13. Spain, J. D., Griffin, A. C., *J. Nat. Cancer Inst.*, 1957, v18, 693.
14. Reid, E., *Cancer Research*, 1962, v22, 398.
15. Clayton, C. C., Abbott, L. D., Jr., *ibid.*, 1958, v18, 94.
16. Smithies, O., *Biochem. J.*, 1959, v71, 585.
17. Boyer, S. H., Fainer, D. C., Naughton, M. A., *Science*, 1963, v140, 1228.
18. Markert, C. L., Ursprung, H., *Develop. Biol.*, 1962, v5, 363.

Received September 2, 1964. P.S.E.B.M., 1964, v117.

Metabolism of Glycerol by the Mammary Gland. (29729)

D. M. CARLSON, DIANE CRIDLER AND R. G. HANSEN
Department of Biochemistry, Michigan State University, East Lansing

Glycerol is known to undergo the following enzymatically catalyzed transformations: 1) phosphorylation by liver preparations to yield L-α-glycerophosphate(1) and 2) dehydrogenation to yield either glyceraldehyde(2, 3), or dihydroxyacetone(3,4).

When glycerol-1,3-C^{14} was injected *in vivo* unilaterally into the pubic artery of the bovine mammary gland, the galactose moiety of milk lactose from the injected side was labeled about 20 time greater than was the glucose moiety(5). Furthermore, 80% of the C^{14} of the galactose was distributed equally in C-4 and C-6 while the labeling of the glucose portion resembled that of blood glucose in that C-1, C-3, C-4, and C-6 had approximately equal activities. The distribution of label in glucose-6-phosphate, uridine diphosphate glucose and uridine diphosphate galactose isolated from the mammary gland tissue was similar to that of the galactose portion of lactose. A consideration of the isotope distribution in the hexose moieties of lactose after injection of labeled glycerol prompted this investigation of the metabolism of glycerol by mammary gland.

Methods. Rats that had been lactating 7-10 days were decapitated and the posterior mammary glands were excised and placed in ice. In most cases an acetone powder was prepared from the cold glands. The acetone powder was ground with 10 volumes of isotonic KCl in a pre-chilled mortar and then stirred for 30 minutes in an ice bath. The resulting extract was centrifuged at 5000 $\times$ *g* for 10 minutes and the precipitate discarded. The supernatant was used as the source of glycerokinase. When fresh tissue was used, it was homogenized in 2 volumes of isotonic KCl in a Waring blendor and centrifuged. The supernatant was filtered through cheesecloth to remove the fat layer and the filtrate used as the enzyme sources.

Results. For measuring the formation of L-α-glycerophosphate, the incubation conditions used were essentially those of Bublitz and Kennedy(1) with the following specific conditions: 25 μmoles KF, 25 μmoles glycerol, 1 μmole $MgCl_2$, 20 μmoles cysteine, 6 μmoles ATP, 61 μmoles Tris buffer, pH 7.4 and 0.2 ml enzyme (2.25 mg protein) in a total volume of 1 ml at 37°. Buffer was substituted for ATP in the control. After 30 minutes, the reaction was stopped by heating at 100° for 3 minutes and the coagulated protein removed by centrifugation.

L-α-glycerophosphate was measured in the incubation supernatant as follows: 0.25 μmoles DPN, 172 μmoles hydrazine buffer, pH 9.4, and 0.05 ml of supernatant were made to a volume of 0.49 ml. After an initial reading, 0.01 ml of L-α-glycerophosphate dehydrogenase was added and the optical density increase at 340 mμ measured. Both rat and bovine mammary gland extracts actively phosphorylated glycerol. Uridine triphosphate and ATP both serve as donors in the phosphorylation of glycerol by either tissue homogenates or acetone powders. This suggests direct phosphorylation by UTP or a phosphate transfer from UTP to endogenous ADP.

To identify the phosphorylated product glycerol-1,3-C^{14} was incubated with the en-

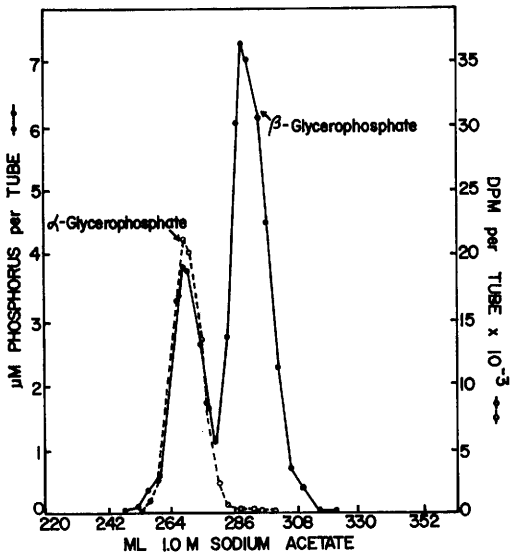

FIG. 1. Column chromatography of α- and β-glycerophosphates. The enzymically phosphorylated product (dashed line) coincides with authentic α-glycerophosphate and is different from β-glycerophosphate.

zyme. After treatment of the incubation mixture with Dowex-50 (H^+), the reaction product was co-chromatographed with authentic DL-α-glycerophosphate on paper using the 2-dimensional system of Bandurski and Axelrod(6). Unlabeled α-glycerophosphate (23.5 μmoles) and β-glycerophosphate (47.0 μmoles) were added to the incubation mixture of the kinase and glycerol-1,3-C^{14} before placing it on an ion exchange column. Upon column chromatography on Dowex-1 (acetate) using the gradient elution method of Bublitz and Kennedy(7) for separation of α and β-isomers the reaction product appeared to be identical to α-glycerophosphate, (Fig. 1). The esters were located by analysis for phosphorus(8). The radioactive peak of C^{14} α-glycerophosphate was located by liquid scintillation counting. Quantitative recovery of L-α-glycerophosphate from similar columns was demonstrated. Thus from the evidence presented L-α-glycerophosphate is the product of phosphorylation of glycerol by mammary gland preparations.

The direct dehydrogenation of glycerol by the enzyme preparation to yield either dihydroxyacetone or glyceraldehyde could not be demonstrated using the assay method of Burton and Kaplan(3), however, dehydrogenation of L-α-glycerophosphate was readily established by following a substrate dependent oxidation of DPNH.

Wood *et al*(5) have postulated a pathway whereby a product of glycerol metabolism could label the galactose moiety of lactose by a transaldolase exchange reaction. This could involve the phosphorylation, dehydrogenation and isomerization of glycerol to yield glyceraldehyde-3-phosphate which would then exchange for carbons 4, 5 and 6 of fructose-6-phosphate. More recently Hansen *et al*(9) have predicted a novel pathway for the formation of galactose or UDP galactose on the basis of unequal labeling of the hexose nucleotides formed from glycerol as a source of carbon.

Summary. Data are presented which suggest that glycerol may be oxidized to an appropriate level after phosphorylation, rather than before, and in this form incorporated into compounds which could give rise to galactose in preference to glucose.

1. Bublitz, C., Kennedy, E. P., *J. Biol. Chem.,* 1954, v211, 951.

2. Wolf, H. P., Leuthardt, F., *Helv. Chim. Acta,* 1953, v36, 1463.

3. Burton, R. M., Kaplan, N. O., *J. Am. Chem. Soc.,* 1953, v75, 1005.

4. Asnis, R. E., Brodie, A. F., *J. Biol. Chem.,* 1953, v203, 153.

5. Wood, H. G., Joffe, S., Gillespie, R., Hansen, R. G., Hardenbrook, H., *ibid.,* 1958, v233, 1264.

6. Bandurski, R. S., Axelrod, B., *ibid.,* 1951, v193, 405.

7. Bubltiz, C., Kennedy, E. P., *ibid.,* 1954, v211, 963.

8. King, E. J., *Biochem. J.,* 1932, v26, 292.

9. Hansen, R. G., Wood, H. G., Peeters, G. J., Jacobson, B., Wilken, J., *J. Biol. Chem.,* 1962, v237, 1034.

Received September 2, 1964. P.S.E.B.M., 1964, v117.