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Effects of Cold Environment on Deposition of Fat in the Liver in Choline Deficiency

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In a preliminary communication (Sellers & You, 1949) it was reported that when rats were exposed to a cold environment the deposition of excess fat in the liver produced by feeding a hypolipotropic diet was effectively prevented. Only one set of experimental conditions had been tested, so that it was of interest to extend the investigation by varying the length of exposure and the type of diet offered, and to study the effects of acclimatization in relation to the phenomenon. Our interest in the effects of the thyroid gland on deposition of fat in the liver (Sellers & You, 1951) also made it desirable to assess the role of this gland in the 'pseudolipotropic' action of cold.

EXPERIMENTAL

In all experiments rats of the Wistar strain, bred in the department, were kept in individual metal cages and were given water *ad lib.* After the experimental period they were killed by cervical dislocation and the livers were removed for chemical and histological examination. Total liver lipids were estimated using the method of Best, Lucas, Patterson & Ridout (1946). The basal diet consisted of peanut meal 30%, casein 6%, salts 4% (Beveridge & Lucas, 1945), sucrose 39%, 'vitamin powder' 1%, beef fat 15%, corn oil 5%, α -tocopherol acetate 0.010%, cod-liver oil concentrate 0.015%. The 'vitamin powder' was of such a composition that the intake per 10 g. of diet was as follows: Biotin 3 μ g., thiamine hydrochloride 50 μ g., riboflavin 25 μ g., pyridoxine

hydrochloride 20 μ g., calcium pantothenate 100 μ g., nicotinic acid 100 μ g., folic acid 5 μ g., 2-methyl-1:4-naphthoquinone 10 μ g., inositol 5 mg., *p*-aminobenzoic acid 1 mg. The cod-liver oil concentrate supplied at least 300 i.u. vitamin A and at least 75 i.u. vitamin D/10 g. of diet. In the high-fat diet the content of fat was increased by adding an additional 20% of beef dripping and 10% of corn oil at the expense of sucrose to make the total content of fat 50%.

Effect of cold environment on acute choline deficiency

(1) Twenty male rats weighing from 170 to 220 g. were divided into two groups and were fed the basal hypolipotropic diet *ad lib.* for 14 days. During the experiment, one group was placed in a cold room maintained at a temperature of $1.5 \pm 1^\circ$, while the other was kept in the usual animal quarters at a temperature of $22 \pm 2^\circ$.

(2) Ten adult female rats (170–216 g.), which had been kept in the cold room for 54 days, were fed the hypolipotropic diet instead of their usual ration for a period of 2 weeks. At the same time ten female rats of similar weight range which had lived at 'room temperature' in the animal colony were exposed to cold and were fed the same hypolipotropic diet for a period of 2 weeks.

(3) The thyroid glands of ten adult female rats were removed surgically and the animals were given subcutaneous injections of 5 μ g. DL-thyroxine (Roche-Organon) daily for 5 days before and during exposure to cold. While in the cold room the animals were fed the basal hypolipotropic diet *ad lib.* The purpose of this procedure was to obviate the increased production of thyroid hormone which may occur after exposure to cold, and also to make possible the survival

of such animals in the cold environment (Sellers & You, 1950). Ten normal female rats were also kept in the cold room and received daily the average amount of basal diet consumed by the thyroidectomized rats. In both groups body weights ranged from 180 to 200 g. at the commencement of cold exposure.

(4) A hypolipotropic diet of high (50%) fat content was given *ad lib.* to two groups of ten female rats for 14 days. One group was maintained at room temperature, and the other was exposed to cold. A third group of ten females was exposed to cold simultaneously but was fed the basal (moderate fat) diet. The average body weight of all rats was 181 g. when the feeding was started.

The first part of this experiment was repeated using thirty female rats of approximately the same weight range. All rats received the same high-fat, low-choline diet *ad lib.*, but eighteen animals were kept in the cold room during the experimental period, while the remaining twelve lived at normal room temperature.

(5) A group of twenty female rats with body weights ranging from 162 to 190 g. were placed in the cold room and were fed the hypolipotropic diet *ad lib.* At the end of each week two animals were sacrificed, the last four rats being killed at the end of 10 weeks' exposure. The liver of each animal was removed and examined histologically. Other animals fed the same diet were sacrificed after a 15-week period of exposure to cold.

RESULTS

The excessive deposition of fat found in the livers of rats fed the hypolipotropic diet at room temperature did not occur in rats which were given the same diet but were kept in the cold environment. This preventive effect was also observed in rats which had previously been acclimatized to the cold environment. Similar results were obtained with thyroidectomized rats which received a constant daily maintenance dosage of thyroxine. The results are summarized in Table 1. Even after 10 and 15 weeks of exposure the fat content of the livers of rats fed the hypolipotropic diet remained low, in some cases within the normal range (Pl. 3).

When the content of fat in the basal diet was increased to 50%, the total lipid content of the livers was found to be higher than that of the group receiving the basal hypolipotropic diet in the cold environment. However, it was lower than the average figure obtained in the groups of rats which were fed the high-fat diet and were kept at room temperature (Table 2, A and B). The food intake of all rats exposed to cold was considerably higher than

Table 1. *Effect of cold environment (2°) on acute choline deficiency (14 days)*

No. of rats	Sex	Group	Environment	Average body wt. (g.)		Average food intake per day (g.)	Average liver wt. (g.)	Average total lipids (% wet liver wt.) $\pm$ S.D.
				Initial	Final			
10	M.	Normal	Cold	195	214	22	12.4	7.2 $\pm$ 1.2
10	M.	Normal	Normal	196	248	15	13.8	24.8 $\pm$ 4.9
10	F.	Acclimatized*	Cold	191	195	19	8.1	7.20†
8	F.	Normal	Cold	185	184	17	9.1	7.50‡
6	F.	Thyroidectomized†	Cold	189	182	16	8.3	6.75‡
6	F.	Normal	Cold	186	180	16	9.7	6.70‡

* Rats were in cold environment for 54 days before commencement of low choline diet.

† Each rat received 5 γ thyroxine per day.

‡ Livers were pooled for fat determination.

Table 2. *Effect of cold environment on fat deposition in the liver with high-fat, choline-deficient diet (14 days)*

No. of rats (female)	Diet	Environment	Average body wt. (g.)		Average food intake per day (g.)	Average liver wt. (g.)	Average total lipids (% wet liver wt.) $\pm$ S.D.
			Initial	Final			
9	Basal	Cold	183	181	16.3	10.7	8.6*
9	High fat†	Cold	179	180	11.5	10.2	19.9 $\pm$ 9.7
8	High fat†	Normal	181	200	8.9	13.0	23.8 $\pm$ 12.2 ($P > 0.10$)
B							
14	High fat†	Cold	168	170	11.76	10.4	18.1 $\pm$ 8.1
12	High fat†	Normal	177	205	10.9	12.2	28.5 $\pm$ 12.0 ($P < 0.02$)

* Livers were pooled for fat determination.

† 50% fat in basal diet.

that of controls, but the rate of growth as measured by increase in body weight was lower than that of the control rats living at room temperature. The increased consumption of food was more marked in the diets with lower content of fat, and therefore with lesser caloric value.

DISCUSSION

When rats are fed diets low in choline and its precursors, the amount of excess lipid deposited in the liver may be influenced by a variety of factors. Some of these (e.g. intake of food, growth rate, age, sex, previous nutritional status, the presence or absence of other dietary constituents) have been appreciated for many years, others (e.g. environmental temperature, hormonal action) have received less attention. In the experiments reported in this paper the effect of lowering the environmental temperature has been studied, and it is apparent that a definite 'pseudolipotropic effect' due to cold took place. This effect was quite obvious when a diet of moderate fat content (20 %) was fed, but was less so with a diet of high (50 %) fat content. It is of interest to consider whether these results may be explained on the basis of one of the mechanisms described previously or whether some new explanation is necessary.

The energy requirement of a rat exposed to a cold temperature rises greatly, and in our experience the caloric intake is always increased. The rate of growth is adversely affected, but some increase in body weight still takes place. Rats of the range of weight studied have been observed in an environment of 1.5° for as long as a year and a half. In such circumstances it would be unreasonable to claim that the animals were failing to meet their energy requirements from dietary sources. At normal room temperature ($22 \pm 2^{\circ}$) the basal hypolipotropic diet described here must be restricted greatly in order to achieve the same lowering of liver lipids, and a failure to gain, or a fall in body weight occurs before excessive deposition of fat in the liver is decreased significantly (authors' unpublished data). From this it is concluded that a decrease in the ratio of caloric intake to energy and growth requirements (inanition) cannot alone explain the result.

The preventive action of the cold environment on deposition of fat in the liver is one of degree, however, for when the high-fat diet was fed, large amounts of lipid material were laid down. The average amount of high-fat (50 %) diet consumed was considerably less than that of the basal (20 %) diet. Because of the greater caloric values of the high-fat diet, this would be expected.

In studying the endocrine system, exposure to a cold temperature has been used frequently to elicit hormonal responses to conditions of 'stress'. Some of the reactions occur rapidly and are associated

with chemical and histological changes in the adrenal cortex. If the pseudolipotropic action of cold were concerned with this type of response, one might expect that the action would be transient in nature. After rats had been fed the basal hypolipotropic diet in the cold for 10 and 15 weeks, the fat content of the liver remained low. In 1950, Sellers, You, Ridout & Best observed that small daily doses (1-2 mg.) of cortisone given to normal rats fed a hypolipotropic diet failed to prevent the deposition of fat in the liver. Within a limited dosage schedule, this finding also applies to cortisone given orally, to deoxycorticosterone acetate and to adrenocorticotrophic hormone (ACTH) given by injection (Sellers, You, Ridout & Best, 1951). Thus it appears unlikely that adrenal stimulation is a principal cause of the effect.

A high dose of thyroid substance (0.8 % in the food) is necessary in order to produce a level of lipid approaching the normal, when this basal hypolipotropic diet is fed to rats. Even with such a high dosage, large, centrilobular extracellular fatty cysts are formed (Sellers & You, 1951), a feature seldom if ever seen in the livers of the animals exposed to cold. Therefore, the results obtained do not support the hypothesis that the thyroid plays an important part in the pseudolipotropic effect observed.

Acclimatization does not appear to alter appreciably the pseudolipotropic effect of cold. Acclimatized animals consumed considerably more food than did controls kept at room temperature, yet on chemical examination had significantly lower levels of fat in the livers.

No adequate explanation of the phenomenon can be advanced at present, yet an analysis of energy balance factors affords a basis for future work. The animal in the cold consumes a greater quantity of food than does the control kept at room temperature but does not grow so rapidly. In order to maintain body temperature in the cold, the energy expenditure is much greater and therefore it is logical to suggest that the greater caloric intake is used in producing heat. Very little dietary choline is available, so the necessary metabolic processes must be carried out (1) without choline, (2) by synthesis of choline within the body, or (3) the limited amount of choline present must be used more efficiently. Whichever of these alternatives is correct, it would appear that exposure to cold has brought about an alteration in normal metabolic pathways, and efforts are being made to investigate the possibilities more fully.

SUMMARY

1. When a hypolipotropic diet of moderate fat content (20 %) is fed to rats exposed to a temperature of $1.5 \pm 1^{\circ}$, excessive deposition of fat in the liver is effectively prevented.

2. This 'pseudolipotropic' action is a matter of degree, however, for with a high-fat (50%), low-choline diet lipids accumulate in the liver. The fat content of the liver is significantly less than in control animals kept at room temperature on the same type of diet.

3. The pseudolipotropic action of cold is demonstrable in acclimatized as well as in normal animals, and is apparently not mediated to a recognizable

extent through either the thyroid or the adrenal gland.

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EXPLANATION OF PLATE 3

- (a) Livers from (left) a rat fed the hypolipotropic diet for 8 weeks in an environment of 1.5°, and (right) from a rat fed the same diet for the same period at room temperature. The liver from the control animal is yellowish in colour and is much larger than that of the rat kept in the cold.
- (b) Photomicrograph of liver tissue (Orange red O $\times$ 135) from a rat fed a hypolipotropic diet for 8 weeks in an environment of 1.5°. The appearance of the section is essentially normal.
- (c) Photomicrograph of liver from rat fed the same diet but kept at room temperature. Note the globules of fat deposited throughout the lobule.

(a)

(b)

(c)

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