Z. Rechtsmedizin 77, 177 - 189 (1976)

Zeitschrift für
Rechtsmedizin

Serum Glucose, Serum Free Fatty Acids and Adipose Tissue Lipids after Fatal Hypothermia of Cold Acclimatized, Reserpine or Propranolol Treated Guinea-Pigs

J. HIRVONEN, Pirkko HUTTUNEN and H. VAPAATALO
Department of Forensic Medicine and Department of Pharmacology
University of Oulu (Finland)

Received July 9, 1975

Summary: Surviving ability in frost (-20°C) was studied in cold acclimatized guinea-pigs given either reserpine, propranolol or saline. Survival time, rectal temperature at death, serum glucose, serum FFA and triglycerides in the interscapular adipose tissue were determined.

Rectal temperature was highest in the reserpine group, in the same animals that endured the frost the shortest time. The survival time had decreased by about a half of that in the controls. Propranolol treatment decreased the living time only slightly. The fact that serum glucose remained high in the reserpine treated animals was obviously related to the short survival time. In the propranolol group glucose values were somewhat higher than in the control group (saline-animals). Reserpine seemed to have inhibited the release of FFA in the warm-acclimatized animals as interpreted from the low serum values. On the other hand, FFA were rather high in the cold-acclimatized reserpine animals. The blocking effect of reserpine reflected also in the higher contents of triglycerides in the adipose tissue both in cold-acclimatized and warm-acclimatized animals. Propranolol prevented slightly the depletion of the triglycerides. Amount of total lipids in the adipose tissue was lower in the cold-acclimatized animals than in the warm-acclimatized ones because of the change of the type of the adipocytes from unilocular to multilocular. The results corroborated the importance of FFA for longer survival in severe cold. Sensitization to reserpine seems to develop during cold-acclimatization. It calls attention to a possible hazard of reserpine treatment in cold environment.

Zusammenfassung: Die Überlebenszeit von kälteadaptierten, mit Reserpin oder Propranolol behandelten Meerschweinchen wurde in -20°C bestimmt. Die Rektaltemperatur, Blutzucker, unveresterte Fettsäuren (UFS) im Serum und Triglyzeride in dem interskapularen Fettgewebe wurden beim Todeseintritt bestimmt.

Die höchste Körpertemperatur beim Tod zeigten die Reserpinbehandelten Tiere, die auch am schnellsten - ungefähr doppelt so schnell wie die Kontrolltiere - in der Kälte starben. Die Lebenszeit der Propranololbehandelten Meerschweinchen war nur wenig abgekürzt.

Der Blutzucker bei Reserpintieren war beinahe im Normalbereich, aber sehr stark erniedrigt bei Propranolol- und Kontrollgruppen. Diese Befunde zeigten eine gute Korrelation mit der Abkürzung der Lebenzeit.

Die Reserpinbehandlung verhinderte die Freisetzung der UFS sowohl im Serum als im Fettgewebe. Propranolol hatte nur einen geringen hemmenden Effekt auf die Lipolyse.

Die Menge der Gesamtlipiden im Fettgewebe war bei den kälteadaptierten geringer als bei den Kontrolltieren, und die Fettzellen zeigten eine Veränderung von unilokular zu multilokular.

Die Ergebnisse zeigen die wichtige Rolle der UFS für den Organismus bei starker Kälte. Reserpin scheint ein gefährliches Medikament für Patienten, die in der Kälte arbeiten, zu sein.

Key words: Hypothermia, fatal - Thermoregulation and drugs

INTRODUCTION

The importance of the sympathetic nervous system for survival during cold stress has been demonstrated (MAICKEL *et al.* 1967). Mobilization of triglycerides from adipose tissue is mediated by noradrenaline from the sympathetic nerve endings.

When rats were kept at -20°C until death a complete depletion of triglycerides from the brown fat cells occurred, but not from the white fat cells (HIRVONEN and ELFVING 1973). In preliminary experiments we found that treatment with reserpine 12-16 hours before cold exposure inhibited this depletion. If propranolol, a β-adrenergic blocking agent, was given, partial depletion occurred. Survival time of the reserpine treated rats was about half of that of the controls and survival time of the propranolol treated rats was about 3/4 of the controls. Phenoxybenzamine, an α-adrenergic blocking drug, had no apparent effect. In similar experiments with non-acclimatized guinea-pigs their interscapular adipose tissues was not depleted of fat. Adrenergic blocking drugs had no noticeable effect (ELFVING *et al.* 1973). In a hypothermic infant depletion of fat from the brown adipose tissue has been noticed at autopsy (AHERNE and HULL 1966). This phenomenon is similar to that seen in the rat.

Multilocular brown fat is rare in adult man and in the adult guinea-pig. In guinea-pigs about half of the interscapular adipose tissue cells are changed to multilocular after a two month acclimatization period at +4°C (HIRVONEN *et al.* 1974). Adrenergic innervation grows stronger simultaneously (HUTTUNEN *et al.* 1975). There are some scattered observations at autopsies that men working outdoors might have retained brown adipose tissue, which would increase their cold endurance.

The preliminary findings in guinea-pigs and rats led us to test the effect of reserpine and propranolol on lipid mobilization and blood glucose in severe cold exposure and thus clarify the role of adrenergic nerves in withstanding cold exposure after acclimatization. The main questions were whether the adipose tissue of guinea-pigs was reacting as brown fat after cold acclimati-

zation and whether its newly grown sympathetic innervation was blockable leading to reduced cold survival. The practical aspect is that adrenergic blocking drugs are widely used in the treatment of cardiovascular disease and hypertonia which could make these patients more susceptible to cold exposure as is the case with patients treated with various antipsychotic drugs.

MATERIALS AND METHODS

Adult guinea-pigs of Dunkin-Hartley strain were used. A group of 26 animals was placed into a cold room (4-5°C) and kept there in separate cages for 2 months of acclimatization. One of these died at the beginning of the period. Another group, 28 animals, served as control and was kept at normal room temperature (20°C). During the acclimatization the guinea-pigs received pelleted food, vegetables and water ad libitum.

The animals were weighed before and after the acclimatization period. 11 of the 25 guinea-pigs in the cold increased weight. The increase ranged from 5 g to 200 g. 13 guinea-pigs out of the 25 lost weight between 25 g and 200 g. One animal maintained constant weight. Of the 28 control guinea-pigs, 24 increased weight (between 25 g and 300 g), only 3 guinea-pigs lost weight, the loss being between 20 g and 50 g. One had constant weight.

Administered drugs

The doses and time of injection were adjusted on the basis of preliminary experiments.

Reserpine: 1 mg/kg of body weight was injected subcutaneously 16-18 hours before the severe cold exposure.

Propranolol: 25 mg/kg of body weight was injected subcutaneously 30 min before the exposure.

Saline (Controls): 0.5 ml was injected subcutaneously 30 min before the exposure.

Exposure to severe cold

Both cold-acclimatized and warm-acclimatized guinea-pigs were exposed to severe cold after drug treatment. Exposure was effected in a cold chamber (-20°C) with a fan. Each animal was kept in its cage until death. The moment of death was established by observing the cessation of heart beat with an ECG-monitor (Olli 201, Ollituote Oy). Rectal temperature at death was also recorded (YSI model 42 sc, Tele-Thermometer, Honeywell).

SPECIMENS AND METHODS

Biochemical assays

Blood was drawn by cardiac puncture through thoracotomy. Serum was immediately separated by centrifugation. Serum glucose was measured according to the

method by HYVÄRINEN and NIKKILÄ (1972) and serum free fatty acids according to LAURELL and TIBBLING (1967).

Interscapular adipose tissue was excised and frozen immediately in liquid nitrogen. The sample was stored at -25°C until analysis. Part of the fat was homogenized in chloroform-methanol mixture (2:1) for extracting the lipids (FOLCH *et al.* 1956). Total lipid content was determined from an aliquot of the extraction by evaporating the solvent and weighing the residue.

Triglycerides were determined using the colorimetric method of MARZO *et al.* (1971). Monoglycerides, diglycerides, triglycerides and free fatty acids of the interscapular adipose tissue were first separated by thin-layer chromatography. The triglyceride spot was eluted and treated with concentrated H_2SO_4 at 200°C. The resulting solution absorbance was measured at 375 nm.

Histology

A separate piece of the interscapular adipose tissue was excised and frozen in liquid nitrogen. Frozen sections were stained with Oil Red O and hematoxylin. Attention was focused on the possible depletion of fat from the multilocular and unilocular adipocytes.

RESULTS

Rectal temperature at death of the animals varied from one individual to another between +11°C and +20°C. Generally, the temperature at death was inversely proportional to the survival time. The lowest mean, + 14°C, was in the cold-acclimatized propranolol group. In the warm-acclimatized propranolol group it was +16°C. In the cold-acclimatized control group the mean was +17°C, in the warm-acclimatized control group +16°C. Between the reserpine groups the difference was two degrees, i.e. +17°C in the cold-acclimatized vs +15°C in the warm-acclimatized (Table 1).

Survival time in the cold varied from 35 minutes for a cold-acclimatized reserpine treated guinea-pig to 670 minutes for a cold-acclimatized control guinea-pig. When the survival time was plotted against the weight change a general trend was noticed. The guinea-pigs having lost weight during cold-acclimatization died faster. The trend was seen in all groups, but it was mist distinct both in the reserpine and propranolol groups.

The mean survival time of the reserpine treated group was less than half of that of the control group both in the case of cold-acclimatized and warm-acclimatized animals. The cold-acclimatized propranolol treated animals lived

Table 1. *Mean and SD of the parameters recorded at the end of the cold exposure. +20°C indicates warm-acclimatized animals, +4°C cold-acclimatized ones. The results of the statistical comparisons between and within the drug groups are given in the following tables 2-4. IAT = interscapular adipose tissue*

Parameters	Reserpine		Propranolol		Control	
	+20°C	+4°C	+20°C	+ 4°C	±20°C	+4°C
Rectal temperature at death °C	15.0 ± 2.6	17.3 ± 3.1	15.9 ± 1.4	13.8 ± 3.4	15.7 ± 3.1	16.7 ± 1.6
Survival-time, min.	155 ± 44	143 ± 49	234 ± 76	343 ± 82	294 ± 76	378 ±183
Serum glucose mmol/l	4.6 ± 2.4	3.9 ± 2.5	1.1 ± 1.3	1.4 ± 1.3	0.7 ± 1.1	0.8 ± 1.1
Serum FFA mmol/l	1.0 ± 0.4	1.3 ± 0.4	1.5 ± 0.4	1.4 ± 0.4	1.6 ± 0.6	1.4 ± 0.6
Total lipid in the IAT, % of the wet weight	67.8 ± 5.8	57.3 ± 8.0	64.4 ± 9.1	54.5 ± 14.2	64.7 ± 4.1	51.6 ± 11.8
Triglycerides in the IAT, mg/g	630.2 ±117.1	532.0 ±121.5	658.4 ±151.3	417.0 ±205.0	539.8 ± 70.1	404.9 ±120.9

on the average 90 % and the warm-acclimatized animals 80 % of that time covered by the controls. Expectedly the cold-acclimatized control animals endured the cold for the longest time, but the difference to the warm-acclimatized control animals was not significant. The means, standard deviation and significance levels from statistical calculations are given in Table 1 and 2.

Serum glucose concentrations at death varied from unmeasureable to 9.1 mmol/l. Highest values were found in the reserpinized guinea-pigs. Low concentrations were seen in all guinea-pigs which survived the cold for longer time, i.e. in propranolol and control animals. Between the last mentioned groups there were no significant differences (Table 1 and 3).

Serum free fatty acid concentration at death was within the limits of 0.7 - 2.4 mmol/l in the cold-acclimatized and 0.5 - 2.8 mmol/l in the warm-acclimatized animals.

Reserpinized animals had the lowest values, the mean of the warm-acclimatized was 1.0 mmol/l and that of the cold-acclimatized 1.3 mmol/l. The means of the propranolol and control groups were near each other and they were 10-50 % higher than in the reserpine groups (Table 1 and 3).

The percentage of *total lipids* in the adipose tissue was about one fifth lower in the cold-acclimatized animals than in the warm-acclimatized in all drug groups. The lipids tended to be scarcer in the control and propranolol groups than in the reserpine group, but no statistically significant differences were found between the groups (Table 1 and 4). In warm-acclimatized animals the reserpine group showed the highest average lipid percentage, but the differences to the propranolol and control groups were not significant (Table 1 and 4).

Triglyceride content in the adipose tissue paralleled the total lipids within the drug groups, i.e. there were remarkably lower values in the cold-acclimatized animals (Table 1 and 4). The control group had the lowest mean 304.9 mg/g, propranolol group had the highest mean 532.0 mg/g. The blocking effect of reserpine on the release of triglycerides became apparent both in the cold- and warm-acclimatized animals. Propranolol also prevented the depletion of triglycerides in the warm-acclimatized animals (658.4 mg/g in the propranolol group vs 539.8 mg/g in the control group, Table 1 and 4).

Histological observations: The estimated proportion of multilocular adipocytes in the adipose tissue varied between 10-50 % in the cold-acclimatized animals. A general observation was that the areas containing multilocular cells had

Table 2. *Significant differences t-test of the mean and SD of rectal temperature and survival-time of cold-acclimatized and warm-acclimatized guinea-pigs. Comparisons made both within and between the groups.* $*p < 0.05, ** p < 0.01, *** p < 0.001$

Parameters	Cold-acclimatized vs. warm-acclimatized	Cold-acclimatized vs. cold-acclimatized	Warm-acclimatized vs. warm-acclimatized
Rectal temperature at death °C	No significant differences within the drug groups	reserpine vs. propranolol* 17 ± 3 14 ± 4	No significant differences within the drug groups
Survival-time, min.	propranolol vs. propranolol** 343 ± 82 234 ± 76	reserpine vs. control*** 143 ± 48 378 ± 183	reserpine vs. control*** 155 ± 44 294 ± 76
		reserpine vs. propranolol*** 143 ± 48 343 ± 82	propranolol vs. control* 234 ± 76 294 ± 76
			reserpine vs. propranolol** 155 ± 44 234 ± 76

Table 3. *Significant differences t-test of the mean and SD of blood glucose and serum FFA of cold-acclimatized and warm-acclimatized guinea-pigs. Comparisons made both within and between the groups.* $* p < 0.05, ** p < 0.01, *** p < 0.001$

Parameters	Cold-acclimatized vs. warm-acclimatized	Cold-acclimatized vs. cold-acclimatized	Warm-acclimatized vs. warm-acclimatized
Serum glucose mmol/l	No significant differences within the drug groups	reserpine vs. control** 3.9 ± 2.5 0.8 ± 1.1	reserpine vs. control*** 4.6 ± 2.4 0.7 ± 1.1
		reserpine vs. propranolol** 3.9 ± 2.5 1.2 ± 1.3	reserpine vs. propranolol*** 4.6 ± 2.4 1.1 ± 1.3
Serum FFA mmol/l	No significant differences within the drug groups	No significant differences between the drug groups	reserpine vs. control** 1.0 ± 0.4 1.6 ± 0.6
			reserpine vs. propranolol*** 1.0 ± 0.4 1.5 ± 0.4

Table 4. *Significant differences (t-test) of the mean and SD of total lipid and triglycerides in the adipose tissue of cold-acclimatized and warm-acclimatized guinea-pigs. Comparisons made both within and between the groups.* $*p < 0.05$. $**p < 0.01$, $***p < 0.001$

Parameters	Cold-acclimatized vs. warm-acclimatized	Cold-acclimatized vs. cold-acclimatized	Warm-acclimatized vs. warm-acclimatized
Total lipid in the IAT %	reserpine vs. reserpine*** 57.3 ± 8.0 67.8 ± 5.8	No significant differences between the drug groups	No significant differences between the drug groups
	propranolol vs. propranolol* 54.5 ±14.2 64.4 ± 9.2		
	control vs. control** 51.7 ±11.8 64.7 ± 4.1		
Triglycerides in the IAT mg/g	reserpine vs. reserpine* 532.0 ±121.5 630.2 ±117.1	reserpine vs. control*** 532.0 ± 121.5 304.9 ± 120.9	reserpine vs. control* 630.2 ± 117.1 539.8 ± 70.1
	propranolol vs propranolol** 417.0 ±205.0 658.4 ±151.3		propranolol vs control* 658.4 ± 151.3 539.8 ± 70.1
	control vs. control*** 304.9 ±120.9 539.8 ± 70.1		

smaller amounts of fat in the control group. In the reserpine and propranolol groups the multilocular adipocytes contained more fat (Fig. 1, 2 and 3).

Fig. 1. Interscapular adipose tissue (IAT) of a cold-acclimatized reserpine guinea-pig. Both multilocular fat cells (in the middle of the lobes) and unilocular cells are full of fat, i.e. no signs of release due to cold stress. 63 x. Oil Red O and hematoxylin

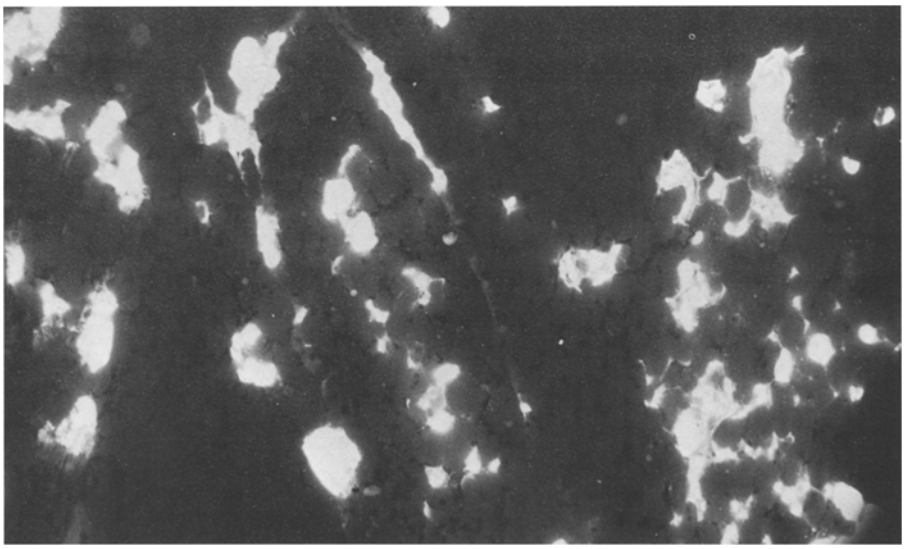

Fig. 2. IAT of a cold-acclimated propranolol guinea-pig. Empty spaces are seen in the central parts of the lobes reflecting release of fat from the multilocular cells. 63 x. Oil Red O and hematoxylin

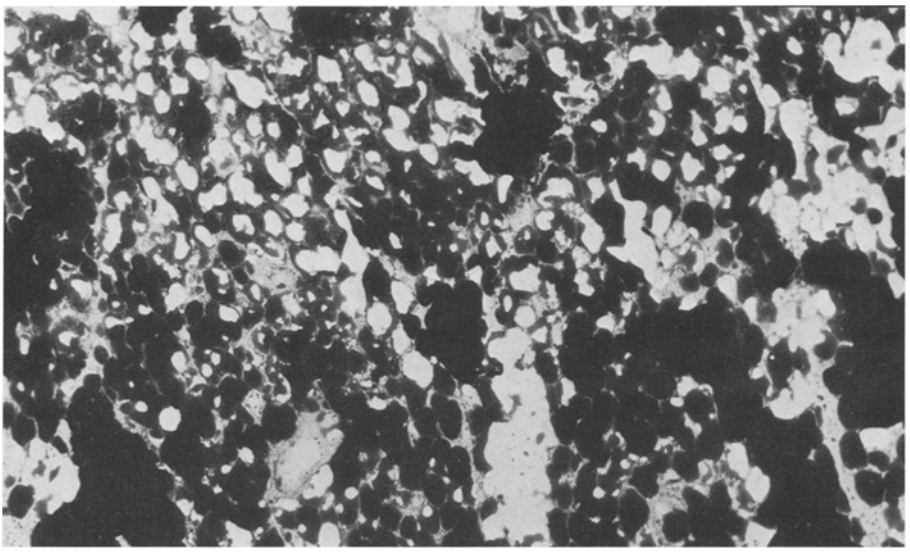

Fig. 3. IAT of a cold-acclimatized control guinea-pig. Release of fat from the cells is clear. Only the unilocular cells at the periphery of the lobes are full of fat. Oil Red O. 63 x

The unilocular adipocytes were full of fat in every group. In the cold-acclimatized control group triglycerides were 42 % lower than in the cold-acclimatized reserpine group and 14 % lower than in the warm-acclimatized reserpine group.

DISCUSSION

Survival-time of the reserpine treated guinea-pigs was very short, which is in accordance with previous results obtained with rats (ZILBERSTEIN 1960; MAICKEL *et al.* 1961). Unexpectedly, the cold-adapted guinea-pigs died faster than the warm-adapted. An explanation as to why there was a stronger effect of reserpine after cold acclimatization may be that the adrenergic nerves had become more sensitive to reserpine possibly due to cold-induced increased turnover of catecholamines. This was followed by more complete depletion of noradrenaline. The animals were then not able to use the acclimatized cells and metabolism for withstanding the cold. Two cold-acclimatized animals died after reserpine injection without cold exposure which also speaks in favor of sensitization. This finding calls attention to a similar possibility in humans. Reserpine might become dangerous in cold enviroment. It can further be kept in mind that its effect on thermoregulation can be both central (hypothalamus) and peripheral (adipose tissue, liver).

Large amounts of triglycerides left in adipose tissue after reserpinization and cold exposure proved the strong inhibiting effect of the drug on lipolysis both in warm- and cold-acclimatized animals. Also the analysis of serum FFA concentration indicated that reserpine had inhibited the release of FFA in the warm-acclimatized guinea-pigs. The lack of FFA for fuel can be regarded as a likely cause of the rapid death of the reserpine animals, because they still had rather high serum glucose levels. The results of the present study are in agreement with the view that the serum FFA are indispensable for longer survival in cold. However, the lack of fuel was probably not the only cause of the rapid death, because there were some glucose and FFA left in the serum. Another mechanism could be uncontrolled loss of heat through vasodilatation caused by blockade of the adrenergic vasoconstrictor nerves by reserpine.

Propranolol had also a tendency to shorten the survival-time. Cold-acclimatized guinea-pigs endured the cold for a longer time than the warm-acclimatized: a normal phenomenon. The dose of propranolol (25 mg/kg) used had earlier been shown to cause an effective beta-blockade of lipolysis in rats (MÄKELÄINEN *et al.* 1973) and a smaller dose, 5 mg/kg, blocked the calorigenic response to cold in new born rabbits (HEIM and HULL 1966). The triglycerides in the adipose tissue of propranolol treated guinea-pigs after cold exposure were depleted but less than in the control animals. The serum FFA concentrations were about the same. Thus partial inhibition of lipolysis had occurred. In hamsters, propranolol should not inhibit lipolysis at all (HISSA and HIRSIMÄKI 1971). The better survival of the cold-acclimatized guinea-pigs might be due to improved shivering thermogenesis, not disturbed by beta-adrenergic blocking agents (NIKKI *et al.* 1972; MÄKELÄINEN *et al.* 1974).

The inhibiting effect of reserpine and propranolol on lipolysis is converged to the adrenergic function, reserpine interacting on the nerve endings, propranolol on the beta-receptors. The fat cell lipases remain thus unstimulated during the administration of both drugs. When comparing the effects of these drugs one can see that inhibition of lipolysis by propranolol is only a fraction of that of reserpine.

In all groups, loss of weight was accompanied by shorter survival-time. The reason could be an inability in storing fat during the acclimatization.

The serum glucose concentration correlated inversely to the survival-time; reserpine animals which died rapidly had high concentrations, control animals which survived longer had low ones. This indicates exhaustion of glycogen stores during the longer lasting cold-exposure. Reserpine and propranolol do

not inhibit adrenergic-stimulated glycogenolysis (WESTERMANN *et al.* 1972; MÄKELÄINEN *et al.* 1973), but obviously glucose alone was not enough to fuel the strained metabolism of the cold exposed guinea-pigs.

Acclimatization to cold causes a change of the type of adipocytes from unilocular to multilocular. The change includes also a shift in the proportion of cytoplasm and fat. The amount of proteins increases and that of fats decreases. Therefore, comparison of warm-acclimatized and cold-acclimatized guinea-pigs within the same drug group is not feasible, because the effect of the precedent cold-acclimatization is likely to disguise the effect of the short exposure. The "multilocular change" also explains the smaller percentage of total lipids and triglycerides in the adipose tissue of the cold-acclimatized guinea-pigs. Acclimatization seems also to make the adipocytes more sensitive to lipolysis or/and combustion of fatty acids. The difference in the reactivity of multilocular and unilocular adipose tissue was seen in the histological investigation. The unilocular fat cells retained their fat inspite of the extreme cold stress. However, a small degree release of triglycerides had occurred according to the biochemical results from the measurements of triglyceride content and the serum FFA. The histochemical and biochemical results were parallel, but only on a large scale. Smaller changes did not become visible in the histological slides, particularly not in the unilocular adipocytes. The change of the interscapular adipose tissue to multilocular type associated with improved reactivity to adrenergic stimulation is one factor in the longer survival in the frost.

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J. HIRVONEN, M.D.
Department of Forensic Medicine
University of Oulu
SF - 90220 Oulu 22
Finland