

POLYMORPHISM IN  $\kappa$ -CASEIN OF COW'S MILK

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The biosynthesis of three variants of  $\alpha_s$ - (Kiddy *et al.*, 1964) and  $\beta$ -caseins (Aschaffenburg, 1961) has been demonstrated to be under the control of three alleles of the respective genes, each of which produces an electrophoretically distinguishable variant. The existence of genetic variants of  $\kappa$ -casein also seemed probable; however, electrophoretically distinguishable variants have not yet been demonstrated. This may be attributable in part to the electrophoretic behavior of  $\kappa$ -casein. In starch-gel electrophoresis (Wake and Baldwin, 1961),  $\kappa$ -casein migrates as a broad unresolved zone and in polyacrylamide-gels it is almost totally retained in the sample slot (Woychik, 1964).

Reduction of the disulfide bonds of  $\kappa$ -casein obtained from pooled milk permitted the resolution of the reduced protein into three major and several minor electrophoretic components (Woychik, 1964). The electrophoretic resolution obtained after reduction suggested the possibility of detecting variations in the electrophoretic patterns of reduced  $\kappa$ -caseins obtained from the milks of individual cows. This communication reports the results of the examination of  $\kappa$ -caseins obtained from the 34 individual cows and the detection of four types of electrophoretic patterns for reduced  $\kappa$ -caseins.

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### Materials and Methods

$\kappa$ -Casein was isolated from whole casein by the urea-sulfuric acid method of Zittle and Custer (1963) and purified by precipitation of small amounts of the other casein components from solution in 50% ethanol by the addition of ammonium acetate (McKenzie and Wake, 1961).

Samples were reduced and prepared for electrophoresis by the addition of 0.01 ml mercaptoethanol to 1.0 ml of a 1% solution in pH 9.2 Tris buffer (Peterson, 1963) containing 7 M urea. The samples were allowed to stand at least 3 hours prior to electrophoresis. Polyacrylamide electrophoresis was done in an E-C Apparatus Company\* vertical electrophoresis cell with gels composed of 7% cyanogum in pH 9.2 Tris - 4.5 M urea. Samples (20  $\mu$ l) were layered in the slots and the gels run at 125 ma for approximately 3 hours at 15° C and then dyed with a solution of Amido Black. Mercaptoethanol had been incorporated into the gels initially (0.5 ml per 150 ml gel solution) to prevent possible reoxidation of the protein during electrophoresis; however, subsequent studies showed this to be unnecessary.

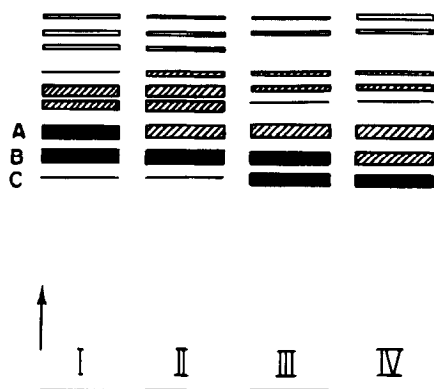
### Results and Discussion

The electrophoretic patterns of reduced  $\kappa$ -caseins consisted of one or two major and five or six minor components, depending on the individual sample examined. Three different major components were observed to occur either singly or in pairs and have been designated A, B and C in the order of decreasing electrophoretic mobilities. The examination of reduced  $\kappa$ -casein from 34 individual cows revealed the four different electrophoretic patterns illustrated in Fig. 1.

When a single major component occurred as in Types II and IV, its intensity was approximately twice that observed when it occurred as one of a pair of major components as in Types I and III. This suggested

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**Fig. 1** Electrophoretic patterns of the four types of reduced  $\kappa$ -caseins obtained with polyacrylamide gels at pH 9.2.

that the reduced  $\kappa$ -casein types could be more properly described with reference to the major components, for example A,B; B,B; etc. Two other possible combinations of major components, A,A and A,C, have not yet been observed. This may be attributable to the limited number of samples examined.

Preliminary studies indicated that both the major and minor components observed in the electrophoretic patterns of reduced  $\kappa$ -casein possess rennin sensitivity and  $\alpha_s$ -casein stabilizing ability. Although these minor components may be artifacts produced during the preparative processes, it is also possible they represent an integral part of the electrophoretic pattern of reduced  $\kappa$ -casein. Further typing and chemical studies will be required to elucidate the nature and significance of these components.

The frequency with which the  $\kappa$ -casein types occurred in the breeds examined is presented in Table I.

The occurrence of the major components either singly or in pairs is similar to that found for the  $\alpha_s$ - and  $\beta$ -casein variants (Kiddy *et al.*, 1964; Aschaffenburg, 1961), while the occurrence of major and minor components in reduced  $\kappa$ -casein is similar to the patterns observed for

Table I  
Occurrence of  $\kappa$ -Casein Types in Different Breeds

Breed	Total No. Examined	Type			
		I	II	III	IV
Holstein	19	6	5	7	1
Guernsey	8	2	3	3	
Jersey	3			3	
Brown Swiss	2	1			1
Ayrshire	2		1	1	
Totals	34	9	9	14	2

cattle transferrins (Ashton, 1959). However, proof of genetic variation and the type of control operative for  $\kappa$ -casein biosynthesis cannot be deduced from the present study, but will require extension of the typing to a larger number of samples.

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