the controls.

The amount of fat in the liver after two or three days' starvation and in the initial phase of refeeding was much more abundant than in the controls. This is in agreement with *Leduc*'s observations. Chronic choline deficiency leads to a marked accumulation of fat in the liver (for references see *Ahlqvist* 1960). In the present study the accumulation of fat in the liver cells was much less, perhaps depending on the energy deficiency in the acutely and completely starved animals using their own stores of fat. The lipid accumulation in the liver observed in this study coincided in time with the highest values for plasma ketone reported in starved animals (*Herrera & Freinkel* 1968).

REFERENCES

Ahlqvist, J.: Liver mast cell counts during development of cirrhosis of the liver in rats on a low protein, high fat diet. Acta path. microbiol. scand. 1960, *50,* Suppl. 152.

Brues, A. M. & B. B. Marble: An analysis of mitosis in liver restoration. J. exp. Med. 1937, *65,* 15—28.

Grande, F.: Man under Caloric Deficiency. Handbook of Physiology. Section 4. Adaption to the environment, p. 913. Amer. physiol. Soc. 1964.

Harrison, M. F.: Effect of starvation on the composition of the liver cell. Biochem. J. 1953, *55,* 204—211.

Herrera, E. & N. Freinkel: Liver acetyl-CoA and citrate during fasting. Biochim. biophys. Acta (Amst.) 1968, *170,* 244—253.

Leduc, E. H.: Mitotic activity in the liver of the mouse during inanition followed by refeeding with different levels of protein. Amer. J. Anat. 1949, *84,* 397—429.

Vilchetz, C. A., I. L. Sadnik & E. G. Bade: Influence of starvation on liver regeneration in the mouse. Naturwissenschaften 1968, *55,* 392—393.

SAMMANFATTNING

Levercellerna i akut svält och återutfodring. En experimentell, histokvantitativ undersökning på möss.

Undersökningen omfattade 52 unga vita hanmöss, som frånhölls foder i 5 dagar. Som kontroller användes 45 normalt utfodrade djur. En grupp vardera försöks- och kontrolldjur avlivades efter resp. 2, 3 och 4 dagars svält och efter 6, 12 och 17 dagars återutfodring. Redan

efter 2 dagar hade svältdjurens vikt och levercell-storleken minskats starkt signifikant, för att återkomma till de ursprungliga värdena efter 12 dagars återutfodring. Hos svältdjur konstaterades en starkt negativ korrelation mellan antalet leverceller per mikroskop-fält och djurens vikt ($r = -0{,}721$, $b = -7{,}520$). Kontrolldjuren visade ingen motsvarande korrelation. Levercellvolymen minskade 61,7 % och kärnornas volym 16,2 % under 4 dagars svält. Efter 2 och 3 dagars svält kunde ofta en förfettning i levercellerna ses. Under regenerationsfasen var mitoserna rikliga vid den tidpunkt, då levercellerna hade återfått kontrollernas storlek.

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