which has not been submitted to flow, few of these fibrils appear.

The electron micrographs of Imhof and Hostettler[3] show that, following a linear aggregation of casein micelles (each micelle containing about 1,000 casein molecules) at the initial coagulation of the milk by rennet, fusion of the micelles occurs during the later stages of the cheesemaking process. The electron micrographs of Baud, Morard and Pernoux[4] seem to indicate the formation of appendages which afterwards serve to join the micelles during coagulation. The fibril formation which we have observed could be explained by the orientation of the separate casein molecules (axial ratio about 8 : 1) and their more or less end-to-end attachment, or more readily by some uncoiling of the polypeptide chains. The flow of the curd mass could itself contribute to this uncoiling, since proteins may be denatured by mechanical shearing action.

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[1] Czulak and Hammond, *Aust. J. Dairy Tech.*, **11**, 58 (1956).
[2] Wolpers and Ruska, *Klin. Wochenschr.*, **18**, 1077, 1111 (1939).
[3] Imhof and Hostettler, *Schweiz. Milchztg.*, **82**, No. 63 (1956).
[4] Baud, Morard and Pernoux, *Z. wiss. Mikr.*, **61**, 290 (1953).

First and Second Cycle Casein in Milk

The component of the casein complex which, according to Waugh and Von Hippel[1], is responsible for the colloid chemical stability of the casein micelles in milk is *k*-casein. The same authors have also given its preparation, as follows. The casein in skim milk is precipitated by addition of calcium, and by subsequent removal of calcium from the precipitate a solution of the so-called 'first cycle casein' is obtained. This first cycle casein contains β-casein and the α/*k*-complex. Reprecipitating first cycle casein with 0·25 *M* calcium chloride at 37° C. results in the splitting of the α/*k*-complex and hence in the chemical instability of the casein micelles in the colloid. The precipitate was named 'second cycle casein', and it no longer contains the protective *k*-casein in stabilizing quantities. The latter can be isolated from the remaining supernatant of second cycle casein. In its physico-chemical properties, such as electrophoretic mobility, diffusion and ultracentrifugation characteristics, *k*-casein was shown to resemble α-casein closely.

The present communication deals with an electrophoretic study of first and second cycle caseins both with and in the absence of the milk-clotting enzyme rennin.

From an earlier investigation by Nitschmann and Lehmann[2] with acid-precipitated casein it is known that the rennin action electrophoretically is characterized by the splitting of the α-peak into two distinct components, namely, α_1- and α_2-casein, with nearly the same mobilities. We have repeated the Nitschmann/Lehmann experiment both with first and second cycle casein.

Table 1

Exp.	Substrate	Rennet	α_1/α_2 split	α_2 (per cent)	$\mu\alpha_1 \times 10^5$ (cm.²/V./sec.)
A	First cycle casein	Heat-inactivated	Occasionally	27	8·98
B	Second cycle casein	Heat-inactivated	Unseparated	13	10·90
C	First cycle casein	Active solution	Very pronounced	29	9·05
D	Second cycle casein	Active solution	Pronounced	14·5	10·70

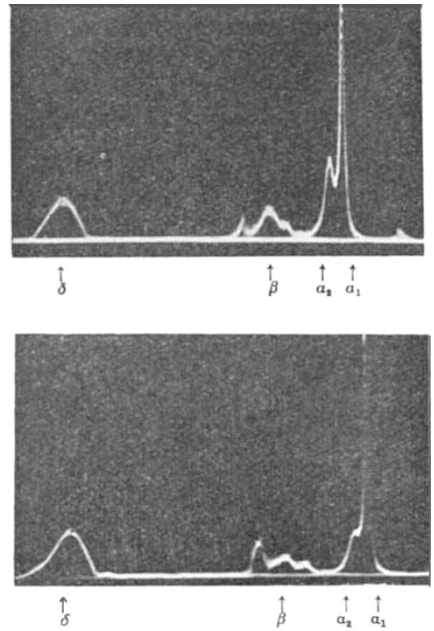

Fig. 1. Ascending electrophoretic patterns of first and second cycle caseins after 4,800 sec. Conditions of experiment: protein concentration, 1·7 per cent; Michaelis buffer pH = 7·3, $\Gamma/2$ = 0·1; field strength, 9·75 V./cm.; 2° C. Above: first cycle casein, active rennet solution added; below: second cycle casein, active rennet solution added

The caseins were incubated at 35° C. with a solution of commercial rennet powder (1 : 100,000) of the N.V. Chemische Industrie Van Hasselt, Amersfoort, Holland. The rennet concentration was chosen such that 10 mgm. rennet were added per gram of casein. In this way its concentration is too low to be distinguished electrophoretically. For comparison heat-inactivated enzyme was also added to first and second cycle casein. The experiments were repeated several times. A typical set of experiments are summarized in Fig. 1 and Table 1, which show that α_2-casein is much less abundant in second cycle than in first cycle casein. These results suggest that α_2-casein is closely related to the *k*-casein of Waugh and Von Hippel: possibly the two components are actually identical.

Additional support for this conclusion is found in a comparison of the mobilities of the α_1-component in first and second cycle casein (Table 1, column 6). The mobility of the α_1-casein is considerably higher in second ($10{\cdot}80 \pm 0{\cdot}1 \times 10^{-5}$ cm.²/V./sec.) than in first cycle casein ($9{\cdot}02 \pm 0{\cdot}03 \times 10^{-5}$ cm.²/V./sec.). Obviously this mobility increase is due to the decrease in α_2-content in the second cycle and the accompanying decrease in α_1/α_2-interaction.

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[1] Waugh, D. F., and von Hippel, P. H., *J. Amer. Chem. Soc.*, **78**, 4576 (1956).
[2] Nitschmann, Hs., and Lehmann, W., *Experientia*, **3**, 153 (1947).