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Related Effects of Acute Exposure to Cold and Fasting on Free Fatty Acid Release by Adipose Tissue.* (29646)

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The exposure of animals to a low environmental temperature causes an increase in oxygen consumption of isolated tissues(1-3). The increase has been associated with high rates of turnover and oxidation of reserve metabolic substrates(4). The accelerated rate of fatty acid synthesis from acetate by adipose tissue from cold-acclimated rats has been postulated by Patkin and Masoro(5) as one source of substrate to meet the energy requirements of these animals. Mallov and Witt(6) demonstrated an increase of plasma free fatty acid (FFA)† to result from cold stress and Mallov(7) observed a stimulation of output of fatty acid by adipose tissue *in vitro* resulting from exposure of the donor animal to cold.

A number of hormones have been shown to increase the rate of release of FFA from adipose tissue(8). Prominent among these are the catecholamines(9,10). These have been found to stimulate heat production in cold-adapted rats(11,12) and urinary excretion of both epinephrine and norepinephrine has been reported to increase rapidly as the result of exposure to cold(13). The existence of mechanisms which link epinephrine and norepinephrine to tissue oxidation and also to the overall response to cold and FFA release from adipose tissue seem to be established. It, therefore, was of interest to compare the effects of fasting and of cold exposure on the production of FFA by adipose tissue, and to determine whether the tissue becomes more sensitive to the stimulating effect of catecholamines as the result of cold stress. To this end, the following experiments were performed.

Methods. Male albino rats of the Holtzman strain, body weight 200-250 g, were fed Purina laboratory chow *ad libitum*. Control animals were housed at a room temperature of 24-25°C. Animals used for exposure to cold were selected at random and were exposed at a temperature of 3-5°C in individual cages in a cold room. At the end of the exposure time, they were sacrificed by an occipital blow and the epididymal fat pad was removed and prepared for incubation in a manner similar to that described by Winegrad and Renold(15). In all experiments the thin distal portion of the fat body was used. Portions of approximately 100 mg were weighed on a Roller-Smith balance and placed in Krebs-Ringer phosphate buffer, pH 7.4, which contained Armour's crystalline bovine serum albumin at a concentration of 5 g/100 ml. Three ml of medium were used in a 25-ml Erlenmeyer flask. The flasks were gassed with oxygen and incubated for 2 hours at 37°C in a Dubnoff metabolic shaker. Samples of the medium before and after incubation were analyzed for FFA by the method of Dole and Meinertz(16). Both right and left epididymal fat pads from each animal were examined. The results obtained in the 2 flasks from each animal were averaged, and this value was used in the final interpretation of the data. The results are reported as

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† The letters FFA will be used to refer to free (unesterified) fatty acids released from adipose tissue.

TABLE I. FFA Release by Adipose Tissue of Fasted Rats Maintained at 24°C or 3-5°C.*

Hours	FFA, μmoles/g/hr 24°C	3-5°C	P‡
0	2.30 ± .12†(6)		
4	3.62 ± .39 (7)	5.56 ± .50 (7)	<.05
8	4.79 ± .20 (7)	6.24 ± .38 (7)	<.01
12	5.05 ± .42 (5)	6.71 ± .52 (5)	<.02
16	5.89 ± .49 (5)	7.45 ± .69 (5)	<.05
20	6.44 ± .33 (5)	8.03 ± .81 (5)	<.05
30	7.38 ± .38 (5)	7.38 ± .71 (5)	>.05

* The fast was begun at 8:00 A.M. in all experiments.
† Mean ± S.E.
‡ Significance by Fisher's "t" test.
No. of animals in parentheses.

micromoles of FFA, expressed as palmitic acid, released per gram of tissue per hour.

Experiment 1. The experiment was begun at 8:00 A.M., at which time all food was removed from the cages. The rats which were exposed to cold and the control animals maintained at room temperature were sacrificed at intervals of 4 to 30 hours (Table I) after the beginning of experiment.

Experiment 2. Food was removed from the cages at 2:00 P.M. The rats were fasted at 24°C for 18 hours followed by 4 hours at 3-5°C, or for 18 hours at 24°C followed by 8 hours at 3-5°C. Their respective controls were fasted 22 and 26 hours at 24°C before sacrifice.

Experiment 3. Food was removed from the cages at 4:00 P.M. The animals were fasted 18 hours at room temperature or at 3-5°C. The effects of epinephrine and norepinephrine (as the bitartrates) were evaluated by the addition of 0.05 μg of the hormone per ml of medium.

Results. The results of Exp. 1 are presented in Table I. The adipose tissue from a non-fasted group of 6 animals produced 2.30 ± 0.12 μMoles of FFA per g of tissue. Tissue from fasted, control animals released from 3.62 ± 0.39 μMoles after 4 hours fast to 6.44 ± 0.33 μMoles after 20 hours. Prolongation of the fast to 30 hours resulted in further increase of FFA to 7.38 ± 0.38 μMoles per g per hour. Tissue from animals exposed to cold while fasting produced from 5.56 ± 0.50 μMoles FFA per g per hour after 4 hours to 8.03 ± 0.81 μMoles per g per hour after 20 hours. However, when the fast was prolonged to 30 hours, there was not only no further increase, but a slight decrease occurred which brought the value to 7.38 ± 0.71 μMoles, fortuitiously equal to that observed for the control animals. The difference between the results from the control and experimental animals was significant at each time period ($P<0.05$) except the last period of 30 hours' fast.

Experiment 2 was done to determine whether a short exposure to cold after fasting at room temperature would give any different results than those observed in Exp. 1 in which the total fast and cold exposure were of the same duration. The animals were fasted 18 hours beginning at 2:00 P.M., after which they were placed in the cold room for 4 to 8 hours. The results indicate that the 18-hour fast begun at 2:00 P.M. was equivalent in effect to a 30-hour fast begun at 8:00 A.M. In neither case was the output of FFA by the tissue of rats exposed to cold any more than that of the control animals fasted for the same period, but maintained at room temperature of 24°C. Table II shows these results.

The results of Exp. 3 are presented in Table III. The 18-hour fast, having begun at 4:00 P.M., was probably almost equivalent to a 30-hour fast begun at 8:00 A.M. The FFA release by adipose tissue from control and experimental animals, 6.56 ± 0.55 μMoles and 6.96 ± 0.17 μMoles per g per hour indicates that this is the case. The tissue from both control and experimental ani-

TABLE II. Effect of Fasting at Room Temperature Plus Additional Fast at 4°C on FFA Release from Adipose Tissue.*

Animal groups†	Preliminary fast at 24°C, hr	Fast at 3-5°C, hr	FFA, μmoles/g/hr‡
Control (10)	22	None	7.19 ± .28
Cold-stressed (10)	18	4	6.12 ± .36
Control (9)	26	None	7.39 ± .70
Cold-stressed (9)	18	8	5.87 ± .26

* Food was removed from the cages at 2:00 P.M.
† No. of animals in parentheses.
‡ Mean ± S.E.

TABLE III. Effects of Epinephrine (E) and Norepinephrine (N) on FFA Release from Tissues of Normal and Cold-Stressed Rats.*

Group	Treatment†	No. of rats	FFA, μmoles/g/hr‡	P§
1	None	8	6.56 ± .55	1-2 <.01
2	.05 μg E	8	9.17 ± .46	1-3 <.01
3	.05 μg N	8	10.69 ± .44	2-3 <.01
4	Cold-stress	8	6.96 ± .17	4-5 <.01
5	" + E	8	9.45 ± .33	4-6 <.05
6	" + N	8	10.93 ± .45	5-6 <.05

* Both groups, those maintained at room temperature and those maintained at 3-5°C, were fasted 18 hr before sacrifice. Food was removed from cages at 4:00 P.M.

† Both epinephrine and norepinephrine were used as the bitartrate added to the medium at a concentration of 0.05 μg/ml as the free hormone.

‡ Mean ± S.E.

§ Significance by Fisher's "t" test.

mals was stimulated by epinephrine and norepinephrine. The former hormone effected a release of 9.17 ± 0.46 and 9.45 ± 0.33 μMoles FFA per g per hour, whereas the latter increased release to 10.69 ± 0.44 and 10.93 ± 0.45 μMoles FFA per g per hour in control and experimental groups, respectively. Exposure to cold under these conditions did not induce a greater response of the adipose tissue *in vitro* to a given quantity of catecholamines in the medium.

Discussion. The results of these experiments indicate that when rats are fasted and exposed to cold at the same time, the release of FFA *in vitro* from the epididymal fat body is greater during the first 20 hours than is FFA release from adipose tissue of animals similarly fasted but maintained at a temperature at 24°C. This difference is maintained up to 20 hours, but beyond that point at some time not precisely determined, the effect of fasting, alone, is as great as that of cold superimposed on fasting. The stimulus for additional release of FFA by the cold-stressed animals may come from the increased output of norepinephrine and/or epinephrine caused by cold exposure as demonstrated by Leduc(13). However, the stimulation of catecholamine production persisted for many days in Leduc's experiments, whereas the effect of cold on FFA production by adipose tissue *in vitro* (Table I) was limited to a few hours. The lack of an effect of a short exposure to cold after a longer fast is also seen in the data presented in Table II. The fasting period at room temperature in this experiment was 18 hours begun at 2:00 P.M., followed by 4 to 8 hours in the cold room, a total of 22 or 26 hours' total fast. This fast, started in the afternoon, probably began at a time when the rats, largely nocturnal feeders, had eaten only sparingly, if at all, since the early morning hours. The short cold exposure under these conditions did not affect the production of FFA by the adipose tissue *in vitro*. Modification of adipose tissue response dependent upon the length of the fasting period has been reported by Marques *et al*(14). They evaluated the tissue as a test object for insulin assay and found that the response to insulin was markedly different depending upon whether the animals had been fasted 2.5, 5, or 24 hours before the insulin effects were measured.

The catecholamines tested both had a stimulating effect on the *in vitro* production of FFA by the fat pad. Food was removed from the cages of these animals at 4:00 P.M. at which time those exposed to cold were placed in the cold room. The close similarity of the FFA production by specimens from Group 1 (room temperature) and Group 4 (cold-stressed) indicates that the period of fasting, or virtual fasting, was longer than the 18 hours which followed removal of food from the cages. Epinephrine and norepinephrine both stimulated the release of FFA, and to practically the same extent in fat pads from animals maintained at 24°C and those exposed to 3-5°C. These results indicate neither an increase nor a decrease of response by adipose tissue to these hormones as a result of acute cold stress.

The failure of cold stress to induce the re-

lease of additional FFA from adipose tissue after a fasting period of 30 hours cannot, therefore, be attributed to a change in its responsiveness to catecholamine stimulation. In the light of Leduc's(13) report of increased excretion of epinephrine and norepinephrine in cold-stressed rats, which lasted for many days, lack of hormone is probably not a factor in the diminution of the cold effect. Apparently the fast, alone, if it is sufficiently prolonged, produces a change in the activity of the adipose tissue which is not further modified by exposing the donor to cold. These findings suggest the presence of more than one source for the FFA, one of which responds rapidly to cold stimulation. The other, apparently less affected by cold, still has a reserve which is responsive to stimulation by the catecholamines, *in vitro*.

The probable existence of more than one fatty acid pool has been suggested several times(8,17,18). The above data suggest that the cold effect is not altogether mediated through stimulation of the lipolytic enzymes (19), but to what other origin the temporarily greater output of FFA by the adipose tissue may be attributed is not apparent.

Summary. Rats deprived of food at the beginning of the experiment were exposed to a cold environment at 3-5°C for periods varying from 4 to 30 hours. The release of FFA by epididymal adipose tissue *in vitro* was compared to that from animals maintained at room temperature of 25°C. FFA was released in significantly greater amounts by the tissue of the animals exposed to cold until the fast had progressed for 30 hours, at which time there was no difference between the two groups. Following a fast of 18 hours, the adipose tissue from the cold-exposed group and from the warm room group responded similarly to epinephrine and norepinephrine.

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Effect of Fasting or Feeding on Release of Free Fatty Acid by the Adipose Tissue of the Cold-Acclimated Rat.* (29647)

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Studies relating the metabolism of adipose tissue through release of free fatty acid (FFA)† to the need for increased energy production during exposure to cold have been

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† Free (unesterified) fatty acid is referred to as FFA in this report.