Incorporation of Intravenously Injected Acetate-2-^{14}C into Tissue Lipids of Hypothermic Hamsters[1] (35509)

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Although numerous studies have been carried out on hibernating and hypothermic animals, little biochemical data have been obtained while the animals were in the hypothermic condition. The available data were reviewed recently by Entenman *et al.* (1). Data were also presented on the incorporation of acetate-2-^{14}C into lipids of the isolated rat liver during perfusions at temperatures of 17, 27, and 37°. Essentially no synthesis of cholesterol, cholesterol esters, triglycerides, or phospholipids occurred at 17°, whereas at 27° considerable lipid synthesis was noted. Additionally, using rat liver slices, it was shown that some incorporation of ^{14}C into fatty acids occurred during a 2-hr incubation with acetate-2-^{14}C at 17° but no synthesis of fatty acids occurred at 7°.

The present data were obtained on the hamster, an animal that is able to hibernate and also is able to sustain hypothermic body temperatures of 5–7° for varying periods (2–5). In the present studies, the body temperatures of the hamsters were reduced to 7° using the helium–oxygen–cold techniques of Fischer and Musacchia (2, 3). Acetate-2-^{14}C was then injected intravenously and tissues were taken for analyses at intervals ranging between 0.5 to 18 hr thereafter. It is shown that the hypothermic hamster tissues can synthesize lipids even at body temperatures of 7°, but the amounts synthesized are considerably less than that in normothermic hamsters.

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[2] It should be noted that 1,2-diglycerides, which accompany the free sterols on the TLC plate are known to be subject to acyl migration; and the 1,3-diglyceride activity reported here probably reflects such acyl migration.

Experimental Methods. The golden hamster, *Mesocricetus auratus,* was used. Animals were taken from a closed colony maintained at the Space Sciences Research Center. Body weights ranged from 90 to 125 g. Both male and females were used.

Several days before the induction of hypothermia, each animal was prepared for cannulation. Under ether anesthesia a polyethylene PE No. 10 tube was introduced through the jugular into the vena cava and exteriorized dorsally at a midpoint between the ears. The polyethylene cannulas were used to introduce the labeled acetate solution directly into the blood stream. Control hamsters were treated similarly but were not made hypothermic.

Hypothermia was induced by exposure to 80% helium and 20% oxygen and to cold. In this method, described in detail by Fischer and Musacchia (2, 3) and Musacchia and Barr (4), animals were placed individually in a glass container with a constant stream of helium and oxygen (150 to 200 liters/min) in a cold room at 0°. During periods of from 4 to 8 hr, often varying with animal size (longer periods for larger animals) there is a fall in body temperature, reduction in cardiovascular and respiratory activity, and a general state of hypothermia ensues. Hypothermic hamsters are limp, relaxed, and breathing is shallow.

When body temperatures reached 8–10°, the hamsters were removed from the cold chambers and placed in household refrigerator at 7–8° until their body temperatures reached 7–8°. At that time, each hamster was injected with 250 μl of an isotonic saline solution containing 50 μCi of acetate-2-^{14}C followed with 250 μl of physiological saline. This later procedure served to rinse the can-

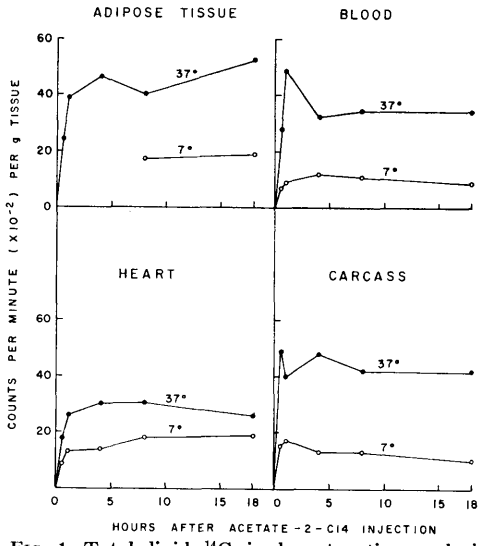

FIG. 1. Total lipid ^{14}C in hamster tissues during the 18-hr period following intravenous injection of acetate-2-^{14}C; each point represents the average value obtained from three hamsters. The body temperature of the hypothermic hamsters were maintained at 7° throughout the experiment; body temperature of normothermic hamsters was 37°.

nula and insure that all of the labeled material entered the blood stream. The polyethylene cannulas were heat sealed after injection of the saline and the hamsters were returned to the refrigerator and maintained at a body temperature of 7°. At periods of 30 min, 1, 4, 8, and 18 hrs after injection of the acetate-^{14}C, animals were sacrificed and tissues were taken. Experimental and control hamsters, three each, were used at each time period. Blood was taken by cardiac puncture, tissues were excised and quickly grabbed with specially constructed brass clamps which had been cooled in liquid air. Each piece of tissue was individually plunged into liquid air, frozen, and stored at —20° until analyzed.

Control hamsters were anesthetized with ether, injected with acetate-2-^{14}C as described above, allowed to awaken, then maintained at room temperature until sacrificed. The order of bleeding, removal of tissue, freezing procedures, and storage were identical as with the hypothermic hamsters. In all cases siblings were used as control and hypothermic pairs in any given experiment.

Analytical Procedures. At the time of analysis, the tissue was homogenized in methanol, then chloroform was added to give a chloroform–methanol (CM) ratio of 2:1 and additional CM (2:1) was added to give a solvent/tissue ratio of 20:1 (v/g). The mixture was heated at 60° in tightly capped culture tubes for 60 min, cooled and filtered through sharkskin paper into a calibrated tube. The solution of lipids was partitioned with 0.2 vol of 0.05% $CaCl_2$, centrifuged, and the upper phase was withdrawn. The lower chloroform phase was then washed twice with a mixture of methanol:water:chloroform (48:47:3, v/v/v). The final lower chloroform phase was used for lipid analysis.

To determine total lipid-^{14}C, an aliquot of the chloroform extract was evaporated to dryness in a counting vial, 10 ml of scintillant (toluene containing 3 g of PPO and 0.1 g of POPOP/liter) were added and the ^{14}C was measured in a Packard Tri-Carb scintillation spectrometer. The data are given in Fig. 1 and 2.

Lipid classes were separated by thin-layer chromatography (TLC) on intestinal extracts only. Other tissues were not sufficiently active to permit accurate determinations of ^{14}C activities. The TLC separations were carried out on 20 × 20-cm plates, layered with a silica gel H–Rhodamine 6 G mixture to a thickness of 250 μ. The developing solvents were (i) ethyl ether:hexane:ethanol:acetic acid (40:50:2:0.2, v/v) to a height of 10 cm; followed by (ii) hexane:ethyl ether:acetic acid (70:30:1, v/v) to a height of 18 cm. The lipid streaks were visualized under UV light, identified by appropriate standards on the same plate, scraped into counting vials, and ^{14}C was determined as described above. After making appropriate quench corrections, the ^{14}C in each lipid class was expressed as a percentage of the total ^{14}C recovered from the TLC plate. The data are recorded in Table I.

For analysis of carcass lipid-^{14}C, the carcass was digested with 30% KOH and lipids were extracted, after acidification, with petroleum ether as described by Entenman (6). ^{14}C activity and lipid mass were determined on aliquots of the petroleum ether extract. The ^{14}C data are recorded in Fig. 1 and carcass fat results are given in the text.

TABLE I. Effect of Hypothermia on Lipid Synthesis by Hamster Small Intestines Following Intravenous Injection of Acetate-2-^{14}C.

Body temp (°)	After acetate-^{14}C injection (hr)	Percentage of ^{14}C recovered from TLC plate found in[a]							
		PL	NL[b]	MG	FS	1,3-DG[c]	FFA	TG	SE
7	.5	13.5	86.5	4.6	8.5	33.3	20.7	5.7	13.8
	1	12.1	87.9	2.8	7.7	41.3	10.5	12.7	12.7
	4	13.8	86.2	6.3	12.2	33.3	24.2	5.4	4.8
	8	15.3	84.7	6.4	11.0	45.1	15.4	6.2	0.6
	18	19.4	80.6	4.4	11.8	42.3	14.1	7.1	0.8
37	.5	45.3	54.7	3.4	6.0	10.1	29.7	4.9	0.5
	1	50.6	49.4	2.9	5.4	8.5	29.2	2.6	0.8
	4	60.5	39.5	1.4	3.8	13.7	19.3	1.1	0.2
	8	62.3	37.7	1.2	2.1	10.0	23.7	0.5	0.3
	18	56.2	43.8	1.3	4.0	19.3	18.0	1.0	0.2

[a] Abbrev used for lipid classes were: PL, phospholipids; NL, neutral lipids; MG, monoglycerides; FS, free sterols + 1,2-DG; 1,3-DG, 1,3-diglycerides; FFA, free fatty acids; TG, triglycerides; and SE, sterol esters.

[b] The lipids were separated by thin-layer chromatography and the ^{14}C value for each lipid was expressed as a percentage of the total lipid-^{14}C recovered from the plate. The percentage of ^{14}C in neutral lipids was calculated as the sum of all lipid-^{14}C on the TLC plate, except phospholipid-^{14}C. The values given are the averages of values found on small intestines from 3 hamsters at each temperature.

[c] See footnote no. 2.

Results and Discussion. The data in Figs. 1 and 2 indicate that the incorporation of ^{14}C from acetate-2-^{14}C into tissue lipids is less for all tissues at 7° than at 37°. The less active tissues at both temperatures (adipose, heart, carcass; Fig. 1) have rapid incorporation of ^{14}C within 1 hr after acetate-2-^{14}C injection, and, except for blood change, little thereafter. The more active tissues (brain, liver, kidney, small intestine) also rapidly incorporate acetate-2-^{14}C within the first 60 min at both temperatures, but lipid-^{14}C in the normothermic hamsters decreases after the first hour, whereas with the 7° hamsters there is a gradual rise in lipid-^{14}C throughout the hypothermic period. The gradual increase is greater with the kidney and liver in which the final lipid-^{14}C activities 18 hr after the acetate-2-^{14}C injection were about the same in both the 7 and 37° hamsters.

One interpretation of these data is that the enzyme systems involved in lipid synthesis in kidney, liver, and small intestine are less affected by the 7° temperature than those in the heart and adipose, and can continue to synthesize lipids from precursors formed early after acetate-2-^{14}C injection. *In vitro* studies will have to be conducted to determine whether the increase in tissue activity over the 18-hr hypothermic period is due to continued uptake and utilization of acetate-2-^{14}C or due to an early uptake of acetate-2-^{14}C and subsequent lipid synthesis. The data on distribution of ^{14}C activity in lipid classes of small intestines (Table I) suggest that there is continued uptake and utilization of acetate-2-^{14}C, since an early buildup of activity in the free fatty acids or any one lipid class was not found.

In vitro studies would also clarify and define the continued functioning of tissues at low temperature, since the possibility of accumulation of radioactive metabolites derived from other tissues and transported to the liver and small intestines cannot be ruled out (Fig. 1). On the basis of these results, it would seem that the liver and kidney are the most active tissues at 7° when the results are expressed as lipid-^{14}C activity per gram of tissue. Further, the data suggest that tissue oxygenation of liver, kidney, and small intestine are quite adequate in the hypothermic

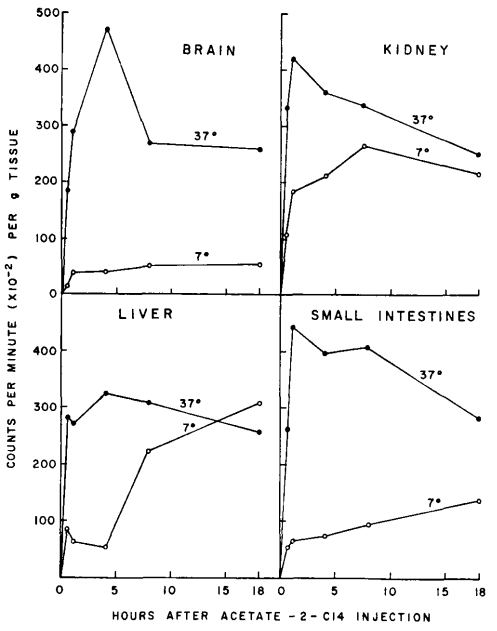

FIG. 2. Total lipid ^{14}C in hamster tissues during the 18-hr period following intravenous injection of acetate-2-^{14}C; each point represents the average value obtained from three hamsters. The body temperature of the hypothermic hamsters were maintained at 7° throughout the experiment; body temperature of normothermic hamsters was 37°.

hamsters since lipid synthesis continues. Lipid synthesis is known to be severely limited in anaerobic conditions. At worst, the level of reduced oxygenation in tissues of helox hypothermic hamsters is only moderate. Recently, Volkert and Musacchia (5) showed that with induction of hypothermia arterial blood P_{O2} is unchanged, venous blood P_{O2} is reduced to about 30% of normal, and Hb–O_2 dissociation is markedly reduced. Those findings are indicative of some level of mild tissue hypoxia. The current results suggest, however, that oxygenation is sufficient to maintain a substantial level of lipid synthesis.

The data presented in Table I express the distribution of ^{14}C activity in lipids in the small intestines of hypothermic and normothermic hamsters. At 7°, the ^{14}C activity in phospholipids constituted 12–19% of the total ^{14}C activity; whereas at 37°, phospholipids-^{14}C comprised 45–62% of the total. Neutral lipid-^{14}C was 81–88% of the total at 7° and 38–55% at 37°. The length of the period of hypothermia did not affect the activity in phospholipids or neutral lipids. It should be appreciated that the total lipid-^{14}C at 37° was much greater than at 7° (Fig. 2), hence the amounts of newly synthesized lipids were always less at 7°. Inspection of the distribution of ^{14}C activity among the neutral lipids reveals that all lipids except FFA, when expressed as percentage of total lipid-^{14}C, were greater at 7° than at 37°. The ^{14}C activity in FFA was about the same at the two temperatures, whereas the percentage of 1,3-DG at 7° was much greater than at 37°. The significance of these changes cannot be ascertained from the data available at this time.[2] The data are not yet conclusive enough to determine whether the increase in diglyceride activity reflects a block in the synthetic pathway of phospholipids due to cold inactivation of enzymes, or if the increases reflect a more active role of neutral glycerides in the energy metabolism of the hypothermic hamster.

Analysis of carcass fat indicated that hypothermia exerted no effect upon total carcass fat. The percentage of fat in the carcass of hypothermic and normothermic hamsters was identical at 13.2%.

Summary. The *in vivo* synthesis of lipids from acetate-2-^{14}C is drastically reduced in hamster tissues at 7 vs 37° body temperature. The most active tissues at the 7° temperature are the brain, liver, kidney, and small intestine, on the basis of amount of ^{14}C activity found in those tissues per gram of tissue. In these tissues, a gradual increase in lipid-^{14}C occurs at a body temperature of 7° following acetate-2-^{14}C injection. At a 7° body temperature a smaller percentage of small intestine phospholipids were synthesized from acetate-2-^{14}C, with a corresponding increase in the percentage of total ^{14}C activity found as neutral lipid-^{14}C. Of the neutral lipids in the small intestines, the greatest percentages of ^{14}C activity appeared in the free fatty acids and diglycerides. There was no discernible change in percentage of carcass fat either between the hypothermic and normothermic hamsters, or among the hypothermic hamsters over the 18-hr time period studied.

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1. Entenman, C., Hillyard, L. A., Holloway, R. J., Albright, M. L., and Leong, G. F., *in* "Depressed Metabolism" (X. J. Musacchia and J. F. Saunders, eds.), p. 159. Amer. Elsevier, New York (1965).

2. Fischer, B. A., and Musacchia, X. J., Amer. J. Physiol. **215,** 1130 (1968).

3. Musacchia, X. J., Fed. Proc., Fed. Amer. Soc. Exp. Biol. **29,** 524 (1970).

4. Musacchia, X. J., and Barr, R. E., *in* "Depressed Metabolism" (X. J. Musacchia and J. F. Saunders, eds.), p. 569. Amer. Elsevier, New York (1969).

5. Volkert, W. A., and Musacchia, X. J., Amer. J. Physiol. **219,** 919 (1970).

6. Entenman, C., Methods Enzymol. **3,** 317 (1957).

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