Linoleic and Linolenic Acids: Their Oxidation by Normal and Diabetic Rats.* (25119)

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The metabolic behavior of saturated and unsaturated fatty acids differs in many important aspects. The β-fatty acids of lecithins isolated from various animals have been shown to be predominantly saturated; those in the α position, mainly unsaturated(1). The fatty acids commonly found in ester linkage with plasma cholesterol are chiefly unsaturated(2-4). Certain of the polyunsaturated fatty acids either are not synthesized by the rat, or are synthesized to a limited extent only (5). Their absence from the diet of young rats may result in a variety of derangements which can be alleviated by addition to diets of small amounts of polyunsaturated fatty acid arachidonic, or its metabolic precursor, linoleic acid. Linolenic acid also relieves deficiency signs, but it is reported to be less effective than the other 2 fatty acids(6). Recently it has been claimed that the 2 types of fatty acids differ in their effects on serum cholesterol in humans, with vegetable oils rich in unsaturated fatty acids lowering serum cholesterol when substituted for the more saturated fatty acids of the diet(7-10). The oxidation of palmitic acid is spared in the intact normal rat by carbohydrate feeding and in the diabetic rat by insulin administrations(11-14). In view of the difference in metabolic behavior of saturated and polyunsaturated fatty acids, and particularly in view of the body's limited (or lack of) capacity to synthesize linoleic acid, it seemed desirable to determine whether catabolism of the 2 principal, naturally-occurring, 18-carbon polyunsaturated fatty acids, linoleic and linolenic, is also controlled by carbohydrate utilization. The effect of insulin upon their utilization in the diabetic rat was also studied because of the interesting observation of Peifer and Holman (15) that depletion of essential fatty acids occurs more rapidly in the diabetic than in the normal rat when they are fed a diet deficient in these fatty acids.

Methods. C^{14}-labeled fatty acids. Linoleic and linolenic acids randomly labeled with C^{14} were obtained as the methyl esters from Dr. Herman Schlenk of the Hormel Institute, Univ. of Minnesota, Austin, and we are greatly indebted to him for a generous supply of both acids. Their photosynthetic preparation and establishment of their purity have been described by Mangold and Schlenk (16). The fatty acids were injected in the form of unesterified fatty acid bound to albumin. They were converted to this form by the following procedure: The methyl esters were saponified with sodium ethoxide at 60° for one hour, the mixture was acidified, and fatty acids were extracted with petroleum ether. The volume of the solvent containing the fatty acids was reduced to a few ml, and the acids were converted to the sodium salts. A few drops of 0.9% saline were added, and volatile solvents were removed in a stream of nitrogen. The sodium salts of the fatty acids were then mixed at 37° with 0.9% saline and rat plasma so as to yield a mixture containing 9 parts plasma to 1 part saline. Plasma proteins were separated by paper electrophoresis in sodium barbital buffer at pH 6, stained with bromphenol blue, and scanned for C^{14}. The albumin fraction was the only one labeled with C^{14}. In one experiment the saline solution of the sodium salt of the fatty acid was divided into 2 parts, one of which was converted to the albumin complex with defatted human albumin, the other, with rat plasma. (The results obtained with these 2 preparations were indistinguishable.) Each of the rats received intravenously 1 ml of the preparation, which contained 0.20 mg of the labeled fatty acid. *Exp. 1.* Long-Evans, normal male rats weighing about 200 g were used. For a few days before the start of the experiment they were fed, by stomach tube, a fluid diet high in carbohydrate, as described elsewhere

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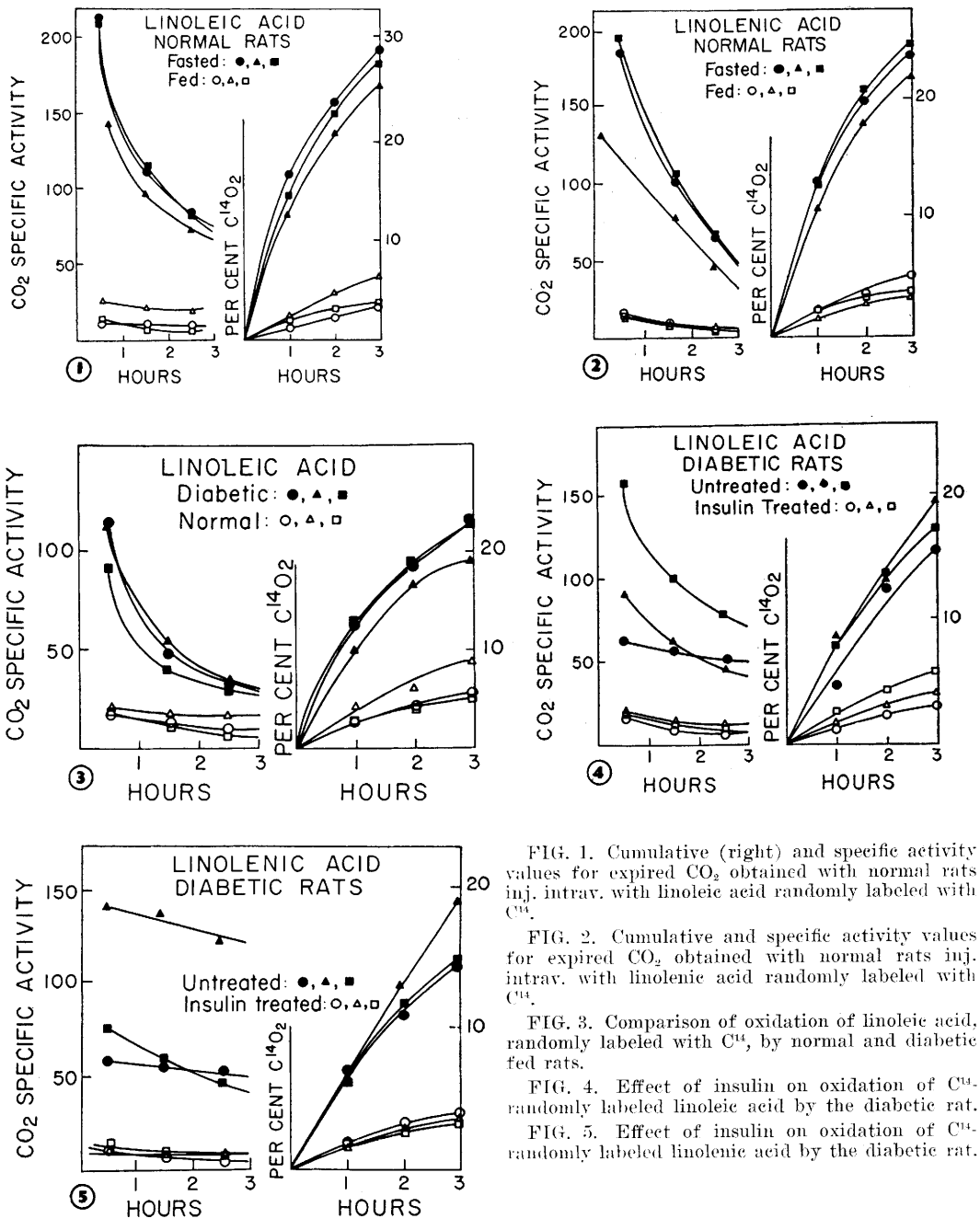

FIG. 1. Cumulative (right) and specific activity values for expired CO_2 obtained with normal rats inj. intrav. with linoleic acid randomly labeled with C^{14}.

FIG. 2. Cumulative and specific activity values for expired CO_2 obtained with normal rats inj. intrav. with linolenic acid randomly labeled with C^{14}.

FIG. 3. Comparison of oxidation of linoleic acid, randomly labeled with C^{14}, by normal and diabetic fed rats.

FIG. 4. Effect of insulin on oxidation of C^{14}-randomly labeled linoleic acid by the diabetic rat.

FIG. 5. Effect of insulin on oxidation of C^{14}-randomly labeled linolenic acid by the diabetic rat.

(11). The fasted rats were fed for the last time 24 hours before start of experiment. The fed rats received their last feeding, 12 ml of a 35% glucose solution, 30 minutes before labeled fatty acids were injected. *Exp. 2.* Diabetes was induced in rats by an intravenous injection of alloxan monohydrate (40 mg/kg of body weight) at least 3 weeks before the rats were used. Daily records of food consumption and urine volume were kept. The rats selected had fasting (6 hours) blood sugar values in excess of 300 mg %, measured a few days before the experiment. Both normal and diabetic rats used in this experiment were fed

TABLE I. Oxidation of Linoleic and Linolenic Acids by Normal, Diabetic, and Insulin-Treated Diabetic Rats.

C^{14}-fatty acid inj.	Specific activities of CO_2				Cumulative % $C^{14}O_2$			
	Hr	Norm. fasted*/Norm. fed	Diab. fed†/Norm. fed	Untreat. diab.‡/Insulin-treat. diab.	Hr	Norm. fasted*/Norm. fed	Diab. fed†/Norm. fed	Untreat. diab.‡/Insulin-treat. diab.
Linoleic	0-1	7.6	6.2	6.0	0-1	5.7	3.4	4.1
	1-2	11.6	4.4	6.1	0-2	9.8	4.3	3.9
	2-3	7.5	3.0	6.4	0-3	5.4	4.4	4.3
Linolenic	0-1	7.9		3.9	0-1	6.0		3.1
	1-2	6.3		4.7	0-2	5.7		3.7
	2-3	6.4		5.3	0-3	5.4		4.1

* Avg of 3 fasted and 3 fed rats (Exp. 1). † Avg of 3 diabetic and 3 normal rats (Exp. 2).
‡ Avg of 3 untreated and 3 insulin-treated diabetic rats (Exp. 3).

ad libitum an adequate stock diet (Diablo abration) until 1 hour before start of experiment; at that time both groups of rats received 6 ml of a 35% glucose solution by stomach tube. *Exp. 3.* Only diabetic rats were used in this experiment, and they were fed, *ad libitum,* a synthetic diet containing 60% glucose(17) for 3 days before start of experiment. The insulin-treated rats received protamine zinc insulin subcutaneously (2 units per 100 g body weight), first at 12 hours and again at 1 hour before labeled fatty acids were injected. *Collection and analysis of expired CO_2.* Immediately after injection of labeled fatty acids, the rats were placed in glass metabolism cages, and the expired air was drawn by reduced pressure into glass columns containing 5N sodium hydroxide. The procedures used for determination of the C^{14} content of expired CO_2 have been described (18).

Results. Figs. 1 and 2 show typical results obtained in Exp. 1 for oxidation of linoleic and linolenic acids by normal rats. In 3 hours the carbohydrate-fed rats converted about one-sixth as much of the injected, labeled, polyunsaturated fatty acids to CO_2 as did the fasted rats. Values for the specific activities (c.p.m. per mg CO_2 carbon) of expired CO_2 were considerably lower in the fed than in the fasted rats at all time intervals.

In Exp. 2, oxidation of linoleic acid was compared in fed normal and diabetic rats. The latter ingest about twice as much food as do normal rats, but in spite of this, the cumulative $C^{14}O_2$ and specific activity values for each time interval in the experiments with diabetic rats were much higher than the corresponding values observed with the normal rats (Fig. 3).

The effect of insulin upon oxidation of linoleic and linolenic acids by diabetic rats was studied in Exp. 3, and typical results are shown in Figs. 4 and 5. Injections of the hormone reduced the conversion of the C^{14} of the 2 labeled, polyunsaturated fatty acids to CO_2 to about the same extent as did carbohydrate feeding in the normal animal.

The results obtained with other rats in each of the 3 experiments are summarized in Table I. Conversion of polyunsaturated to saturated fatty acids has been shown to occur to only a limited extent(19). In view of the short period involved in our experiments, it would therefore seem that we are dealing with the actual oxidation of the administered fatty acids.

Discussion. Despite the fact that the capacity for linoleic acid synthesis is either severely limited or lacking in the rat, the characteristics of the oxidation of this fatty acid resemble those of palmitic acid. Thus, it is more rapidly oxidized in the fasted than in the carbohydrate-fed rat; its oxidation in the fed diabetic rat greatly exceeds that in the fed normal rat; and the increased oxidation in the diabetic rat can be reduced by insulin administrations. The evidence obtained here indicates that oxidation of linoleic and linolenic acids, like that of palmitic, is tied directly to glucose utilization and indirectly to insulin

availability. Our findings on the comparative rates of oxidation of linoleic acid-1-C^{14} by the fed normal and diabetic rats are compatible with the observations of Peifer and Holman (17), that the alloxan-diabetic rat is more rapidly depleted of essential fatty acids than is the normal rat.

Summary. 1. Oxidation of linoleic and linolenic acids, both randomly labeled with C^{14}, to CO_2 was compared in a) fasted and glucose-fed normal rats; b) normal and diabetic fed rats; and c) insulin-treated and untreated, fed diabetic rats. 2. Administration of glucose spared oxidation of both linoleic and linolenic acids. 3. Oxidation of linoleic acid by *ad lib.*-fed diabetic rat exceeded that by similarly fed normal rat. 4. Insulin spared oxidation of polyunsaturated fatty acids in the diabetic rat.

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Conversion of Serum Proteins into Tissue Proteins.* (25120)

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Although elimination of isotopically labeled plasma proteins from the circulation has been investigated by numerous authors(1-9), very little is known on their conversion into tissue proteins. The purpose of the experiments described here was to gain more insight into this process. Whipple and his coworkers(1,10) in their classical work with C^{14}-lysine containing plasma proteins found incorporation of C^{14}-lysine into the tissue proteins. It is not possible, however, in experiments with proteins containing a single labeled amino acid to decide whether incorporation into tissue protein involves transfer of large peptide fragments, or whether total breakdown to the amino acid stage is a prerequisite for incorporation. External labels such as I^{131} are use-

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