mechanisms may also be involved in restoring normal permeability of the blood vessels to red cells following platelet transfusion. A direct chemical or physical-chemical interaction between platelets and blood vessel structures could, for example, be involved. Under the conditions of our study it appeared that circulating platelets may be required for such interaction.

It is remarkable that the average output of red blood cells in the lymph of animals treated with human platelets continued to decrease despite the rapid decline of the platelet levels (Fig. 1B). This partial clearing of red cells from lymph in the absence of adequate levels of circulating platelets resembles experiments reported previously(2) on dogs treated with freeze-dried platelets. Reduction of red cell diapedesis into the lymph of thrombocytopenic dogs has also been described following infusions of soybean phosphatides(11). Further studies to determine whether the phospholipids of human platelets disintegrating *in vivo* have contributed to this reduction may, therefore, be warranted.

Summary. Fresh human platelets transfused into thrombocytopenic rats are removed from the circulation within 2 to 3 hours. The output of red blood cells into the lymph is reduced (-70%) despite the declining platelet count. The effects of fresh rat platelets are both more marked and more consistent. Species-specificity may perhaps account for the lesser activity of heterologous platelets.

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Received March 30, 1962. P.S.E.B.M., 1962, v111.

Conversion of Glycine to Fatty Acids by Adipose Tissue.* (27693)

D. D. FELLER AND E. FEIST (With the technical assistance of J. Prendergast)
Radioisotope Service, Veterans Administratoin Hospital and Department of Medicine, University of Washington, Seattle

Whereas the metabolism of carbohydrates and lipids in adipose tissue has been exhaustively studied, the metabolism of amino acids in this tissue has received little attention. Our recent studies have demonstrated that this tissue converts the carbon chain of alanine, isoleucine, leucine, serine and valine to CO_2, fatty acids and certain intermediate compounds(1-3). Continuing this program, the present study was undertaken to determine whether glycine can be metabolized by adipose tissue and act as an initial substrate for fatty acid biosynthesis. Carbon-14-labeled glycine, glyoxylate, sarcosine or malate have been incubated with epididymal fat pads and evidence is presented to show that adipose tissue oxidizes glycine as well as utilizes its carbons for biosynthesis.

Method. Adult white Swiss mice were fed a diet of Purina laboratory chow *ad libitum* until time of sacrifice. The mice were killed by separation of the cervical vertebrae and the epididymal fat deposit rapidly removed.

* Aided by grants from Nat. Inst. of Arthritis Metab. Dis., U.S.P.H.S. and from Wesson Fund for Medical Research and Education.

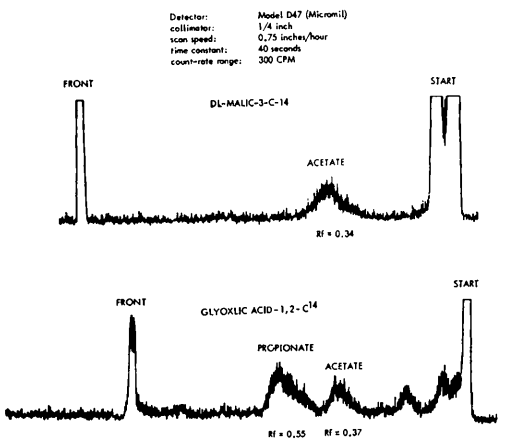

FIG. 1. Autoradiograms of extracts obtained from mouse adipose tissue. Supernatant and combined washings were electrodialyzed, extracted with ether, made basic with NH_4OH and chromatographed on Whatman No. 1 paper for 20 hr with ethanol-NH_4OH-H_2O. Tissues were incubated with glycine-1-C^{14} and glycine-2-C^{14}. Rf values obtained for controls at the points indicated are as follows: propionate, 0.55; acetate, 0.38 and glyoxylate, 0.23.

The tissue was incubated in Krebs-bicarbonate buffer containing unlabeled glucose (0.011M) and succinate (0.01M) as described elsewhere(1). Two to 8 μc of the C-14 labeled compounds were added to each flask at a final concentration of 0.001M. Glycine-1-C^{14}, glycine-2-C^{14}, glyoxylate-1,2-C^{14}, sarcosine-1-C^{14} and DL-malate-3-C^{14} usually with specific activities greater than 1 mc per m mole were purchased from commercial sources.

Procedures for collection of CO_2, separation and isolation of fatty acids, preparation of extracts for paper chromatography and radiochromatographic procedures have been described(1). Methods for separation and identification of volatile acids, preparation of para-bromophenacyl esters of volatile acids, preparation of 2, 4-dinitrophenylhydrazone of glyoxylate and radioassay procedures are presented elsewhere(2). Other experimental details are listed in the tables and figures. For each experiment substrate levels of unlabeled intermediates of glycine metabolism (4,5) were tested in regard to their influence on the metabolism of the labeled substrate. Thus, glyoxylate, formate, malate, as well as acetate or propionate were added at a final concentration of 0.01M.

Results. Several C^{14}-labeled compounds were recovered by extraction from tissue after incubation with C^{14}-labeled glycine, glyoxylate and malate (Fig. 1, 2). Acetate and propionate were separated on a celite column and their para-bromophenacyl esters yield melting point ranges of 83-85° and 61-63° respectively, which agrees with the literature (6). The hydrazone derivative of glyoxylate (melting point: 199-202°C) served to characterize this intermediate(7). Although not illustrated, radioactive succinate was also identified by radiochromatography.

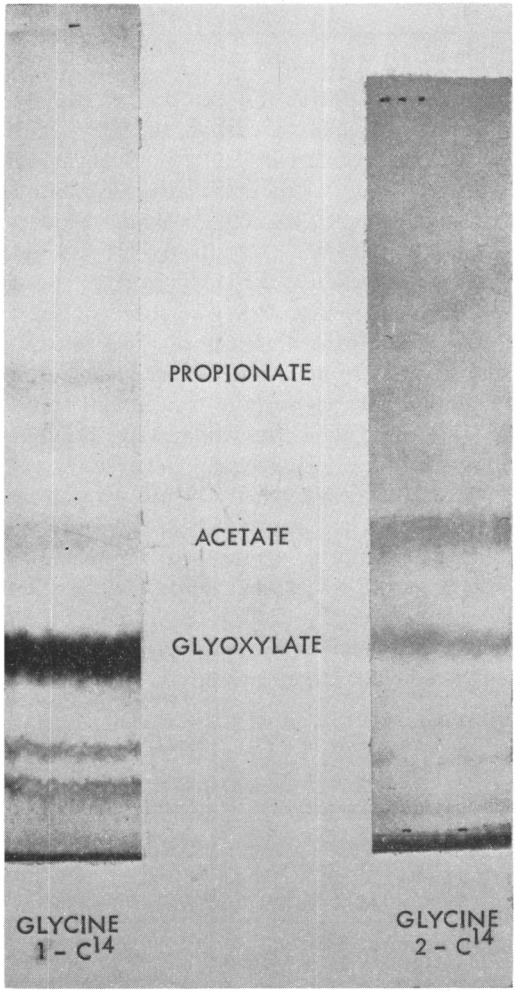

FIG. 2. Scans of paper chromatograms of extracts prepared from mouse adipose tissue as described in the legend to Fig. 1. Paper strips were counted on Nuclear-Chicago Actigraph II unit. Radioactive ink was added at the starting mark and at the solvent front for identification.

TABLE I. Recovery of C^{14}-Fatty Acids and C^{14}-CO_2 after Incubation of Epididymal Adipose Tissue with C^{14}-Labeled Glycine, Glyoxylate, Malate and Sarcosine. (1.4 g of tissue were incubated for 3 hr at 37.5°C in 14.0 ml of buffer containing 14 μmoles of C^{14}-labeled substrate and unlabeled glucose and succinate at final concentrations of 0.011 M and 0.01 M respectively.)

C^{14}-labeled compounds added	No. of exp.	Added dose (μc)	Fatty acids (% of added dose)	CO_2 (% of added dose)
			avg	
Glycine-1-C^{14}	4	5.83	.68 ±.04*	.36 ±.13
Glycine-2-C^{14}	4	7.80	.24 ±.01	1.30 ±.50
Glyoxylate-1,2-C^{14}	4	4.67	.19 ±.03	.47 ±.04
Sarcosine-1-C^{14}	4	4.67	.09 ±.01	.15 ±.06
Malate-3-C^{14}	3	1.56	.15 ±.02	.10 ±.00

* Stand. error of mean.

The incorporation of glycine, glyoxylate, sarcosine and malate carbon to fatty acids and CO_2 is recorded in Table I. Recovery of radioactivity in fatty acids after incubation with glycine-1-C^{14} was 0.68% while recovery in CO_2 was 0.36%. Glycine-2-C^{14} was oxidized more readily than glycine-1-C^{14} and less of its carbons were converted to fatty acids. Significant amounts of C^{14} were recovered in fatty acid and CO_2 fractions after incubation with glyoxylate, malate and sarcosine, but, in general, the values were less than those observed for glycine. Between 1 and 1.5% of the radiolabeled glycine was recovered as fatty acids and CO_2. Herrera and Renold(8) reported conversion of glycine to protein in rat epididymal adipose tissue that exceeded conversion to CO_2 by 5-fold. No attempt to recover non-utilized glycine was attempted in our experiments.

The results of experiments in which unlabeled formate, acetate, propionate, glycine, glyoxylate and malate were incubated with the C^{14}-labeled compounds is shown in Table II. Addition of unlabeled formate had no effect on conversion of glycine and glyoxylate to fatty acids. On the other hand, addition of acetate and propionate caused a significant reduction in fatty acid synthesis from glycine, sarcosine and malate. Added unlabeled acetate also reduced conversion of malate-3-C^{14} to fatty acids. The conversion of glycine-1-C^{14} to fatty acids was reduced after addition of unlabeled glyoxylate. The conversion of glycine-2-C^{14} was lowered after addition of glyoxylate but the difference from the control value was not considered significant as judged by the *t* test.

In 2 cases, recovery of C^{14} in fatty acid fraction was greater after addition of unlabeled substrate. Addition of unlabeled glycine increased incorporation of sarcosine-1-C^{14} to fatty acids and addition of unlabeled malate increased conversion of glycine-1-C^{14} to fatty acids.

Discussion. One possible path for metabolism of glycine in adipose tissue is by conversion to serine. This is accomplished by addition of "active" formaldehyde to the alpha carbon of glycine(4). By this mechanism, glycine-1-C^{14} would yield serine-1-C^{14}. It was previously observed(2) that serine-3-C^{14} is an efficient precursor to fatty acid synthesis while serine-1-C^{14} is less so. Only 0.05% of added serine-1-C^{14} was recovered as fatty acids compared to 0.68% recovered after in-

TABLE II. Effect of Added Unlabeled Substrates on Conversion of Glycine, Glyoxylate, Malate and Sarcosine to Fatty Acids by Adipose Tissue. (Incubation conditions are as described for Table I. Calculations are based on avg values obtained from 3 to 4 experiments. Unlabeled substrates were added to a final concentration of 0.01 M.)

Added unlabeled substrates	Relative incorporation into fatty acid, Control = 100				
	Glycine-1-C^{14}	Glycine-2-C^{14}	Glyoxylate-1,2-C^{14}	Sarcosine-1-C^{14}	Malate-3-C^{14}
Formate	96 (.60)	129 (.24)	89 (.60)		
Acetate	4*(<.001)	29*(<.001)	132 (.16)	8*(.002)	47*(.04)
Propionate	63*(.001)	67*(.01)		56*(.05)	
Glycine				144*(.047)	73 (.33)
Glyoxylate	67*(<.001)	64 (.21)			
Malate	133*(.004)	107 (.70)	137 (.11)		

No. in parentheses are *p* values.

* Values considered significantly different from the control at the 5% probable error level.

cubation with glycine-1-C^{14}. From these facts, one would conclude that the glycine-serine path is probably of little quantitative significance. This has been shown to be true for liver(4).

A more likely path for glycine catabolism is initially by way of glyoxylate. Two possible pathways for dissimilation of glyoxylate have been demonstrated in liver tissue: (a) conversion to oxalic acid and formic acid and (b) glyoxylate cycle(4,5). The conversion of glycine to glyoxylate in adipose tissue has been established by the data reported here, namely, recovery of C^{14}-glyoxylate from C^{14}-glycine and reduction of C^{14}-fatty acids from C^{14}-glycine after addition of unlabeled glyoxylate. Breakdown of glyoxylate to formate is probably of little importance since addition of unlabeled formate did not alter conversion of glycine or glyoxylate to fatty acids. Previously, formate has been shown to be a poor precursor for fatty acid biosynthesis in adipose tissue(9).

Of special interest is the finding that glycine-1-C^{14} was converted to fatty acids more readily that glycine-2-C^{14}. Previous studies (10,11) have shown that acetate-1-C^{14} was more readily converted to fatty acids in adipose tissue than acetate-2-C^{14}. Although not yet proven, a new pathway has been proposed (9) which explains these unexpected findings of acetate metabolism in this tissue. The recovery of C^{14} as acetate and propionate and the reduction of conversion of glycine to fatty acids after addition of unlabeled acetate and propionate suggests the existence of other pathways not yet described for glycine. It has been recently demonstrated that the metabolism of glycine in adipose tissue is both hormonally controlled and modified by the availability of metabolic substrates(8).

Summary. Epididymal adipose tissue was incubated with glycine-1-C^{14}, glycine-2-C^{14}, glyoxylate-1,2-C^{14}, sarcosine-1-C^{14} and malate-1-C^{14}; CO_2 and fatty acids were isolated and analyzed for radioactivity. Glycine-1-C^{14} was converted to labeled fatty acids to a greater extent than glycine-2-C^{14}. Radioactive acetate, propionate, glyoxylate and succinate were recovered as intermediate products of glycine metabolism. Addition of unlabeled acetate, propionate and glyoxylate reduced conversion of C^{14}-glycine to fatty acids. Results show that glycine can be a precursor in the biosynthesis of fatty acids in adipose tissue. The path of conversion of glycine to fatty acids is discussed.

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