Conversion of glutamate carbon to fatty acid carbon via citrate in rat epididymal fat pads

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SUMMARY The incorporation of the C^{14} of glutamate-2-C^{14} and glutamate-5-C^{14} into fatty acids by epididymal fat pads and liver slices suggests the operation of the following metabolic pathway: α-ketoglutarate → isocitrate → *cis*-aconitate → citrate → acetyl CoA + oxalacetate (referred to as the backward reaction of the Krebs cycle).

Fatty acid-C^{14} recoveries from glutamate-2-C^{14}, glutamate-5-C^{14}, and acetate-1-C^{14} in experiments with epididymal fat pads from rats fed a stock diet were increased by the addition of glucose to the incubation medium, and further increased by in vitro addition of insulin. From these experiments it was calculated that, of the total amount of glutamate metabolized via the Krebs cycle, 6% proceeded by way of the backward reaction in the absence of glucose, 17% in the presence of glucose, and 35% when both glucose and insulin were added to the incubation medium. Fatty acid-C^{14} yields from glutamate-5-C^{14} and acetate-1-C^{14} were highest when the epididymal fat was taken from rats that had been fasted and then refed a 60% glucose diet. The in vitro addition of glucose or glucose plus insulin to the latter type of fat pad had no appreciable effect on the fatty acid-C^{14} yields.

In previous reports (1, 2) we demonstrated that the C^{14} of glutamate-2-C^{14} and of glutamate-5-C^{14} appears in fatty acids when either is incubated with lactating rat mammary gland slices. If the labeled glutamates had been metabolized via α-ketoglutarate, succinate, fumarate, malate, oxalacetate, pyruvate, and acetyl CoA (a pathway that we have referred to as the forward reaction of the Krebs cycle), then carbon atoms 2 and 5 of the glutamate would have been lost as CO_2 and would not have appeared in the isolated fatty acid fraction. We therefore concluded that a portion of the glutamate must have been metabolized via the following backward reaction of the Krebs cycle: α-ketoglutarate → isocitrate → *cis*-aconitate → citrate → acetyl CoA + oxalacetate. The oxalacetate can also, via pyruvate, give rise to acetyl CoA. A detailed discussion of these pathways in lactating rat mammary gland appears in (2). A similar mechanism for glutamate metabolism in the perfused liver has been proposed by D'Adamo and Haft (3).

Methods have been presented (2) for estimating the total amount of α-ketoglutarate metabolized in the Krebs cycle via both the forward and backward reactions and via the backward reaction alone. In the case of lactating rat mammary gland slices, the addition of glucose to the incubation medium was shown to increase the percentage of the glutamate metabolized in the Krebs cycle via the backward reaction.

The present report deals with the relative importance of the two reactions of the Krebs cycle in another rat tissue, the epididymal fat pad. The influence of the in vitro addition of glucose and insulin on these pathways in adipose tissue was also studied.

EXPERIMENTAL METHODS

Preparation of Tissue and Incubation Procedures

Male rats of the Long-Evans strain, weighing 225–275 g, and raised and maintained on an adequate stock diet (Wayne Lab-Blox), were used. The animals were killed by a blow on the head, after which the neck was cut and the spinal medulla destroyed in order to stop reflex activities. The abdomen was opened, and the epididymal fat pads were quickly excised and cut into four pieces of approximately 100 mg each. Each piece was quickly weighed on a torsion balance, and immediately thereafter incubated as described below.

All incubations were carried out in specially designed flasks provided with a center well and closed with a

See (2) for paper I in this series.

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TABLE 1 EFFECT OF GLUCOSE ON THE METABOLISM OF GLUTAMATE AND ACETATE IN EPIDIDYMAL ADIPOSE TISSUE

Pieces of epididymal fat pads weighing about 100 mg, and obtained from normal rats fed a stock diet were incubated at 37° for 90 min in 2.5 ml of Krebs-Henseleit bicarbonate buffer (pH 7.3–7.4) containing labeled and unlabeled substrates as indicated below. Gas phase: 95% O_2–5% CO_2. The medium contained 10 μmoles of sodium acetate and 5 μmoles of DL-glutamate. When glucose was present, its concentration was 10 μmoles/ml. The values are expressed as mμatoms of C^{14} incorporated per 100 mg tissue. Other experimental details are given in text.

C^{14} Recovered As	Rat No.	Glutamic Acid-1-C^{14}			Glutamic Acid-2-C^{14}			Glutamic Acid-5-C^{14}			Acetate-1-C^{14}		
		−Glucose	+Glucose	Glucose Effect	−Glucose	+Glucose	Glucose Effect	−Glucose	+Glucose	Glucose Effect	−Glucose	+Glucose	Glucose Effect
		mμmoles	*mμmoles*	%	*mμmoles*	*mμmoles*	%	*mμmoles*	*mμmoles*	%	*mμmoles*	*mμmoles*	%
Fatty acids	1	0	0		0.6	7.1	+1083	2.3	12.7	+452	44	103	+134
	2	0	0		0.6	1.1	+83	0.6	23.3	+3783	11.3	103	+812
	3	0	0		1.6	14.2	+788	4.8	18.6	+288	145	274	+89
	4	0	0		0.4	3.9	+875	0.7	13.5	+1829	11.8	75.5	+540
	5	0	0		1.4	3.9	+179	0.8	4.2	+425	4.4	66.0	+1400
	6	0	0		1.1	1.4	+27	0.2	4.2	+2000	7.1	93.6	+1218
Fatty acids	Mean						+506			+1462			+699
CO_2	1	181	213	+18	121	145	+18	77	121	+57	104	111	+7
	2	112	208	+86	109	122	+12	128	190	+48	67	98	+46
	3	159	265	+67	256	263	+3	149	186	+25	199	106	−47
	4	84	138	+64	56	79	+41	57	71	+25	59	62	+5
	5	109	130	+19	65	103	+58	54	71	+31	53	78	+47
	6	92	194	+111	68	122	+79	64	64	0	70	75	+7
CO_2	Mean			+61			+35			+31			+11

rubber serum stopper (4). The incubation medium consisted of 2.5 ml of the Krebs-Henseleit bicarbonate buffer (pH 7.3–7.4) (5) containing 5 μmoles of DL-glutamate and 10 μmoles of sodium acetate. Twenty-five micromoles of glucose and/or 0.25 unit of insulin were added to the incubation medium, as indicated in the tables, along with the labeled substrates used. The incubation media were equilibrated with a 95% O_2–5% CO_2 gas mixture at 37° before the incubations. The tissues were placed in the medium and the incubation vessels were flushed with the gas mixture for 15–20 sec. The flasks were mechanically agitated at 37°. At the end of the incubation period the tissue was inactivated by addition of 0.2 ml of 5 N H_2SO_4.

Because of the very small amount of acetate taken up by each 100-mg portion of adipose tissue, acetate uptakes were measured in separate experiments in which larger amounts of tissue were used as described in Fig. 1. The other experimental conditions were unchanged.

Analytical Procedures

The analytical procedures were the same as those described in previous reports (2, 6, 7). The fatty acids isolated from adipose tissue were methylated, and analyzed in that form by gas–liquid chromatography as described in (7).

Materials

DL-Glutamic acid-1-C^{14} and acetate-1-C^{14} were obtained from New England Nuclear Corporation, Boston, Mass.; DL-glutamic acid-2-C^{14} from Tracerlab, Boston, Mass.; and DL-glutamic acid-5-C^{14} from Isotope Specialties, Burbank, Calif. Glucagon-free, crystalline zinc-insulin (24.5 U/mg) was a gift from Dr. W. R. Kirtley, Eli Lilly and Company, Indianapolis, Ind. It was dissolved in 0.0017 N HCl to yield a concentration of 25 units/ml. The composition of the 60% glucose diet is described in (8).

RESULTS

As noted by other workers (9–11), the metabolic activity of epididymal fat pads excised from different rats varies widely. Hence, whenever a new condition was studied, control tissue was taken from the same rat.

Effects of Glucose and Insulin

Adipose tissue incorporated the carbons from the 2- and 5-positions of glutamate into fatty acids (Table 1). When glucose was added to the incubation medium, this incorporation was increased, except for one case, from 1.8- to 40-fold. Under the same incubation conditions, glucose increased the conversion of the C^{14} of acetate-1-C^{14} to fatty acids from about 2- to 14-fold. In 5 of 6 experiments the glucose effect was more pronounced on the C^{14}-fatty acid synthesis from glutamate-5-C^{14} than on that from acetate-1-C^{14}. No radioactivity was recovered in the fatty acid fraction when glutamic acid-1-C^{14} served as substrate, regardless of whether glucose was added to the medium (Table 1).

TABLE 2 EFFECTS OF INSULIN IN THE PRESENCE OF GLUCOSE ON THE METABOLISM OF GLUTAMATE AND ACETATE BY EPIDIDYMAL ADIPOSE TISSUE

In addition to 10 μmoles of sodium acetate and 5 μmoles of DL-glutamate, the medium contained 25 μmoles of glucose in a total volume of 2.5 ml. When insulin was added, its concentration was 0.1 unit/ml. The values are expressed as mμatoms of C^{14} incorporated per 100 mg tissue. All other experimental conditions are given in Table 1 and in the text.

C^{14} Recovered as	Rat No.	Glutamic Acid-1-C^{14}			Glutamic Acid-2-C^{14}			Glutamic Acid-5-C^{14}			Acetate-1-C^{14}		
		−Insulin	+Insulin	Insulin Effect	−Insulin	+Insulin	Insulin Effect	−Insulin	+Insulin	Insulin Effect	−Insulin	+Insulin	Insulin Effect
		mμmoles	*mμmoles*	%	*mμmoles*	*mμmoles*	%	*mμmoles*	*mμmoles*	%	*mμmoles*	*mμmoles*	%
Fatty acids	7	0	0		3.2	4.8	+50	9.1	19.9	+119	104.1	101.8	−2
	8	0	0		4.9	19.0	+288	10.9	30.6	+181	136.9	282.2	+106
	9	0	0		2.0	6.2	+210	5.2	18.4	+254	39.5	127.7	+223
	10	0	0		8.3	59.0	+611	44.2	84.0	+90	341	609	+79
	11	0	0		14.6	61.0	+318	27.8	121.5	+337	238	665	+179
	12	0	0		7.1	28.8	+306	39.5	35.7	−10	182	227	+25
Fatty acids	Mean						+297			+162			+102
CO_2	7	146	157	+8	92	85	−8	66	67	+1	70	64	−9
	8	187	154	−18	116	121	+4	91	71	−22	87	79	−9
	9	132	159	+20	80	85	+6	60	54	−10	66	55	−17
	10	295	292	−1	199	208	+4	258	180	−30	154	98	−36
	11	192	247	+29	202	210	+4	150	189	+26	135	98	−27
	12	174	313	+80	153	151	−1	149	153	+3	90	62	−31
CO_2	Mean			+20			+2			−5			−22

While addition of glucose to the incubation medium had no consistent effect on $C^{14}O_2$ production from acetate-1-C^{14}, such addition did increase the $C^{14}O_2$ production from the labeled glutamates (Table 1).

The effects of insulin, in the presence of glucose, on the metabolism of acetate-1-C^{14} and glutamate-1-, -2-, and -5-C^{14} are shown in Table 2. The C^{14} incorporation into fatty acids from both glutamate-2-C^{14} and -5-C^{14}, as well as from acetate-1-C^{14} was increased, the increase being more pronounced for the glutamate carbons than for the acetate carbon. While insulin decreased somewhat the $C^{14}O_2$ production from acetate-1-C^{14}, it had no consistent effect on the $C^{14}O_2$ yields from glutamate, regardless of the position of the C^{14} in the glutamate.

Acetate uptake was determined, with the results shown in Fig. 1. Since the uptake was proportional to the amount of tissue incubated, the uptake by 100 mg of adipose tissue was estimated from the uptakes by the larger amounts of tissue. Acetate uptake by 100 mg of epididymal adipose tissue incubated for 90 min was calculated to be 179 mμmoles in the absence of glucose and insulin in the medium, 288 mμmoles when glucose was present, and 547 mμmoles when both glucose and insulin were added to the incubation mixture. We assume that these values for acetate uptake approximate closely the production of acetyl CoA from added acetate by the tissue, as acetyl CoA formation is the first step in acetate metabolism.

We have used these acetate uptake values and the mean values shown in Tables 1 and 2 to calculate the quantitative significance of the backward reaction of the Krebs cycle in epididymal adipose tissue by means of the formulas previously presented (2).[1] The results of these calculations are given in Table 3. Addition of glucose to the medium increased the amount of glutamate carbon utilized via the backward reaction of the Krebs cycle, both when expressed as mμmoles and when expressed as a percentage of the total amount of glutamate metabolized by the Krebs cycle reactions (forward plus backward). The addition of insulin to incubation media containing glucose resulted in a 2-fold increase in the participation of the backward reaction in glutamate metabolism.

Effect of Previous Nutritional State of the Rat

The incorporation of the C^{14} of glutamate-5-C^{14} and of acetate-1-C^{14} into fatty acids was greater in the experi-

[1] C^{14}-acetyl CoA from glutamic acid-2-C^{14} =

$$\frac{\text{Acetyl CoA from acetate} \times \text{FA from glutamic acid-2-C}^{14}}{\text{FA from acetate}};$$ and,

C^{14}-acetyl CoA from glutamic acid-5-C^{14} =

$$\frac{\text{Acetyl CoA from acetate} \times \text{FA from glutamic acid-5-C}^{14}}{\text{FA from acetate}},$$

where FA stands for fatty acid recoveries.

The total amount of glutamate metabolized in the Krebs cycle was estimated as: $C^{14}O_2$ from glutamic acid-1-C^{14} plus 0.5 (acetyl CoA from glutamic acid-5-C^{14} minus acetyl CoA from glutamic acid-2-C^{14}). The assumptions upon which these calculations are based are given in (2).

These calculations were based on the assumption that acetyl CoA, formed by (*a*) activation of acetate taken up from the medium (*b*) citrate-cleavage, or (*c*) pyruvate decarboxylation, mixes in a common pool and that its subsequent fate is independent of its origin.

TABLE 3 QUANTITATIVE EVALUATION OF THE BACKWARD REACTION OF THE KREBS CYCLE IN EPIDIDYMAL ADIPOSE TISSUE

Glutamate Metabolism	Addition to Incubation Medium		
	None*	Glucose†	Glucose plus Insulin‡
Via backward reaction, mμ-moles per 100 mg	8	35	84
Total (backward plus forward), mμmoles per 100 mg	125	202	238
% of total via backward reaction	6	17	35

* The fatty acid recoveries used for these calculations are shown in Table 1.

† The fatty acid recoveries used for these calculations are shown in Tables 1 and 2.

‡ The fatty acid recoveries used for these calculations are shown in Table 2.

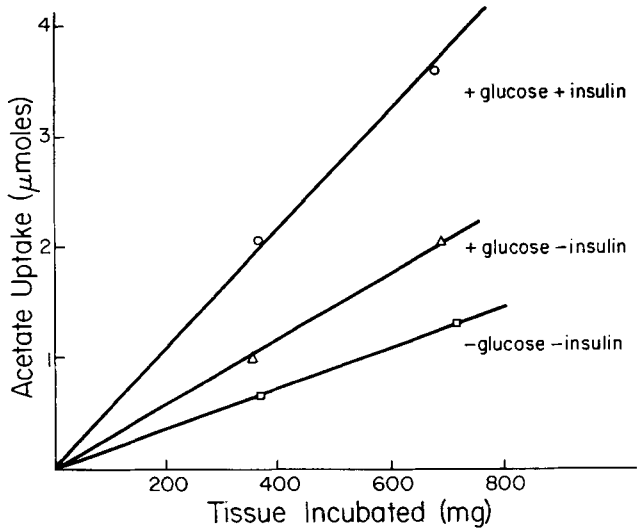

FIG. 1. Acetate Uptake by Rat Epididymal Adipose Tissue. Adipose tissues from 8 animals were pooled and incubated, so that tissue from all animals were represented in each flask. The incubation medium was 2.5 ml of Krebs-Henseleit bicarbonate buffer (pH 7.3–7.4) containing 10 μmoles of sodium acetate-1-C^{14} and 5 μmoles of DL-glutamate. To some flasks were added 25 μmoles of glucose and to others, 25 μmoles of glucose plus 0.25 unit of insulin. Gas phase: 95% O_2–5% CO_2. Incubation for 90 min at 37°. At the end of the incubation period the tissue was inactivated by addition of 0.5 ml of 5 N H_2SO_4. Acetate-1-C^{14} remaining in the medium was determined as described in (2). The acetate uptake (added acetate minus recovered acetate) was plotted as a function of the amount of tissue incubated. In flasks to which no adipose tissue was added, acetate did not disappear from the medium.

ments with rats fed the 60% glucose-containing diet than in those with rats fed the stock diet (compare Tables 1 and 2 with Table 4). In the absence of glucose, the incorporation of the C^{14} of glutamate-5-C^{14} into fatty acids by the adipose tissue from these rats was of the same order as that found when tissues of animals fed the stock diet were incubated in the presence of glucose and insulin. With acetate-1-C^{14} as the labeled substrate, and in the absence of glucose, the incorporation of C^{14} into fatty acids by fat pads excised from the fasted, refed rats was even higher than that observed when the pads from animals fed the stock diet were incubated with glucose and insulin (cf Tables 2 and 4).

With epididymal adipose tissue obtained from fasted, refed rats it was not possible to demonstrate the glucose (or glucose plus insulin) effect on C^{14} incorporation into fatty acids from acetate-1-C^{14} that we found in the experiments with animals fed the stock diet (cf Tables 1 and 2 with Table 4). Similarly, the effect of glucose on the incorporation of C^{14} from glutamate-5-C^{14} into fatty acids was not discernible.

The $C^{14}O_2$ production from acetate-1-C^{14} was of the same magnitude in the experiments with epididymal fat

TABLE 4 EFFECTS OF GLUCOSE AND INSULIN ON THE METABOLISM OF GLUTAMATE AND ACETATE BY EPIDIDYMAL ADIPOSE TISSUE FROM RATS FASTED AND REFED A DIET CONTAINING 60% GLUCOSE

The animals were fasted for 48 hr and then refed a 60% glucose diet (9) for 48 hr. The values are expressed as mμatoms of C^{14} incorporated per 100 mg tissue. Experimental conditions are given in Tables 1, 2, and in the text.

Rat No.	Labeled Substrate	mμmoles C^{14} Recovered in Fatty Acids				mμmoles C^{14} Recovered in $C^{14}O_2$			
		−Glucose −Insulin	−Glucose +Insulin	+Glucose −Insulin	+Glucose +Insulin	−Glucose −Insulin	−Glucose +Insulin	+Glucose −Insulin	+Glucose +Insulin
13	Glutamic acid-5-C^{14}	106		109	190	252		186	242
14		32	34	67	77	158		173	103
15		48	39	48	82	249	208	133	109
16		54	59	75	81	266	274	57	39
17		46	43	30	22	160	218	31	40
Mean	Glutamic acid-5-C^{14}	57	44	66	90	217	233	116	107
13	Acetate-1-C^{14}	443		904	970	156		100	108
14		755	724	508	636	84	95	60	59
15		651	841	617	547	129	131	75	61
16		748	955	1201	811	91	98	91	86
17		415	574	542	492	89	107	100	91
Mean	Acetate-1-C^{14}	602	774	754	691	110	108	85	81

pads from fasted, refed animals as in those with pads from rats fed the stock diet, and was not markedly influenced by the presence of glucose or glucose plus insulin in the incubation medium. Glutamic acid-5-C^{14}, however, yielded 2–3 times more $C^{14}O_2$ when incubated with fat pads excised from the fasted, refed rats in the absence of glucose than it did in similar experiments with adipose tissue from animals fed the stock diet. If either glucose or glucose plus insulin was added to the incubation mixture, the $C^{14}O_2$ recoveries from glutamate-5-C^{14} were of the same order in the experiments with rats fed the stock diet as in those with fasted, refed animals (Table 4).

The addition of insulin to the incubation medium in the absence of glucose induced no change in either the production of fatty acid-C^{14} or of $C^{14}O_2$ from glutamate-5-C^{14} or from acetate-1-C^{14} (Table 4).

Nature of the Fatty Acids Synthesized from Glutamate-2- and -5-C^{14}, and from Acetate-1-C^{14} by Adipose Tissue

The chain lengths of the fatty acids synthesized by adipose tissue from glutamate-2-C^{14}, -5-C^{14}, and acetate-1-C^{14} are shown in Table 5. The major portion of the C^{14} was found in the five fatty acids 16:0, 16:1, 18:0, 18:1, and 18:2. In general, the C^{14} labeling patterns of the fatty acids formed were similar, regardless of whether glutamate-2-C^{14}, -5-C^{14}, or acetate-1-C^{14} was used.

DISCUSSION

The observation that C^{14} from glutamic acid-2-C^{14} and -5-C^{14} is incorporated into fatty acids strongly suggests that what we refer to as the backward reaction of the Krebs cycle operates in adipose tissue. The additional finding that the C^{14} from glutamate-5-C^{14} was more readily incorporated into fatty acid than was that from glutamate-2-C^{14} indicates that neither the glyoxylate shunt (12) nor the glutamate β-methylaspartate pathway (13, 14) plays a significant role in the formation of acetyl CoA from glutamate in this tissue. It should be noted that Winegrad (15) has reported the incorporation of C^{14} from α-ketoglutarate-5-C^{14} into fatty acids in rat epididymal fat pads.

Because of the great variation in metabolic activity from one sample of adipose tissue to another, the extent of operation of the backward reaction was estimated on the basis of mean values. Using mean values from 6–12 experiments with epididymal fat pads excised from different rats in the equations previously reported (2), we calculated that, in the absence of added glucose, 6% of the glutamate metabolized in the Krebs cycle proceeded by the backward pathway. The corresponding value for the experiments in which glucose was added to the incubation medium was 17%. Previously we showed that, of the glutamate metabolized via the Krebs cycle by lactating rat mammary gland slices, 3–7% is handled by the backward reaction in the absence of glucose in the medium (2). The values calculated from the results of experiments in which glucose was added to the medium were between 20 and 30% (2).

Glucose metabolism of the epididymal fat pad is markedly accelerated by insulin (9, 10); thus the addition of insulin to the incubation mixture would be expected to enhance further the effects of added glucose. This was indeed found to be the case in our experiments. The percentage of glutamate metabolized via the backward reaction rose from 17 to 35 by addition of insulin to the medium.

Shapiro and Wertheimer (16) have shown that the incorporation of deuterium into the fatty acids of adipose tissue incubated with D_2O is increased if the tissue is obtained from rats that had been fasted and then refed a diet rich in carbohydrate. Lyon, Masri, and Chaikoff (17) and Tepperman and Tepperman (18) found a highly increased incorporation of acetate-1-C^{14} into the lipid fraction of liver slices from rats that had undergone a similar dietary treatment. Thus, it was of interest to investigate the effect of fasting plus refeeding on the metabolism of glutamate and acetate. The data presented here indicate that fatty acid synthesis by the epididymal adipose tissue obtained from the fasted, refed rats was operating at near-maximal capacity since the fatty acid recoveries from acetate-1-C^{14} and glutamate-5-C^{14} (*a*) were higher in the experiments with the adipose tissue of fasted, refed rats than in those with the tissues of rats fed the stock diet, even in the presence of glucose or glucose plus insulin, and (*b*) were not significantly influenced by addition of either glucose or glucose plus insulin in the experiments with the adipose tissue of fasted, refed rats.

TABLE 5 INCORPORATION OF C^{14} FROM C^{14}-LABELED GLUTAMATE AND ACETATE INTO FATTY ACIDS OF DIFFERENT CHAIN LENGTHS BY EPIDIDYMAL ADIPOSE TISSUE

Fatty Acid Chain Length*	C^{14} Fatty Acids from Glutamate-2-C^{14}	Glutamate-5-C^{14}	Acetate-1-C^{14}
	% of C^{14} recovered from GLC column		
<14	12	7	4
14:0	9	5	6
>14, <16	4	4	3
16:0, 16:1, <18	34	50	50
18:0, 18:1, 18:2	21	24	22
18:3	4	2	2
>18:3	15	9	13

* The figure after the colon represents the number of double bonds in the fatty acid.

Whether this level of fa 'ty acid synthesis in epididymal adipose tissue was caused by adaptive enzyme changes induced by the dietary treatment of the rats or by an accumulation of carbohydrate in the tissue cannot be ascertained from our data. It is well known that a high-carbohydrate diet increases the glycogen content of the adipose tissue (19), which might have brought about this effect.

Our finding that incorporation of the C^{14} of glutamate-5-C^{14} into fatty acids by epididymal adipose tissue from the fasted, refed rats did not decrease when glucose plus insulin was added to the incubation medium, whereas the $C^{14}O_2$ production decreased by about one-half, suggests that the percentage of glutamate carbon metabolized via the backward reaction is increased by the presence of glucose and insulin.

Because of the stereospecificity of the citrate-cleavage enzyme, glutamate-5-C^{14} will give rise to carboxyl-labeled acetyl CoA, and glutamate-2-C^{14} will yield oxalacetate-3-C^{14} via the backward reaction of the Krebs cycle. Oxalacetate-3-C^{14} may be converted to C^{14}-labeled acetyl CoA via pyruvate. Hence the yield of C^{14}-fatty acids from glutamate-5-C^{14} should be the same as that from glutamate-2-C^{14} if all of the oxalacetate were converted to acetyl CoA through pyruvate. However, in the presence of glucose, the values for the ratio (C^{14} FA from glutamate-5-C^{14})/(C^{14} FA from glutamate-2-C^{14}) were about 1.5 for lactating rat mammary gland slices (2), about 3 for epididymal adipose tissue, and considerably higher for liver slices. In the two experiments with liver slices from the rats fed the stock diet, the values for this ratio were about 17, while the value was 6 with the fasted, refed rat liver slices.[2] Such ratios indicate that a much smaller fraction of the oxalacetate produced by the citrate-cleavage enzyme is converted to acetyl CoA by the liver than by either the lactating rat mammary gland or by the epididymal adipose tissue.

[2] Unpublished observations.

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