

Polymorphism in the Red Protein isolated from Milk of Individual Cows

GENETICALLY determined polymorphism of human and other primate transferrins, the iron-binding proteins in blood, has been established by starch-gel electrophoresis^{1,2}. The transferrin in cattle differs from that in humans since cattle homozygous for an allele have been shown to contain more than one transferrin^{3,4}. The red protein, also called lactotransferrin, is an iron-binding protein found in cow's milk and has now been found to be polymorphous by gel electrophoresis. Although serum transferrin resembles the red protein in size, absorption spectra, and amount of iron bound per mole of protein, it differs in electrophoretic mobility and amino-acid content^{5,6}.

Red protein isolated from the milk of individual cows in the experimental herds at Beltsville, Maryland, was examined by zone electrophoresis. Since the red protein is a minor protein in milk, it must be isolated before electrophoretic typing can be carried out. Also, it should be reasonably pure, since a number of other minor proteins with similar mobilities are found associated with the red protein fraction.

Zone electrophoresis by the Raymond method⁷ at pH 9.0 has recently been applied to milk proteins⁸ and was found to give good resolution of the red protein when used at 5 per cent gel concentration. For most comparisons shown here the red protein was isolated from the casein fraction⁵ or the whey fraction of milk⁹. Good agreement in electrophoretic patterns was found for proteins prepared by either method.

Variation in gel-electrophoretic patterns at alkaline pH of the isolated red protein is shown in Fig. 1, numbers 1 through to 5. The photographic reproduction does not show some of the minor bands as clearly as they could be seen in the original gel. The bands are marked according to their mobility—*A* for the fastest and *D* for the slowest. Band *D* generally stains heavily, with each succeeding zone becoming lighter; however, with number 3 the *D* band is faint. Number 5 is typed (*C*), *D*, the parenthesis indicating little protein in the *C* zone. Numbers 1, 2, and 4 are typed *B*, *C*, *D*, while 3 is *A*, *B*, *C*, (*D*). The protein in 1 and 2 was isolated from the whey and casein fractions of milk from a single cow. A tabulation of the types according to breed is shown in Table 1.

As some of the bands might result from the binding of varying amounts of iron, electrophoretic determinations were made on protein solutions to which excess iron ions were added. The resulting electrophoretic patterns were unchanged. In another experiment the iron was dissociated from the red protein by adjusting a protein solution

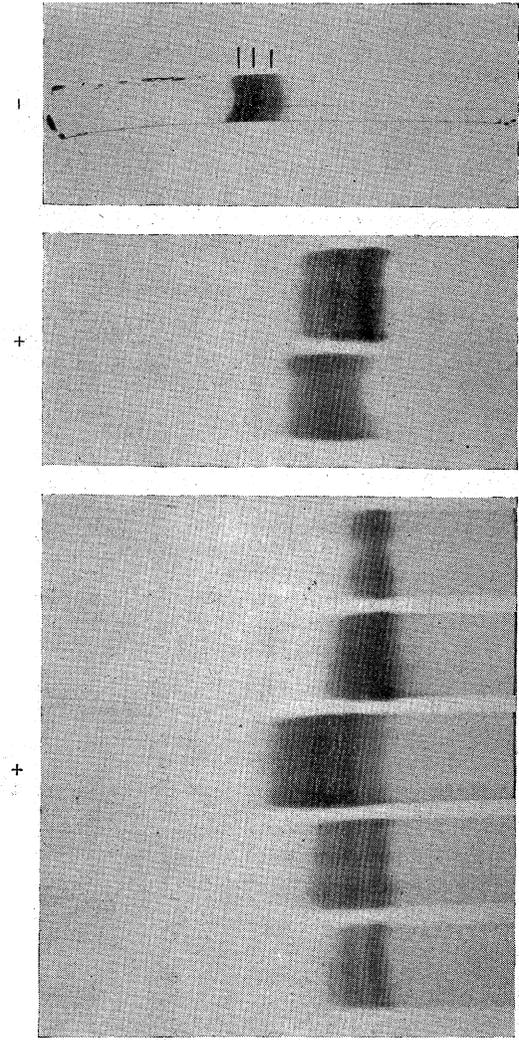


Fig. 1. Gel-electrophoretic patterns of the red protein. Vertical gel, 9-10: 1-5, protein from individual cows; 6, apoprotein; 7, control. Disk gel, pH 4-8: 8, protein from individual cow

4
p
C
D

Table 1. A COMPARISON OF THE ELECTROPHORETIC TYPE OF RED PROTEIN WITH BREED

Breed	Total	Electrophoretic zone*			
Ayrshire	2†, ‡		(C)	D	
Ayrshire	1†	B	C		D
Brown Swiss	2†		(C)		D
Brown Swiss	3(2†, 1‡)	B	C		D
Guernsey	3(1†, 2‡)		B	C	D
Holstein	1†, ‡	(A)	B	C	D
Jersey	1†	(A)	B	C	D
Jersey	1†			C	D
Jersey-Brahman	1§		B	C	D
Holstein-(mixed breed)	1 , †	A	B	C	(D)

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* Letters in parenthesis indicate a very small amount of protein.

† Red protein prepared from casein fraction.

‡ Red protein prepared from whey fraction.

§ Red protein prepared from β -lactoglobulin fraction.

|| Red protein prepared from colostrum milk of this cow.

to pH 2.0 and removing the iron with a 'Dowex 50' resin in the chloride form. An electrophoretic comparison (Fig. 1, numbers 6 and 7) shows that the zones for the apoprotein, although less sharp, have a greater mobility and are out of phase with those of the control. The consistency of the mobilities of the various red protein types and the fact that the addition of iron does not change the electrophoretic patterns suggest that a small variation in iron will not significantly alter the patterns.

Polymorphism in the red protein has also been found at acid pH, as shown in Fig. 1, number 8. Disc electrophoretic determinations were made in 7.5 per cent gel concentration, pH 4.3, according to Reisfeld *et al.*¹⁰. A current of 60 m.amp (12 tubes) was applied long enough for the protein to move through about 70 per cent of the lower gel. In contrast to the variation found at alkaline pH, all the proteins examined show a major fraction of 3 closely-related bands.

Electrophoretic differences at alkaline pH in the red protein isolated from milk of individual cows suggest the existence of a genetically controlled polymorphism. Final proof will require a study of larger numbers of individual animals than is practical with the present isolation procedures.

Preliminary peptide maps indicate differences in a few peptides, and this will be the subject of future work.

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