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FATTY TISSUE IN STARVED LAMBS

A HISTOQUANTITATIVE AND STATISTICAL STUDY

By

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OKSANEN, AILI and H. G. OSBORNE: *Fatty tissue in starved lambs. A histoquantitative and statistical study.* Acta vet. scand. 1972, *13*, 340—347. — The kidney fat was histologically examined of 27 spontaneously dead lambs of which 14 had starved. The lambs were born with mature fat, but in young animals a starvation period of more than 24 hrs. reduced the fat tissues and changed its cells towards the embryonal type built up of preadipocytes. These cells were smaller than the mature fat cells. Nucleus was large, round and situated in the centre of the cell. The slight eosinophilic, strongly diminished cytoplasm was some granulated and had some small fat droplets. The starvation changes of fat cells did not depend on the weight of animals or on the age of lambs less than two weeks.

lamb; starvation; fatty tissue; embryonal fat.

Histologically in embryonal development of white adipose tissue primitive organs are seen built up of preadipocytes. These cells have a nucleus of reticular cell type and later on small fat droplets in the cytoplasm (*Simon* 1965). Histologically different from the mature fat is the brown fat met in hibernants and new-born non-hibernants characterized by multilocularity of fat droplets in the cells (see for ref. *Smith & Horwitz* 1969). Another from normal fat different fat tissue is the "serous atrophy" in starved adult animals described in pathological textbooks (*Nieberle & Cohrs* 1967).

A great deal of lamb mortality is caused by starvation, and in starved lambs the kidney fat is scanty, oedematous and discoloured (*McFarlane* 1965 and *Dennis & Nairn* 1970). This study is presented because as far as we know no description on the histology of fatty tissue in young starved animals has been given. In this study is described the histology of fatty tissue of starved lambs and the relationship to the age and weight of the lambs.

MATERIAL AND METHODS

Twenty-seven spontaneously dead or stillborn lambs, 22 of which were born in an experiment studying nutritional muscle degeneration and five in different farms, were necropsied for diagnosis and their kidney fat was examined histologically.

As starved animals were counted: 1. Five cases of primary starvation. These lambs had lived longer than 24 hrs. and had no milk coagulate in the abomasum and no sign of food absorption in the intestinal lymph ducts as white emulsion (*McFarlane* 1965). 2. Seven lambs dead in colisepticaemia and starved because they had fasted during their illness. 3. Two lambs with a wasting chronical disease.

As unstarved controls were used 1. Six stillborn, or lambs which died during the first 24 hrs. (here called: dead born) and 2. Seven lambs suffering from nutritional muscular degeneration (NMD). The numbers of lambs, their ages and weights (mean and standard deviation) in the different groups and in the whole material are seen in Table 1.

The lambs were necropsied and samples of kidney fat were fixed in 10 % neutral formalin, sectioned to 8 μm and stained with Scharlachrot. The paraffin-embedded samples were sectioned to 4 μm and stained with haematoxylin-eosin. From the slides the number of preadipocytes per every 10 cells (preadipocyte density) was estimated in two random places of the fatty tissue. If the results differed, the mean was recorded. Cell density was estimated by counting with oil immersion the cells in two areas of 80×80 μm (count of cells per field). All cells, of which more than half of the cell was inside the area, were counted.

The mean and standard deviation for the weight and age of the animals and the density of preadipocytes and the count of cells per field in the fatty tissue were calculated for all diagnostic groups and for the whole material. The Student's t-test was employed to compare these values of the starved and not starved animals. Correlation and regression analyses were undertaken in order to compare weight and age of the animals and cell counts per field with the density of preadipocytes in fatty tissue. The results were evaluated as follows:

$P < 0.05^{*}$ — almost significant difference
$P < 0.01^{**}$ — statistically significant difference
$P < 0.001^{***}$ — highly significant difference

RESULTS

Kidney fat tissue in non-starved lambs was macroscopically white, divided in fat-lobuli, rather soft in new-born animals and firmer in older animals. In starved animals it was scanty, oedematous and reddish brown. In non-starved animals the cytoplasm of fat cells was filled by a large drop of fat. The nuclei were small, dense in chromatine, crescent shaped and situated in the margin of the cell (Fig. 3). Fat cells of starved animals were smaller. Their cytoplasm was rather homogenous, eosinophilic and occasionally had small vacuoles resp. fat droplet. Their large oval nuclei were in the center of the cell (Figs. 1 and 2). These cells resembled the preadipocytes (*Simon* 1965).

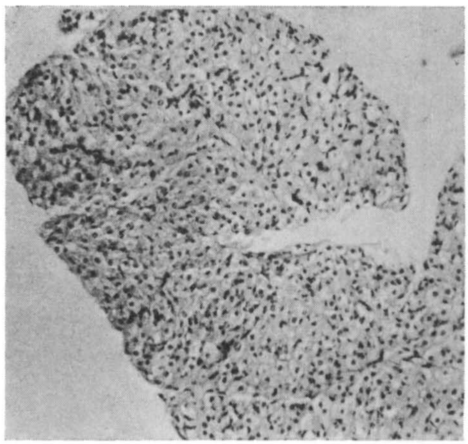
Mean and standard deviation for the preadipocyte density and the cell count per field in different diagnosis groups and in the whole material are shown in Table 1.

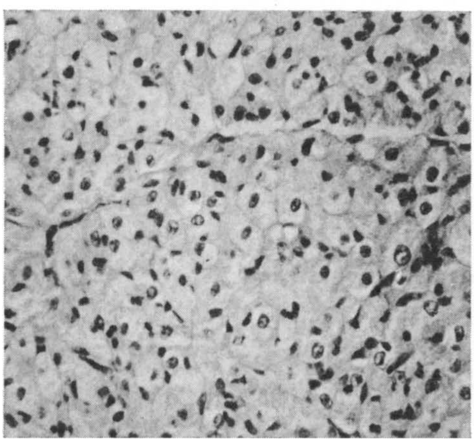
Table 1. Mean and standard deviation of preadipocyte density and cells per field in fat tissue and age and weight of lambs in different conditions.

	Number of animals	Age (d) m. ± s	Weight (kg) m. ± s	Preadipocyte density m. ± s	Cells per field 80 × 80 µm m. ± s
Starvation	14	4.1 ± 4.9	2.2 ± 1.9	7.5 ± 2.2	28.0 ± 2.0
primary starvation	5	1.6 ± 0.9	2.1 ± 0.9	9.2 ± 1.8	30.4 ± 4.6
coli sepsis	7	2.9 ± 2.0	2.2 ± 0.3	5.9 ± 1.8	24.7 ± 2.5
secundary starvation	2	14.5 ± 4.9	2.4 ± 0.4	9.0 ± 1.4	37.0 ± 5.6
No starvation	13	9.8 ± 15.0	3.3 ± 3.1	1.4 ± 2.3	12.2 ± 2.0
born dead	6	0 ± 0	1.7 ± 0.6	2.7 ± 1.3	16.8 ± 1.0
muscular dystrophy	7	18.3 ± 16.8	4.7 ± 3.9	0.3 ± 0.5	8.1 ± 3.6
Whole material	27	6.9 ± 10.9	2.7 ± 10.4	4.6 ± 3.6	20.6 ± 9.8

Results of the Student's t-test are seen in Table 2. The density of preadipocytes in starved animals was highly significantly greater than in non-starved animals ($P < 0.001$). This goes for the cell count per field in fatty tissues as well ($P < 0.001$), but there was no detectable difference regarding age and weight of starved and non-starved animals.

The results of the regression analysis are seen in Table 3. Cell count per field increased by increasing of the preadipocyte





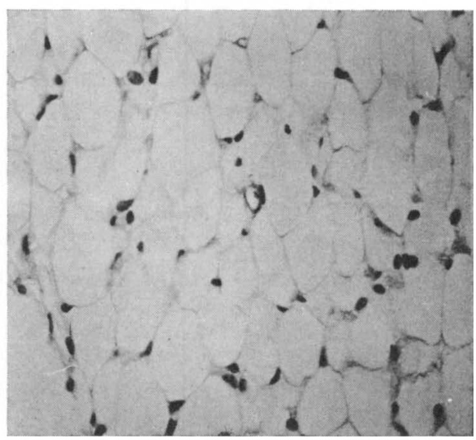


Figure 1. A lobulus of preadipocytic tissue of a starved lamb. H.E. × 135.

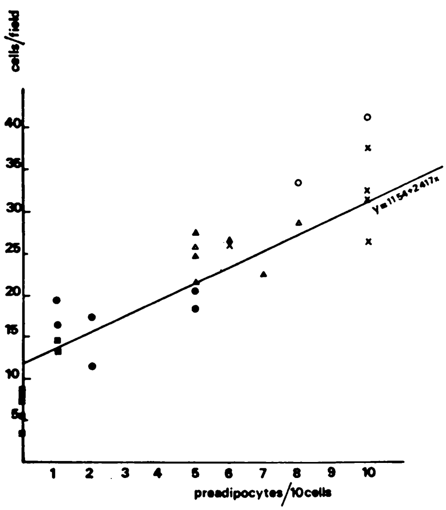
Figure 2. The same as Fig. 1. Note small cells with central, roundish nuclei and rather homogenous cytoplasm. × 312.

Figure 3. Mature fat tissue. H.E. × 312.

Table 2. Differences in Student's t-test of density of preadipocytes, cell count per field, and age and weight of animals between starved and not starved lambs.

	d. f.	t	P
Preadipocyte density	25	6.78	0.001***
Cells per field	25	7.26	0.001***
Age of animal	25	1.307	not significant
Weight of animal	23	0.36	not significant

density (r = 0.891, b = 2.417) (Fig. 4). In the whole material there was a slight negative correlation and regression between the age of the animals and the preadipocyte density (r = —0.386, b = —1.17) (Fig. 5). But if only the more homogenous age group,



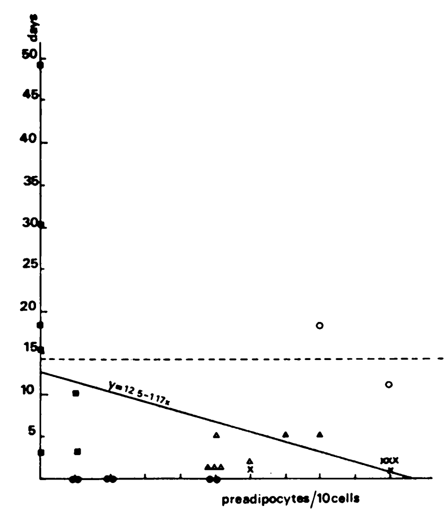


Figure 4. Correlation and regression between count of cells per field and density of preadipocytes. r = 0.891, b = 2.417, a = 11.54, P = 0.001***.

Figure 5. Correlation and regression between age of lambs (in days) and the preadipocyte density. r = —0.385, b = —1.17, a = 12.5, P = 0.05*. If counted animals less than 2 weeks only: r = —1.106, P = not significant.

× primary starvation
○ secondary starvation
△ coli-septicaemia
● born dead
■ NMD

Table 3. Correlation-regressions between cell count per field in fat tissue, weight and age of lambs and preadipocyte density in fat tissue.

	d. f.	r	P	b	a
Cells per field — preadipocyte density	25	0.891	0.001***	2.417	11.52
Weight of lambs — preadipocyte density	23	0.367	not sign.		
Age of lambs — preadipocyte density (all animals)	25	—0.386	0.05*	—1.17	12.5
Age of lambs > 2 weeks — preadipocyte density	20	—0.106	not sign.		

younger than two weeks, was taken into account, there was no observable correlation. So there was no correlation either between the preadipocyte density and the weight of animals.

DISCUSSION

Fatty tissue of starved lambs is histologically similar to the "preadipocytic tissue" described by *Bell* (1909) and included to the reticuloendothelial organs by *Wassermann* (1926). There are some similarities between preadipocytic fatty tissue and the "brown fat" seen in hibernants and new-born non-hibernants (see for ref. *Smith & Horwitz* 1969). However, the preadipocytic fatty tissue differs from brown fat: the cytoplasm of preadipocytes does not possess numerous small vacuoles after fat droplets which is typical for "brown fat". Neither do preadipocytes, like brown fat cells, occur in new-born animals, but they exist in very young animals after starvation. The adipose tissue in starved lambs does neither resemble the "atrophia serosa" with fatless fat cells filled with serous fluid seen in chronically ill cachectic adult animals (*Nieberle & Cohrs* 1967).

The preadipocytes in fatty tissue of starved animals were smaller than the mature fat cells of non-starved animals. The mean of number of cells per field in corresponding fatty tissue had increased to 28.0 ± 2.0 in the starvation group from 12.2 ± 2.0 in the group on non-starved animals. The difference in cell size is seen, too, by the strong positive regression between the cell count per field and the density of preadipocytes ($r = 0.891$, $b = 2.417$). The size of the adipocytes and the fat tissue diminishes, when the fat is consumed during starvation. The cells get the appearance of preadipocytes typical for the embryological fatty tissue. The size of the fat cells depends on the amount of fat stored in their cytoplasm, which is in agreement with the observation of *Hirsch & Han* (1969) in adult rats.

In this study it was observed that the new-born lamb already has a rather mature fatty tissue. The preadipocyte density in new-born animals was low (2.7 ± 1.3). During a primary starvation it increased quickly (9.2 ± 1.8).

In this study no correlation between preadipocyte density and the weight of young lambs was observed. The weight of the lambs was not depending on the amount of stored fat in the fatty tissue. Neither was there any correlation between preadipocyte density and the age of lambs less than two weeks old. This depends on the fact that the smallest preadipocyte density i.e. the most mature fat was seen in the youngest and the oldest lambs of the material (Fig. 4). There was a slight negative correlation between the age of all the examined animals and the preadipocyte density, depending on the fact that the fat in some older lambs with muscular degeneration was of mature type.

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SAMMANFATTNING

Fettvävnaden hos svultna lamm, en histokvantitativ och statistisk undersökning.

Histologiskt undersöktes njurfett från 27 självdöda lamm, av vilka 14 hade svultit. Lammen föddes med fullt utvecklad fettvävnad. Hos unga djur reducerade en svältperiod på 24 timmar starkt fettvävnadens mängd och gav den ett histologiskt utseende av embryonalt fett, som var uppbyggt av preadipocyter. Dessa celler var mindre än mogna fettceller. Kärnan var stor och rund och var belägen centralt i cellen. Den svagt eosinofila, starkt förmindskade cytoplasman var något granulerad och visade några små fettdroppar. Hos lamm under 2 veckor var fettcellernas svältförändringar inte beroende på djurets vikt eller ålder.

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