containing fraction was eluted from a DEAE-cellulose column by using a buffer gradient extending across two liters of eluate, the composition of which ranged from 0.04 M Tris-phosphate, pH 8.6, to 0.5 M Triphosphate, pH 4.65.

When this material was rechromatographed on a column of Sephadex G-200, the active fraction passing through the column had kininogen activity equivalent to 0.65 μg bradykinin per milligram dry protein, representing the most active preparation obtained so far.

From the position of the kininogen fraction in the effluent from the Sephadex G-200 column, it is estimated that the kininogen in milk has a molecular weight of approximately 70,000. If this is true and assuming one molecule kinin per molecule of kininogen, the best preparation we have made is approximately 1% pure.

However, it is apparent from our work that the milk of the cow does contain a significant amount of protein which, on incubation with trypsin or snake venom, will release a material having the kinin-like ability to contract smooth muscle. The composition of this material and its similarity to colostrokinin awaits the isolation of both these materials.

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Thiol-disulfide Interchange in Formation of β-Lactoglobulin-κ-Casein Complex

When milk, or a solution of β-lactoglobulin and κ-casein, is heated, a complex involving these two proteins is formed. Available evidence (3) implicates thiol or disulfide groups in formation of this complex, but such groups affect tertiary structure and may influence the positioning of adjacent molecules and, therefore, could influence complex formation without forming a covalent linkage between the two complexing proteins.

Identification of the β-lactoglobulin-κ-casein complex in the presence of denatured but uncomplexed β-lactoglobulin is difficult. We have observed that development, for each sample, of two polyacrylamide gel electrophoretic patterns with urea-tris buffers (1), one in the absence and the other in the presence of mercaptoethanol, provides reasonably conclusive evidence of the presence of the complex in β-lactoglobulin-κ-casein mixtures (Fig. 1A and B). In the absence of mercaptoethanol, presence of the complex (Fig. 1A, slot 6) is indicated by a decrease in intensity of staining, relative to the unheated control, of the β-lactoglobulin zones and sometimes of the κ-casein zones, and an increase in intensity of staining in the region between β-lactoglobulin and the starting slot. The position of the zone(s) of maximum staining intensity in this region varies with the κ-casein preparation used and with conditions of heating (compare Fig. 1A, slot 6 and Fig. 3A, slot 4). In the presence of mercaptoethanol (Fig. 1B), the increased intensity of staining caused by the presence of the complex disappears and is replaced by zones characteristic of uncomplexed β-lactoglobulin and κ-casein (Fig. 3B).

We have used this test to determine the presence or absence of β-lactoglobulin-κ-casein complex in mixtures of these two proteins, and modifications of them, under a variety of conditions. The following observations are of significance:

1) No complex formed when β-lactoglobulin and alkylated (S-carboxyamidomethyl) κ-casein were heated together.

2) Complex formed to a limited extent when heated and recooled β-lactoglobulin solution was mixed with κ-casein solution (Fig. 2, slot 4) (note the decreased intensity of the β-lactoglobulin bands and of the κ-casein band adjacent to the slot, and the increased intensity in the region

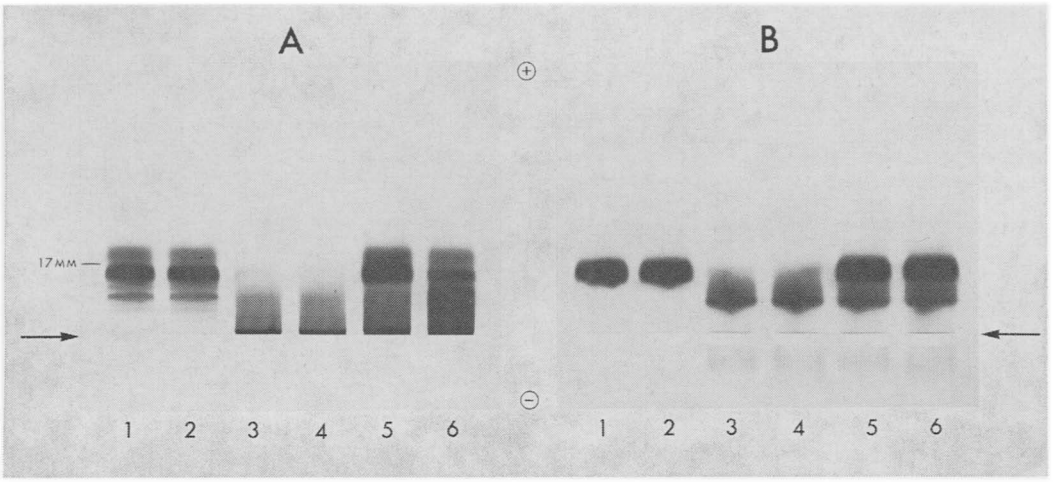

FIG. 1. β-Lactoglobulin-κ-casein complex formation shown by electrophoretic patterns: A in the absence, and B in the presence of mercaptoethanol; 6 hr at 20 ma. 1) β-lactoglobulin; 2) β-lactoglobulin heated 70 C, 60 min; 3) κ-casein; 4) heated κ-casein; 5) 1:1 mixture of β-lactoglobulin and κ-casein; 6) heated mixture.

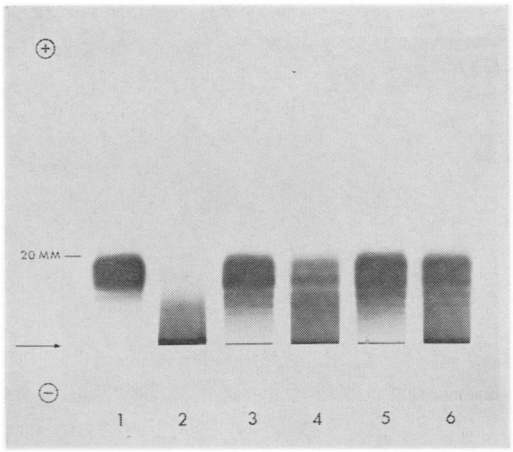

FIG. 2. Effect of alkylation of heated β-lactoglobulin on complex formation; mercaptoethanol absent; 6.25 hr at 20 ma.
1) β-lactoglobulin; 2) κ-casein; 3) heated (100 C, 5 min) β-lactoglobulin; 4) 1:1 mixture of heated β-lactoglobulin and κ-casein; 5) alkylated, heated β-lactoglobulin; 6) 1:1 mixture of alkylated, heated β-lactoglobulin, and κ-casein.

around 10 mm from the slot.) However, when the heat activated thiol groups of the heated β-lactoglobulin were alkylated with iodoacetamide in the absence of urea and of reducing agent, very little complex was formed (Fig. 2, slot 6). β-lactoglobulin contains two thiol and four disulfide groups per mole (2), and these can rearrange by (probably) random interchange when β-lactoglobulin solution is heated. A number of molecular types would, therefore, be present in the heated, recooled solution. Complex formation when κ-casein is added to heated, recooled β-lactoglobulin solution, but not when it is added to such solutions after alkylation, suggests that the reactivity of the thiol groups, and not their influence on molecular configuration or position, is the major factor influencing complex formation.

3) As previously shown (3), complex formation is inhibited in β-lactoglobulin-κ-casein mixtures by added N-ethylmaleimide (NEM). This is clearly indicated in Fig. 3, where extensive complex formation is indicated by the virtual disappearance of the β-lactoglobulin bands and the presence of a dense zone adjacent to the slot in A, slot 4. No such changes occur in slot 6, though the mobility of the β-lactoglobulin has been increased by heating with NEM. A similar inhibition appeared to occur in milk, although the complexity of the gel patterns made interpretation difficult (Fig. 4). In this figure, β-lactoglobulin zones cannot be distinguished, but complex formation is suggested by decreased intensity of staining in the κ-casein region and the narrow dark band on the edge of the slot in slot 4, as compared to slot 3. Slots 5 and 6 do not differ markedly in these areas, probably because NEM prevented complex formation.

4) Regardless of the conditions of formation, β-lactoglobulin-κ-casein complex was separated into components having the electrophoretic properties of β-lactoglobulin and κ-casein by mercaptoethanol in the presence of urea.

These observations lead us to conclude that the complex between β-lactoglobulin and κ-casein is formed by thiol-disulfide interchange, and that the principal chemical linkage responsible for its stability is the disulfide bond.

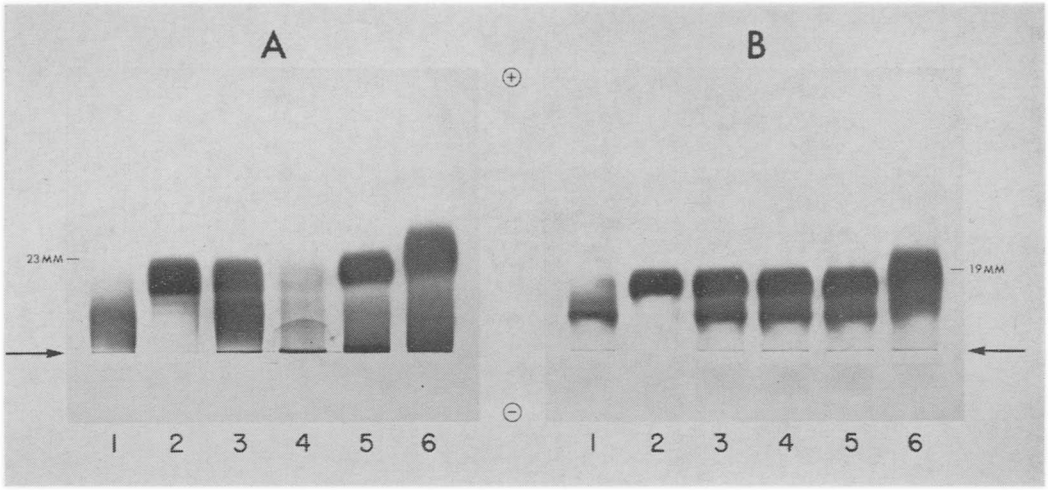

FIG. 3. Effect of NEM on complex formation: (A) mercaptoethanol absent, (B) mercaptoethanol present; 6 hr at 20 ma. 1) κ-casein; 2) β-lactoglobulin; 3) 1:1 mixture; 4) heated (100 C, 5 min) mixture; 5) mixture plus NEM, 3 mM/liter; 6) mixture plus NEM, heated.

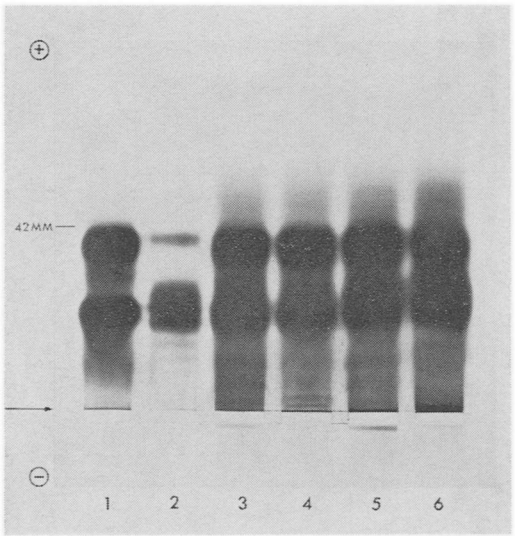

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FIG. 4. Formation of β-lactoglobulin-κ-casein complex in milk and milk plus NEM; mercaptoethanol absent; 11.25 hr at 20 ma. 1) acid casein; 2) β-lactoglobulin; 3) skimmilk; 4) heated (100 C, 5 min) milk; 5) milk plus 3 mM NEM; 6) milk plus NEM, heated. The gel has been deliberately overloaded, so as to emphasize changes in the area between β-casein and the slot.