Metabolism of D- and L-glyceraldehyde in adipose tissue: a stereochemical probe for glycerokinase activity

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ABSTRACT Distributions of ^{14}C have been determined in free glycerol, in glycerol from triglycerides, in glucose from glycogen, and in lactate after incubation of D-glyceraldehyde-3-^{14}C and L-glyceraldehyde-3-^{14}C with rat adipose tissue. The distributions are interpreted in terms of presently accepted possible reactions for the initial metabolism of glyceraldehyde.

Formation of glycerol-1-^{14}C from D-glyceraldehyde-3-^{14}C indicates that in adipose tissue glyceraldehyde is reduced to glycerol. Incorporation of ^{14}C from D-glyceraldehyde-3-^{14}C into carbon 3 of the glycerol of triglyceride indicates that D-glyceraldehyde is either phosphorylated or oxidized to D-glyceric acid, or both, in its initial metabolism. Incorporation of ^{14}C from L-glyceraldehyde-3-^{14}C into carbon 3 of glycerol indicates that L-glyceraldehyde is reduced to glycerol, which is phosphorylated and (or) converted to D-glyceric acid via L-glyceric acid. Some ^{14}C from L-glyceraldehyde-3-^{14}C is incorporated into carbon 1 of glycerol of triglycerides and carbon 4 of glycogen; the explanation for this incorporation is uncertain.

SUPPLEMENTARY KEY WORDS phosphorylation · reduction · glycerol · triglycerides · lactate · glycogen

THE INITIAL REACTIONS have not been established for the metabolism of glyceraldehyde by adipose tissue. By analogy with studies in rat liver (2) several reactions could exist. D-Glyceraldehyde (Fig. 1) could be (*a*) reduced to glycerol which could be phosphorylated to L-glycerophosphate and this could be dehydrogenated to form dihydroxyacetone phosphate, (*b*) phosphorylated to D-glyceraldehyde-3-phosphate, or (*c*) oxidized to D-glyceric acid which could be phosphorylated to phosphoglyceric acid. Since these phosphates are intermediates in the Embden–Meyerhof pathway, they would then be metabolized via this pathway.

L-Glyceraldehyde (Fig. 2) could be (*a*) reduced to glycerol which could be phosphorylated, (*b*) phosphorylated to L-glyceraldehyde-3-phosphate for which no further metabolism is known, or (*c*) oxidized to L-glyceric acid which via hydroxypyruvate could be converted to D-glyceric acid (3) with further metabolism as noted above.

The present study had two objectives. The first was to establish the absence or presence of these possible pathways in white adipose tissue of the rat. This has been achieved to some degree. The second objective was to use this information to quantify the extent of phosphorylation of glycerol by adipose tissue to form triglyceride. Other investigators who have concluded that phosphorylation of glycerol by adipose tissue is negligible have been unable to demonstrate glycerolkinase activity in homogenates of the tissue (4). However, such activity has recently been demonstrated (5). Phosphorylation has also been considered to be negligible because of the very small quantity of ^{14}C from glycerol-^{14}C incorporated by adipose tissue in vitro (4). Dilution of glycerol-^{14}C by endogenous glycerol, or failure of glycerol-^{14}C to penetrate into the tissue, could mean that greater phosphory-

Terminology: The numbering of the carbons of ^{14}C-labeled glycerol is that proposed by Hirschmann (1).

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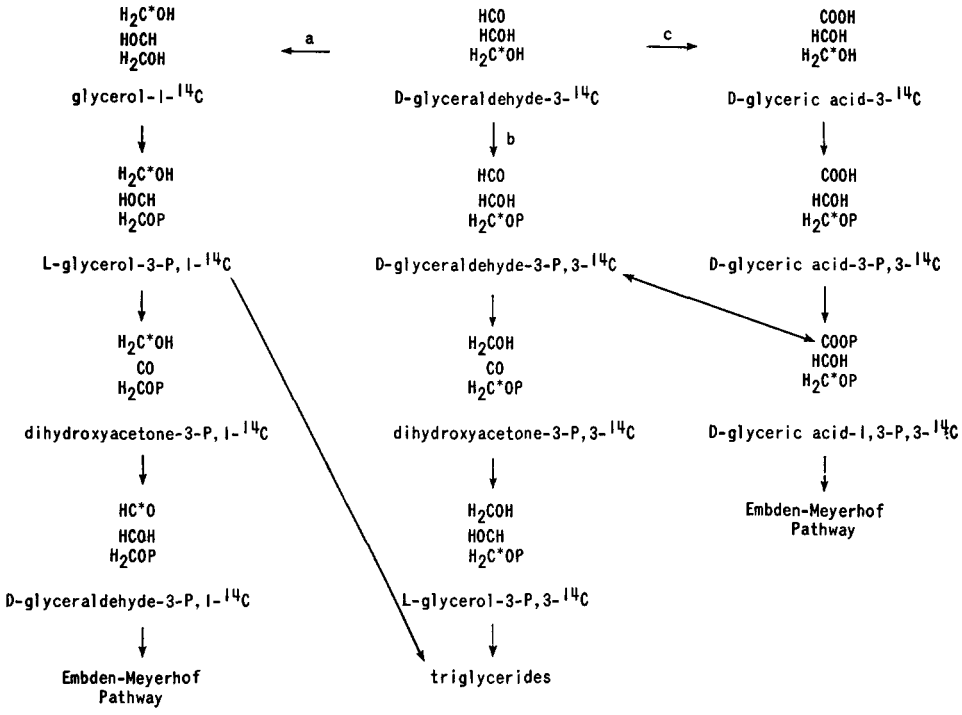

FIG. 1. Initial steps of D-glyceraldehyde metabolism with expected ^{14}C-labeling pattern indicated (*) for D-glyceraldehyde-3-^{14}C.

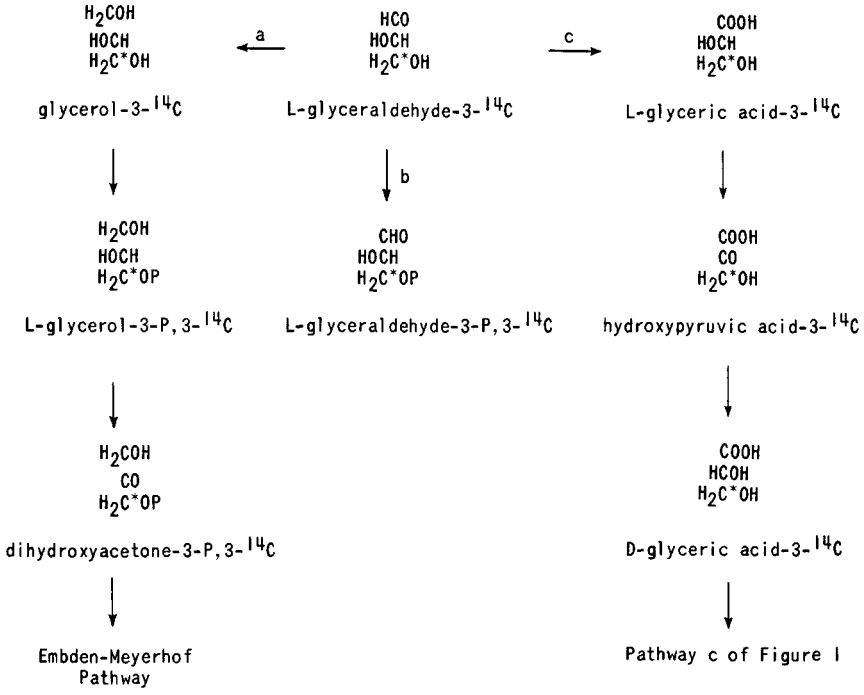

FIG. 2. Initial steps of L-glyceraldehyde metabolism with expected ^{14}C-labeling pattern indicated (*) for L-glyceraldehyde-3-^{14}C.

lation occurs than that concluded from such incorporation.

Our approach to measuring phosphorylation of glycerol to form triglyceride depends upon the fact that only via pathway *a* of Fig. 1, (phosphorylation of glycerol formed from D-glyceraldehyde), can carbon 3 of D-glyceraldehyde be incorporated into carbon 1 of triglyceride-glycerol. If it is assumed that glycerol is formed in this

manner in the fat cell and that a single pool of glycerol exists in the cell, the specific activity of carbon 1 of the glycerol that is free inside the cell and released from the cell will be the specific activity of the pool. The quantity of glycerol converted to triglyceride can then be estimated from this specific activity and the total ^{14}C incorporated into carbon 1 of glycerol of triglyceride. Thus in this approach, glycerol is formed inside the cell to eliminate the problems of restricted penetration and of dilution by endogenous substrate. The latter problem may still not be completely eliminated, if there is more than one metabolic pool of glycerol. In the experiments to be described, our expectation that ^{14}C from D-glyceraldehyde-3-^{14}C would be incorporated into carbon 1 of glycerol of triglyceride only via pathway *a* was not established, so that our second objective, estimating the extent of phosphorylation, was not achieved. However, we present the method since it is perhaps applicable to other tissues, substrates, and conditions.

MATERIALS AND METHODS

Materials

D- and L-Glyceraldehyde-3-^{14}C were prepared from D-fructose-6-^{14}C and L-sorbose-6-^{14}C respectively, by a small-scale modification of the method of Perlin and Brice (2, 6). The glyceraldehydes were purified by descending chromatography on Whatman 3MM paper in *n*-butanol–acetic acid–water 40:10:50 after addition of nonradioactive fructose and sorbose to dilute any unreacted, labeled hexose. The glyceraldehydes and hexoses were located on the chromatograms by means of appropriate standards run simultaneously. Despite the spreading of glyceraldehyde it was well separated from the hexoses. The R_f values were 0.23 for sorbose, 0.25 for fructose, and 0.40–0.63 for glyceraldehyde. The area of paper corresponding to the glyceraldehyde guide spots was removed and eluted with water, and the eluate was lyophilized. Essentially all the ^{14}C in the lyophilizate was established by degradation to be in carbon 3 of glyceraldehyde (2).

These preparations of D- and L-glyceraldehyde were used in the first four experiments, designated G1 through G4. The results of these experiments raised the possibility that dihydroxyacetone-1,3-^{14}C might be a contaminant in the preparations. Glyceraldehyde has been reported to partially isomerize to dihydroxyacetone during chromatography (7). By spectrometric assay (8), dihydroxyacetone could have been present to the extent of 5% although the assay is not specific and a similar percentage was obtained on assaying a commercial preparation of crystalline D,L-glyceraldehyde.

An additional purification step was introduced before the subsequent experiments. The preparations of D- and L-glyceraldehyde were chromatographed on Whatman 3MM paper in ethyl acetate–formic acid–water 70:20:10 (7). In this system the R_f values are 0.18 for glyceraldehyde and 0.52 for dihydroxyacetone. The area corresponding to the R_f of dihydroxyacetone contained 7% of the total radioactivity and yielded glycerol-1,3-^{14}C on incubation with adipose tissue. L-glyceraldehyde purified by this chromatographic step was checked before experiment G5 by rechromatographing a small aliquot. Only a single peak with the mobility of glyceraldehyde was present. Seven months later, before experiments G7, aliquots of the D- and L-glyceraldehyde preparations were chromatographed. D-glyceraldehyde had a single radioactive component, but approximately 10% of the total radioactivity of the L-glyceraldehyde preparation was in an area of the chromatogram corresponding to dihydroxyacetone. The entire samples of D- and L-glyceraldehyde were rechromatographed and the resulting preparations were used in experiment G7.

Incubation

Segments of epididymal fat pads from Wistar rats weighing 150–250 g were incubated in a modified Krebs bicarbonate buffer at 37°C (9). The rats were either fasted and refed or maintained on a stock diet (Purina Rat Chow, Ralston Purina Company, Inc., St. Louis, Mo.) (Table 1). Except for experiment G3, each flask contained 3 ml of medium and 1–1.5 g of tissue. D-glyceraldehyde-3-^{14}C was present at a concentration of 0.27, 0.5, or 2 mg/ml and L-glyceraldehyde-3-^{14}C at 0.5 mg/ml. Between 1 and 4 μc of ^{14}C was added to each flask. Incubation was for 2 or 3 hr. Glucose (2 mg/ml), gelatin (2 mg/ml), bovine albumin (40 mg/ml), and epinephrine (20 μg/ml) were added as indicated (Table 1).

Incubations were terminated by addition of perchloric acid. In some experiments the ^{14}C evolved as $^{14}CO_2$ was collected and assayed (9). The segments of fat pads were rinsed with water and immersed in chloroform–methanol 2:1. Glycogen was isolated from the residues remaining after extraction of the fat by digesting the residues in 30% KOH and precipitating the glycogen from the digests with ethanol. After hydrolysis of the glycogen the resulting glucose was degraded and the individual carbons, isolated as CO_2, were assayed for ^{14}C activity (9). The extracts containing the fat were washed by the method of Folch, Lees, and Sloane Stanley (10). The glycerol from the triglycerides was isolated (9), and was degraded with *Aerobacter aerogenes* to obtain the specific activity of each of its three carbons (11). In three experiments the total incorporation of ^{14}C into lipid and glycerol was determined as described previously (9).

TABLE 1 CONDITIONS OF INCUBATION

Exp. No.	Rat Diet	Optical Isomer	Glyceraldehyde Concentration	Incubation Time	Glucose 2 mg/ml	Albumin 40 mg/ml	Gelatin 2 mg/ml	Epinephrine 20 μg/ml
			mg/ml	*hr*				
1A	Fasted-refed	D	0.27	2	−	−	+	−
1B	Fasted-refed	D	0.27	2	+	−	+	−
2A	Stock	D	0.27	2	−	−	+	−
2B	Stock	D	0.27	2	+	−	+	+
G1A	Stock	D	2	2	−	−	+	−
G1B	Stock	D	2	2	−	−	+	+
G2A	Stock	D	0.5	3	−	+	−	−
G2B	Stock	D	0.5	3	−	+	−	+
G3*	Stock	L	0.5	3	−	+	−	+
G4	Stock	L	0.5	3	−	+	−	+
G5	Stock	L	0.5	3	−	+	−	+
G6	Stock	D L	0.5	3	−	+	−	−
G7	Stock	D L	0.5	3	−	+	−	+

* In all experiments except G3, each flask contained 3 ml of medium and 1–1.5 g of tissue. In Exp. G3, volume was 10 ml and 3 g of tissue were incubated.

The medium was neutralized with KOH and the potassium perchlorate that precipitated was removed by centrifugation. The supernatant fraction was combined with the Folch washings and free glycerol was isolated as follows. The solution was deionized by passage through the mixed-bed ion exchanger Amberlite MB-3 (Rohm & Haas Co., Philadelphia, Pa.). Over 90% of the ^{14}C of the glyceraldehyde-^{14}C in the medium was retained on the resin even after thorough washing of the column with water. We do not know the reason for the retention of the glyceraldehyde on this ion exchanger. Carrier glycerol (4 mg) was added to the effluent, which was concentrated, applied to Whatman 3MM paper, and chromatographed in *t*-butanol–methyl ethyl ketone–formic acid–water 40:30:15:15 (17). The area containing glycerol, as identified by guide spots, was eluted and 2 mmoles of glycerol was added as carrier. The solution was concentrated to 10 ml, made 1 N in NaOH, and refluxed for 3 hr to destroy residual dihydroxyacetone and glyceraldehyde (12). The solution was deionized on another Amberlite MB-3 column and the effluent and aqueous washings were evaporated. The glycerol was purified further through its tribenzoate and degraded, and the individual carbons were assayed for ^{14}C. Lactate was isolated in experiments G2A and G2B and was also degraded and assayed for ^{14}C (9).

RESULTS

Free glycerol formed from D-glyceraldehyde-3-^{14}C was labeled primarily in carbon 1. L-Glyceraldehyde-3-^{14}C yielded glycerol labeled almost completely in carbon 3 (Table 2).

TABLE 2 DISTRIBUTION OF ^{14}C IN FREE GLYCEROL FROM D- AND L-GLYCERALDEHYDE-3-^{14}C

Optical Isomer	Exp. No.	Activity in Carbon 1	2	3	Recovery*
			%		%
D	G2B	92.2	0.9	6.9	100.4
	G6	64.2	1.8	34.0	96.2
	G7	90.3	3.1	6.6	95.5
L	G3	7.2	3.6	89.2	90.8
	G6	12.8	4.1	83.1	91.7
	G7	7.7	8.8	83.5	91.0

* In this and succeeding tables, the sum of the activities in the individual carbons has been taken as 100% and the percentage in each of the carbons is recorded. The total ^{14}C in the compound (here glycerol) was determined by total combustion of an aliquot. The value in the last column is that percentage of the total ^{14}C that was found in the degraded compound (sum of ^{14}C in each carbon). These recoveries serve as a measure of the adequacy of the degradation.

Glycerol of triglyceride formed from D-glyceraldehyde-3-^{14}C contained little ^{14}C activity in carbon 2 (Table 3); about one-fourth of the activity was in carbon 1 with the remainder in carbon 3. ^{14}C was distributed similarly in lactate (Table 4). Glucose from glycogen (Table 5) was labeled primarily in carbons 4 and 6 with about one-third as much ^{14}C in carbon 4 as in carbon 6. Glycerol from the triglycerides when L-glyceraldehyde-3-^{14}C was substrate had a distribution similar to that obtained with D-glyceraldehyde-3-^{14}C (Table 6).

When the concentration of D-glyceraldehyde-3-^{14}C was 2 mg/ml, about 0.5% of the added counts were recovered in CO_2 and 2% in lipid (about half of this percentage being in glycerol). When the concentration of the D-isomer was 0.5 mg/ml, 3% was in CO_2 and 8%

TABLE 3 DISTRIBUTION OF ^{14}C IN TRIGLYCERIDE-GLYCEROL FROM D-GLYCERALDEHYDE-3-^{14}C

Exp. No.	Activity in Carbon 1	Activity in Carbon 2	Activity in Carbon 3	Recovery
		%		%
1A	23.2	2.3	74.5	83.3
1B	34.4	4.6	70.0	78.3
2A	24.1	1.1	74.8	98.9
2B	26.7	5.3	68.0	104
G2B	29.3	1.4	69.3	92.1
G6	17.2	2.5	80.3	103
G7	16.3	2.4	81.3	94.1
G7*	38.3	1.6	60.1	118.5

* Unlabeled dihydroxyacetone (0.5 mg/ml) added to medium.

TABLE 4 DISTRIBUTION OF ^{14}C IN LACTATE FROM D-GLYCERALDEHYDE-3-^{14}C

Exp. No.	Activity in Carbon 1	Activity in Carbon 2	Activity in Carbon 3	Recovery
		%		%
G2A	21.1	2.5	76.4	88.3
G2B	17 5	2.6	79.9	91.3

in lipid. With L-glyceraldehyde at a concentration of 0.5 mg/ml, 5% of the added counts were in CO_2 and 7% in lipid. In experiments G2A and G2B, 8.6% and 10.0% respectively of the ^{14}C in the glyceraldehyde-3-^{14}C were recovered in lactate. In experiment G2B, 1.6% was recovered in free glycerol.

DISCUSSION

Only by the reduction of D-glyceraldehyde-3-^{14}C to glycerol (pathway *a* of Fig. 1) can carbon 1 of glycerol become labeled. The incorporation of ^{14}C into carbon 1 of the glycerol (Table 2) indicates that this reaction does occur in adipose tissue and is evidence for the presence of glyceraldehyde hydrogenase activity in the tissue.

As indicated in the Introduction, if the above reaction were the only one by which ^{14}C from D-glyceraldehyde-3-^{14}C was incorporated into carbon 1 of glycerol of triglycerides, the extent of glycerol phosphorylation could be estimated from the specific activity of carbon 1 of the free glycerol and the total quantity of ^{14}C incorporated into carbon 1 of the glycerol from the triglycerides. However, in view of our finding (Table 6, discussed later) that in incubations with L-glyceraldehydehyde-3-^{14}C, ^{14}C is incorporated into carbon 1 of glycerol of triglyceride, the incorporation of ^{14}C into carbon 1 with D-glyceraldehyde-3-^{14}C as substrate cannot be ascribed with certainty to the phosphorylation of glycerol-1-^{14}C.[1]

Incorporation of ^{14}C into carbon 3 of free and triglyceride glycerol with D-glyceraldehyde-3-^{14}C as substrate could arise either by phosphorylation of the D-glyceraldehyde to D-glyceraldehyde phosphate or via its oxidation to D-glyceric acid (pathways *b* and *c* of Fig. 1). Glycerol-3-phosphate, formed from both of these compounds, can yield free glycerol either on hydrolysis through the action of a phosphatase (13) or via formation of glycerides and their hydrolysis.

The greater incorporation of ^{14}C into carbon 3 than into carbon 1 of triglyceride-glycerol would indicate that these reactions (i.e. pathways *b* and *c*, Fig. 1) predominate over the phosphorylation of glycerol-1-^{14}C formed from glyceraldehyde. One would expect to find more ^{14}C in carbon 1 of the free glycerol than in carbon 3 since glycerol-1-^{14}C would be formed directly from the D-glyceraldehyde-3-^{14}C, while the glycerol-^{14}C derived from glycerol-3-phosphate and triglyceride would first be diluted by the pools of these unlabeled compounds in the tissue.

The isolation of glycerol-3-^{14}C when L-glyceraldehyde-3-^{14}C was substrate is in accord with pathways *a* and *c* of Fig. 2. An NADPH-dependent D-glyceraldehyde hydrogenase isolated from liver has been reported to have less activity toward D,L-glyceraldehyde than toward D-glyceraldehyde, but its action on L-glyceraldehyde was not reported (14). An NADH-dependent dehydrogenase catalyzes the reduction of D,L-glyceraldehyde (15), and L-glyceraldehyde has been shown to be preferentially formed from glycerol by an NADH-dependent alcohol dehydrogenase from horse liver (16).

None of the reactions thus far considered can account for the incorporation of ^{14}C from L-glyceraldehyde-3-^{14}C into carbon 1 of triglyceride glycerol (Table 6). Whatever the sequence of reactions may be, they could also lead to the incorporation of ^{14}C from D-glyceraldehyde-3-^{14}C into carbon 1 of triglyceride-glycerol (Table 3)

[1] In experiment G2B the quantity of free glycerol in the medium combined with the Folch washings was 10.3 μmoles by assay with glycerol dehydrogenase (Worthington Biochemical Corp., Freehold, N. J.). From the recoveries of the glycerol during its purification and the dilution factors due to addition of carrier glycerol, the specific activity of carbon 1 of the free glycerol was determined. From this activity and the total ^{14}C activity in carbon 1 of the triglyceride-glycerol, we calculated that 10.6 μmoles of glycerol had been converted to triglyceride-glycerol via phosphorylation of glycerol. In experiments G6 and G7, in which the activity into carbon 1 was about 60% of that in experiment G2B (Table 3), if we assume that all else was similar, approximately 6 μmoles would be estimated to have been phosphorylated. This estimation is offered as a maximum value since it is assumed that all the activity in carbon 1 was incorporated into the triglyceride-glycerol via pathway *a* of Fig. 1. The quantity of ^{14}C that was incorporated into carbon 1 of triglyceride-glycerol and then released in free glycerol can be calculated from the ^{14}C in carbon 3 of the free glycerol and the activity in carbon 1 relative to carbon 3 if the assumption is made that the activity in carbon 3 of free glycerol arises only through hydrolysis of triglyceride-glycerol.

TABLE 5 DISTRIBUTION OF ^{14}C IN GLUCOSE FROM GLYCOGEN DERIVED FROM D-GLYCERALDEHYDE-3-^{14}C

Exp. No.	Activity in Carbon						Recovery
	1	2	3	4	5	6	
			%				%
1A	1.4	0.7	1.4	24.7	3.4	68.4	95.7
1B	10.5	4.9	3.8	23.5	1.2	56.1	100.5
2B	2.6	3.5	0.0	23.1	0.6	70.2	86.9

TABLE 6 DISTRIBUTION OF ^{14}C IN TRIGLYCERIDE-GLYCEROL FROM L-GLYCERALDEHYDE-3-^{14}C

Exp. No.	Activity in Carbon			Recovery
	1	2	3	
		%		%
G3	36.9	3.5	59.6	96.7
G4	42.1	1.9	56.0	93.0
G5	21.7	7.2	71.1	107.3
G6	23.4	2.4	74.2	100.6
G7	7.0	2.0	91.0	101.7
G7*	12.3	0.0	87.7	113.2

* Unlabeled dihydroxyacetone (0.5 mg/ml) added to medium.

as well as into carbon 4 of glycogen (Table 5). We examined the possibility that this incorporation was due, in the first four experiments, to contamination of our preparation with dihydroxyacetone-1,3-^{14}C (see Materials section). However, ^{14}C from purified L-glyceraldehyde was also incorporated into carbon 1 (experiments G5–G7 of Table 6), although the incorporation was perhaps one-half as much as in experiments G3 and G4. A decrease in incorporation of ^{14}C from D-glyceraldehyde-3-^{14}C into carbon 1 may also have occurred as a result of the purification (Table 3). Addition of relatively large quantities of unlabeled dihydroxyacetone to the preparations did not reduce incorporation into carbon 1 (Tables 3 and 6). Glucose labeled only in carbons 1 and 6 has been obtained on incubating L-glyceraldehyde-3-^{14}C with slices of liver (2) and the D-glyceraldehyde-3-^{14}C preparation with intestinal segments (17). When glyceraldehyde was incubated in the absence of tissue, no dihydroxyacetone was formed as measured by a spectrophotometric method (8). The possibility exists that in adipose tissue dihydroxyacetone is formed from glyceraldehyde; this would explain the incorporation of ^{14}C into carbon 1.

The ^{14}C recovered in CO_2, lipid, and lactate in the first experiments cannot be derived primarily from dihydroxyacetone-1,3-^{14}C, since its amount exceeds that present in the contaminant at the time of chromatography. Incorporation of ^{14}C into triglyceride-glycerol cannot be due primarily to dihydroxyacetone-1,3-^{14}C since incorporation into carbon 3 markedly exceeded that into carbon 1 in most of the experiments. Glycerol from dihydroxyacetone-1,3-^{14}C should give equal labeling in carbons 1 and 3.

Possibly, ^{14}C was incorporated into carbon 1 of the triglyceride-glycerol via dephosphorylation of dihydroxyacetone phosphate and subsequent rephosphorylation. There is evidence that these reactions occur in propionic acid bacteria (18). Dihydroxyacetone is utilized by the bacteria, triokinase and triose phosphatase activities have been shown to be present, and incubation of the bacteria with glycerol-1-^{14}C and glycerol-3-^{14}C gives distributions of ^{14}C in propionate consistent with the incorporation of the ^{14}C via a symmetrically labelled intermediate.

L-Glyceraldehyde-3-^{14}C could also be converted to D-glyceraldehyde-3-P via pathways *a* and *c* of Fig. 2. If there is in adipose tissue a phosphatase which can convert D-glyceraldehyde-3-phosphate, 3-^{14}C to D-glyceraldehyde-3-^{14}C, incorporation of ^{14}C into carbon 1 of glycerol could then have occurred via pathway *a* of Fig. 1. If this were the case the method for estimating glycerol phosphorylation in adipose tissue would be applicable in spite of the incorporation of carbon 3 of L-glyceraldehyde into carbon 1 of glycerol, since only pathway *a* of Fig. 1 would be responsible for the incorporation into carbon 1.

Another possible explanation for incorporation into carbon 1 of glycerol and carbon 4 of glycogen is fixation of $^{14}CO_2$ formed by oxidation of ^{14}C-labeled glyceraldehyde. Pyruvate-1-^{14}C formed by this fixation and by the reactions of the Krebs cycle could then introduce ^{14}C into carbon 1 of glycerol and carbons 3,4 of glycogen. This seems unlikely in view of the small quantity of $^{14}CO_2$ formed and its probable dilution by the large pool of CO_2 present in the incubation system. Moreover, the incorporation of the carbon of CO_2 into glycerol by adipose tissue is relatively small (19, 20). In addition, if glyceraldehyde-3-phosphate,3-^{14}C were metabolized in the Krebs cycle and pyruvate-^{14}C were then formed, one would expect to find ^{14}C in carbon 2 of glycerol and in carbon 5 of glycogen (20).

That the distributions of ^{14}C in lactate, in carbons 4, 5, and 6 of glucose from glycogen, and in triglyceride-glyceride are similar to one another is in accord with these compounds' being derived from triose phosphates

of a similar ^{14}C distribution. This means that glycerophosphate in the tissue from which glycerol was derived was equilibrated with the tissue glyceraldehyde-3-phosphate from which the lactate and carbons 4, 5, and 6 of the glucose were derived. The negligible activity in carbons 1 and 3 of glucose from glycogen probably indicates that incorporation of ^{14}C into the glucose occurred via the transaldolase reaction (21, 22) in which carbons 1, 2, and 3 are from the hexose-6-phosphate in the tissue and the carbons of glyceraldehyde-3-phosphate exchange with carbons 4, 5, and 6 of the hexose-6-phosphate.

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