

# Effects of epinephrine on glucose transport and metabolism in adipose tissue of normal and hypothyroid rats

GEORGE A. BRAY and H. M. GOODMAN\*

New England Medical Center Hospitals and Department of Medicine, Tufts University School of Medicine, and The Department of Physiology, Harvard Medical School, Boston, Massachusetts 02111

**ABSTRACT** Epinephrine increases the oxidation of glucose in adipose tissue even when its lipolytic effects are markedly reduced or abolished by propranolol, nicotinic acid, ouabain, or thyroidectomy. In order to locate the site(s) at which epinephrine stimulates glucose utilization, we studied the effects of epinephrine on the oxidation of various metabolites of glucose.

Epinephrine neither increased the production of  $^{14}\text{CO}_2$  from 1- or 3- $^{14}\text{C}$ -pyruvate nor affected pyruvate conversion to glyceride-glycerol. To assess the possibility that epinephrine might accelerate the entry of glucose into adipocytes, we studied the accumulation of the nonmetabolized sugar L-arabinose in the intracellular water of adipose tissue. Epinephrine increased arabinose penetration into adipocytes to a degree comparable with that caused by 0.1 mU/ml of insulin.

Virtually identical results were obtained in tissues from thyroidectomized rats in which the lipolytic effects of epinephrine were significantly reduced. It is concluded that epinephrine increases glucose oxidation by promoting its entry into adipose tissue and that the effect is independent of lipolysis.

**SUPPLEMENTARY KEY WORDS** lipolysis · glucose oxidation · ouabain · propranolol · nicotinic acid · insulin · L-arabinose

**I**N ADDITION to its well-known effects of lipolysis, epinephrine has been repeatedly demonstrated to increase the utilization of glucose in adipose tissue. The production of  $\text{CO}_2$  from glucose-6- $^{14}\text{C}$  is affected to a greater

extent by epinephrine than that from glucose-1- $^{14}\text{C}$  (1, 2). The studies of Cahill, Leboeuf, and Flinn (2) suggested that the stimulation of glucose oxidation by epinephrine might be secondary to the accumulation of free fatty acids (FFA) in the tissue during lipolysis, for they showed that the addition of high concentrations of palmitate to the incubation medium bathing the adipose tissue modified the pattern of glucose utilization in a manner similar to that seen with epinephrine. This observation has received recent independent confirmation (3).

While it is true that FFA produced by lipolysis may increase the oxidation of glucose under certain conditions, the stimulation of glucose oxidation by epinephrine (or other agents) occurs even in the absence of lipolysis. For example, Love, Carr, and Ashmore (4) showed that DL-1-(2',4'-dichlorophenyl)-2-*t*-butylaminoethanol simulated the effects of isoproterenol on glucose utilization but did not enhance lipolysis. Moreover, blockade of lipolysis with propranolol did not prevent the epinephrine-induced stimulation of  $^{14}\text{CO}_2$  formation from glucose- $^{14}\text{C}$  (5). The present experiments were stimulated by our finding (6) that thyroidectomy diminished the lipolytic effects of epinephrine without diminishing its effects on the oxidation of radioactive glucose to  $^{14}\text{CO}_2$ . We have investigated the effects of epinephrine on glucose utilization in adipose tissue from normal and hypothyroid rats.

## METHODS AND MATERIALS

### *Animals*

Male rats (Holtzman Breeding Farms, Madison, Wis.) weighing 150–350 g were housed in a constant-temperature room and fed Purina Laboratory Chow (Ralston

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Abbreviation: FFA, free fatty acids.

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Purina Co., St. Louis, Mo.) and tap water. Thyroidectomy was performed as described previously (7), and the effectiveness of the operation in inducing hypothyroidism was assessed by observing the impairment of growth (8).

### Experimental Procedures

**Incubation of Fat Pads with Radioactive Substrates.** Rats were killed by a blow on the head, and the epididymal fat bodies removed and divided into 6–8 segments. Tissues weighing 50–100 mg were placed in incubation vials containing 1 ml of Krebs–Ringer bicarbonate buffer, pH 7.4, with 40 mg of albumin (Fraction V, Armour Pharmaceutical Co., Chicago, Ill.). This quantity of albumin contained 0.4  $\mu\text{g}$  of extractable acids. Substrates for the incubations were either glucose (1 mg/ml) uniformly labeled with  $^{14}\text{C}$  or specifically labeled at the 1 or 6 position, or pyruvate (1 mg/ml) labeled with  $^{14}\text{C}$  at either the 1 or 3 position. Each vial contained approximately 0.2  $\mu\text{c}$  of radioactivity. After incubation for 1 hr in an atmosphere of 95% oxygen, 5%  $\text{CO}_2$ , 0.5 ml of 0.5 N sulfuric acid was added to the medium and 0.5 ml of *p*-(diisobutylcresoxyethoxyethyl) dimethyl benzyl ammonium hydroxide (“hydroxide of Hyamine 10X,” Packard Instrument Company, Inc., Downers Grove, Ill.) was added to a polyethylene cup suspended from the cap of the incubation vial. The vials were shaken for an additional hour and the polyethylene cup containing the  $^{14}\text{CO}_2$  was then transferred to a liquid scintillator for assay of the radioactivity (9). For determination of  $^{14}\text{C}$  converted into lipid components, the fat pad was removed, rinsed, and homogenized in the extraction fluid described by Dole (10). Triglycerides were extracted into the heptane phase, and 1 ml of this phase was added directly to liquid scintillation counting vials for assay. A 2 ml aliquot of the heptane phase was saponified in 10% alcoholic KOH, acidified, and extracted three times with 3 ml of heptane or diethyl ether. The pooled extracts were evaporated to dryness in counting vials and the liquid scintillator was added. Incorporation of label into glyceride-glycerol was determined by subtraction of the counts incorporated into fatty acids from the total radioactivity in the initial heptane layer. FFA in the medium and tissues were determined by the method of Dole (10).

**Measurement of Sucrose and L-Arabinose Space.** The incubation of segments of epididymal fat was similar to that described above, but the substrates were sucrose- $^{14}\text{C}$ , 1 mg/ml (0.2  $\mu\text{c}$ /ml), or L-arabinose-1- $^{14}\text{C}$ , 1 mg/ml (0.2  $\mu\text{c}$ /ml). The tissues were incubated for 30 min at 37°C and then carefully removed, blotted, and weighed in tared glass liquid scintillation vials. The tissues were dried to constant weight and then digested at 60°C in closed counting vials containing 1.0 ml of Hyamine

hydroxide. A detailed description of the procedure and calculations has been presented elsewhere (11).

### Materials

All radiochemicals were obtained from New England Nuclear Corp. (Boston, Mass.) and were claimed to be chromatographically pure. L-Arabinose was purchased from the National Bureau of Standards. Nicotinic acid (Nutritional Biochemicals Corporation, Cleveland, Ohio) was diluted in saline before use. Propranolol (Inderal, Ayerst Laboratories, New York), supplied by Dr. Sahigian-Edwards, was diluted with 0.15 M saline before use. Epinephrine hydrochloride (Parke, Davis & Co., Detroit, Mich.) was diluted from vials containing 1 mg/ml. Ouabain was purchased from Calbiochem, Los Angeles, Calif.

## RESULTS

The production of  $^{14}\text{CO}_2$  and FFA under basal conditions showed considerable variation from one experiment to the next (Table 1). In previous studies (12) we had found that the variation in day-to-day basal activity of adipose tissue was greater than the variation within any given experiment, and for this reason we routinely studied control tissues with each experiment. Epinephrine stimulated the accumulation of FFA within the fat pad and increased the release of FFA into the medium (Table 1). The conversion of glucose- $^{14}\text{C}$  to  $^{14}\text{CO}_2$  in adipose tissue was also enhanced by epinephrine; the effect was larger with glucose-6- $^{14}\text{C}$  than with glucose-1- $^{14}\text{C}$  (Table 2). Even when lipolysis was reduced or abolished by various inhibitors, the stimulation of  $^{14}\text{CO}_2$  production from glucose- $^{14}\text{C}$  was affected slightly, if at all. Nicotinic acid increased the conversion of radioactive glucose to  $^{14}\text{CO}_2$  without raising the concentration of FFA in the fat pad (Table 1). The effect of epinephrine on  $^{14}\text{CO}_2$  formation was not diminished in the presence of nicotinic acid, although the concentration of FFA in the fat pad rose only half as much as in control tissues incubated with epinephrine alone. Propranolol prevented the rise in FFA in the fat pads in the presence of epinephrine. The increase in glucose oxidation was still evident although reduced by half in these tissues. Thyroidectomy, like the other treatments studied, diminished the lipolytic response to epinephrine, but had no effect at all on the stimulation of  $\text{CO}_2$  formation from glucose.

Epinephrine increased the formation of  $^{14}\text{CO}_2$  from glucose regardless of the position of the labeled carbon and regardless of whether the tissues were obtained from normal or thyroidectomized rats (Table 2). However, lipolysis, as assessed by the release of glycerol, was greater in the tissues from the normal rats. When adipose tissue was incubated with pyruvate- $^{14}\text{C}$ , epinephrine had no

TABLE 1 EFFECT OF EPINEPHRINE ON LIPOLYSIS AND ON THE INCORPORATION OF RADIOACTIVITY FROM GLUCOSE-<sup>14</sup>C INTO CO<sub>2</sub>\*

Expt. No.	Rat Condition	Addition to Medium	FFA				<sup>14</sup> CO <sub>2</sub> Production	
			Medium		Tissue		C	E
			C	E†	C	E	C	E
			<i>μeq/g</i>				<i>cpm/mg/hr</i>	
1	Normal	None	2.34 ± 0.15†	9.15 ± 0.70	2.88 ± 0.18	7.53 ± 0.66	5.58 ± 0.71	16.34 ± 1.85
		Ouabain§	1.61 ± 0.27	5.26 ± 0.77	2.76 ± 0.22	4.33 ± 0.36	7.14 ± 1.32	20.09 ± 1.80
		Nicotinic Acid¶	1.92 ± 0.50	2.84 ± 0.19	2.38 ± 0.20	3.46 ± 0.21	10.38 ± 1.17	19.15 ± 1.62
2	Normal	None	-1.36 ± 0.67	11.8 ± 1.26	6.3 ± 0.48	17.6 ± 0.80	14.4 ± 1.9	35.8 ± 2.8
		Propranolol	-0.92 ± 0.42	-1.46 ± 0.35	5.4 ± 0.40	5.0 ± 0.33	10.8 ± 1.3	20.3 ± 1.3
3	Normal	None	0.39 ± 0.21	4.56 ± 0.50	4.47 ± 0.25	9.23 ± 0.40	1.94 ± 0.12	4.54 ± 0.65
	Hypothyroid	None	-0.55 ± 0.14	0.88 ± 0.19	3.94 ± 0.31	6.57 ± 0.50	2.39 ± 0.25	5.86 ± 0.84

C, control; E, epinephrine.

\* Pieces of adipose tissue were incubated for 60 min at 37°C in Krebs-Ringer bicarbonate buffer containing 40 mg/ml albumin and 5.6 mM uniformly labeled glucose-<sup>14</sup>C.

† Epinephrine 1 μg/ml except for experiment with propranolol, where 30 μg/ml was used.

‡ Mean ± SEM for eight observations.

§ Ouabain, 150 μg/ml.

¶ Nicotinic acid, 10 μg/ml.

|| Propranolol, 100 μg/ml.

TABLE 2 EFFECT OF EPINEPHRINE ON LIPOLYSIS AND ON THE CONVERSION OF RADIOACTIVITY FROM GLUCOSE AND PYRUVATE INTO CO<sub>2</sub> AND GLYCERIDE-GLYCEROL BY ADIPOSE TISSUE OF NORMAL AND HYPOTHYROID RATS\*

	Glycerol Release			
	Normal		Hypothyroid	
	<i>μmoles/g/hr</i>			
Control	1.56 ± 0.29†		1.90 ± 0.45	
Epinephrine‡	7.69 ± 0.64		4.61 ± 0.65	
	Incorporation of Radioactivity			
	Normal		Hypothyroid	
	CO <sub>2</sub>	Glyceride-Glycerol	CO <sub>2</sub>	Glyceride-Glycerol
	<i>cpm/mg/hr</i>			
Glucose-1- <sup>14</sup> C				
Control	8.7 ± 0.7†	10.0 ± 3.2	13.2 ± 3.3	23.8 ± 2.6
Epinephrine	24.0 ± 8.7	71.0 ± 7.6	22.4 ± 4.3	65.5 ± 7.5
Glucose-6- <sup>14</sup> C				
Control	3.6 ± 0.5	15.2 ± 3.0	5.4 ± 1.2	18.7 ± 2.4
Epinephrine	20.4 ± 1.3	63.2 ± 9.4	20.4 ± 3.7	50.3 ± 3.6
Pyruvate-1- <sup>14</sup> C				
Control	49.3 ± 5.7	11.0 ± 1.5	54.8 ± 7.6	15.1 ± 2.0
Epinephrine	53.2 ± 7.2	11.0 ± 1.2	51.5 ± 6.2	16.6 ± 2.7
Pyruvate-3- <sup>14</sup> C				
Control	9.1 ± 2.6	29.4 ± 4.0	6.0 ± 0.6	29.1 ± 5.0
Epinephrine	7.2 ± 0.5	26.8 ± 4.8	6.4 ± 0.5	33.1 ± 2.6

\* Pieces of epididymal fat were incubated 60 min at 37°C in 1 ml of Krebs-Ringer bicarbonate containing 40 mg/ml of albumin and 1 mg/ml of glucose uniformly labeled or labeled specifically at C-1 or C-6, or pyruvate labeled either at C-1 or C-3.

† Mean ± SEM for eight observations per group.

‡ Epinephrine, 1 μg/ml.

effect on the production of <sup>14</sup>CO<sub>2</sub> from either carboxyl or methyl-labeled carbon. The radioactivity incorporated into glyceride-glycerol was similar in adipose tissue from normal and hypothyroid rats, whether or not epinephrine was present in the incubation medium. However, only half as much radioactivity appeared in glyceride-glycerol

in the tissues incubated with pyruvate-1-<sup>14</sup>C as in those incubated with pyruvate-3-<sup>14</sup>C. More than five times as much radioactive pyruvate-1-<sup>14</sup>C was converted to <sup>14</sup>CO<sub>2</sub>.

To investigate the possibility that epinephrine might increase the metabolism of glucose by accelerating its



entry into adipocytes, we incubated segments of adipose tissue in the presence of L-arabinose-1-<sup>14</sup>C and measured its distribution in the water of adipose tissue. L-Arabinose was chosen for this purpose because the steric configuration of its first 3 carbons is identical to that of glucose and because L-arabinose shares a common transport system with glucose in red blood cells and muscle (13). The distribution of uniformly labeled sucrose-<sup>14</sup>C in replicate tissues was taken as a measure of the extracellular volume. Fig. 1 shows that epinephrine significantly increased the percentage of tissue water in which radioactive arabinose was distributed. Similar results were obtained for adipose tissue from hypothyroid animals (Fig. 2). Epinephrine was not observed to have these effects on the distribution of sucrose. The increase in arabinose space caused by epinephrine (1 μg/ml) was comparable to that obtained with 0.1 mU/ml of insulin (Fig. 1).

### DISCUSSION

The present experiments have shown a dissociation between changes in the concentration of FFA within adipose tissue and the stimulation of CO<sub>2</sub> production from radioactively labeled glucose. Ouabain, nicotinic acid, and thyroidectomy all reduced the accumulation of

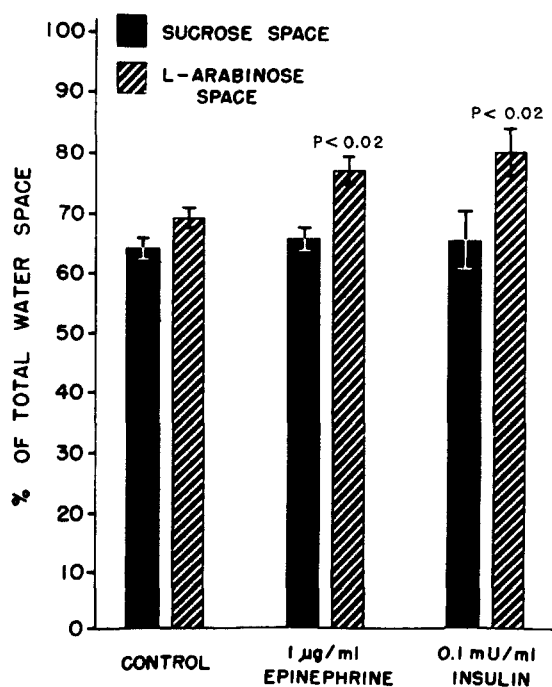


FIG. 1. Effect of epinephrine and insulin on the distribution of sucrose-<sup>14</sup>C and L-arabinose-<sup>14</sup>C in the water of adipose tissue of normal rats. 50–100 mg pieces of adipose tissue were incubated in 1 ml of Krebs–Ringer buffer containing 1 mg/ml of sucrose-<sup>14</sup>C or L-arabinose-<sup>14</sup>C for 30 min. Each bar represents the mean ± SEM for eight observations. Both insulin and epinephrine significantly increased the volume of distribution of L-arabinose.

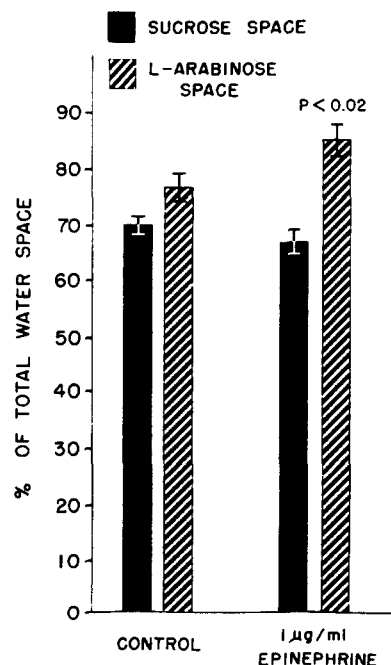


FIG. 2. Effect of epinephrine on the sucrose and L-arabinose space of adipose tissue from hypothyroid rats. Incubations were similar to those in Fig. 1. Each bar represents the mean ± SEM for eight observations.

FFA in segments of adipose tissue incubated with epinephrine, but none of these experimental maneuvers diminished the stimulation of CO<sub>2</sub> production from glucose. This dissociation was brought out most clearly in these and previous experiments (5) by the use of propranolol, which completely prevented the rise in FFA in adipose tissue, but did not prevent the increase in glucose oxidation. Although others have suggested (2, 3) that the pattern of glucose utilization in adipose tissue evoked by epinephrine might result from increased levels of FFA in the fat cell, we must conclude that at least part of the stimulation of glucose oxidation by epinephrine is independent of changes in tissue levels of FFA.

The results of our studies with radioactively labeled intermediates are consistent with the suggestion that epinephrine alters glucose utilization by affecting some reaction prior to the production of pyruvate. Indeed, the effect would appear to precede formation of triose-phosphates. Epinephrine augmented the incorporation of radioactivity from glucose into glyceride-glycerol. If the effect of epinephrine had been on the conversion of triose-phosphates into glycerol 3-phosphate, one might have expected epinephrine to increase the incorporation of radioactivity from pyruvate into glyceride-glycerol, but this did not happen.

Studies with pyruvate labeled at the 1 or 3 position permit us to estimate the relative contribution of different pathways of pyruvate metabolism to the production of <sup>14</sup>CO<sub>2</sub>. Radioactive pyruvate that enters the

adipocyte may be metabolized by two routes: formation of acetate by decarboxylation, or formation of oxaloacetate by the addition of a carboxyl group (14). The  $^{14}\text{CO}_2$  that is evolved may arise from direct decarboxylation of pyruvate, from the decarboxylation of oxaloacetate after randomization of the label (15), or from the Krebs cycle. Our data suggest that more than half of the  $^{14}\text{CO}_2$  arises from the direct decarboxylation of pyruvate, and that this reaction may contribute at least four times as much  $^{14}\text{CO}_2$  as the Krebs cycle (see Appendix). Our data also show that epinephrine and thyroidectomy (16) do not affect any of these reactions.

The experiments with L-arabinose- $^{14}\text{C}$  suggest that epinephrine may accelerate the penetration of sugar into adipocytes and thus make glucose available to the intracellular enzymatic machinery. This effect of epinephrine on the entry of glucose into the adipocyte is in harmony with the earlier studies which showed that epinephrine increased the permeability of rat diaphragm (17) and frog sartorius muscle (18, 19) to sugars. Our findings are in disagreement with the conclusions of Rodbell (20), who reported that insulin stimulated glucose transport in adipose cell ghosts. Neither epinephrine nor ACTH had this effect, but neither epinephrine nor ACTH stimulated the conversion of glucose to  $\text{CO}_2$  in his preparation. Thus, adipose cell ghosts apparently differ from intact adipose cells or tissue in their response to epinephrine, a finding which suggests that some cellular component essential for the effects of epinephrine on glucose metabolism is lost in the preparation of the ghost.

Although both insulin and epinephrine increase the utilization of glucose by adipose cells, presumably by increased entry of glucose, two quite different patterns are produced. Insulin has been repeatedly shown to increase the synthesis of long-chain fatty acids (21) while epinephrine generally depresses lipogenesis (1, 2). Insulin significantly stimulated  $\text{CO}_2$  production through the pentose cycle whereas epinephrine reduced the activity of this pathway. One explanation for the differences in glucose oxidation produced by epinephrine and insulin may be found in the observations that epinephrine increases the intracellular concentration of cyclic AMP (22) and insulin decreases it (23). Theophylline and dibutyryl cyclic AMP produce a pattern of glucose utilization similar to epinephrine but do not increase glucose uptake. Furthermore, dibutyryl cyclic AMP produces these effects at concentrations too low to increase lipolysis (24). Since epinephrine and insulin both augment the entry of glucose into the adipocytes, the differences they produce in the metabolism of glucose may result, at least in part, from the differences in intracellular concentration of cyclic AMP. For example, cyclic AMP activates phosphofructokinase in adipose tissue (25) and might thus lower the concentration of glucose-6-phos-

phate. Such a reduction in glucose-6-phosphate, which is known to be produced by epinephrine (26), might signal carrier-mediated transport of glucose. Further experiments will be necessary to determine whether or not the effects of epinephrine on glucose transport are secondary to changes in cyclic AMP.

Thyroidectomy has been shown by several groups of investigators (6, 27-29) to decrease the lipolytic effects of epinephrine on adipose tissue, and the present studies confirm this finding. Our data show, in addition, that the impaired lipolytic response does not extend to the effect of epinephrine on glucose utilization (6). Particularly important for the present series of observations is the fact that adipose tissue from hypothyroid rats shows an increase in the intracellular distribution of L-arabinose in the presence of epinephrine, just as does the adipose tissue from normal rats. These observations on adipose tissue from rats with different degrees of sensitivity to the lipolytic effects of epinephrine support our thesis that the effects of epinephrine on glucose oxidation involve a change in entry of glucose into the adipocyte, and are not secondary to the accumulation of FFA within the adipose cell or in the incubation medium.

#### APPENDIX

The relative quantities of radioactivity from pyruvate-1- $^{14}\text{C}$  and pyruvate-3- $^{14}\text{C}$  incorporated into glyceride-glycerol will depend on the extent of randomization in oxaloacetate (30). With no randomization the yields of radioactivity in glyceride-glycerol would be identical; with complete randomization half as much radioactivity would be incorporated into glyceride-glycerol from pyruvate-1- $^{14}\text{C}$  as from pyruvate-3- $^{14}\text{C}$ . The data in Table 2 indicate nearly complete randomization and support the conclusion of Leveille (30) that randomization in oxaloacetate is essentially complete. With complete randomization in oxaloacetate, the quantity of radioactivity from pyruvate-1- $^{14}\text{C}$  that appears in  $^{14}\text{CO}_2$  would equal the amount that appears as glyceride-glycerol (i. e., 11 cpm/mg per hr in the normal and 15 cpm/mg per hr in hypothyroid). By subtracting these numbers from the  $^{14}\text{CO}_2$  produced by tissues incubated with pyruvate-1- $^{14}\text{C}$  we can estimate the quantity of  $\text{CO}_2$  produced by decarboxylation of pyruvate and in the tricarboxylic acid (TCA) cycle ( $49.3 - 11.0 = 38.3$  cpm/mg per hr in the normal and  $54.8 - 15.1 = 39.7$  cpm/mg per hr in the hypothyroid group). An estimate of the contribution of the TCA cycle can be derived from the  $\text{CO}_2$  formed by tissues incubated with pyruvate-3- $^{14}\text{C}$ . Radioactivity from pyruvate-3- $^{14}\text{C}$  could enter the TCA cycle after entering the pool of acetyl CoA or after forming oxaloacetate. If one assumes that there is no pyruvate decarboxylation, then all of the  $^{14}\text{CO}_2$  produced from pyruvate-3- $^{14}\text{C}$  should come from the entry of oxaloacetate into the TCA cycle. This assumption provides a maximum estimate for the  $^{14}\text{CO}_2$  produced. Similar quantities of  $^{14}\text{CO}_2$  would arise by the entry of pyruvate-1- $^{14}\text{C}$  into the TCA cycle as oxaloacetate. By subtracting the  $^{14}\text{CO}_2$  formed from decarboxylation of oxaloacetate and from the

oxidation of oxaloacetate in the TCA cycle we can estimate the minimal contribution from pyruvate decarboxylation. This would be 29 cpm/mg per hr for the normal and 33 cpm/mg per hr in the hypothyroid group. This quantity of CO<sub>2</sub> is the minimum amount that one would expect to arise from decarboxylation of pyruvate directly and is four to five times the quantity formed in the TCA cycle. A similar conclusion on the importance of pyruvate decarboxylation as a source in CO<sub>2</sub> in adipose tissue has been arrived at by Katz, Landau, and Bartsch (31).

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