Location of butyric acid in bovine milk triglycerides

Investigations in several laboratories have shown that the volatile fatty acids of ruminant milk fat are synthesized in the mammary gland from acetate and β-hydroxybutyrate[1,2]. GLASCOCK, DUNCOMBE AND REINUS[3] have reported that the formation of the volatile fatty acids by the chain shortening of the unsaturated acids of the blood glycerides occurred only to a negligible extent. Substitution of a part of the unsaturated acids of the precursor triglycerides by the volatile acids has been suggested by HILDITCH[4] to account for the characteristic glyceride pattern of the milk fat.

Determination of the location of the volatile fatty acids in the ruminant-milk triglycerides was expected to provide a clue regarding the mechanism of their incorporation in the milk fat. Accordingly, advantage was taken of the specificity of pancreatic lipase for the fatty acids attached to the α-carbon atoms of glycerol[5]. Butyric acid was considered as typifying the short-chain fatty acids.

Butter fat, the molar butyric acid content of which was determined by the chromotographic procedure of KEENEY[6], was subjected to the action of pancreatic lipase. At the end of the incubation period the reaction was stopped by lowering the pH to 2.0 with 1 *M* HCl. The free fatty acids liberated and the glycerides remaining were extracted with alcohol–ether (1:2) mixture. The extract was washed free of the fatty acids with 1 % Na_2CO_3, the excess of Na_2CO_3 was washed out and the neutral glycerides were isolated. The molar proportion of butyric acid in this fraction was determined as before. The data are shown in Table I. It is seen that there is a significant loss of butyric acid from the glycerides. This finding shows that the acid

TABLE I

BUTYRIC ACID CONTENT OF BUTTER FAT GLYCERIDES AFTER LIPASE ACTION

2.0 g of fat (butyric acid, 10.2 molar %) were emulsified with 0.15 g sodium glycotaurocholate in tris(hydroxymethyl)aminomethane buffer, 2 ml of 45 % $CaCl_2$ was added and pH adjusted to 7.0. The mixture was incubated with 0.15 g pancreatic lipase at 39° for 3 min with vigorous stirring.

Expt.	*Volume of the medium (ml)*	*Molar % of butyric acid in the resulting glycerides*
1	80	4.3
2	160	3.2
3	360	2.4

TABLE II

FRACTIONATION OF THE GLYCERIDES AFTER LIPASE ACTION AND THEIR BUTYRIC ACID CONTENT

The procedure was the same as described under Table I.

Glycerides	*% recovered*	*Molar % of butyric acid*
Tri-	48.7	4.25
Di-	36.1	5.16
Mono-	11.9	0.0
Total	96.7	

is located predominantly at the α-carbon atoms of the glyceride. The greater loss of the acid with increasing dilution is undoubtedly a reflection of the inhibition of the exchange reaction between long-chain glyceride fatty acids and the free acids of the medium[5].

In further experiments the glyceride fraction isolated after lipase action was fractionated into mono-, di- and triglycerides by the procedure of QUINLIN AND WEISER[7] and the butyric acid content was determined in each species. The data obtained in one experiment are presented in Table II. Each of the experiments with varying incubation periods and enzyme and substrate concentrations yielded monoglycerides with no detectable butyric acid.

These results show that the butyric acid in the bovine milk fat is attached exclusively at the α-carbon atoms of the glyceride. This and the negligible exchange of the lower fatty acids between the different species of glycerides in the presence of lipase would suggest that they are incorporated into α, β-diglycerides, most probably by the mechanism suggested by WEISS, KENNEDY AND KIYASU[8].

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L-Xylulokinase and L-xylulose 5-phosphate–L-ribulose 5-phosphate 3-epimerase in *Aerobacter aerogenes*

Growth of *Aerobacter aerogenes* on L-xylose evokes the formation of a cobalt-activated isomerase which produces L-Xu fom L-xylose[1], and two additional new enzymes, a kinase and a 3-epimerase, which effect the further metabolism of L-Xu. The L-xylulokinase has been purified 500-fold from cell extracts by protamine, ammonium sulfate, alumina Cγ, and DEAE-cellulose fractionations. Of the four ketopentoses, only L-Xu was phosphorylated. The product of L-Xu phosphorylation was prepared by incubating 1 mmole L-Xu, 1 mmole ATP, 100 μmoles NaGSH, 80 μmoles ethylenediaminetetraacetate, and 2 mmoles $MgCl_2$ with 633 units kinase* (before DEAE-

Abbreviations: Xu, xylulose; Ru, ribulose; -P, phosphate; NaGSH, sodium glutathione; DEAE-cellulose, diethylaminoethylcellulose; ATP, adenosine triphosphate; TPN, triphosphopyridine nucleotide; DPNH, reduced diphosphopyridine nucleotide.

* The pH was maintained at 6.8 to 7.5 with 0.1 N NaOH. 1 unit = 1 μmole phosphorylated/h.