EFFECT OF MUSCULAR EXERCISE ON THE FAT CONTENT OF THE LIVER

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TWO FIGURES

It is well known that fat deposition in the liver may be produced by inadequate food, intoxication with various drugs, anemia and numerous other stimuli (Best and Taylor, '37). It appeared of interest therefore, to establish whether this fat deposition is the specific result of all these agents or whether it is a part of the non-specific response of the organism to damage, as such, which expresses itself in the 'alarm reaction' (Selye, '37, '38 a, b; Karady et al., '38; Masson, '38; Leblond and Segal, '38; MacKay and Clark, '38). In order to answer this question, a series of experiments was performed on the guinea pig in which the characteristic symptoms of the alarm reaction were elicited by excessive muscular exercise, exposure to cold or subcutaneous formaldehyde injections. It was observed that although in the doses given, all these agents elicited the typical symptoms of the alarm reaction (acute involution of the lymphatic organs, gastro-intestinal ulcers, water retention in tissues, hemoconcentration, etc.) only the animals performing muscular exercise showed marked fatty infiltration of the liver; in the other groups fat deposition was only very slight if present at all. In some of the exercised guinea pigs, hemorrhagic necroses appeared in certain parts of the hepatic parenchyma (Foglia and Selye, '38).

Since it is usually more difficult to produce fatty livers in the rat than in most other laboratory animals it seemed of

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interest to establish whether exercise would have the same effect in this species as it has in the guinea pig. Preliminary experiments showed this to be the case and indicated that in the rat, as well as in the guinea pig excessive muscular exercise is much more active in eliciting fat deposition in the liver than are other damaging agents. We decided, therefore, to make a detailed study of this fat deposition both from the morphological and the chemical point of view in an attempt to gain a better understanding of the mechanism by which muscular work produces this effect on the liver.

METHODS

For all the experiments reported in this communication 'hooded' black and white rats were used. These animals came from a stock inbred for many years and were raised on 'purina' fox chow.

For the histological studies, the livers were fixed in 4% formaldehyde, cut on a frozen section microtome and stained with Sudan III.

Since it is difficult to make quantitative estimations of the fat content from histological evidence only, we made chemical determinations of the total alcohol-ether soluble material in the liver. The determinations were performed as follows: 1 gm. of liver tissue, freed as far as possible from the large blood vessels, was ground up with sand in a mortar. This material was then rinsed into an Erlenmeyer flask with 35 cc. of a mixture containing 3 parts of alcohol to 1 part of ether. The flask was then heated on a hot water bath for 15 minutes. The supernatant fluid was decanted through filter paper into a 100 cc. volumetric flask. Thirty-five cubic centimeters of the alcohol-ether mixture were added to the residue in the flask and heated again for 15 minutes. This was repeated once more, after which the volumetric flask was filled up to the 100 cc. mark with the same mixture. The 100 cc. of extract were then transferred to a beaker which had previously been weighed. The solvent was evaporated under low pressure and the residue with the beaker weighed again. The weight

of the alcohol-ether soluble fraction was obtained by subtracting the weight of the empty beaker from the latter figure. The results are expressed in the tables as the per cent of wet weight of liver represented by this fraction.

While it is true that in using this method some of the liver constituents that are not fat will also be extracted, the error is very insignificant in the case of liver tissue, as indicated by control experiments in which other methods are used.

OBSERVATIONS

In our first series fifty-one 6-month-old female rats weighing 168 to 230 gm. were used. Three of these were killed as controls. Their livers showed no morphological signs of fatty infiltration, and chemical determinations showed that the average fat content was only 5.2%. Since previous experiments have shown that fasting increases fat deposition in the liver under other experimental circumstances (Selye et al., '35; Collip et al., '35) we wanted to see whether withdrawal of food would increase the fatty infiltration of the liver caused by muscular exercise. For this purpose three rats were fasted for 24 hours during which time they were forced to run for four 1-hour periods in motor driven drum cages having a diameter of 12 inches and revolving at the rate of 18 to 22 revolutions per minute. The last exercise period was started 23 hours after the beginning of the experiment and the animals were killed immediately following the 1-hour run. Three control rats treated in the same manner, but allowed food between the exercise periods, were also killed after the last run. Table 1 summarizes the results of all the experiments of this series and shows that the average fat content of the liver of rats fasted during this 24-hour period was 9.0% as compared with the average of 7.0% in the exercised but fed controls. It appears that although feeding does not completely prevent fat deposition in the liver, it decreases it to some degree.[1]

[1] After this paper was ready for the press, Kaunitz and Selzer (Z. exper. Med., Bd. 103, S. 638; 1938) published a report of experiments which also indicates that excessive muscular exercise may increase the fat content of the rat liver as judged by chemical determinations.

Histological examination of the livers of these exercised rats showed marked fat deposition especially in the peripheral portions of the lobules, while the cells in the immediate vicinity of the central veins remained relatively free of fat. Figure 1 shows the appearance of a lobule of an unexercised, fasted, control rat of this series stained with Sudan III. Only two cells in this field (marked by arrows) contain lipid granules. Figure 2 shows a lobule of the liver of a similar rat forced to perform exercise during fasting. There is intense fatty infiltration especially in the peripheral parts of the lobule. The Kupffer cells, which are barely visible at this magnification are also loaded with sudanophilic gran-

TABLE 1

Effect of muscular exercise on the fat content of the liver in female rats 6 months old

	Per cent of fat in the liver of							
Time	*Fasted animals*				*Fed animals*			
0 hours	10.0	9.2	7.9	Average 9.0	7.5	6.5	7.0	Average 7.0[1]
2 hours	9.1	8.7	9.98	Average 9.3	6.9	8.5	8.3	Average 7.9
8 hours	7.0	7.7	9.0	Average 7.9	8.8	9.0	8.5	Average 8.7
24 hours	6.0	6.7	6.9	Average 6.5	7.1	8.1	6.8	Average 7.3
48 hours	5.9	4.9	5.7	Average 5.5	6.2	7.1	6.0	Average 6.4
72 hours	4.9	4.7	4.5	Average 4.7	4.3	4.7	4.8	Average 4.6

[1] All animals represented in this table except this group were fasted during the 24 hours of intermittent exercise which immediately preceded the time marked '0 hours,' when determinations were started.

ules. It should be mentioned in this connection that in many of these exercised animals, the epithelial cells of the proximal convoluted tubules of the kidney also contained numerous lipid granules.

The remaining rats were used to determine the rate at which the fat deposited under the influence of muscular exercise would disappear from the liver. For this purpose thirty rats, 6 months of age, were exercised and fasted during 24 hours in the same manner as those used for the previous experiment; then they were divided into two groups. Fifteen of them were given food and water, after the last exercise period, while the other fifteen were given water but no food. It was assumed that at the end of 24 hours of intermittent exercise

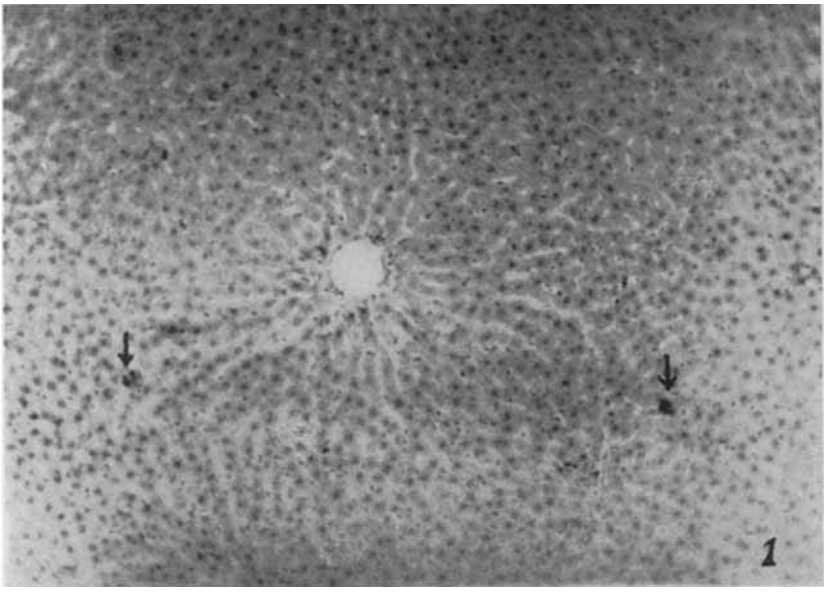

Fig. 1 Frozen section of a liver lobule from an un-exercised control rat (stained with Sudan III). Only the two cells marked by arrows contain sudanophilic granules.

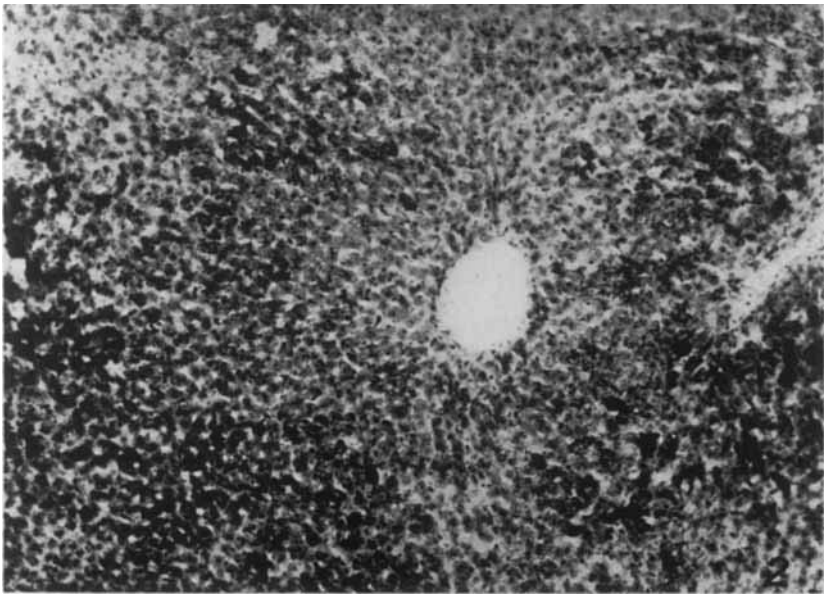

Fig. 2 Frozen section of a liver lobule from a rat that performed strenuous muscular exercise (Sudan III). Note the fatty infiltration of the peripheral cells in this lobule and the sudanophilic granules in the Kupffer cells.

their livers would contain about 9% fat just as in those of fasting animals. The rate of disappearance was determined by killing three fasted and three fed rats at 2, 8, 24, 48 and 72 hours following the last exercise period. Table 1 shows that during this time there is a gradual decrease in the fat concentration of the liver, which returns to normal between the forty-eighth and the seventh-second hour. It should be emphasized that both the fasted and the fed rats represented in this table were fasted during the exercise period so as to obtain the maximum fat deposition of about 9%. The table shows that with the exception of the 2-hour group, in which for some reason the fat content is lower in the fed series, the decrease in the fat content of the liver is actually more rapid in the fasted animals. This was confirmed by the macroscopical appearance of the liver which was always more yellow in the fed group. Most recent investigators, assume that the source of the lipids in acute fatty infiltration of the liver are the fat deposits of the body. If one accepts this interpretation one might explain our findings by the assumption that if the animal receives food during the exercise period it can more easily satisfy its caloric requirements from the ingested food and does not have to draw upon its fat reserves. Consequently less fat will be discharged into the blood and available for deposition in the liver. On the other hand, if food is given after the fat is already deposited in the liver, this will decrease the necessity for drawing upon the liver fat as a source of energy.

Fasting in itself may cause fatty infiltration of the liver in certain animal species as several investigators have shown, and we can confirm this on the basis of experiments on the mouse. In the rat, however, the increase in the fat content of the liver resulting from fasting is only slight. Table 2 summarized the results which we obtained in this connection. The maximum average increase was from 5.1 to 6.3 following a 48-hour fasting period. After 72 hours, it was reduced to the initial level and stayed there even after 96 hours of fasting. If we compare the animals of table 1 with those of

table 2, we must bear in mind that the rats represented in table 1 were fasted during the 24 hours of intermittent exercise before the first group was killed so that with respect to fasting the 72-hour groups of table 1 must be compared with the 96-hour group of table 2. Such a comparison shows that 72 hours after the exercise period the fat content is actually slightly below normal, irrespective of whether the animals receive food or not.

TABLE 2

Effect of fasting on the fat content of the liver in female rats 6 months old

Fed controls	5.0	5.1	5.4	Average 5.1%
Fasted 24 hours	5.8	6.4	6.5	Average 6.2%
Fasted 48 hours	6.2	6.1	6.8	Average 6.3%
Fasted 72 hours	5.1	4.1	6.0	Average 5.0%
Fasted 96 hours	5.6	5.0	5.1	Average 5.2%

In many of our early experiments on this subject we noticed that males are less likely to develop fatty livers as a result of exercise than females. While at that time we had not as yet made quantitative determinations, sections stained with Sudan III revealed an obvious sex difference in the responsiveness of our animals. It seemed advisable, however, to confirm this observation by more quantitative methods.

TABLE 3

Effect of muscular exercise on the fat content of the liver in normal and castrate male and female rats 2 months old

Exercised	Males	6.9	6.9	6.3	5.1	7.0	5.9	Average 6.3%
Exercised	Male castrates	6.6	6.6	5.8	6.2	6.3	6.0	Average 6.2%
Exercised	Females	5.6	7.6	8.0	7.0	7.0	7.0	Average 7.0%
Exercised	Female castrates	6.5	5.6	6.5	5.9	6.6	7.1	Average 6.4%
Un-exercised controls	Males	5.0	5.3	5.5				Average 5.2%
Un-exercised controls	Male castrates	6.8	5.5	5.4				Average 5.8%
Un-exercised controls	Females	5.6	6.0	5.0				Average 5.5%
Un-exercised controls	Female castrates	6.5	6.3	5.0				Average 6.3%

Table 3 summarizes the results of chemical determinations made on a 2-month-old rats. The females in this series weighed 150 to 171 gm., the males 187 to 246 gm. We also

added one group of male and one group of female castrates in which the gonads were removed 14 days before the experiment. All these animals were fasted during the exercise period and killed immediately following the last run. They were exercised in the same manner as the rats summarized in table 1. Three normal males, three normal females and three castrate males and three castrate females were not exercised and served as controls, being killed after a 24-hour fasting period. The results shown in table 3 indicate that although the un-exercised control females and the castrate females have possibly a slightly higher liver fat content than the normal or castrate males, the difference is not very striking. Following exercise the most marked average rise occurs in the normal females, although a slight increase in fat content is also seen in the other groups. However, on the whole, the increase in fat content in this group of 2-month-old rats is much less marked than in the 6-month-old animals represented in table 1.

TABLE 4

Effect of muscular exercise on the fat content of the liver in normal and castrate male and female rats 1 year old

Male	12.0	9.0	8.5	9.5	8.0	Average 9.4%
Male castrates	5.8	9.3	7.2	7.2	7.4	Average 7.4%
Female	13.5	14.0	9.4	11.8	9.0	Average 11.5%
Female castrates	10.3	13.5	12.8	8.0	12.2	Average 11.3%

In order to obtain a clearer picture of the influence of sex on this response, we performed another experiment on animals 1 year old since we thought that possibly adult animals would react still better than those 6 months of age. Table 4 summarizes the results obtained on a group of ten males weighing 200 to 235 gm. and ten females weighing 185 to 241 gm., all of which were exercised in the same manner as the rats of the previous groups during a 24-hour fasting period and killed immediately after the last run. Half of the males and half of the females were gonadectomized 14 days prior to the experiment. It should be mentioned that although we did

not make any determinations upon unexercised adult animals for this particular experiment, numerous observations made in connection with other research subjects show that the average fat content of the liver in a normal adult male rat of our colony is about 5.5% and in an adult female 6.1%. The results of table 4 indicate that our suspicion was correct inasmuch as the adult rat responds with more marked fatty infiltration following muscular exercise than either the 2-month or 6-month age group. While this is true both of the male and the female, the fat infiltration of fat is again much more pronounced in the latter. Judged by the results shown in table 3 and table 4, castration does not seem to influence this response very much but both the male and the female castrates are perhaps slightly less responsive than the normals of the same sex.

SUMMARY

Experiments on the rat show that excessive muscular exercise causes a considerable increase in the lipid content of the liver. This increase is more marked if the animals are fasted during the period of exercise than if they are fed.

The rate of disappearance of lipids from a liver in which the fat content has been increased by muscular exercise is somewhat more rapid in animals fasted throughout the recovery period than it is in animals that receive food following the performance of exercise. It takes about 48 to 72 hours before the fat content returns to normal in such experiments.

The increase in the fat content of the liver following muscular exercise is more marked in the adult than it is in the young animal, and among animals of the same age, it is more marked in females than it is in males. This sex difference in the response seems to be relatively independent of gonadal hormones as castration does not abolish it.

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