

PHENOTYPING OF CASEINS OF COW'S MILK: COLLABORATIVE
EXPERIMENT

Aschaffenburg has contributed materially to our current knowledge of the genetic heterogeneity of the proteins of cow's milk. In 1957 he and Drewry (4) described the genetics of the occurrence of two forms of β -lactoglobulin which they termed A and B. As a result of this study, many laboratories have been engaged in research concerning the chemical and physical properties of these proteins. By the use of starch-gel electrophoresis, a more searching tool for resolving heterogeneous mixtures than the paper electrophoresis technique employed by Aschaffenburg and Drewry, Bell (5) discovered a third β -lactoglobulin variant, C, which migrated more slowly than B. In 1961, Aschaffenburg (1) reported the presence of three forms of β -casein which could occur singly (A, B, and C) or in pairs (AB, AC, or BC) in the milk of individual cows. Thompson et al. (9) confirmed these studies and drew the same conclusion as Aschaffenburg (2) that the variation was breed specific.

Concurrent with the studies of Aschaffenburg, Thompson et al. (10) and Kiddy et al. (6) reported the presence of three forms of α_s -casein [now termed α_{s1} -casein (8)], A, B, and C, in the milk of individual cows. The mode of inheritance parallels that of β -casein; i.e., the production of these caseins is controlled by three allelic autosomal genes with no dominance. Further, breed differences in the occurrence of these proteins exist.

Certain genetic variants of α_{s1} - and β -casein occur only in low frequency. α_{s1} -A has been found only in the milk of a single blood line of Holstein cows (6), and it would not be expected to be found in a random sampling of the breed. β -Casein C has been reported only in the milk of Guernsey (2) and Brown Swiss cows (9).

The discovery of genetic variants in β - and α_{s1} -caseins has emphasized the need for uniformity of techniques for their detection. The author was prompted to submit standard phenotypes and unknown samples to a number of laboratories to determine (a) if, by using different electrophoretic techniques, the genetic variants could be detected; and (b) if there was substantial agreement on the typing of the unknown samples. Similar studies have been performed on blood proteins; i.e., transferrins. The following individuals collaborated in this study:

Dr. R. Aschaffenburg, National Institute for Research in Dairying, Shinfield, Reading, England; Dr. J. King, Animal Breeding Research Organization, Edinburgh, Scotland; Drs. J. Garnier and G. Moequot, Station Centrale de Recherches Laitieres et de Technologie des

Produits Animaux, Jouy-en-Josas (S. & O.), France; Drs. T. A. J. Payens and D. G. Schmidt, Netherlands Institute for Dairy Research, Ede, The Netherlands; Dr. C. A. Kiddy, Dairy Cattle Research Branch, Animal Husbandry Research Division, ARS, Agricultural Research Center, U. S. Department of Agriculture, Beltsville, Maryland; Dr. Robert Jenness, Department of Biochemistry, University of Minnesota, St. Paul, Minnesota.

In most instances a sample set of the six known phenotypes each of α_{s1} - and β -caseins was sent as standard samples. These and eight unknown samples, to be typed for α_{s1} - and β -caseins, were shipped as isoelectric caseins dried with organic solvents. However, genetic typing can be accomplished with equal ease on whole milk samples (3, 9). The unknown samples were typed at the author's laboratory as follows:

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|---------------------------------------|---------------------------------------|
| (1) α_{s1} -B, β -B | (5) α_{s1} -A, β -A |
| (2) α_{s1} -BC, β -A | (6) α_{s1} -BC, β -A |
| (3) α_{s1} -AC, β -A, or | (7a) α_{s1} -B, β -A, or |
| (3a) α_{s1} -BC, β -A | (7b) α_{s1} -B, β -BC |
| (4) α_{s1} -BC, β -AB | (8) α_{s1} -BC, β -AC |

Each laboratory used its own preferred gel electrophoresis method. The methods included starch-gel electrophoresis (SGE) at pH 8.6 in 7.0 M urea (11), polyacrylamide-gel electrophoresis (PAE) at pH 8.6, veronal buffer, in 5 M urea by the method of Aschaffenburg (3) or PAE at pH 9.1-9.3, 4.5 M urea (7, 9). The samples were typed in the author's laboratory using both SGE and PAE, whereas one of the collaborating laboratories used both SGE and PAE, one SGE, and four PAE. Results of typing by the several collaborating laboratories showed that, with the exception of one sample (β -AB for β -A), the typing of the eight unknowns was exactly the same as in the author's laboratory. Those laboratories using SGE often found difficulty in discerning between α_{s1} -BC. However, those laboratories which encountered difficulty with SGE found that with PAE the resolution of the α_{s1} -B and α_{s1} -C zones was improved considerably although the electrophoretic differences between these components is, in fact, very small. α_{s1} -A casein is easily detected because of its rapid mobility upon gel electrophoresis. The α_{s1} -A variant may be a mutant of recent origin and thus not widespread. Nevertheless, one should be aware of its occurrence, and be cognizant of the possibility of discovering other α_{s1} -variants, and also additional β -casein variants. However, genetic polymorphs, which do not differ in net negative charge, may yet be discovered, in which case present electrophoretic methods

would not be of much value. Further identification by amino acid analyses or peptide mapping, or both, would be necessary.

In general, less difficulty was encountered with the typing of β -casein, even with the AB heterozygote. Per unit distance of migration, β -caseins are far better resolved than are the α_{s1} -caseins. Published patterns (9) show that PAE is superior to SGE in resolving the β -casein variants.

This study shows that, with the proper use of zonal electrophoresis methods, the genetic variants of α_{s1} - and β -caseins are readily detected, and excellent agreement was accomplished on the typing of unknown samples. Both zonal electrophoresis methods used were acceptable, but PAE seemed the more desirable. That agreement in phenotyping exists is valuable in (a) establishing a uniform nomenclature of the genetic variants, (b) searching for particular genetic variants for study, and (c) studying relationships among breeds as to casein phenotypes.

MARVIN P. THOMPSON
Eastern Regional Research Laboratory¹
Philadelphia, Pennsylvania

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¹Eastern Utilization Research and Development Division, Agricultural Research Service, U. S. Department of Agriculture.