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Dissociation of Casein Micelles to Soluble Casein Complexes

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The internal cohesion of the various protein components within the intact casein micelle was investigated by removal of the colloidal calcium phosphate and the soluble salts of milk under mild conditions to cause dissociation of the casein micelles to soluble casein complexes.

On fractionation at 5° of skim milk on Sepharose 2B (Pharmacia, Uppsala, Sweden) columns, the casein micelles were eluted at the void volume. As with Sephadex G-200, the molecular-weight exclusion limit of Sepharose 2B for proteins is likely to be at least four times the value (2.5×10^7) quoted for dextrans by the manufacturers. Consequently the molecular size of casein micelles appears to exceed 10^8 , in agreement with results obtained by electron microscopy (Nitschmann, 1949; Carroll, Thompson & Nutting, 1968). To prevent micellar disintegration during gel filtration, similar to that encountered by Yaguchi & Tarassuk (1967), columns were equilibrated with a buffer identical in composition with milk ultrafiltrate.

On removal of the colloidal calcium phosphate from milk by the method described by Pyne & McGann (1960), the casein micelles are dissociated to smaller complexes, which were eluted from Sepharose 2B columns in the expected position for a globular protein of mol.wt. approx. 2×10^6 .

These soluble complexes contain α_s -, β - and κ -casein apparently in aggregated form, and it is suggested that they may constitute the subunits (Rose, 1969) of casein micelles in which they are bonded together by colloidal calcium phosphate.

When the soluble salts of milk are removed together with colloidal calcium phosphate by dialysis in the cold against 0.2–1 M-NaCl, the casein micelles are solubilized. As indicated by gel filtration on Sepharose 4B columns equilibrated with various concentrations of NaCl, the molecular weights of the casein aggregates present in these preparations vary with the concentration of NaCl used for dialysis. The smallest aggregates were obtained by dialysis against 0.5 M-NaCl.

The relationship of these various submicellar complexes to the intact casein micelle and its subunits will be discussed.

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DEMONSTRATION

Clinical Biochemical Analysis: The Baird and Tatlock Analmatic Instrument

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This is an automatic analyser of the so-called 'discrete' type, processing serum analyses in batches of 100. Methods are available for a variety of tests and some of these will be demonstrated.