Note

Variation in Casein from Individual Human Milk Samples

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It is known that casein from bovine colostrum gives the characteristic pattern of α_{s1} -, β - and κ -casein by starch-gel electrophoresis as that from mature milk does,¹⁾ similarly.

However, the casein composition in human milk changes markedly with the progression of lactation,²⁾ and human whole casein from individual mature milk samples gives different patterns by starch-gel electrophoresis.³⁾

The acid coagulability of human casein is different in character from that of bovine casein. Toyoda and Yamauchi⁴⁾ classified human milk into three types with respect to acid-coagulability, i.e. acid-coagulable types and acid-noncoagulable type I and II.

We have studied the variation in human casein at various stages of lactation by Disc-polyacrylamide-gel electrophoresis (Disc-PAGE) and SDS-polyacrylamide-gel electrophoresis (SDS-PAGE).

Human lactoferrin (Lf) coprecipitated with human casein at pH 4.6⁵⁾ was also identified by SDS-PAGE and

immunodiffusion.

Individual milk samples at various stages of lactation were collected respectively from three mothers and mature milk samples from 14 mothers. After being defatted, they were stored at -20°C .

Human whole casein was prepared according to the method of Nagasawa *et al.*⁶⁾ The pH of the skim milk was adjusted to 4.6 with *N* HCl and after being left to stand for 1 or 2 hr the precipitate was obtained by centrifugation at approximately $35,000 \times g$ at 30°C for 10 min. The precipitate was suspended in water and dissolved by the addition of *N* NaOH. The pH of this solution was

FIG. 1. Disc-PAGE Patterns of Human Whole Casein from a Mother at Various Stages of Lactation.

1, first day of lactation; 2, second day of lactation; 3, 5th day of lactation; 4, 10th day of lactation; 5, 40th day of lactation.

FIG. 2. SDS-PAGE patterns of Human Lactoferrin (Lf) and Human Whole Casein Corresponding to the Samples Analyzed in Fig. 1.

a, CB-staining; b, PAS-staining.

readjusted to 4.6 with *N* HCl and the precipitate was obtained by centrifugation at approximately $1,000 \times g$ at 30°C for 10 min. The precipitate was suspended in water and the pH was adjusted to 7.0. The casein was then lyophilized. In this paper, casein was defined as the proteins precipitated at pH 4.6. Disc-PAGE was performed by the method of Davis,⁷⁾ SDS-PAGE was by the method of Weber and Osborn⁸⁾ and immunodiffusion was according to the method of Ochterlony.⁹⁾

The yield of casein from colostrum (first and second day of lactation) was approximately 0.04% (w/v) on average. From the milk after 5th day, the average of the yield was 0.4% (w/v). The exact content of casein was, however, not clear because whey proteins such as Lf were contaminated in the casein fraction obtained by the method used. So the yield was assumed to be half of the above values or less, as suggested by Hambræus.¹⁰⁾

Figure 1 shows the typical Disc-PAGE patterns of human whole casein from a mother at various stages of lactation. Similar patterns were obtained in the case of the other samples. It is said that β -casein, the major component of human casein, consists of six components which have the same protein phosphorylated at different levels and shows the characteristic six bands on gel electrophoresis in the presence of urea.^{11,12)} At the beginning of lactation, the characteristic pattern of β -casein was not observed. The result agrees with that of Nagasawa *et al.*²⁾ The casein from the milk of 5th day of lactation showed the full six bands of β -casein for the first time (Fig. 1).

On Disc-PAGE κ -casein could not be identified, so we performed the analysis on SDS-PAGE to identify κ -casein as well as β -casein. Figure 2 shows the SDS-PAGE patterns corresponding to the casein samples analyzed in Fig. 1.

On SDS-PAGE of whole casein from mature milk (40th day of lactation), a band was observed between the bands of Lf and β -casein. It was stained well by periodic acid-schiff reagent (PAS¹³⁾) rather than by coomassie brilliant blue (CB). This broad band was confirmed to be human κ -casein.¹⁴⁾ By PAS staining, the band was slightly detected in the patterns of caseins from transitional milk (after 5th day of parturition) but colostrum (first and second day of lactation) did not give this band.

It was made clear by Guérin *et al.*¹⁾ that casein from bovine colostrum at the beginning of lactation gave the characteristic patterns of β - and α_{s1} -casein, and, 60 hours later, the characteristic band of κ -casein could be detected on starch-gel electrophoresis.

In human colostrum of first and second day of lactation, the amount of precipitates at pH 4.6 was very little and the patterns of precipitates on SDS-PAGE showed mainly human Lf and a component with the same mobility as Ig A. Human Lf, one of the major whey proteins,¹⁵⁾ which was contaminated in the casein fraction was identified by SDS-PAGE (Fig. 2-a) and immunodiffusion with anti-human Lf serum (Fig. 3).

FIG. 3. Immunodiffusion Pattern of Human Lactoferrin.

1, anti-human lactoferrin; 2, human lactoferrin; 3, human casein fraction.

FIG. 4. SDS-PAGE Patterns of Human Whole Casein from Individual Mature Milk Samples.

1, acid-noncoagulable type I; visible coagulation was formed when reprecipitated at pH 4.6; 2 and 3, acid-noncoagulable type II; hardly reprecipitated at pH 4.6; 4, acid-coagulable type; visible coagulation was observed when the pH of the skim milk was adjusted to 4.6.

Precipitation of casein from human milk by acid is often difficult.¹⁶⁾ Toyoda and Yamauchi⁴⁾ classified human whole casein into three types based on the acid-coagulability. In our study, five of 14 individual samples (all were collected a month or more after parturition) were, as they said, acid-noncoagulable type. No precipitate was observed when the pH of the skim milk was adjusted to 4.6. Two of them showed the similar patterns of casein as the acid-coagulable type did on SDS-PAGE, but three of them gave the PAGE-patterns where the bands of β -casein were absent and low molecular peptides were detected instead (Fig. 4). There was no close relationship between acid coagulability and the components of casein, but the

casein of acid-noncoagulable type was likely to show a tendency to be lacking in β -casein for some reason. It is unknown whether the lack of β -casein is due to digestion by proteases present in human milk or incomplete biosynthesis.

In either case, it may be not indispensable for the neonate to intake the intact casein. It would be interesting to make this clear from the aspects of sitology and physiology.

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