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The effect of lowered environmental temperature on lipid metabolism in rats fed on normal and high-fat, high-cholesterol diets

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1. Prolonged intermittent exposure to reduced environmental temperature (+2°) produced in rats given a nutritionally well-balanced diet a decrease in the concentration of esterified fatty acids in the blood serum, liver and epididymal fat tissue. In the last there was a significant increase in the unesterified:esterified fatty acid ratio. The hepatic synthesis of fatty acids from [1-^{14}C]acetate remained unchanged. A decrease in the concentration of cholesterol was found in the blood serum, liver and lungs of animals exposed to cold. 2. When a high-fat, high-cholesterol diet was given, exposure to cold increased the mobilization of lipids; this was indicated by the elevation of the unesterified fatty acid levels in the blood serum and in the epididymal fat tissue. In rats given the high-fat diet the lipotropic action of cold on the liver was confirmed. This action was characterized by a decrease of esterified fatty acid levels and by an increase of glycogen concentration in the liver. This effect is probably due to a lowering of hepatic lipogenesis and to increased oxidation of fatty acids in the liver tissue. In rats given the high-fat diet, cold exposure produced an increased cholesterol accumulation in the tissues and more pronounced morphological changes in the myocardium.

Papers published in recent years have brought a wealth of new data on changes in metabolism, produced by prolonged exposure of warm-blooded animals to lowered environmental temperature. Acclimatization to cold is associated with increased food intake and with a general acceleration of metabolism (Smith, 1962), thus enabling the organism exposed to cold to meet the increased requirement for energy. These changes cause increased mobilization of reserve fat from depot tissues into the circulation (Gordon, 1960; Mallov & Witt, 1960; Mallov, 1963) and an increased oxidation of fatty acids and metabolically related substances in the liver (Felts & Masoro, 1959; Denyes & Hasset, 1960; Denyes & Carter, 1961). The relationship of these changes in lipid metabolism to the pathogenesis of atherosclerosis remains unexplored; many of the authors believe that exposure to cold enhances the processes which lead to atherosclerosis (Sellers & You, 1956; Wilgram, 1959; Sellers & Baker, 1960; Sodeman & Logue, 1960).

All the papers published on this subject so far have been concerned with animals that have been continuously exposed to cold. We have considered it important to investigate the metabolism of animals acclimatized to intermittent exposure to lowered environmental temperatures. Our research work concerns mainly lipid metabolism and its possible relationship to the pathogenesis of atherosclerosis.

EXPERIMENTAL

In our three experiments white male Wistar rats with body-weights from 180 to 220 g were used. In each experiment there were four groups of fifteen animals. The animals of the first two groups in each experiment were given the nutritionally well-balanced Larsen diet (Fábry, 1955) whereas the animals of the remaining two groups of each experiment were given a high-fat, high-cholesterol diet (Bobek & Ginter, 1965). Half of the animals given these two diets served as controls and were maintained at a temperature of 22–23°. The other half of the experimental animals were for 8 h every day exposed to a temperature of $+2 \pm 1°$ in a cold room. The experimental conditions and procedures were similar in all three experiments except that in Expts 1 and 3 the duration of the experiment was 13 weeks whereas it was only 7 weeks in Expt 2.

At the end of Expt 1 the animals of all the groups were killed by decapitation, and subsequently the concentration of cholesterol (Hořejší, 1964), esterified fatty acids (Stern & Shapiro, 1953), lipid phosphorus (Stewart & Hendry, 1935) and the various lipoprotein fractions (Swahn, 1953) were determined in the blood serum. The lipids were extracted from the livers and lungs by the method of Folch, Lees & Stanley (1957) and the concentrations of cholesterol, esterified fatty acids and lipid phosphorus were determined in the lipid extracts by the above-mentioned methods. For the histological examination of the heart and of the thoracic aorta the following staining techniques were applied: haematoxylin–eosin, Sudan IV, Mallory, PAS, Hotchkiss, Halle and alcian blue. The concentration of unesterified fatty acids (Novák, 1961) was determined in the blood serum and epididymal fat tissue. In Expt 2 concentrations of the lipid fractions were determined in the adrenal glands and in the epididymal fat by the same methods. In addition, the concentration of glycogen was determined in the liver (Good, Kramer & Somogyi, 1933). In Expt 3 the animals received intraperitoneally, 3 h before they were killed, a solution of sodium [1-^{14}C]-acetate diluted in physiological saline solution. The dose administered was 40 μc/100 g body-weight. After the animals had been killed, the cholesterol from the liver and adrenal glands was isolated according to the method of Sperry & Webb (1950). Total fatty acids were isolated from the liver by the method of Tretyakova & Grozdensky (1959). In these tissues the concentration of cholesterol (Sperry & Webb, 1950) and the concentration of total fatty acids (van de Kamer, Huinink & Weyers, 1949) were determined. The activity of samples which were applied in thin layers on small aluminium plates was measured by means of thin-wall Geiger–Müller tubes. The results obtained were expressed as specific activities and evaluated statistically by means of the *t* test.

RESULTS

In the course of all three experiments there was a gradual increase of the body-weight of rats given the Larsen diet and kept at room temperature. In rats exposed to cold and given a nutritionally well-balanced food there was a retardation of growth in the 1st week of the experiment; thus, during the remainder of the experiment the weight of the rats exposed to cold was significantly lower than that of the control rats

($P < 0{\cdot}001$). In rats given the high-fat diet and maintained at room temperature there was no significant change in body-weight during the experiment. The body-weights of these rats were significantly lower ($P < 0{\cdot}001$) than those of the rats given the Larsen diet in the 10th and 13th week of the experiment. In animals given the high-fat diet, cold exposure caused a decrease in body-weight between the 2nd and 5th week ($P < 0{\cdot}001$); body-weight then remained constant up to the end of the experiment (Fig. 1). Exposure to cold resulted in an increased mortality in this group (33%). The mean mortality of all other groups in the three experiments was 7%.

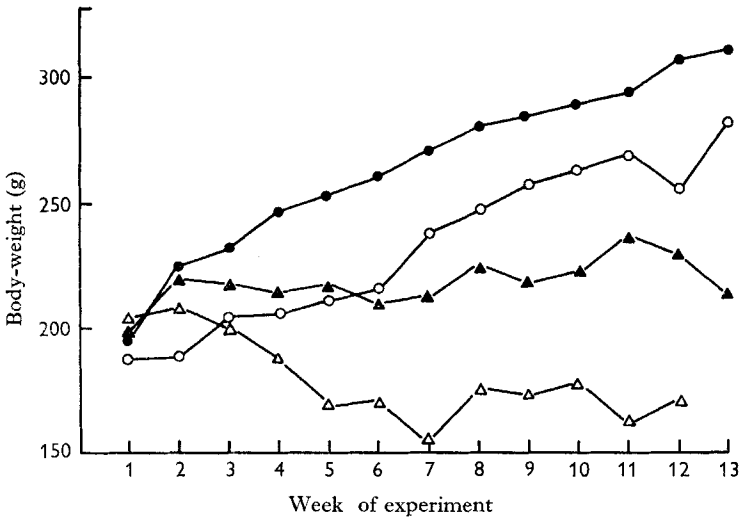

Fig. 1. Expt 1. The effect of exposure to cold on weight curves of rats fed on a nutritionally well-balanced or on a high-fat, high-cholesterol diet. ●—●, well-balanced diet, temperature of 22–23°; ○—○, well-balanced diet, temperature of +2°; ▲—▲, high-fat diet, temperature of 22–23°; △—△, high-fat diet, temperature of +2°.

Table 1. *Expt 1. Effect of exposure to cold on the concentrations of lipid fractions in the blood serum of rats fed on a nutritionally well-balanced or a high-fat, high-cholesterol diet*

(Mean values with their standard errors)

	Well-balanced diet		High-fat, high-cholesterol diet	
Temperature (° C)...	22–23	+2±1	22–23	+2±1
No. of determinations	14	14	12	8
Cholesterol (mg/100 ml)	58±3	48±2*	271±34	255±30
Esterified fatty acids (m-equiv/100 ml)	0·76±0·05	0·66±0·05	0·83±0·05	0·85±0·06
Phospholipids (mg/100 ml)	120±5	87±5***	127±7	130±2
Non-esterified fatty acids (m-equiv./1000 ml)	1·41±0·14	1·56±0·12	0·86±0·09	1·99±0·16***
β-lipoproteins (relative %)	49·1±1·2	42·7±0·6***	56·7±2·8	56·2±1·0

In Tables 1–5 the symbols *, **, and *** indicate that the effect of temperature was significant at the 5%, 1% and 0·1% levels respectively.

In rats given the Larsen diet, cold exposure caused a general decrease of the lipid fractions of the blood serum, with the exception of the unesterified fatty acid pattern.

In the rats given the high-fat diet, cold exposure did not result in any significant changes in the concentration of blood lipids, with the exception of the unesterified fatty acids, the concentration of which increased markedly under cold conditions (Table 1). In the livers of rats given the Larsen diet, exposure to cold resulted in decreases in the concentrations of cholesterol and esterified fatty acids. The decrease in the concentration of esterified fatty acids in the liver under the influence of a cold environment was found also in the group given the high-fat diet. In rats given the

Table 2. *Expts* 1 *and* 2. *Effect of exposure to cold on the concentrations of cholesterol, esterified fatty acids and phospholipids* (*Expt* 1) *and glycogen* (*Expt* 2) *in the livers of rats fed on a nutritionally well-balanced or a high-fat, high-cholesterol diet*

(Mean values with their standard errors)

	Well-balanced diet		High-fat, high-cholesterol diet	
Temperature (° C)...	22–23	+2±1	22–23	+2±1
No. of determinations	14	13	13	8
Cholesterol (mg/100 g)	403±15	328±25*	10048±783	10796±1496
Esterified fatty acids (m-equiv./100 g)	17·1±1·0	13·8±1·2*	21·9±1·1	16·4±0·8***
Phospholipids (mg/100 g)	4225±125	4075±100	3400±125	3450±150
Glycogen (g/100 g)	2·7±0·2	4·8±0·3***	0·6±0·1	3·2±0·2***

See footnote to Table 1.

Table 3. *Expt* 1. *Effect of exposure to cold on the concentrations of lipid fractions in the lungs of rats fed on a nutritionally well-balanced or a high-fat, high cholesterol diet*

(Mean values with their standard errors)

	Well-balanced diet		High-fat, high-cholesterol diet	
Temperature (° C)...	22–23	+2±1	22–23	+2±1
No. of determinations	14	13	13	8
Cholesterol (mg/100 g)	583±17	508±8***	620±19	831±58**
Esterified fatty acids (m-equiv./100 g)	6·7±0·3	6·5±0·3	9·1±0·4	8·4±0·5
Phospholipids (mg/100 g)	2575±75	2525±125	2825±100	3000±175

See footnote to Table 1.

normal or the high-fat diet cold exposure caused a sharp increase of the liver glycogen level, whereas phospholipid concentration remained unchanged (Table 2). In the lungs there were no significant changes in the concentrations of esterified fatty acids and phospholipids. In the animals given the normal diet exposure to cold resulted in only a moderate decrease in the concentration of cholesterol in the lungs, but in the rats given the high-fat diet exposure to cold gave rise to a marked increase in the cholesterol level in the lungs (Table 3). In the epididymal fat tissues of rats given the Larsen diet, cold exposure caused a very sharp decrease in the concentration of esterified fatty acids (Table 4). In this tissue, cold exposure resulted in an increase in the concentration of unesterified fatty acids. In the group given the high-fat diet, exposure to cold caused an increase in the concentration of cholesterol in the epididymal fat

tissues. In the adrenal glands of rats given the Larsen diet, and also in the adrenals of rats given the high-fat diet, acclimatization to cold produced an increase in the concentration of cholesterol and a significant decrease in the concentration of phospholipids. The concentration of esterified fatty acids in the adrenal glands was not significantly influenced by exposure to cold (Table 5).

Table 4. *Expts* 1 *and* 2. *Effect of exposure to cold on the concentrations of non-esterified fatty acids* (*Expt* 1) *and cholesterol and esterified fatty acids* (*Expt* 2) *in the epididymal fat tissue of rats fed on a nutritionally well-balanced or a high-fat, high-cholesterol diet*

(Mean values with their standard errors)

	Well-balanced diet		High-fat, high-cholesterol diet	
Temperature (° C)...	22–23	+2±1	22–23	+2±1
No. of determinations	10	11	10	8
Cholesterol (mg/100 g)	136±8	125±7	234±16	332±29**
Esterified fatty acids (m-equiv./100 g)	215·9±15·6	30·9±2·6***	119·4±13·9	124·0±29·8
Non-esterified fatty acids (m-equiv./100 g)	0·47±0·04	0·57±0·04	0·52±0·04	1·28±0·17***

See footnote to Table 1.

Table 5. *Expt* 2. *Effect of exposure to cold on the concentrations of lipid fractions in the adrenal glands of rats fed on a nutritionally well-balanced or a high-fat, high-cholesterol diet*

(Mean values with their standard errors)

	Well-balanced diet		High-fat, high-cholesterol diet	
Temperature (° C)...	22–23	+2±1	22–23	+2±1
No. of determinations	9	12	10	8
Cholesterol (mg/100 g)	2962±190	4258±340*	8829±794	11335±865*
Esterified fatty acids (m-equiv./100 g)	41·6±3·4	38·7±2·6	38·0±5·3	27·6±1·4
Phospholipids (mg/100 g)	4925±175	4425±150*	4075±200	1450±150***

See footnote to Table 1.

In the animals given the Larsen diet, exposure to cold environment tended to decrease the incorporation of [1-^{14}C]acetate into the liver and adrenal cholesterol. However, this decrease was not statistically significant. Similarly, the incorporation of [1-^{14}C]acetate into the liver fatty acids was not significantly influenced in these conditions. In the animals given the high-fat diet the endogenous synthesis of cholesterol in the liver and adrenals had decreased to such an extent that the activity of the isolated cholesterol was practically zero. In the rats given the high-fat diet the mean specific activity of liver fatty acids appeared to decrease as a result of exposure to cold but this decrease was not statistically significant (Table 6).

Histological examination revealed no pathological alterations in the myocardium and aorta of rats in the group given the Larsen diet and kept at room temperature. In two of the animals exposed to cold and given the Larsen diet, small postnecrotic foci were found. The neighbouring muscle fibres exhibited increased acidophilia and

disappearance of striature. In the other animals the histological findings appeared to be normal. The high-fat diet caused, in some animals kept at room temperature, small subepicardial necrotic foci with surrounding vacuolization of muscle fibres. Small branches of the coronary arteries were oedematously permeated and vacuolized. They gave a feebly positive reaction with PAS and with alcian blue. In the aorta, findings were negative except for one animal in which moderate vacuolization of the arterial wall was found. Staining for fats was negative. Cold exposure in conjunction with a high-fat diet caused more marked changes, namely subendothelial necrotic foci in the septum, with associated leukocytic and histiocytic reactions, and remnants of muscle cells with cloudy degeneration. The edge of arterial rami and the surrounding muscle fibres were oedematous.

Table 6. *Expt* 3. *Effect of exposure to cold on the specific activities of total cholesterol in the livers and adrenal glands and of total fatty acids in the livers of rats fed on a nutritionally well-balanced or a high-fat, high-cholesterol diet*

(Mean values with their standard errors)

	Well-balanced diet		High-fat, high-cholesterol diet	
Temperature (° C)...	22–23	+2±1	22–23	+2±1
No. of determinations	10	14	9	10
Cholesterol				
Liver (counts/min mg)	626±149	304±69	—	—
Adrenals (counts/min m-equiv.)	282±106	226±60	—	—
Total fatty acids of liver (counts/min m-equiv.)	12039±2468	12397±4130	5442±2349	1674±325

DISCUSSION

In rats given a nutritionally well-balanced diet, exposure to a cold environment inhibited growth during the 1st week of the experiment and for this reason the weights of the animals of this group remained less than those of the control animals in the course of the entire experiment. This finding is consistent with the physiological process of acclimatization to cold (Cottle & Carlson, 1956). In rats given a high-fat diet and kept at 22° there was no change in body-weight during the experiment, but a cold environment under these dietary conditions caused a decrease in body-weight, associated with increased mortality.

In rats given the Larsen diet and exposed to cold, a decrease in the concentration of cholesterol was found in the blood and in most of the tissues studied. Concomitantly, the β-lipoprotein concentration in the serum was lowered. One reason for this decrease might be a decreased cholesterol biosynthesis, since in the livers of the animals exposed to cold we have observed a tendency towards a reduced incorporation of labelled acetate into cholesterol. The results obtained by other investigators in this field are not uniform: in conditions of acute exposure to cold no substantial decrease of cholesterol biosynthesis has been found in experiments in vitro (Masoro, Cohen & Panagos, 1955). Mefferd, Nyman & Webster (1958) have found in rats acclimatized to cold an increased incorporation of labelled acetate into total body cholesterol. The

tendency towards a lowered hepatic synthesis of cholesterol observed under our experimental conditions may be explained by the fact that in rats exposed to cold an elevated oxidation of acetate occurs through the Krebs cycle (Denyes & Carter, 1961). There is thus less acetate available for cholesterol synthesis. We cannot, however, exclude the possibility that cold exposure exerts an influence on cholesterol catabolism. A heavy accumulation of cholesterol occurred in most of the tissues of the animals given the high-fat diet. This cholesterol is apparently of exogenous origin, since—in accordance with other workers—we have demonstrated that the ingestion of large amounts of cholesterol substantially lowers the endogenous synthesis of cholesterol. In animals exposed to cold and given a high-fat diet we have observed a tendency for the deposition of cholesterol to be increased in some tissues.

Cold exposure caused a decrease in the concentration of esterified fatty acids in the blood serum and livers of rats fed on the Larsen diet. Simultaneously there was a sharp decrease in the concentration of esterified fatty acids in the epididymal fat tissue, and also in the esterified:unesterified fatty acid ratio in this tissue (51 against 432 in the control group), which demonstrates an increased mobilization of fatty acids from depot tissues (cf. Mallov & Witt, 1960; Schönbaum, Sellers & Rimmer, 1962; Mallov, 1963). Since at the same time the rate of biosynthesis of fatty acids from labelled acetate in the liver was unaltered, the decrease of esterified fatty acid concentration in the liver may be a reflection of the elevated catabolism of fatty acids in the livers of animals exposed to cold. This conclusion is in accordance with the finding of an increased oxidation of ^{14}C-labelled palmitate in the livers of rats acclimatized to cold (Felts & Masoro, 1959), and it confirms the assumption of a total acceleration of lipid metabolism in animals acclimatized to cold (Young & Cook, 1955; Mefferd *et al.* 1958). When the rats were given the high-fat diet, exposure to cold resulted in a decrease in the concentration of the esterified fatty acids in the liver. Under these conditions, too, exposure to cold accelerated the mobilization of lipids from reserve tissues, as illustrated by the very significant elevation of the unesterified fatty acid levels both in the blood serum and in the epididymal fat tissue. Since these changes were accompanied by a tendency towards a lowered transformation of labelled acetate into hepatic fatty acids, we assume that the lipotropic action of a cold environment on the liver, described previously by other authors (Treadwell, Flick & Vahouny, 1955, 1957, 1958), may be explained both by the enhanced oxidation of fatty acids by the liver tissue, and by a decreased hepatic lipogenesis.

The lipotropic effect of a cold environment on the liver is further confirmed by the significant elevation of the liver glycogen level in rats exposed to cold and given either the normal or high-fat diet. The mechanism of this phenomenon is unexplained. Although various workers have studied the glycogen metabolism in animals acclimatized to cold, the results published so far are very conflicting. While Kaufman, Gavan & Hill (1958) and Depocas (1962) have found decreased hepatic glycogen levels in cold-acclimatized rats, Felts & Masoro (1959) have reported elevated levels of glycogen in the livers of animals exposed to cold. In connexion with our finding of a significant increase in the concentration of glycogen in the livers of rats exposed to cold (irrespective of the kind of diet), it should be noted that the glucokinase reaction

may be inhibited under these conditions (Denyes & Carter, 1961). It is known that an organism exposed to cold has only small quantities of ATP available, and that this is a consequence of the lowered degree of oxidative phosphorylation (Masoro & Felts, 1959; Hannon, 1959; Lianides & Beyer, 1960).

It appears that exposure to cold does not exert a substantial influence on phospholipid metabolism. The only exception is the very sharp decrease of lipid phosphorus in the adrenal glands of rats exposed to lowered environmental temperature; this decrease occurred irrespective of dietary treatment. The reason for this finding remains unexplained.

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