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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absorbance at 260 nm of 0.5 to 0.8 U/ml. Samples were equilibrated at 20°C for 10 min and then heated at a rate of 1°C per min.

The thermal denaturation profiles were measured in 0.15 *M* and 0.3 *M* KCl in the presence and in the absence of 0.01 *M* magnesium acetate, all solutions being buffered by 0.01 *M* sodium cacodylate buffer pH 7.0 In F gure 1 the effect of varying the concentration of spermine on the thermal denaturation profiles of rRNA in 0.3 *M* KCl and in the absence of magnesium ions is shown. The maximum stabilization of rRNA is obtained in the presence of 5 m*M* spermine; further stabilization is not achieved by 10 m*M* spermine. The intracellular concentration of spermine in *B. stearothermophilus* is about 2 m*M*[9]. In Figure 2a–d the effects of 2 m*M* spermine on the thermal denaturation profiles of rRNA in 0.15 *M* and 0.3 *M* KCl and in the presence and in the absence of magnesium ions are shown. In all 4 cases spermine stabilizes rRNA. Greatest stabilization by spermine is achieved in the absence of magnesium ions (Figure 2 a and c), but spermine greatly increases the cooperativity of thermal denaturation in the presence of 0.01 *M* magnesium acetate and 0.15 *M* KCl (Figure 2b). The corresponding results obtained using ribosomes are shown in Figure 3 a–d. Ribosomes have much more cooperative thermal denaturation profiles than isolated rRNA. In the absence of magnesium ions spermine still has a pronounced effect on the thermal denaturation profiles of ribosomes (Figure 3 a and c), but in the presence of 0.01 *M* magnesium acetate and 0.3 *M* KCl, where thermal denaturation is highly cooperative, spermine has no effect (Figure 3d).

Although it is possible to measure the total concentrations of cations within cells it is difficult to estimate accurately the proportion of the various ions which are in free solutions and those which are firmly associated with intracellular polyanions. Potassium ions and magnesium ions appear to be the two most important intra-

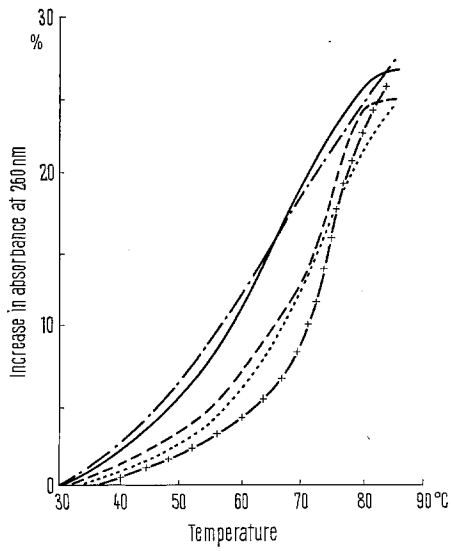

Fig. 1. The effect of spermine on the thermal denaturation profile of rRNA from *B. stearothermophilus* measured in 0.3 *M* KCl-0.01 *M* cacodylate buffer: —, no spermine; -----, 1 m*M* spermine; ..., 2 m*M* spermine; -×-×-, 5 m*M* spermine; and ---, 10 m*M* spermine.

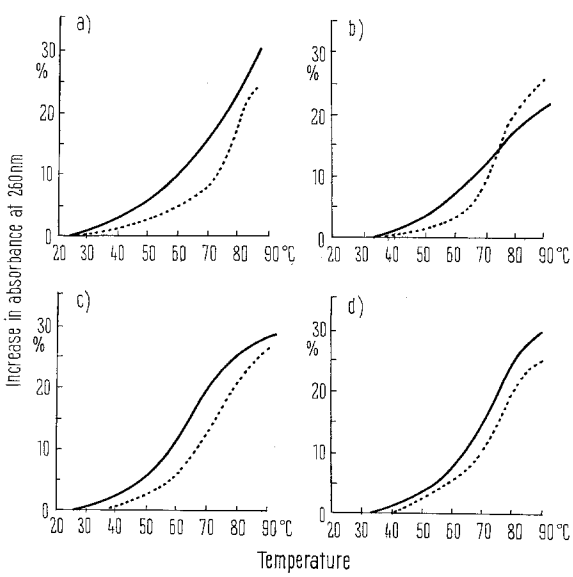

Fig. 2. The effect of 2 m*M* spermine on the thermal denaturation profiles of rRNA from *B. stearothermophilus:* a) in 0.15 *M* KCl-0.01 *M* cacodylate buffer; b) in 0.15 *M* KCl-0.01 *M* magnesium acetate-0.01 *M* cacodylate buffer; c) in 0.3 *M* KCl-0.01 *M* cacodylate buffer; d) in 0.3 *M* KCl-0.01 *M* magnesium acetate-0.01 *M* cacodylate buffer.–, the profiles obtained in the absence of spermine, and ..., the profiles obtained in the presence of 2 m*M* spermine.

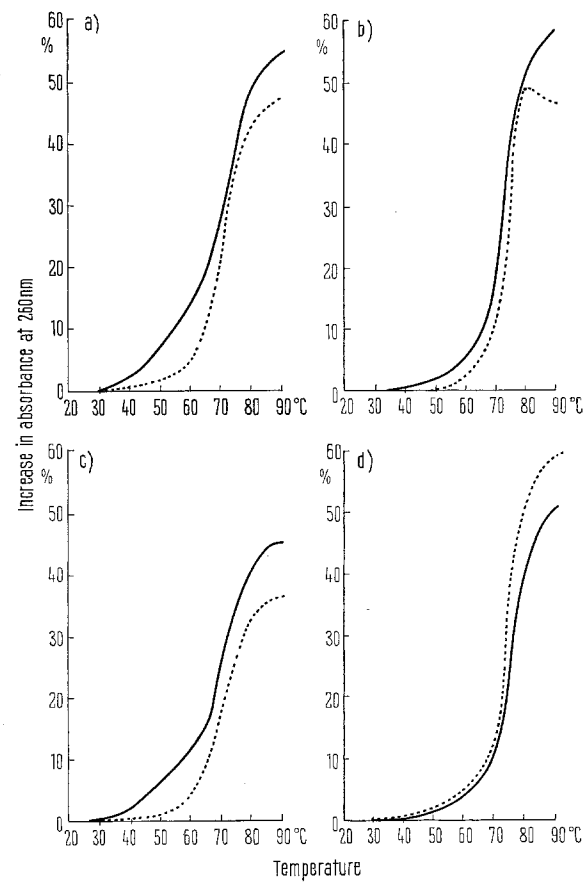

Fig. 3. The effect of 2 m*M* spermine on the thermal denaturation profiles of ribosomes from *B. stearothermophilus:* a) in 0.15 *M* KCl-0.01 *M* cacodylate buffer; b) in 0.15 *M* KCl-0.01 *M* magnesium acetate-0.01 *M* cacodylate buffer; c) in 0.3 *M* KCl-0.01 *M* cacodylate buffer; d) in 0.3 *M* KCl-0.01 *M* magnesium acetate-0.01 *M* cacodylate buffer. —, the profiles obtained in the absence of spermine, and ..., the profiles obtained in the presence of 2 m*M* spermine.

cellular cations in bacteria. Their concentrations are strictly controlled and are related to the overall growth rate of the cells and the latter is in turn related to the concentrations of ribosomes[10]. Although there may be a substantial intracellular concentration of sodium ions this appears to be less strictly controlled and much more dependent on the concentration of sodium ions in the growth medium[11]. The intracellular concentration of potassium ions in bacteria, excluding halophiles, ranges from 0.05 M to 0.6 M[9,11,12,14,16] and most of this appears to be unbound[15]. The intracellular concentration of magnesium ions is usually about 0.03 M[17–19] of which about 10% is unbound[18], and the intracellular sodium ion concentration varies between 0.005 M and 0.2 M[9,11,14,15].

Since the effects of sodium ions and of potassium ions on the stabilization of the secondary structure of nucleic acids are similar[20], in the present work thermal denaturation profiles were measured in the presence of potassium ions as the only monovalent ion. The monovalent ion concentrations used here, 0.15 M and 0.3 M, thus represent the middle of the range of physiological concentrations of monovalent ions, and the magnesium ion concentration, 0.01 M, probably represents the upper range of concentration for unbound magnesium which is likely to occur in a bacterial cell. Under these conditions a physiological concentration of spermine stabilizes rRNA against thermal denaturation, but it has a less pronounced effect on ribosomes, the extent of which depends particularly on the concentration of magnesium ions. The presence of spermine in a procaryotic organism appears to be unusual, but, in *B. stearothermophilus* the occurrence of spermine may be related to the ability of this organism to grow at high temperatures, and more particularly, the ability to assemble ribosomes from rRNA at these temperatures.

Zusammenfassung. Nachweis, dass Spermin die rRNS von *B. stearothermophilus* bei Thermaldenaturierung in Gegenwart physiologischer Konzentrationen von Kalium- und Magnesiumionen stabilisiert.

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Flavonoids of *Metrosideros polymorpha* (Myrtaceae)

In the course of a survey of some Hawaiian plants for flavonoids we were made aware, by Professor S. Siegel and Miss Caroline Corn of the University of Hawaii, of the existence of distinct varieties of 'Ohia' (*Metrosideros polymorpha* Gaud. (Myrtaceae)), which is endemic to the islands. One variety bears shovel-shaped leaves with a white, tomentose dorsal surface and a second, which we also examined, has smooth, ovate leaves. The only previous studies on the chemistry of this genus are concerned with the identification of the triterpenoid, arjunolic acid, in *M. umbellata*[1] and the identification of methyl gallate and gallic, ellagic, ursolic and betulic acids in flowers of *M. excelsa*[2].

Fresh leaves of *M. polymorpha* wer first extracted with 70% ethanol followed by fractionation on a polyamide column using water with increasing concentrations of ethanol as an eluent. The column fractions were further fractionated and purified by paper chromatography yielding 5 major and 2 minor flavonoids. Acid hydrolysis of 3 of the major components yielded quercetin (3,3',4',5,7-pentahydroxyflavone), while the remaining major components gave myricetin (3,3',4',5,5',7-hexahydroxyflavone) as the aglycones. The sugar moieties of the 3 quercetin glycosides were identified as arabinose, galactose and rhamnose, respectively. The 2 myricetin glycosides gave on acid hydrolysis arabinose and rhamnose. Ultraviolet data indicated that glycosylation of all 5 flavonoids occurred in position 3. This was further confirmed by H_2O_2 oxidation[3] giving rise to the same monosaccharides previously mentioned. The glycosides were thus identified as quercetin-3-arabinoside, quercetin-3-galactoside, quercetin-3-rhamnoside, myricetin-3-arabinoside and myricetin-3-rhamnoside.

The 2 minor flavonoids gave, on acid hydrolysis, quercetin and myricetin, respectively, as well as galactose in both cases. The UV-spectra of both flavonoids were similar to those of the 3-galactosides of quercetin and myricetin but, at the same time, exhibiting the presence of a strong acyl group. Alkaline hydrolysis failed to afford any recognisible acylating groups. The chromatographic properties of these compounds are not indicative of polyglycosides. Although larger amounts of plant material (5 kg) were re-extracted and fractionated, only trace amounts of both flavonoids were obtained which prevented any further studies. We found that all 7 flavonoids are present in both varieties of *Metrosideros* and that therefore this feature of their chemistry is not useful in distinguishing them.

Résumé. Dans deux variétés de *Métrosideros polymorpha* Gaud. (Myrtaceae), nous avons trouvé les favanoides suivants: arabinoside-3, galactoside-3, rhamnoside-3-quercetine, et arabinoside-3, thamnoside-3-myricetine; en outre, deux glycosides en moindre quantité.

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