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Milk Fat Synthesis from Acetate in Mammary Gland of the Cow. (23059)

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Previous publications from our laboratory have been concerned with the roles of short chain fatty acids, bicarbonate and glucose as precursors of milk-constituents(1-9). These have been studied by intravenous injection of various metabolites appropriately labeled with C^{14}. Acetate is the principal metabolite derived from fermentation of carbohydrates in the rumen(10), and we have shown that it is a precursor of all milk-constituents, as well as being extensively catabolised to meet energy requirements. As might be expected, plasma acetate is a potent precursor of the fatty acid moiety of milk fat, but acetate carbon is also utilized in synthesis of the glycerol moiety. In view of the large amount of acetate available to cow's tissues, this latter synthesis is probably an important source of glycerol. The evidence indicates that it takes place via the tricarboxylic acid cycle as well as via fixation of carbon dioxide derived from acetate oxidation(8,9).

The work of Popjak, Folley *et al.*(11,12) with perfused udders has shown that a considerable part of milk fat synthesis from small molecules takes place in the mammary gland itself. In this experiment 2-C^{14} labeled acetate was injected into the cistern of one quarter of the mammary gland of an intact cow, and radioactivities of milk fat components from that quarter were compared with those from the other 3 quarters and with those from a similarly labeled acetate injection made intravenously.

Methods. The technic for conducting tracer experiments with intact cows has been described by Kleiber(1). Following injection of labeled acetate, continuous samples of respired carbon dioxide were taken for the first 3 hours and then for short periods at intervals up to 35 hours. The cow was milked at 3, 10, 22, and 35 hours after injection. Details of injections and characteristics of the cows are tabulated in Table I. The fats were separated from milk samples and 4 crude fractions prepared from each. These were the glycerol, water-soluble steam-volatile fatty acids, water-insoluble steam-volatile fatty acids, and non-volatile fatty acids. Details of separation are described by Rogers (9). A sample of each fraction was combusted and the resultant carbon dioxide was trapped as barium carbonate. This was planchetted and counted at infinite thickness in a flow-gas counter. Udder injection of acetate was made into the right front quarter by passing a large blunt needle up the teat canal and depositing the acetate in the milk cistern.

TABLE I. Details of Animals Used and Isotope Injected.

	Udder inj. exp.	Intrav. inj. exp.
Wt of cow	478.5	470
Milk production, kg/day	7.4	7.6
Fat, %	4.8	5.7
Isotope inj.	1.20 mc $C^{14}H_3COOH$	3.86 mc $C^{14}H_3COOH$

TABLE II. Integrated Radioactivities of Milk Fat Constituents.

	$\sum_0^{35} \lambda_s \Delta t$				$\int_0^{35} \rho_s \, dt$
	Glycerol	Sol. VFA	Insol. VFA	NVFA	
Udder injection RFQ	30.51	249.6	431.04	171.78	3.96
" " 3Q	16.07	43.3	61.75	15.44	
Intrav. injection	27.9	79.7	83.1	16.8	62.78

This quarter was subsequently milked out separately from the others, and the data referring to this milk are henceforth indicated by the abbreviation RFQ. A similar abbreviation for the other 3 quarters is 3Q, and the intravenous injection data are indicated by the abbreviation i.v.

Results. The results are summarized in Table II and Fig. 1. Radioactivity of each sample was determined as "Standardized Specific Activity λ_s"(2) of which the units are μc/g atom carbon : μc injected/kg body weight. By dividing specific activity by administered dosage, it is possible to compare results from experiments in which different dosages were injected into animals of different weights. As metabolic processes take place at different rates, the specific activity of a milk-constituent is not necessarily a guide to the extent to which it is synthesized from injected metabolite. The best indication of the potency of an injected metabolite as pre-

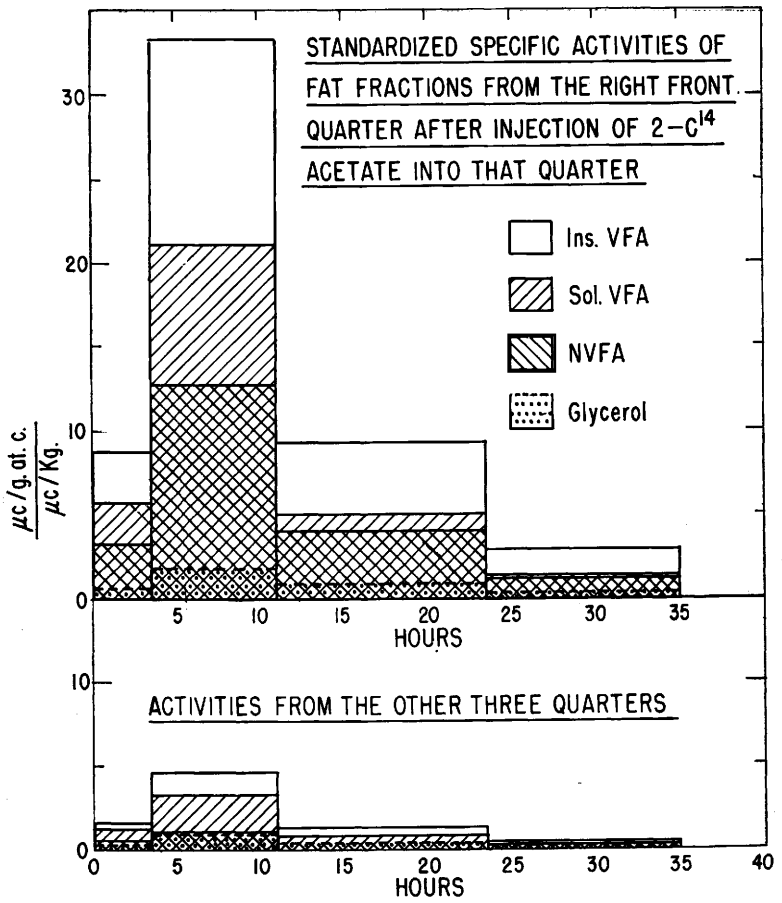

FIG. 1.

cursor of a milk-constituent is given by the time integral of specific activity of that constituent. This is easily determined since specific activity of a milk-constituent from any milk sample is the average specific activity of that constituent which has been secreted since the previous milking. Therefore, each average radioactivity can be multiplied by the number of hours in its milking period to give the integral for that period, and the sum of these will be the integral for the entire period $\sum_0^{35} \lambda_s \Delta t$. The time integral of the respired carbon dioxide activity is designated $\int_0^{35} \rho_s \, dt$.

The graphs in Fig. 1 illustrate the remarkably different activities of fat fractions from the RFQ and those from the 3Q. The fact that there is a difference shows that at least some of each fraction is synthesized in the mammary gland itself. The magnitudes of the differences are illuminating. Glycerol is only twice as radioactive from the RFQ as from the 3Q, whereas the 3 fatty acid fractions are more radioactive by factors of 6, 7, and 11 for soluble-volatile, insoluble-volatile and non-volatile fatty acids, respectively. In the RFQ the glycerol has much lower radioactivity than fatty acid fractions associated with it. In the 3Q, however, the relative difference is smaller and the ratio of radioactivities of glycerol and fatty acids is of a similar order to that seen previously where the labeled acetate was injected intravenously. From these 2 observations one can infer that there is less glycerol synthesis from acetate in the mammary gland than there is fatty acid synthesis. It is also apparent that mammary tissue cells are extremely active in synthesis of fatty acids of all chain lengths.

One of the most striking results from the udder injection experiment is that labeled acetate seemed to stay in the mammary gland and did not distribute throughout the body of the cow. This is clearly shown by the very small time integral of radioactivity of respired carbon dioxide, which is only 6.4% of that following a similar intravenous injection. It might be concluded from this that certainly less than 10% of injected acetate found its way into the circulation despite the vascularity of the udder. Similarly, the enormously greater radioactivity of milk fat constituents in the RFQ than in the 3Q and the persistence of the difference through 35 hours suggest that diffusion through the extravascular fluid is also limited. This apparent immobilization of injected acetate is not at all consistent with other observations concerning diffusion of small molecules in fluids of the body. An explanation is suggested by some other observations. As was previously reported(9), the most radioactive fat was recovered from the second milk sample after intravenous injection of a labeled precursor. The peak radioactivities of lactose and other milk constituents were seen in the first milk sample(2-8). The same phenomenon was observed even where labeled acetate was injected into the mammary gland, showing that the delay is not due to slow transport of fat to the udder from other synthetic sites. The work of Popjak, Folley *et al.*(11,12) with surviving slices of mammary tissue shows that actual synthesis of fatty acids from acetate is rapid. Furthermore, delay of peak radioactivity is observed in the glycerol moiety of fat as well, but peak of radioactivity in the lactose(8,13) is seen in the first milk sample in all trials, including this udder experiment. Since glycerol and lactose syntheses from acetate have common pathways, it is evident that the delay is not one of synthesis. Therefore, we have additional evidence for a previous suggestion(9) that the fat from the second milk sample is always more radioactive than the first because of a delay in actual secretion of fat.

If it is assumed, therefore, that injected acetate is rapidly utilized by cells adjacent to the milk cistern for fat synthesis and that this fat is only secreted several hours later, the apparent immobility of injected acetate seems less paradoxical. Continuance of the difference of the radioactivities of fat fractions between RFQ and 3Q even after 35 hours is understandable if the mammary gland is still secreting fat which was actually synthesized very shortly after injection of labeled acetate into the RFQ. The obviously small amount of injected acetate that diffused away from the RFQ can be accounted for in two ways. First, the rate of its incorporation

into fat molecules may have been much faster than the possible passive diffusion of acetate through the extravascular fluid. Second, the rate of acetate utilization for biosynthesis and catabolism in cells adjacent to the milk cistern might be so great that there is a steep diffusion gradient down towards that area, so that the number of acetate molecules moving in the opposite direction is small.

Summary. 1. 2-C^{14} labeled acetate was injected into the milk cistern of the right front quarter of a lactating cow. This quarter was subsequently milked separately from the other 3 quarters and radioactivities of milk fat constituents from each were determined. 2. The data confirm that there is a delay of several hours between synthesis and secretion of milk fat. 3. A surprisingly small amount of the injected acetate diffused into the other 3 quarters or into the rest of the body. This can be accounted for by its rapid utilization for milk synthesis near the site of injection. 4. Fatty acids of all chain lengths and glycerol seem to be synthesized from acetate in the mammary gland itself. The glycerol synthesis, however, is on a smaller scale.

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Further Studies of Prothrombin Derivatives.* (23060)

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Activation of purified prothrombin to thrombin can be achieved with the use of several combinations of materials separated from natural sources. All these "activator" factors are dispensable for even in 25% sodium citrate solution thrombin activity develops by autocatalytic mechanisms(1) thus indicating that the site on the prothrombin molecule which gives rise to thrombin activity is a structure arising only from changes in the prothrombin molecule itself. In addition to thrombin other derivatives can be obtained (2,3,4). Certain of these have a function in clotting of blood and it is likely that factor VII and plasma thromboplastin component (PTC) are derivatives of prothrombin(2,3). One derivative can again be reconverted to prothrombin by means of liver mitochondria preparations(5,6).

In the presence of thrombin, fibrinogen activates and subsequently polymerizes; and synthetic substrates, such as tosylarginine methyl ester (TAMe), are hydrolyzed(7). Thrombin can thus be regarded as an esterase, and in accordance with that view we have recently found that it hydrolyzes tributyrin quite effectively. Equally interesting is the discovery, reported here, that clotting activity of thrombin may decrease while the esterase activity is retained. Thus, we have

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