A Mendelian polymorphism underlying quantitative variations of goat α_{s1}-casein

F. GROSCLAUDE, Marie-Françoise MAHÉ, Ghislaine BRIGNON *,
Liliana DI STASIO ** and R. JEUNET ***

*I.N.R.A., Laboratoire de Génétique Biochimique, Centre de Jouy-en-Josas,
F 78350 Jouy-en-Josas*

* *I.N.R.A., Laboratoire de Biochimie et Technologie Laitières, Centre de Jouy-en-Josas,
F 78350 Jouy-en-Josas*

** *Osservatorio di Genetica Animale, Via Pastrengo, 28, 10128 Torino, Italie*

*** *I.N.R.A., Station Expérimentale Laitière, B.P. 94, F 39800 Poligny*

Summary

Using SDS-polyacrylamide gel electrophoresis and rocket immunoelectrophoresis, 3 new alleles, designated α_{s1}-Cn$^{B^-}$, α_{s1}-Cn^F and α_{s1}-Cn^O, were identified at the goat α_{s1}-Cn locus, in addition to alleles α_{s1}-Cn^A, α_{s1}-Cn^B and α_{s1}-Cn^C previously reported by BOULANGER *et al.* (1984). Alleles α_{s1}-Cn^A, α_{s1}-Cn^B and α_{s1}-Cn^C are associated with a high content of α_{s1}-casein (approximate mean contribution of each allele being 3.6 g/l) compared to α_{s1}-Cn^F with a low content (0.6 g/l) and α_{s1}-Cn$^{B^-}$ with an intermediate content (1.6 g/l) ; α_{s1}-Cn^O appears to be a true null allele. In a sample of 213 *Alpine* females from 49 flocks in West Central France, the frequencies of the 6 alleles were : α_{s1}-Cn^A = 0.14 ; α_{s1}-Cn^B = 0.05 ; α_{s1}-Cn^C = 0.01 ; α_{s1}-Cn$^{B^-}$ = 0.34 ; α_{s1}-Cn^F = 0.41 ; and α_{s1}-Cn^O = 0.05. In a sample of 159 *Saanen* females from 52 flocks of the same region, the frequencies were : α_{s1}-Cn^A = 0.07 ; α_{s1}-Cn^B = 0.06 ; α_{s1}-Cn^C = 0 ; α_{s1}-Cn$^{B^-}$ = 0.41 ; α_{s1}-Cn^F = 0.43 ; α_{s1}-Cn^O = 0.03. Additional data confirm that loci α_{s1}-Cn and α_{s2}-Cn are closely linked.

Preliminary investigations indicated a significant superiority in casein content of milks from goats possessing the allele α_{s1}-Cn^A, as compared to that of milks from goats of genotypes α_{s1}-Cn^F/ α_{s1}-Cn^F and α_{s1}-Cn$^{B^-}$/α_{s1}-Cn^F and, in a large herd (N = 251), a strong correlation was observed between the α_{s1}-casein content and the rennet-casein content of milk (r = 0.68 ; b = 0.64).

Key words : Goat, α_{s1}-casein, α_{s2}-casein, polymorphism, null type, genetic linkage, quantitative variations.

Résumé

Un polymorphisme mendélien sous-jacent aux variations quantitatives de la caséine α_{s1} caprine

A l'aide d'électrophorèses en gel de polyacrylamide SDS et d'immuno-électrophorèses « rocket », 3 allèles, appelés α_{s1}-Cn$^{B^-}$, α_{s1}-Cn^F et α_{s1}-Cn^O, ont été identifiées au locus α_{s1}-Cn de la chèvre, en plus des allèles α_{s1}-Cn^A, α_{s1}-Cn^B et α_{s1}-Cn^C déjà détectés par BOULANGER *et al.* (1984). Les allèles α_{s1}-Cn^A, α_{s1}-Cn^B et α_{s1}-Cn^C sont associés à un taux élevé de caséine α_{s1} (contribution approximative de chaque allèle : 3,6 g/l), l'allèle α_{s1}-Cn^F a un taux faible (0,6 g/l) et l'allèle α_{s1}-

Cn^B a un taux intermédiaire (1,6 g/l). Dans un échantillon de 213 femelles *Alpine* provenant de 49 troupeaux du centre-ouest de la France, les fréquences des 6 allèles actuellement identifiés étaient les suivantes : $\alpha_{s1}\text{-}Cn^A = 0,14$; $\alpha_{s1}\text{-}Cn^B = 0,05$; $\alpha_{s1}\text{-}Cn^C = 0,01$; $\alpha_{s1}\text{-}Cn^{B^-} = 0,34$; $\alpha_{s1}\text{-}Cn^F = 0,41$ et $\alpha_{s1}\text{-}Cn^O = 0,05$. Dans un échantillon de 159 femelles *Saanen* provenant de 52 troupeaux de la même région, les fréquences étaient : $\alpha_{s1}\text{-}Cn^A = 0,07$; $\alpha_{s1}\text{-}Cn^B = 0,06$; $\alpha_{s1}\text{-}Cn^C = 0$; $\alpha_{s1}\text{-}Cn^{B^-} = 0,41$; $\alpha_{s1}\text{-}Cn^F = 0,43$; $\alpha_{s1}\text{-}Cn^O = 0,03$. Des données supplémentaires confirment que les loci $\alpha_{s1}\text{-}Cn$ et $\alpha_{s2}\text{-}Cn$ sont étroitement liés.

Des investigations préliminaires révèlent que le taux de caséine des laits des chèvres possédant l'allèle $\alpha_{s1}\text{-}Cn^A$ est significativement supérieur à celui des laits des chèvres de génotype $\alpha_{s1}\text{-}Cn^F/\alpha_{s1}\text{-}Cn^F$ ou $\alpha_{s1}\text{-}Cn^{B^-}/\alpha_{s1}\text{-}Cn^F$; par ailleurs, dans un grand troupeau (N = 251), une forte corrélation a été observée entre le taux de caséine α_{s1} et le taux de matières azotées coagulables (r = 0,68 ; b = 0,64).

Mots clés : Chèvre, caséine α_{s1}, caséine α_{s2}, polymorphisme, type nul, liaison génétique, variations quantitatives.

I. Introduction

BOULANGER *et al.* (1984) demonstrated by starch gel electrophoresis that goat α_{s1}-casein exhibited a rather complex polymorphism, which appeared to result from the combination of 2 forms of heterogeneity, both being controlled by locus α_{s1}-Cn. These were a classical electrophoretic polymorphism with 3 variants, α_{s1}-Cn A, B and C, and a quantitative variation, apparently only associated with the α_{s1}-Cn B variant. While it was considered that the inheritance of α_{s1}-Cn A and α_{s1}-Cn C had been established, an analysis of the genetic basis for the quantitative variations associated with variant α_{s1}-Cn B remained to be done.

The present paper provides additional results on the genetic control of goat α_{s1}-casein polymorphism and on the associated quantitative variations.

II. Materials and methods

A. *Origin and preparation of milk samples*

Individual milk samples were collected at one milking from goats born of artificial insemination and their mothers. A total of 888 samples, representing 476 dam-daughter pairs and subdivisible into 24 sire families were collected at the end of June 1985 from 101 private farms located in West Central *départements* of France (mainly Deux-Sèvres and Vienne, but also Loir-et-Cher, Maine-et-Loire, Charente and Haute-Vienne). The numbers of dam-daughter pairs per sire varied from 5 to 64, with only 10 sires having more than 20 pairs. For the quantification of the caseins, individual samples were collected again in August 1985 from animals (112 samples from 43 farms) chosen according to their genotype.

To estimate the relationship between the α_{s1}-casein contents of milks and their protein compositions, individual samples were obtained from the « Station de Testage Caprin » near Moissac (48110 Sainte-Croix Vallée Française) during April, 1986.

To all the samples (15 to 20 ml) were added 2-3 drops of a solution of potassium dichromate (10 g/l), 2-3 drops of a 10mM solution of Phenylmethylsulfonidefluoride (Serva) in isopropanol, and 2-3 drops of a 10mM aqueous solution of ε-amino-n-caproïc acid (Serva). The preserved samples were stored at − 20 °C pending chemical analysis.

The biochemical analysis of a F α_{s1}-casein type was carried out on a fresh individual milk sample provided by the « Domaine Expérimental de Brouessy » (I.N.R.A., 78470 Magny-les-Hameaux).

B. *Electrophoresis techniques*

Acid starch gel electrophoresis (SGE) was carried out as described by BOULANGER *et al.* (1984).

SDS-Polyacrylamide gel electrophoresis (SDS-PAGE) was performed on LKB glass plates (size of the gel : 18 × 15 × 0.15 cm) using a LKB 2117 Multiphor apparatus under conditions derived from those described by LAEMMLI (1970). Gels containing 3 p. 100 (stacking gel) and 14 p. 100 (separation gel) acrylamide were prepared from a stock solution of 30 p. 100 by weight of acrylamide and 0.8 p. 100 by weight of bis-acrylamide (Serva). The final concentrations in the separation gels were as follows : 0.39 M tris-HCl (Prolabo), pH 8.9 and 0.1 p. 100 SDS (Serva). The gels were polymerized by the addition of 0.04 p. 100 by volume of tetramethylethylenediamine (TEMED) and 2.5 p. 100 by volume of a 10 p. 100 by weight solution of ammonium persulfate. The stacking gels (approximately 4 cm) contained 0.06 M tris-HCl, pH 6.8, and 0.2 p. 100 SDS ; they were polymerized by addition of 0.2 p. 100 TEMED and 6 p. 100 of ammonium persulfate solution. The electrode buffer contained 0.049 M trizma base (Sigma), 0.38 M glycine (Prolabo) and 0.1 p. 100 SDS. The denaturing solution for diluting milk samples was made of 12.5 ml of the tris solution, pH 6.8, 10 ml glycerol, 2.3 g SDS, 125 mM dithiothreitol (Merck), 0.1 ml of a 1 p. 100 solution of Coomassie blue G (Serva) adjusted to 100 ml with distilled water. Samples of milk (4 µl) were diluted with the denaturing solution (17 µl) followed by addition of 2 µl of β-mercaptoethanol (Prolabo) and boiling for 5 min in a water-bath.

Electrophoresis (8 samples per plate) was carried out with a current of 180 V and approximately 65 mA per plate for 4 hours at 15 °C. The gels were stained for 2-3 h at room temperature with a 0.3 p. 100 by weight Coomassie brilliant blue G solution made up in 50 p. 100 methanol and 10 p. 100 acetic acid (Prolabo). The gels were destained by repeated washing for 16 h in a 30 p. 100 methanol, 7.4 p. 100 acetic acid, 10 p. 100 glycerol solution.

C. *Preparation of antibodies specific for individual caseins*

β-casein was isolated by the precipitation of α_{s1}-, α_{s2}- and κ-caseins in 3.3 M urea at pH 4.7 (THOMPSON & KIDDY, 1964) and α_{s1}-casein was then separated by ethanol precipitation of α_{s2}- and κ-caseins (ZITTLE & CUSTER, 1963). The 4 caseins were purified from the above fractions by DEAE-Cellulose chromatography (MERCIER *et al.*, 1968). Their purities were assessed by electrophoresis of high concentrations of proteins in acid and alkaline SGE and SDS-PAGE.

Antibodies were produced by the immunizations of rabbits with solutions of 0.5 mg casein per ml of a pH 7.0 buffer (0.1 M KH_2PO_4, 0.13 M NaCl, 2 mM KCl). Rabbits were injected subcutaneously, 15 days apart, at 20 points on both sides of the vertebral column, with 2 doses of 200 μl casein solution mixed with 200 μl complete Freund adjuvant. Fifteen days later, a 3rd dose was injected intramuscularly. Rabbits were bled 8-10 days later from the ear arteries.

The specificities of the antibodies were assessed by the double diffusion technique of OUCHTERLONY (1967) and immunoelectrophoresis (GRABAR & WILLIAMS, 1953).

D. *Electroblotting of α_{s1}-casein*

The caseins were transferred from a SDS-PAGE gel onto a nitrocellulose membrane (Biorad, 162-0115) in a Transphor unit, model TE 50 (Haefer scientific Instruments, Bioblock) using a pH 8.3 buffer containing 25 mM tris-HCl, 150 mM glycine and 5 p. 100 SDS (w/v), under a current of 1.2-1.5 A, for 2 h at 4 °C (TOWBIN *et al.*, 1979). After transfer, the membrane was immersed overnight in a phosphate-saline buffer pH 7.0 (10 mM NaH_2PO_4, 0.15 M NaCl, 0.1 p. 100 Tween 20) containing 5 p. 100 (w/v) BSA (Sigma), and then washed 2-3 times for 1/4 h in the phosphate saline buffer. For fixation of the antibody, the membrane was incubated for 3 h at 37 °C in 60 ml phosphate saline buffer containing 600 μl of anti-α_{s1}-casein antiserum and then washed 3-4 times for 1/2 h at room temperature. Subsequently the membrane was incubated at 37 °C for 2-3 h in a solution of 100 μl peroxidase labelled goat anti-rabbit IgG (H = L) antibodies (Institut Pasteur) in 50 ml phosphate buffer, washed 2-3 times for 1/2 to 1 h at room temperature and the peroxidase activity revealed with a solution of diaminobenzidine.

E. *Rocket Immunoelectrophoresis*

Rocket immunoelectrophoresis (LAURELL, 1966) was performed on pretreated LKB plates in a gel (8.5 × 9 cm ; volume : 12 ml) containing 1 p. 100 (w/v) agarose (LKB) and 0.025 M veronal buffer pH 8.6 with 600 μl antiserum being added per gel. Casein samples were diluted in the veronal buffer : 1/100 w/v for α_{s2}-casein and κ-casein ; 1/300 for β-casein and 1/20, 1/50 or 1/100 for α_{s1}-casein depending on the genotype ; 3 or 4 μl of casein solution were applied in 1.5 mm diameter wells under a 30 V and 8mA current. The electrode vessel contained veronal buffer 0.05 M pH 8.6. A 150 V current was applied overnight at 15 °C. Washing, drying, staining and destaining of the gels were carried out according to WEEKE (1976). Quantification of proteins was made by measurement of the peak heights with reference to standards of known concentrations.

F. *Preparation and amino-acid composition of α_{s1}-casein of type F*

Aliquots of 2 ml of a 5 p. 100 solution of whole casein of type F, reduced according to WOYCHIK (1964) were chromatographed on a cation exchanger Mono S HR 10/10 column (1 × 10 cm ; Pharmacia) using the FPLC system of Pharmacia. Elution was carried out at room temperature in a 75 mM formate-7.5 M urea, pH 4.0, buffer with a NaCl linear gradient (0.1 to 0.26 M ; 1.5 ml/min) for 60 min. A

repetition of 15 successive chromatographies was necessary to obtain 13 mg of fraction α_{s1}-Cn F (yield : 0.9 p. 100 of the whole casein).

The amino-acid composition of this fraction was established using a Biotronik LC 5000 analyzer.

G. *Quantification of milk proteins*

Individual lactosera were prepared immediately after milking by rennet coagulation followed by paper filtration. Total rennet-casein content (TC) was determined with a Milko-Scan 104 equipment and was obtained as the difference between the nitrogen content of milk and that of lactoserum with a specific calibration of the apparatus for the 2 measures. The reference method used for calibration was the Kjeldahl method (FIL-IDF-E-Doc 214-1985).

III. Results

A. *Identification of allele* α_{s1}-Cn^F

With only a few exceptions, all samples which were collected for genetic investigations were tested in both acid SGE and SDS-PAGE. Patterns obtained by SDS-PAGE could therefore be interpreted with reference to the nomenclature proposed by BOULANGER *et al.* (1984) using SGE. In SDS-PAGE, the 2 known variants of α_{s2}-casein, α_{s2}-Cn A and B, were separated and all samples showed the same faster migrating minor band (m) of α_{s2}-casein (fig. 1). The α_{s1}-Cn C and α_{s1}-Cn B variants were not separable in SDS-PAGE [their common band will hereafter be designated by (B)] while α_{s1}-Cn A migrated faster and was well resolved (fig. 1). Milks classified according to BOULANGER *et al.* (1984) as null types had no α_{s1}-casein fraction in this region. However, all these samples but one (designated later as α_{s1}-Cn^O/α_{s1}-Cn^O) showed a faint double band, designated F, in front of β-casein, which was also present in some of milks possessing either the α_{s1}-Cn A or the α_{s1}-Cn (B) band.

Fraction F, which was purified by FPLC, had the following amino-acid composition which is comparable to that given by BOULANGER *et al.* (1984) for goat α_{s1}-casein B : Asx : 19.0 (19) ; Thr : (7) ; Ser : (12) ; Glx : 35.3 (35) ; Pro : 21.7 (22) ; Gly : 9.7 (10) ; Ala : 11.0 (11) ; Val : 9.4 (9) ; Met : 4.4 (4) ; Ile : 8.6 (9) ; Leu : 17.2 (19) ; Tyr : 9.2 (9) ; Phe : 8.7 (9) ; His : 6.1 (6) ; Lys : 12.8 (13) ; Arg : 6.3 (6). On immunoblotting, the double band F reacted with anti-α_{s1}-casein antibodies (fig. 2).

These results suggested that the double band F was under control of an allele of locus α_{s1}-Cn, called α_{s1}-Cn^F, a hypothesis supported by the following. Firstly, the double band F has not been observed in milks from animals of genotype α_{s1}-Cn^A/α_{s1}-$Cn^{(B)}$. Secondly, segregation data in families with heterozygous dams, shown in table 1, fit with the expected Mendelian ratios (the other dam's genotypes may include the α_{s1}-Cn^O allele described hereafter). Thirdly, in the progeny of sires transmitting band (B) or bands F to their daughters, thus supposed to be of genotype α_{s1}-$Cn^{(B)}$/α_{s1}-Cn^F, band (B) was transmitted 66 times, bands F, 72 times, which is not different from the expected 1 : 1 ratio. The occurrence of milks with a low amount of α_{s1}-casein, improperly called
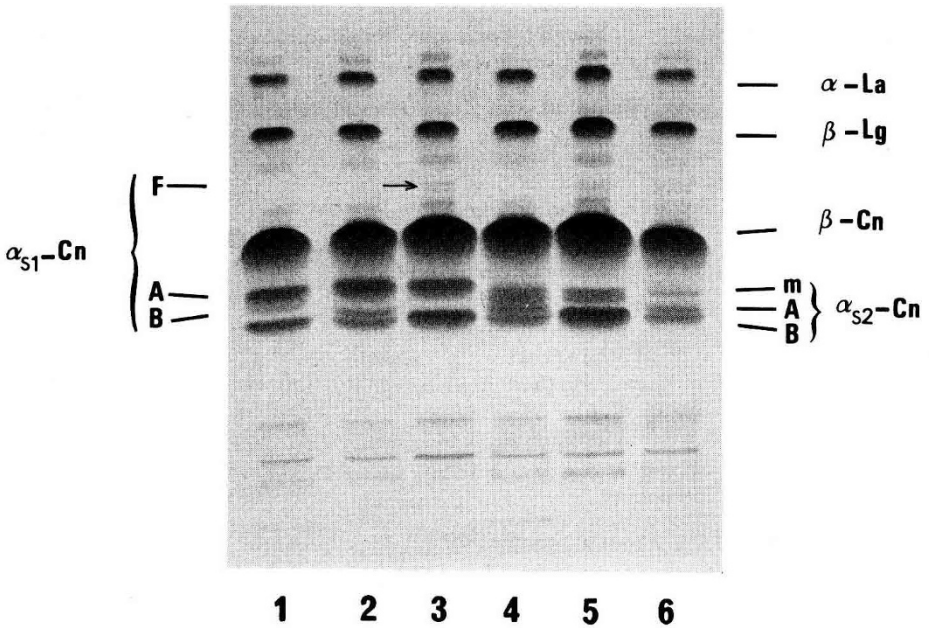
FIG. 1

SDS-polyacrylamide gel electrophoresis of 6 individual goat milks. Samples 2, 4 and 6 are heterozygous at locus α_{s2}-Cn (α_{s2}-Cn^A/α_{s2}-Cn^B) ; m = minor α_{s2}-casein fraction. The α_{s1} casein phenotypes are as follows : 1 = AB$^-$; 2 = A ; 3 = AF ; 4 = B$^-$; 5 = B$^-$F ; 6 = F.
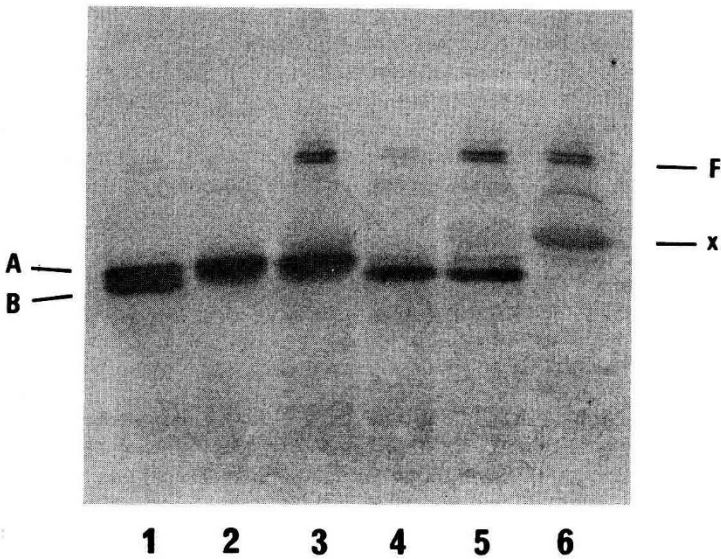

FIG. 2

Immunoblotting of α_{s1} casein for the same milk samples as in fig. 1 ; x = fraction only present in some of α_{s1}-Cn F phenotypes (see text).

TABLE 1

Segregation of α_{S1}-casein polymorphism in sire-dam-daughter families.

a

Phenotype of dams	Phenotype of daughters					
	A	A(B)	AF	(B)	(B)F	F
A	4					
A(B)	2	2				
(B)	4 *	8				
(B)F		11	10			
(F)	3 *		11			

b

Phenotype of dams	Phenotype of daughters					
	A	A(B)	AF	(B)	(B)F	F
A		3	2			2 *
A(B)		2	7	5	4	
AF		5	2		4	9
(B)				16	8	1 *
(B)F				15	41	15
F				1 *	15	22

c

Phenotype of dams	Phenotype of daughters					
	A	A(B)	AF	(B)	(B)F	F
A		2		1 *		
A(B)		1				
AF		3			5	
(B)				25		
(B)F				17	26	1 **
F				2 *	13	

In this table (B) = B or C.
a : one sire, of genotype α_{s1}-Cn^A/α_{s1}-Cn^A.
b : 9 sires, all of genotype α_{s1}-Cn^B/α_{s1}-Cn^F.
c : 4 sires, all of genotype α_{s1}-Cn^B/α_{s1}-Cn^B.
* Data explained by the existence of allele α_{s1}-Cn^O.

The proportions in lines a-AB, a-(B)F, c-AF and c-(B)F ($\chi^2 = 1.88$ with 1 df) are not significantly different from 1:1 ratio (in line c-(B)F : ** is interpreted as an error of parentage) ; the proportions in lines b-A(B) and b-AF are not significantly different from 1:1:1:1 ratio, in line b-(B)F, from 1:2:1 ($\chi^2 = 1.7$ with 2 df).

« null types » by BOULANGER *et al.* (1984) was thus accounted for by the existence of allele α_{s1}-Cn^F. Some of the milks showed an additional band (x) which migrated slightly slower than β-casein and reacted with anti-α_{s1}-casein antibodies (fig. 2). The nature of this fraction which curiously was present only in the milk of certain dams, and was not transmitted to their daughters, remains to be established. The immunoblotting results demonstrated the apparent presence of the double band of type F in all milks, but it was much weaker in those milks without a visible protein band in this region of the SDS-PAGE pattern.

B. *Identification of allele α_{s1}-Cn^O*

Segregation data in 14 families of table 1 (4.5 p. 100 of all families, marked with an asterisk) could not be explained under the 3 allele hypothesis (α_{s1}-Cn^A, α_{s1}-$Cn^{(B)}$, α_{s1}-Cn^F). Although errors in registration of parentages do occur, it is unlikely that they would account for the 4.5 p. 100 exceptions. In 11 of the 14 cases, the daughter had inherited allele α_{s2}-Cn^B from the dam, a proportion not due to chance since the frequency of α_{s2}-Cn^B in the sample was only 0.08. Because loci α_{s1}-Cn and α_{s2}-Cn are closely linked, as suggested by BOULANGER *et al.* (1984) and confirmed in this paper, these observations led one to suspect the existence of a true null allele, α_{s1}-Cn^O, associated in a haplotype with α_{s2}-Cn^B. In fact, the only dam homozygous for α_{s2}-Cn^B and therefore also possibly homozygous for α_{s1}-Cn^O, had no double band of type F or any other α_{s1}-casein (fig. 3). In addition, one sire (Saxon 7903981036 ; family not
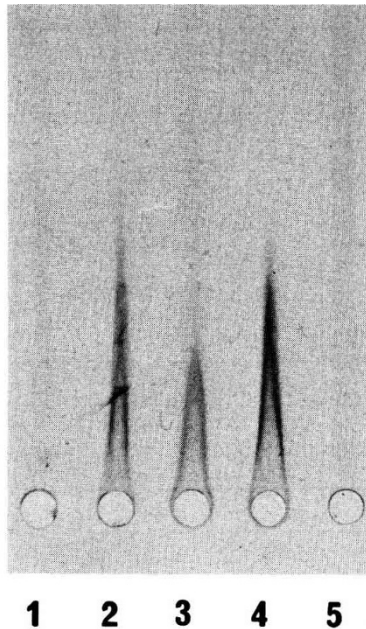

1   2   3   4   5

FIG. 3

Rocket immunoelectrophoresis of milk from the individual of genotype α_{s1}-Cn^O/α_{s1}-Cn^O (1 = dilution 1/20 ; 5 = 1/30) compared to milk from individuals of phenotypes A (2 = 1/100), F (3 = 1/20) and B⁻ (4 = 1/50) using anti-α_{s1}-casein antibodies.

included in table 1) of genotype α_{s2}-Cn^A/α_{s2}-Cn^B, obviously possessed haplotype α_{s1}-Cn^O-α_{s2}-Cn^B, because he transmitted α_{s2}-Cn^B to 7 of his 13 daughters, with no detectable α_{s1}-casein fraction. The existence of a true null and formally recessive allele was thus
• established.

C. Identification of allele α_{s1}-Cn$^{B^-}$

BOULANGER et al. (1984) concluded that α_{s1}-Cn B existed in at least 2 variant forms differing in their synthesis rates and with one of the forms being present in the so called « null types ». The results of the present paper do not support the latter conclusion in that α_{s1}-Cn F does not have the electrophoretic properties of α_{s1}-Cn B. Nevertheless, the SDS-PAGE patterns still reveals 2 quantitative levels of α_{s1}-Cn B, called α_{s1}-Cn B and α_{s1}-Cn B$^-$. The concentration of α_{s1}-Cn B is comparable to that of α_{s1}-Cn A and α_{s1}-Cn C, while that of α_{s1}-Cn B$^-$ is much lower. Although the discrimination between the 2 levels was not easy in a few milks, the B and B$^-$ patterns were repeatable within and between lactations and transmitted in a Mendelian fashion.

In conclusion, the polymorphism of goat α_{s1}-casein is under control of a minimum of 6 alleles, α_{s1}-Cn^A, α_{s1}-Cn^B, α_{s1}-Cn^C, α_{s1}-Cn$^{B^-}$, α_{s1}-Cn^F and α_{s1}-Cn^O.

D. Allelic frequencies

The frequencies of α_{s1}-Cn^A, α_{s1}-Cn^{B+C}, α_{s1}-Cn$^{B^-}$, α_{s1}-Cn^F and α_{s1}-Cn^O were estimated from SDS-PAGE data by the iterative method of CEPPELLINI et al. (1956) and the frequency of α_{s1}-Cn^C by gene counting from acid SGE data. In the group of 213 Alpine dams from 49 flocks of West Central France, the frequencies were : α_{s1}-Cn^A = 0.14 ; α_{s1}-Cn^B = 0.05 ; α_{s1}-Cn^C = 0.01 ; α_{s1}-Cn$^{B^-}$ = 0.34 ; α_{s1}-Cn^F = 0.41 ; α_{s1}-Cn^O = 0.05. In the group of 159 Saanen dams from 52 flocks from the same area, the frequencies were : α_{s1}-Cn^A = 0.07 ; α_{s1}-Cn^B = 0.06 ; α_{s1}-Cn^C = 0 ; α_{s1}-Cn$^{B^-}$ = 0.41 ; α_{s1}-Cn^F = 0.43 ; α_{s1}-Cn^O = 0.03.

E. Linkage of α_{s1}-Cn and α_{s2}-Cn

BOULANGER et al. (1984) suggested linkage between loci α_{s1}-Cn and α_{s2}-Cn because, in 12 informative sire-dam-daughter families, the sires transmitted only 2 haplotypes (α_{s1}-Cn^A-α_{s2}-Cn^B and α_{s1}-Cn$^{(B)}$-α_{s2}-Cn^A). In the present study, 10 additional informative cases were observed in the family of one sire which transmitted only 2 haplotypes : α_{s1}-Cn$^{B^-}$-α_{s2}-Cn^B (6 times) and α_{s1}-Cn^F-α_{s2}-Cn^A (4 times). This brings to 22 the number of informative families without recombinants, a result confirming that loci α_{s1}-Cn and α_{s2}-Cn are closely linked in the goat as in the cow (GROSCLAUDE et al., 1978).

F. Preliminary results on quantitative aspects

Figure 4 represents the distribution of the α_{s1}-casein contents of 251 individual milks collected in April 1986 at the « Station de Testage caprin » near Moissac. The 1st mode corresponds essentially to phenotypes F, the 2nd one to phenotypes B$^-$ and B$^-$F,
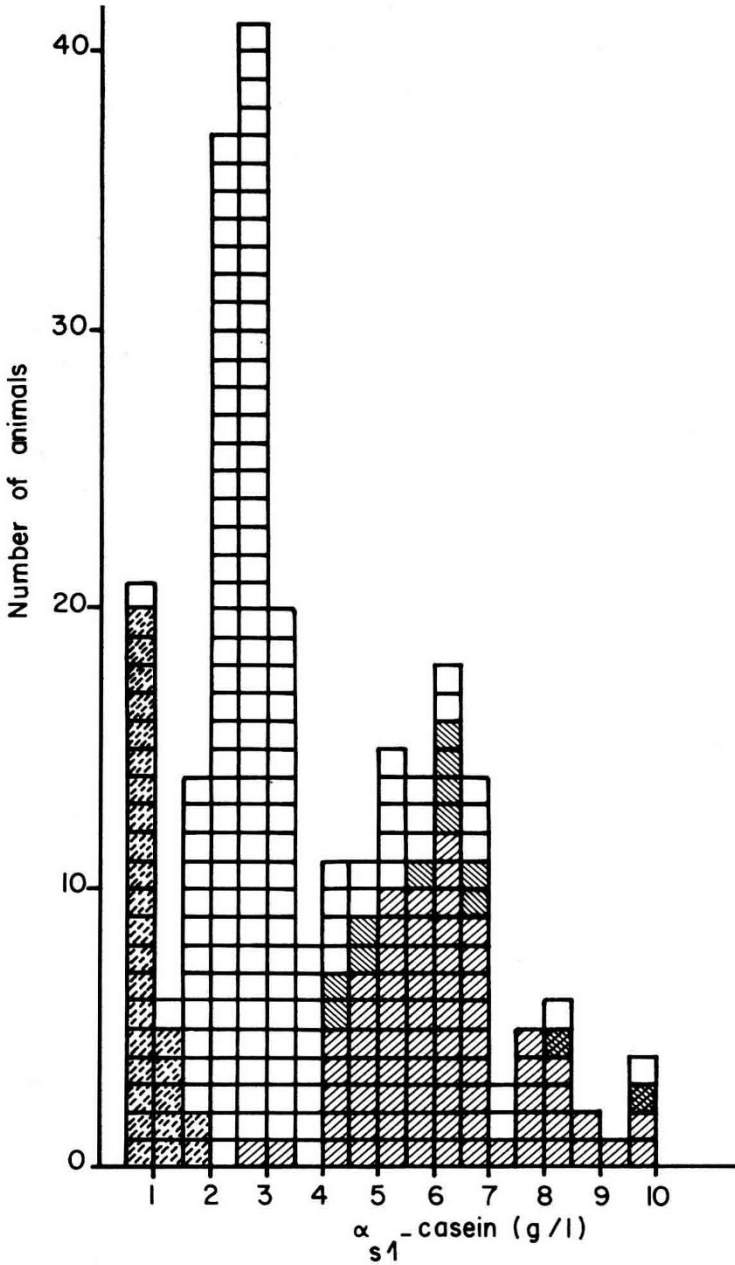
FIG. 4

Distribution of the α_{s1}-casein contents of individual milks in a large flock.

: *individuals of phenotype F* ; : *individuals possessing allele α_{s1}-Cn^A* ; : *individuals possessing allele α_{s1}-Cn^C* ; : *1st and 2nd modes : individuals of phenotype B F or B⁻* ; *other modes : individuals of phenotype BF or B. There is no individual homozygous for allele α_{s1}-Cn^O.*

the 3rd to genotypes including alleles α_{s1}-Cn^A, α_{s1}-Cn^B or α_{s1}-Cn^C and the genotypes with 2 doses of the latter alleles being found at the extreme right of the distribution. The rather clear cut distinction between different phenotypes, illustrated by the plurimodality of the histogram, is a straightforward confirmation of the visual interpretation of electrophoregrams. This holds true in particular for the discrimination between phenotypes B and B$^-$, but also for the fact that a few B type phenotypes are difficult to classify. Note also that individuals with one α_{s1}-Cn^A or one α_{s1}-Cn^C allele are found in the very same zone.

With the same milk samples, a correlation of 0.68 was found between the α_{s1} and the rennet-casein contents (for Ho = 0, $P < 10^{-4}$). The increase of rennet-casein content as a function of α_{s1}-casein content is given by TC = 19.59 + 0.64 α_{s1} (in g/kg ; standard error of b = 0.04).

The contents of the individual caseins in milks from animals of different genotypes were estimated by rocket immunoelectrophoresis. Table 2 gives the results obtained for 4 genotypes (α_{s1}-Cn^F/α_{s1}-Cn^F = 25 individuals from 14 different farms ; α_{s1}-Cn$^{B^-}$/α_{s1}-Cn^F = 18,14 ; α_{s1}-Cn^A/α_{s1}-Cn^F = 20,11 ; α_{s1}-Cn^A/α_{s1}-Cn$^{B^-}$ = 24,11). The numbers of individuals with other genotypes were too low for statistical analyses. The F test for heterogeneity of means was highly significant for α_{s1}-casein (F : 74.26 ; $P < 10^{-4}$), α_{s2}-casein (F : 13.79 ; $P < 10^{-4}$) and total casein (F : 7.51 ; $P < 2.10^{-4}$) but non significant for β-and κ-caseins. The Student-Newman-Keuls test confirms the significant superiority in milk α_{s1}-casein content of genotype α_{s1}-Cn$^{B^-}$/α_{s1}-Cn^F as compared to genotype α_{s1}-Cn^F/α_{s1}-Cn^F, as well as the significant superiority in milk α_{s1}-, α_{s2}- and total casein content of the 2 genotypes possessing allele α_{s1}-Cn^A as compared to the 2 genotypes without this allele. Assuming an additive contribution of each allele, and based on values obtained for the first 3 genotypes of table 2, the contribution of the alleles considered to the milk α_{s1}-casein content is as follows : α_{s1}-Cn^F = 0.6 g/l ; α_{s1}-Cn$^{B^-}$ = 1.6 g/l ; α_{s1}-Cn^A = 3.6 g/l. Because of the relatively small sample size these values should only be considered as approximative. Note that the α_{s1}-casein contents of milks from genotypes α_{s1}-Cn^A/α_{s1}-Cn$^{B^-}$ and α_{s1}-Cn^A/α_{s1}-Cn^F are not significantly different, a result which needs further confirmation.

TABLE 2

Content (g/l) in α_{s1}-, α_{s2}-, β-, κ- and total casein of milks from goats of 4 different genotypes at locus α_{s1}-Cn (means and standard deviations).

Caseins	Genotype at locus α_{s1}-Cn and number of individuals (N)			
	F/F N = 25	B$^-$/F N = 18	A/F N = 20	A/B$^-$ N = 24
α_{s1}	1.21 ± 0.70 a	2.24 ± 0.37 b	4.26 ± 0.95 c	4.03 ± 1.01 c
α_{s2}	3.94 ± 1.43 a	4.12 ± 1.52 a	5.76 ± 1.47 b	6.11 ± 1.29 b
β	10.29 ± 3.50 a	9.56 ± 2.26 a	11.22 ± 2.12 a	10.96 ± 2.62 a
κ	7.30 ± 2.80 a	6.51 ± 1.97 a	7.72 ± 1.74 a	7.66 ± 1.77 a
Total	22.74 ± 7.54 a	22.43 ± 5.22 a	28.96 ± 5.28 b	28.76 ± 5.77 b

For F/F, read : α_{s1}-Cn^F / α_{s1}-Cn^F, etc. ; a, b, c = comparison of means by the Student-Newman-Keuls test : 2 identical letters in the same line correspond to non significantly different means, 2 different letters to significantly different means.

Along the same line, the higher α_{s1}-casein content of milk from genotype α_{s1}-Cn$^{B^-}$ / α_{s1}-Cn^F as compared to that from genotype α_{s1}-Cn^F/α_{s1}-Cn^F, which illustrates the superiority of allele α_{s1}-Cn$^{B^-}$ upon allele α_{s1}-Cn^F, appears to be balanced by a decrease (although not statistically significant) in β- and κ-casein contents. However the full significance of this result is questionable, knowing that withdrawing individuals possessing allele α_{s1}-Cn^A from the group of Moissac animals, which increases the stastistical weight of allele α_{s1}-Cn$^{B^-}$, doesn't affect the regression coefficient of TC on α_{s1} (TC = 19.53 + 0.66 α_{s1}, with standard error of b = 0.06).

IV. Discussion

The occurrence at the caprine α_{s1}-casein locus of alleles associated with a high (α_{s1}-Cn^A, α_{s1}-Cn^B, α_{s1}-Cn^C), an intermediate (α_{s1}-Cn$^{B^-}$), a weak (α_{s1}-Cn^F) and a null (α_{s1}-Cn^O) synthesis rate, which has no counterpart in the cow, is of special interest. The existence, in or near the caprine α_{s1}-Cn locus, of a structural feature responsible for the generation of abnormal allelic forms can be suspected. In this respect, an analysis of the goat casein cluster of genes at the DNA level should be all the more informative as the alterations specific to alleles α_{s1}-Cn$^{B^-}$, α_{s1}-Cn^F and α_{s1}-Cn^O have dramatically different effects and thus possibly affect distinct DNA regulatory sites. Among the variants, α_{s1}-Cn F shows atypical electrophoretic properties. A biochemical analysis of this casein fraction could contribute to the identification of the nature of the underlying mutation.

It is interesting to note that a mutation affecting the activity of the α_{s1}-Cn locus may also influence that of the closely linked α_{s2}-Cn locus. More research is needed to measure accurately the rates of synthesis of the 4 casein genes in the different haplotypes. But here again, an analysis at the DNA level will probably be necessary to understand the mecanisms involved.

The 6 recognized α_{s1}-Cn alleles produce as many as 21 genotypic combinations. However, the differences between the rates of synthesis of the 4 allele groups are such that the distribution of the α_{s1}-casein contents of milks shows a remarkable plurimodality. In addition, our results suggest a strong positive correlation between the α_{s1}-casein content and the casein content of milk. The superiority in casein contents of milks from animals possessing alleles associated with a high level of α_{s1}-casein, particularly the most frequent one, α_{s1}-Cn^A, strongly favours the investigation and feasibility of a selection based on these alleles. However, a thorough analysis has still to be done, taking more particularly into account the expected effects of such a selection on the quantity and on the technological properties of milk. Because this selection would increase the proportion of individuals homozygous for α_{s1}-Cn^A (which is presently as low as 1 to 2 p. 100), the properties of milk from such individuals should be carefully investigated.

BOULANGER *et al.* (1984) pointed out that the predominance in the populations of alleles associated with a decreased α_{s1}-casein synthesis rate is difficult to explain. The existence of individuals possessing allele α_{s1}-Cn^A among males selected on progeny-test does not support the hypothesis of a close linkage between α_{s1}-Cn^A and an unfavourable allele at another locus. Further investigations are needed to account for the exceptional situation found at the α_{s1}-Cn locus of the goat.

Acknowledgements

References

BOULANGER A., GROSCLAUDE F., MAHE M.F., 1984. Polymorphisme des caséines α_{s1} et α_{s2} de la chèvre *(Capra hircus)*. *Génét. Sél. Evol.*, **16**, 157-176.

CEPPELLINI R., SINISCALCO M., SMITH C.A.B., 1956. The estimation of gene frequencies in a random mating population. *Ann. Hum. Genet.*, **20**, 97-115.

GRABAR P., WILLIAMS C.A., 1953. Méthode permettant l'étude conjuguée des propriétés électrophorétiques et immunochimiques d'un mélange de protéines ; applications au sérum sanguin. *Biochim. Biophys. Acta*, **10**, 193-194.

GROSCLAUDE F., JOUDRIER P., MAHÉ M.F., 1978. Polymorphisme de la caséine α_{s2} bovine : étroite liaison du locus α_{s2}-Cn avec les loci α_{s1}-Cn, β-Cn et κ-Cn ; mise en évidence d'une délétion dans le variant α_{s2}-CnD. *Ann. Génét. Sél. Anim.*, **10**, 313-327.

LAEMMLI U.K., 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*, **227**, 680-685.

LAURELL C.B., 1966. Quantitative estimation of proteins by electrophoresis in agarose gel containing antibodies. *Analyt. Biochem.*, **15**, 45-52.

MERCIER J.C., MAUBOIS J.L., POZNANSKI S., RIBADEAU-DUMAS B., 1968. Fractionnement préparatif des caséines de vache et de brebis par chromatographie sur DEAE-cellulose, en milieu urée et 2-mercaptoéthanol. *Bull. Soc. Chim. Biol.*, **50**, 521-530.

OUCHTERLONY Ö, 1967. Immunodiffusion and immunoelectrophoresis. *In :* WEIR D.M. (ed.), *Handbook of experimental immunology*, **1**, 655-706, Blackwell, Oxford and Edinburgh.

THOMPSON M.P., KIDDY C.A., 1964. Genetic polymorphism in caseins of cow's milk. III. Isolation and properties of α_{s1}-caseins A, B and C. *J. Dairy Sci.*, **47**, 626-632.

TOWBIN H., STAEHELIN T., GORDON J., 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets : procedure and some applications. *Proc. Natl. Acad. Sci., USA*, **76**, 4350-4354.

WEEKE B., 1976. General remarks on principles, equipment, reagents and procedures. *In :* AXELSEN N.H., KROLL J., and WEEKE B. (ed.), *A manual of quantitative immunoelectrophoresis*, 15-35, Universitetsforlaget, Oslo.

WOYCHIK J.H., 1964. Polymorphism in κ-casein of cow's milk. *Biochem. Biophys. Res. Commun.*, **16**, 267-271.

ZITTLE C.A., CUSTER J.J., 1963. Purification and some of the properties of α_s-casein and κ-casein. *J. Dairy Sci.*, **46**, 1183-1188.