Differences in the Fatty Acid Patterns Synthesized from ^{14}C-Acetate by Functional and Dedifferentiated Bovine Mammary Cells

Fatty acid synthetase (FAS) which is very active in lactating bovine mammary tissue is characterized by its tendency to synthesize short chain fatty acids from acetate[1–3]. There is some evidence that stearic and oleic acid can also be synthesized from acetate in bovine mammary tissue[1,4,5] though the preponderance of these is derived from lipoproteins[4,6]. Bovine mammary cells cultured in vitro synthesize labeled stearic and oleic acid from acetate especially when these cells have dedifferentiated.

Materials and methods. Fresh cells from a lactating Guernsey cow were dispersed by the method of EBNER et al.[7], and cultured as described[3,7–9]. The old bovine cells originally prepared from a lactating cow had been subcultured for two years. Approximately 1×10^7 cells were incubated in media containing 50 nM of sodium 1,2 ^{14}C acetate (1 μCi) for 18 h at 37°C. The lipids were extracted from the cells by the method of FOLCH et al. (1957). The lipids were fractionated and analyzed by thin layer and radiogas chromatography as described[8,9]. Radioactivity was quantified using a liquid scintillation spectrophotometer (Packard TriCarb).

Results. The fresh and dedifferentiated cells converted 54 ± 6 and $9 \pm$% of the acetate into lipids, respectively. Most of the label was incorporated into typical milk fatty acids by functional cells whereas subcultured cells, which had undergone dedifferentiation, synthesized predominantly long chain fatty acids (Table). There was synthesis of unsaturated fatty acids C10:1, C12:1, C14:1 and C16:1 by the freshly prepared cells (Figure 1). The radioactive peaks occurring between the major acids include methyl ketones and fatty acids with uneven carbon chain lengths. Fresh cells from the lactating mammary synthesized stearic and oleic acid, though these rarely contained more than 4% of the radioactivity incorporated.

The pattern of fatty acids synthesized by dedifferentiated cells was very different compared to fresh cells (Figure 2). The short chain and unsaturated fatty acids were absent and there was a greater incorporation of acetate into stearic and oleic acid.

Discussion. The pattern of incorporation of labeled acetate into fatty acids by freshly dispersed cells was generally similar to that observed previously[3,9] except that there was a relatively greater incorporation of acetate into unsaturated acids and stearic acid. Like the goat, rat and rabbit[10–12] the fatty acid synthetase of the cytosol is responsible for the characteristic fatty acids synthesized by functional bovine mammary tissue[13]. However a mechanism which accounts for the consistent presence of short chain fatty acids in bovine milk fat has yet to be elucidated. The specificity of chain termination by bovine mammary FAS may be substrate dependent i.e., similar to that of rat and rabbit mammary and yeast

The percentage distribution of radioactivity in fatty acids isolated from bovine mammary cells following incubation with 1,2^{14}C-acetate

Fatty acid	Lactating cells	Dedifferentiated cells
C4:0	2.0	—
C6:0	2.2	—
C8:0	3.4	—
C10:0	12.2	—
C10:1	3.0	—
C12:0	12.0	—
C12:1	2.0	—
C14:0	18.8	9.1
C14:1	6.4	—
C16:0	26.0	39.3
C16:1	8.3	2.7
C18:0	2.4	32.8
C18:1	1.3	16.1

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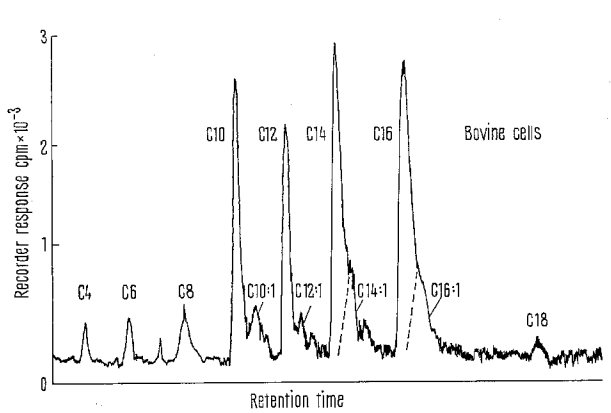
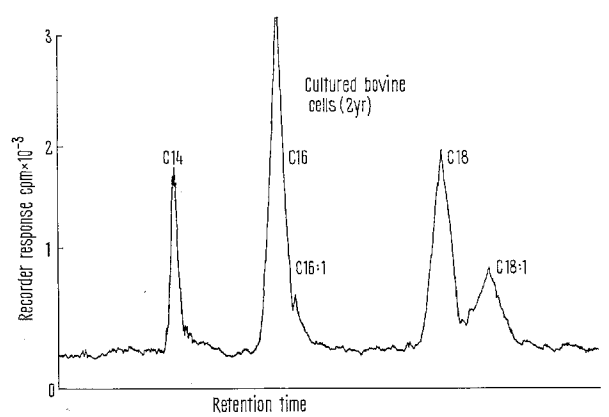


Fig. 1. Radio gas chromatogram of the labeled fatty acids synthesized from Na,1,2^{14}C-acetate by fresh bovine mammary cells in vitro. Fatty acids are denoted by their carbon chain length.



F. 2. Radiogas chromatogram of the labeled fatty acids synthesized from Na-1,2^{14}C acetate by bovine mammary cells subcultured for 2 years.

FAS[11–14] where the concentrations of acetyl CoA and malonyl CoA markedly influence the chain lenght of the fatty acids synthesized by FAS. Thus, the high concentration of acetate available to the lactating bovine mammary gland[15] may result in a high intramammary acetyl CoA/malonyl CoA ratio and consequently the production of short chain fatty acids is enhanced. The biosynthesis of labeled stearic and oleic acid from acetate by the mammary cells confirms the observations of GERSON et al.[4]. The mechanism and intracellular site of synthesis of these long chain fatty acids is not known. The soluble FAS may synthesize these acids de novo similar to FAS from rabbit mammary tissue[12] or they could be produced by the chain elongation mechanism of the mitochondrial membranes[16,17]. SMITH and MCCARTHY[17] reported that mitochondria of bovine mammary tissue synthesized stearic acid from acetate.

The mode of synthesis of the unsaturated fatty acids from acetate by mammary cells has not been studied. The present observation indicates that these are synthesized de novo. An active stearyl desaturase is present in lactating bovine mammary cells[8] but its specificity toward fatty acids of shorter chain length has not been determined. The suggestion that the unknown radioactive peaks are methyl ketones is plausible because these occur in milk fat[18] and the precursor β keto-acids are synthesized from acetate by bovine mammary tissue[19]. SUMPER et al.[14] showed that when NADPH is limiting for yeast fatty acid synthetase, β-keto acyl derivatives are the predominant products. In cultured mammary cells the incorporation of acetate into unsaturated fatty acids and methyl ketones may result from inadequate NADPH levels.

The observation that the subcultured bovine cells failed to utilize acetate for synthesis of short chain fatty acids is further evidence of the dedifferentiated state of these cells and confirms the findings of LARSON[20] that bovine mammary cells lose their specialized functions when cultured in vitro. The speculation as to whether the same FAS enzyme whose chain terminating specificity is altered by variation in substrate levels, is functioning in lactating and dedifferentiated cells or if discrete and different FAS enzymes are active in both cell types warrants further study. If the activities of different FAS species is dependent on the particular physiological state of populations of mammary cells in vivo it may account for some variation in the source of milk stearic acid[5].

Résumé. Les cellules de lactation des tissus mamellaires, lorsqu'elles sont cultivées in vitro, perdent leur capacité de synthétiser les acides gras à courte chaîne propres au lait de vache – les cellules et peut-être la synthétase, enzyme des acides gras, se dédifférencient et produisent, à partir de l'acétate exogène radioactif, des acides gras à longue chaîne, c'est-à-dire les acides palmitique, stéarique et oléique.

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The Effect of Spermine on the Thermal Denaturation Profiles of Ribosomal RNA and of Ribosomes from *Bacillus stearothermophilus* in the Presence of Physiological Concentrations of Cations

The naturally occuring polyamines, spermine, spermidine and putrescine, can stabilize the secondary structure of RNA against thermal denaturation[1–4]. The extent to which polyamines stabilize RNA and also DNA against thermal denaturation depends on the presence of other cations in solution[3,5,6]. In previous studies on the effects of polyamines on the thermal denaturation profiles of RNA and ribosomes measurements have been made at ionic strengths below those which occur in physiological conditions[1–4]. Most bacteria contain spermidine and putrescine[7], but the thermophile *Bacillus stearothermophilus* contains spermine and spermidine[8]. We have therefore studied the effect of spermine on the thermal denaturation profiles of rRNA and of ribosomes from *B. stearothermophilus* in the presence of physiological concentrations of other cations, in order to determine whether the presence of spermine in this organism affects the stability of its ribosomes and rRNA in vivo.

B. stearothermophilus strain 8923 was grown as described previously[9]. Ribosomes were prepared from exponentially growing cells and endogenous spermine was removed from ribosomes by dialysis against 1 *M* KCl-0.01 *M* *Tris*-HCl buffer pH 7.6 followed by dialysis against 0.01 *M* magnesium acetate-0.01 *M* *Tris*-HCl buffer pH 7.6[8]. rRNA was extracted from ribosomes using guanidinium chloride[10]. The thermal denaturation profiles, which are the means of 4 determinations, were measured in a Unicam SP800 spectrophotometer having a temperature programmer SP876. Ribosomes or rRNA were diluted about 50-fold in the appropriate buffered salt solution to give an

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