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Effect of Starvation on the Composition of the Liver Cell

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It has long been known that starvation causes the liver as a whole to shrink, but it is only with the development of analytical and histological techniques during the last 100 years that this reduction in size has been attributed to a decrease in the size of the individual liver cells, caused by a rapid loss of glycogen and a slow but persistent loss of protein and water. Numerous descriptions of the changes produced by fasting in the livers of many species have appeared and many authorities have discussed and reviewed them (Benedict, 1915; Kosterlitz & Campbell, 1945; Peters & Van Slyke, 1946). Most workers were interested principally in the organic matter of the liver (Davidson, 1947; Davidson & Waymouth, 1944, 1946; Dible, 1932; Addis, Poo & Lew, 1936) or, more recently, in its enzymes (Miller, 1948; Lightbody & Kleinman, 1940), and few paid attention to the minerals. Moreover, no one has analysed the changes which take place in the liver cell itself, although Elman, Smith & Sachar (1943) and Kosterlitz (1947) correlated their chemical findings with the histological appearance of the liver.

A method by which the results of a fairly complete chemical analysis of samples of whole liver can be used to deduce the mass and composition of the liver cell was described in detail in a previous paper and is given here in outline (Harrison, 1953*a*). This method is based upon the assumption that the masses of the liver cells, like those of their nuclei, are dependent upon the number of chromosomes which they contain and are therefore related to their content of deoxynucleic acid. Moreover, the average mass of the tetraploid cell, whether it contains the tetraploid number of chromosomes in a single nucleus or is binucleate, is twice that of the diploid cell, containing the diploid number of chromosomes in its nucleus. Similarly, the octoploid cell is four times as large as the diploid cell. It follows that the average mass of the diploid cell, which is independent both of the degree of polyploidy and of the

proportion of binucleate cells, may be obtained from the ratio of the amount of deoxynucleic acid (DNA) in a diploid nucleus to that in a unit weight of liver tissue. The amount of DNA in the diploid nucleus was determined in the kidney, a tissue known to contain only diploid nuclei. The average mass of the diploid cell determined in this way was considered a better unit for comparison than the mass of the 'mean liver cell', the average for all the cells in the liver. A correction was made for that portion of the mass of the liver which lies outside the cells, which was assumed to be equal to the mass of water, protein and minerals in the blood, lymph, bile and interstitial fluid, and was deduced from the chloride space of the liver and the composition of the serum. The water, protein and minerals in the extracellular fluids of the liver were subtracted from values for whole tissue to determine the composition of the cells; it was assumed that all of the neutral lipids, glycogen, phospholipids, ribonucleic acid (RNA), DNA, inorganic iron, copper and zinc was intracellular.

This method has now been used to follow the changes in the liver cells of adult male and female rats during a 6-day period of starvation and to define differences in the responses of the two sexes to fasting.

EXPERIMENTAL

Animals. Thirty-five male and thirty-five female black and white hooded rats of the Lister strain were used. The male and female rats were from the same litters and were 3-4 months old. Until the fast began they were fed on a stock diet consisting of bread, milk, whole wheat, yeast, fishmeal, oatflakes and bran. The rats were weighed twice each week for 3 weeks before the fast to ensure that they were growing at a normal rate. The males and females were divided into five comparable groups of seven rats, according to their mean weights on the 3 days before the fast, and to their increase in weight during the three pre-experimental weeks. One group of either sex was killed at the start between 11 a.m. and 12 noon and served as controls. Their food had been removed 2 hr. previously. The remaining rats were kept in cages which were raised to prevent coprophagy.

Table 2. *The inorganic constituents in the livers of male and female rats during a 6-day period of starvation*

(Values for groups of seven rats expressed/100 g. liver.)

Sex	Length of fast (days)	Total iron (mg.)	Inorganic iron (mg.)	Copper (mg.)	Zinc (mg.)	Potassium (m-equiv.)	Sodium (m-equiv.)	Chloride (m-equiv.)	Total P (g.)	Inorganic P (mg.)
Male	0	17	13.2	0.42	4.2	8.10	3.30	2.55	0.37	49
	1	19	14.8	0.55	7.0	8.55	4.30	2.74	0.38	54
	2	20	18.3	0.61	6.6	8.10	4.35	3.00	0.39	52
	4	22	19.9	0.61	7.3	8.30	4.25	2.82	0.39	46
	6	25	22.8	0.61	7.7	8.20	4.05	2.69	0.41	45
Female	0	46	44	0.52	3.6	8.67	3.65	2.64	0.36	34
	1	55	53	0.64	6.3	8.90	3.82	2.76	0.39	43
	2	52	50	0.65	7.8	8.90	4.09	2.97	0.41	48
	4	64	63	0.63	—	8.85	3.61	2.71	0.41	45
	6	72	70	0.65	9.9	8.10	3.61	2.74	0.40	40

considered entirely intracellular components; hence the average amount of each of these constituents in a cell was equal to the amount in the combined mass *b/h*. Allowance was made for extracellular water, protein and minerals in deducing the amount of water, protein and K within the diploid cells.

No attempt has been made to draw smooth curves through the points in any figure. They have been joined by straight lines which are not meant to represent the changes during the intervening periods but merely to indicate general trends.

Table 3. *The concentration of protein, sodium and chloride in the serum of adult male and female rats during starvation*

Sex	Length of fast (days)	Protein (g./100 ml. serum)	Sodium (m-equiv./l. serum)	Chloride (m-equiv./l. serum)
Male	0	7.18	152	103
	1	7.12	160	104
	2	7.00	158	102
	4	6.88	159	103
	6	6.75	157	105
Female	0	7.68	154	105
	1	7.62	160	105
	2	7.55	161	104
	4	7.55	158	106
	6	6.68	156	106

RESULTS

Composition of liver and serum

Tables 1-3 contain the results from the analysis of the liver and serum during the 6-day period of starvation. The values in these tables, together with those illustrated in Fig. 1 for the volume of ECW, have been used to calculate the mass and composition of the diploid liver cell, on the assumption that the amount of DNA in the diploid nucleus was $5.7 \times 10^{-6} \mu\text{g}$. (Harrison, 1953*a*). Fig. 1 suggests that starvation was accompanied by a rise in the volume of ECW and conversely a fall in that of ICW during the first 48 hr. of the fast, but thereafter until the end of the fast the changes were reversed.

The same proportion of cells (0.3) was binucleate in suspensions of liver cells from the normal male and female rats. Fasting produced no change in this value in either sex and this agreed with the histological appearance of the livers. No evidence of cell

Fig. 1. Volume of ECW, lower curves, and ICW, upper curves, in the livers of male and female rats during a fast of 6 days. O---O, Male; ●—●, female.

necrosis was observed in sections of the livers after 6 days' fasting; moreover, the total amount of DNA in the liver remained constant throughout the fast ($18.3 \pm 0.3 \text{ mg.}$ in the male rats, and $15.8 \pm 0.3 \text{ mg.}$ in the female rats).

Mass of the cell and of its nucleus

Fig. 2 illustrates the changes in the mass of the diploid liver cell. The average mass of the female cell was initially 80% of that of the male cell. During the first 48 hr. the mass of the female cell

Table 4. The effect of starvation on the average amount of DNA/liver nucleus, and on the mass and 'protein' content of the diploid liver nucleus

Sex	Length of fast (days)	Body weight		Liver weight (g.)	DNA/liver nucleus (10^{-6} μ g.)	'Protein'/diploid nucleus (10^{-6} μ g.)	Mass of diploid nucleus (10^{-4} μ g.)
		Initial (g.)	At death (g.)				
Male	0	270	270	8.1	9.6	32	1.5
	6	265	204	5.3	9.6	31	1.5
Female	0	205	205	6.5	10.0	27	1.2
	6	210	155	4.3	9.9	27	1.1

decreased to three-quarters of its initial value, and from then until the end of the fast it slowly fell to 73 % of its initial value. The male cell, on the other hand, lost a quarter of its weight in the first 24 hr.; by the end of the fast it had lost 37 %. Thus, although the male liver cell initially had a larger mass than the

each time. These findings are consistent with the hypothesis of Leuchtenberger & Schrader (1951) that the volume of the nucleus is determined by the amount of protein in it. Furthermore, since there was no change in the size or number of nuclei in the liver and only a small proportionate change in its extracellular phase, such alterations as occurred must have been largely confined to the cytoplasm. The specific gravities of the nucleus and of the cell were assumed to be unaltered by the fast in calculating the nucleo-cytoplasmic ratio (see Harrison, 1953*a*); the ratio increased from 8 % in the normal livers of both sexes to 13 % in the males and 12 % in the females.

Composition of the cell

The amounts of each constituent of the cell are listed in Table 5, and the percentage of the cell mass which each contributes is represented in Fig. 3. The greater mass of the normal male cell is due to larger amounts of water, protein, glycogen, neutral lipids and phospholipids. The normal female cell, however, contains a greater percentage of water and this it maintained throughout the experiment. The water in the liver cell fell in the course of the first 24 hr. of the fast by 3.2×10^{-4} μ g. in the male and 1.9×10^{-4} μ g. in the female, so that the male cell had lost 1.7 times as much water as the female. The greater loss of water by the male liver cell may have been associated with its larger initial store of glycogen, which was almost completely lost during the first 24 hr. Fenn (1939) has suggested that the breakdown of glycogen was accompanied by a simultaneous loss of water from the cells. More water was lost during the first day while glycogen was disappearing than at any other period of the fast, the amounts lost being equivalent to 25 and 18 % of the initial amounts in the male and female cells; at the end of the fast the water had decreased to 61 and 69 % of the initial values, respectively. These changes caused the percentage of water in the cells to decrease during the first 2 days by a little more than 2 % in the males and a little less in the females; thereafter it tended to rise, but fell again at the end of the fast.

There was a similar percentage of protein in the normal cells of both sexes, and in the course of the first 2 days this increased, although the cells were

Fig. 2. Mass of the diploid liver cell of male and female rats during a 6-day period of starvation. O---O, Male; ●—●, female.

female cell, their average masses were not very different at the end of the fast (12.8 , cf. 11.6×10^{-4} μ g. respectively). On the other hand, Table 4 shows that the mass of the nucleus was almost the same at the beginning and end of the fast in both sexes. The amounts of DNA and 'protein' in the nucleus were also unchanged by the fast. Whether the average amount of protein found in the nucleus represents the amount present *in vivo* is not certain (Dounce, Tishkoff, Barnett & Freer, 1950; Harrison, 1953*a*), but since the same procedure was always followed in preparing the nuclear suspensions, it is probable that the same proportion of protein was recovered

Table 5. *The composition of the diploid liver cell of male and female rats during a 6-day period of starvation*

(Results expressed as $\mu\text{g.} \times 10^{-4}$)

Sex	Length of fast (days)	Water	Glycogen	Protein	Neutral lipids	Phospho-lipids	RNA	DNA
Male	0	12.5	1.17	4.35	0.41	0.76	0.26	0.06
	1	9.4	0.02	4.26	0.25	0.66	0.24	0.06
	2	8.7	0.02	4.12	0.57	0.68	0.24	0.06
	4	8.0	0.10	3.58	0.44	0.56	0.20	0.06
	6	7.7	0.15	3.39	0.40	0.57	0.20	0.06
Female	0	10.3	0.87	3.56	0.13	0.59	0.24	0.06
	1	8.4	0.02	3.38	0.27	0.57	0.22	0.06
	2	7.5	0.01	3.18	0.43	0.62	0.21	0.06
	4	7.5	0.03	3.06	0.57	0.57	0.18	0.06
	6	7.1	0.06	2.68	0.85	0.50	0.17	0.06

losing protein. This they did at a fairly constant rate so that on the 6th day the male and female cells contained 75 and 78 % of their initial amounts of protein. The protein in the female cell fell slightly more between the 4th and 6th days than that in the males, and this greater fall coincided with a fall in the concentration of protein in the female serum.

female cell gained them, so that at the end of the day both cells contained the same amount. During the following day both cells stored neutral lipids, and this the female cell continued to do at a uniform rate until the end of the fast when neutral lipids made up 7 % of its mass. This store was probably not pathological for there was no sign of fat in histological sections. Furthermore, the value of 7 % was derived from the analytical value of 5.4 g. neutral lipid/100 g. liver, which is less than Glynn, Himsworth & Lindan (1948) found in mildly fatty livers. Meanwhile, the neutral lipids in the male cell fell with its mass, so that from the 2nd to the 6th day there was almost a constant percentage of neutral lipids within it (3 %). During this period the male cell had accumulated twice as much glycogen as the female cell, although both contained very little compared with normal cells. The percentage of phospholipids in the female cell rose distinctly in the first 2 days and thereafter fell again, whereas in the male cell it remained practically unchanged.

The male cell contained 1.1 times as much RNA as the female cell, and during the fast a little more was lost from the female cell. Although RNA is found principally in the cytoplasm the amount present in the cell is apparently not a satisfactory index of the mass of the cytoplasm. In calculating the composition of the cells, it was assumed that the amount of DNA per diploid nucleus was the same in both sexes, and remained constant throughout the fast. This made the percentage of DNA a little higher in the female than in the male cell, and both values increased during the fast.

Minerals in the cell

In Table 6 are listed the amounts of iron, copper and zinc in the liver cells of both sexes, assuming that all the inorganic iron, copper and zinc in the liver were intracellular. The inorganic iron does not represent all the iron located within the cells for they possess enzymes containing haematin, but the iron contributed by these is negligible compared with

Fig. 3. Percentage composition of the liver cells of male and female rats during a 6-day period of starvation. $\bigcirc$ --- $\bigcirc$, Male; $\bullet$ --- $\bullet$, female.

The metabolism of fats in the cells of the male and female liver appeared to respond to starvation in a slightly different manner. Initially the male cell contained about 3 times as much neutral lipid as the female cell, both values being quite small. In the first day the male cell lost neutral lipids, whereas the

Table 6. The amounts of iron, copper, zinc and potassium in the diploid liver cell of male and female rats during a 6-day period of starvation

Sex	Length of fast (days)	Iron (10 ⁻⁷ μg.)	Copper (10 ⁻⁸ μg.)	Zinc (10 ⁻⁷ μg.)	Potassium (10 ⁻⁸ m-equiv.)
Male	0	3.5	1.1	1.1	2.1
	1	3.1	1.1	1.5	1.7
	2	3.9	1.3	1.4	1.6
	4	3.7	1.1	1.3	1.4
	6	4.1	1.1	1.2	1.3
Female	0	9.6	1.2	0.8	1.8
	1	9.6	1.2	1.2	1.5
	2	8.8	1.1	1.3	1.4
	4	10.0	1.0	—	1.3
	6	10.1	1.0	1.6	1.2

Fig. 4. Percentage of water (▽, ▼), protein (○, ●), and potassium (×) retained in the diploid liver cell after fasting for 1, 2, 4 and 6 days, for the livers of (a) male, and (b) female rats.

that 'stored' in the liver. The liver cells of the normal male and female rats contained similar amounts of copper and zinc whereas those of the female contained 3 times as much iron as those of the male. Fasting for 6 days produced little change in the 'storage' of these minerals within the cells, although there seemed to be a tendency for zinc to accumulate in the female cell. The copper in the cell was located principally, if not entirely, in the nucleus (Harrison, 1953a); at the end of the fast there was about the same amount of copper in the nucleus as at the beginning of the experiment, 0.9×10^{-8} , cf. $0.8 \times 10^{-8} \mu\text{g.}$ for the male cell, and 1.0×10^{-8} , cf. $1.2 \times 10^{-8} \mu\text{g.}$ for the female cell.

The normal male liver cell contained a little more potassium than the female (see Table 6), and during the fast the potassium fell in the liver cells of both

sexes. There was a drop of 19% in the male cell and of 17% in the female cell by the end of the first day; thereafter the potassium fell more slowly, but with larger losses between the 4th and 6th days in the female. By the end of the 6th day the liver cells of the male and female rats had lost 38 and 33% of their initial contents of potassium, and these losses corresponded with their losses of water (39 and 31% respectively). Fenn (1939) suggested that the disappearance of glycogen from liver cells was associated with the removal of potassium as well as of water, and conversely that the deposition of glycogen was associated with a return of water and potassium. He also found shifts of water with changes in the protein content of the liver, but he made no mention of movements of potassium with these changes. Fig. 4 shows the percentages of

water, protein and potassium which remained in the diploid liver cell at successive stages of the fast; the graphs for the liver cells of the male rats are given on the left-hand side of the figure whereas those for the female rats are on the right. It will be recalled that the glycogen had almost disappeared from the liver cells during the first 2 days of the fast, after which there were small amounts of glycogen, twice as much in the male cell as in the female cell. If the movement of potassium from the liver cell were linked only with the movement of glycogen, then between the 2nd and 4th days the potassium in the cells should have increased slightly. However, this did not occur, and from the 1st to the 6th days the graphs showing losses of protein and potassium are almost parallel. This would indicate that changes in the content of protein were accompanied by comparable changes in that of potassium. It might be a fair summing up to state simply that losses of both glycogen and protein reduced the amount of intracellular fluid, and with it the amount of its principal cation, potassium.

DISCUSSION

The changes in the composition of the cells were practically confined to the cytoplasm and were similar in both sexes during the first 4 days; both cells lost a considerable portion of their water, protein, glycogen, phospholipids and RNA. Thereafter the female cells tended to accumulate neutral lipids, whereas those of the males accumulated glycogen. Glycogen and neutral lipids do not accumulate simultaneously in the liver, and the normal liver accumulates glycogen in preference to neutral lipids. Hence it would appear that the normal functions of the female liver cell were the more disturbed, and this was borne out by the fall in the serum protein during the last 2 days of the fast. The female rats seemed to have reached a more advanced stage in the syndrome of starvation than the males at corresponding times. This was a little surprising for younger rats are thought to have somewhat less resistance to fasting than older ones because of the greater demands of growth. The male rats which were considerably heavier than the females, were a little younger since the experiment on the males was performed 2 weeks before that on the females. Furthermore, the male rats were growing considerably faster, at least until the growth of both was arrested by fasting. The difference between the sexes was however of no great magnitude.

There seemed to be no impairment in the ability of the liver to retain its stores of iron, copper and zinc, for the intracellular stores of these minerals were unchanged in both sexes throughout the fast. Whether they would have been drawn upon or would alternatively have been increased during

a longer fast remains undemonstrated. Visible deposits of iron have been found in livers of undernourished persons (Sherlock & Walshe, 1951) and of South African natives with pellagra (Gillman & Gillman, 1945), presumably as a result of breakdown of the muscles and red blood corpuscles. It is doubtful, however, if the changes which occur are comparable with those during fasting, for the changes found in protein deficiency (Kosterlitz, 1947; Elman *et al.* 1943) and after feeding reduced rations (Wertheimer & Ben-Tor, 1950) were not those found in starvation.

SUMMARY

1. A method for the determination of the composition of the liver cells from the results of chemical analysis of samples of whole liver has been employed to investigate the effects of starvation upon the size and composition of the liver cells of male and female rats.

2. Thirty-five male and thirty-five female young adult rats were divided into five similar groups. Seven rats of each sex were killed for analysis of their livers before the commencement of the experiment, and on the first, second, fourth and sixth days of the fast. Similar rats submitted to a precisely similar fast were used for analysis of the liver cell nuclei at the beginning and at the end of the fast.

3. As the animals lost weight, their livers became smaller, but the size and composition of the nuclei did not alter, and neither did the amount of nuclear material in the liver. It follows that the reduction in size of the whole liver was the result of the loss of cytoplasmic constituents from its cells.

4. Glycogen almost disappeared from the liver cells of both sexes during the first 24 hr. Thereafter the amount increased somewhat in the male cell but not in that of the female, which showed instead an increasing amount of neutral lipid until the end of the fast.

5. The cells lost protein throughout the fast, and a somewhat greater loss from the female liver cells during the final 2 days was accompanied by a fall in serum protein.

6. Potassium and water varied with the mass of the cells.

7. The amounts of iron, copper and zinc in the hepatic cells of both sexes were unchanged by the fast.

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The Presence of Potassium as a Source of Inaccuracy in the Chemical Estimation of Sodium in Urine

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Most methods for the chemical estimation of sodium in urine are based on precipitation of the metal quantitatively as the triple salt, sodium zinc uranyl acetate. This is subsequently estimated gravimetrically, colorimetrically, or by titration with alkali.

An early study of this method was made by Barber & Kolthoff (1928), and many subsequent modifications have been suggested (McCance & Shipp, 1931; Hoffman & Osgood, 1938; Bradbury, 1946; Albanese & Lein, 1948). In our hands, one version of the method, that of Dreguss (1939), has proved reliable and accurate for simple solutions of a sodium salt and for blood serum.

Difficulties were encountered, however, in applying the method to urine. The apparent sodium content was higher than expected on a basis of equivalence with the chloride, and higher than that obtained by flame photometry (Holiday & Preedy, 1953), especially in urines of low chloride content. This finding was investigated further.

EXPERIMENTAL

The method used was that of Dreguss (1939) with the following minor modifications. The solution was not heated after the addition of ethanolic zinc acetate; it was then centri-

fuged, not filtered. The volumes of supernatant fluid and of zinc uranyl acetate solution were 2 ml. each, instead of 1.5 ml. The solution in which precipitation took place was left for 3-24 hr. at room temperature. A filter stick with a tuft of asbestos was used for washing the precipitate, instead of repeated centrifuging.

Calculation. From the equation and calculation given by Dreguss, 1.0 ml. 0.1 N-NaOH used in titration is equivalent to 0.256 mg. Na. In our hands, this formula gave accurate results on the standard NaCl solution. (Dreguss used the formula 1.0 ml. 0.1 N-NaOH $\equiv$ 0.243 mg. Na, apparently on account of Na impurities in his samples of zinc uranyl acetate.)

Apparatus for flame spectroscopy. The flame photometer of Holiday & Preedy (1953) was used. The lens and filter system was dismantled, and a Hilger monochromator, Type D. 246, with glass prism, was interposed between the flame source and the image converter. Spectral analysis of the flame emission was carried out by scanning the spectrum between 400 and 800 m μ .

RESULTS

Accuracy of the chemical method

A standard solution of NaCl was prepared, containing 3 mg. Na/ml. Duplicate estimations of Na in this solution were carried out whenever a batch of urines was analysed. The relevant statistics of these