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Nutrition Studies in the Cold. II. Curative Effect of Cold on Fat Fatty Livers.* (23766)

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The comparative response of rats maintained at 25° and at 1° to variations in protein and fat content of the diet has been described recently(1). Diets of a low protein or high fat content produced elevated levels of liver fat at 25° while at 1° the liver fat was within the normal range at all protein and fat levels which were employed. It has been demonstrated that in addition to the preventive effects of supplementary choline and methionine in hypolipotropic diets, these lipotropic factors have a curative effect on fat fatty livers, *i.e.*, will decrease an elevated level of liver fat due to previous ingestion of a hypolipotropic diet(3).

It became of interest to determine if cold also had a curative effect on fat fatty livers† as well as the preventive effect described recently(1). The data presented below clearly demonstrate that cold exhibits both of these characteristic effects of the other lipotropic factors.

Methods. Young male rats (50-80 g) of the Carworth strain were used. The care of the animals and the general experimental conditions were the same as described earlier(1). Three diets (Table I) which in our earlier experiments produced fatty livers at 25° were used. Choline and inositol were omitted from the vitamin mixture so that the lipotropic factors of the diets were supplied entirely by the methionine of the dietary casein. At the end of the appropriate periods, as described below, the animals were sacrificed and the livers were removed, weighed, and analyzed for total lipids essentially as described by Dury and Treadwell(4). Twenty-two or 23 rats were placed on each of the 3 diets for 21 days at 25°. At the end of this first period 7 rats on each diet were sacrificed. Those remaining on each diet were divided into 2 groups and maintained on the same diet for an additional 28 days; during this second period, one group was continued at 25°, the other group was kept at 1°. At the end of this 28-day period all rats were sacrificed and the livers analyzed for total lipids. This experimental design provided an evaluation of the curative effect of cold (level of liver lipids after 21 days at 25° compared with that after additional 28 days at 1°) and confirmation of our earlier results on the preventive

TABLE I. Experimental Diets.

Constituents	Diets, g/100 g No. 1	No. 2	No. 8
Casein*	5	10	20
Salt mixture†	5	5	5
Cellu flour	2	2	2
Starch	33	31	16
Sucrose	33	30	15
Vitamin mix‡	2	2	2
Lard	18	18	38
Cod liver oil	2	2	2

* Vitamin-free casein.
† Hubbell, Mendel, and Wakeman(5).
‡ For composition see Treadwell *et al.*(1).

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† Several types of fatty livers have been distinguished, principally on mode of dietary production. Fat fatty liver is produced by ingestion of a diet consisting of purified protein, carbohydrate, salts, vitamins, and some easily available form of neutral fat. This type of diet supplemented with sufficient cholesterol produces the cholesterol fatty liver(2).

TABLE II. Curative and Preventive Effects of Cold.

Diet No.	No. of rats	% survival	Wt change per day, g	Food intake per day, g	Protein efficiency ratio*	Liver, g/100 g body wt	
						Wt	Total lipids
			First period—25°, 21 days				
1	7	100	−.4 ± .1†	6.8 ± .5	−1.2 ± .3	6.2 ± .4	.80 ± .13
2	7	"	1.5 ± .2	9.0 ± .6	1.6 ± .2	5.9 ± .4	.97 ± .22
8	7	"	4.0 ± .4	9.8 ± .7	2.0 ± .1	7.0 ± .3	1.64 ± .19
			Second period—25°, 28 days				
1	7	100	.9 ± .1	7.1 ± .4	2.4 ± .3	8.1 ± .6	2.10 ± .30
2	7	"	1.1 ± .2	8.4 ± .6	1.3 ± .4	6.6 ± .4	1.50 ± .16
8	7	"	3.6 ± .2	11.7 ± .3	1.5 ± .0	5.3 ± .4	.98 ± .18
			Second period—1°, 28 days				
1	9	78	1.2 ± .1	12.7 ± .5	1.8 ± .1	4.8 ± .2	.46 ± .05
2	8	75	1.5 ± .2	15.0 ± .5	1.0 ± .1	4.3 ± .3	.53 ± .14
8	8	100	2.6 ± .2	15.2 ± .5	.8 ± .2	4.7 ± .1	.49 ± .06

* g change in wt/g protein ingested. † Mean ± stand. error.

effect of cold (level of liver lipids at 1° compared with that at 25° after additional 28 days).

Results. The data are summarized in Table II. Individual values are the averages for the number of animals indicated. At 25° the survival was 100% for all 3 dietary groups and for both periods; at 1°, during the second period, there was a decreased survival on the low protein diets 1 and 2. The loss in weight and negative protein efficiency ratio on Diet 1 at 25°, the greater weight gain on Diets 1 and 2 at 1°, and the lower liver weights at 1° are all in agreement with our earlier findings(1). The animals on Diets 1 and 2 which were maintained for the additional 28 days at 25° exhibited increased levels of liver lipids over those sacrificed at the end of the first period. On Diet 8 at 25° the liver lipids were decreased slightly at the end of the second period in comparison to the level after 21 days. In this group the food intake increased and the growth rate and protein efficiency ratio decreased during the second period so that more protein was probably available for lipotropic action which would account for the slight decrease in liver lipids. Comparison of the dietary groups maintained at 25° and at 1° during the second period shows that the cold prevented the increase in liver lipids which occurred at 25°. With all 3 diets the levels of the liver lipids were lower after the additional 28 days at 1° than they were after the initial 21 days at 25°. This finding demonstrates that cold has a curative effect on the fat fatty liver as well as a preventive one. This curative effect of cold has not been reported previously. It is now apparent that cold exhibits both of the characteristic effects (preventive and curative) of the lipotropic factors choline and methionine. As suggested previously(1) and confirmed by the present data, using the level of liver lipids as a criterion, the rat at 1° has an increased capacity for metabolizing fat even when there is an inadequate supply of dietary lipotropic factors. Cold appears to decrease the dietary requirement for lipotropic factors. Possible explanations for this effect of cold have been discussed earlier(1).

Summary. 1) Three groups of young male rats received one of 3 different choline and inositol-free hypolipotropic diets for 21 days at 25°. Seven rats of each group were then sacrificed. The remainder were continued on the same diet but divided into two subgroups; one was maintained at 1°, the other at 25° for an additional 28 days. The livers of all animals were analyzed for total lipids. 2) Data on food intake, growth rate, protein efficiency ratio, and liver weight confirmed earlier findings. The levels of liver lipids after 28 days at 1° were lower in the 3 dietary groups than in either the comparable groups at 25° after the initial 21 days or after the following 28 days. The results demonstrate that cold has a curative effect on the fat fatty liver and confirm earlier observations on the preventive effect of cold.

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Host Resistance in Hemorrhagic Shock. XIV. Induction of Shwartzman Reaction by Shock Plasma and Tissues.* (23767)

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The plasma of an animal in hemorrhagic shock contains a toxin which is not present in normal plasma(1). Certain properties of this toxin, shared with bacterial endotoxins, are: 1. Ability to reduce the phagocytic activity of normal phagocytes and macrophages *in vitro*(2). 2. Induction of "flight of leukocytes," *i.e.* granulocytopenia with segregation of granulocytes, primarily in capillaries of the lung, liver, and spleen(3). 3. Production of a characteristic febrile reaction in trained rabbits, with development of resistance to the pyrogen following repeated daily administration(4). 4. Development in such resistant animals of tolerance of prolonged hemorrhagic shock, with recovery following a transfusion which is not curative in non-resistant animals (4). The presence of this toxin in shock plasma is not simply an incidental abnormality, for it has been shown to be responsible for the failure of animals in prolonged shock (6 hours) to respond to transfusion; and it is capable of converting reversible shock, in which transfusion of normal plasma leads to full recovery, into a state of shock which is not responsive to any therapy(1). The thesis that this toxin is of bacterial origin stems from two observations: 1. Demonstration, already referred to, that resistance to hemorrhagic shock, induced by making animal resistant to a known bacterial endotoxin, is also induced by the toxin in shock plasma(4). 2. Finding that broad-spectrum non-absorbable antibiotics, administered orally so as to nearly sterilize the gastro-intestinal tract, prevent prolonged shock from becoming irreversible to transfusion, and render blood in prolonged shock free of the toxin(1,5). Two further lines of investigation into the properties of this toxin are presented in this paper to provide additional evidence that the circulating toxin in shock is similar to, or identical with, bacterial endotoxin. The first is the demonstration that the plasma and tissues of the shocked animal elicit a generalized Shwartzman reaction under conditions that may be considered virtually specific for endotoxin(6), while normal plasma and tissues, under identical test conditions, fail to do so. The second is the observation that this activity of shock plasma and tissues resides within a polysaccharide fraction extractable with trichloroacetic acid, in the manner employed by Boivin and Mesrobeanu(7) for the isolation and purification of bacterial endotoxins.

Method. Hemorrhagic shock was induced in dogs and rabbits, which were exsanguinated after the shock was shown to be irreversible to transfusion(5).

The blood from rabbits was drawn with sterile precautions into a heparinized syringe, and centrifuged in a refrigerated sterile container. Ten ml of the plasma was then injected intravenously into young (1 kg) rab-

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