Variation in size of goat milk casein micelles related to casein genotype

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Summary — Casein (CN) micelle size was determined in 2 goat milks from animals with different genotype for αs₁CN. One milk lacked αs₁CN, the other had an αs₁CN content of 4.7 g kg⁻¹. In comparison, the goat bulk milk has 3.3 g kg⁻¹ αs₁CN. The mean diameter of micelles in milk without αs₁CN was found to be 280 nm, while the diameter was 199 nm in milk with a high content of αs₁CN, i.e. a decrease by 29%. Micellar sizes were estimated by photon correlation spectroscopy (PCS) and by transmission electron microscopy (TEM) associated with negative staining. Micellar volume distribution according to size showed that milk without αs₁CN had the typical distribution of goat milk, i.e. a large spread of diameters from 20 to 270 nm without any clear maximum in frequency and a high proportion of casein forming the large micelles. In contrast, milk with a high level of αs₁CN showed a narrow unimodal distribution of frequency. The chemical analysis of milks, total casein, individual caseins, total and soluble minerals showed that the only difference is the αs₁CN level. The results might imply a role of αs₁CN in micelle size regulation.

goat milk / casein / micelle / αs₁casein / size / TEM microscopy / photon correlation spectroscopy

Résumé — Taille des micelles de caséine du lait de chèvre en relation avec la variation génétique de la composition du lait. La taille des micelles de caséine a été déterminée dans 2 laits provenant de chèvres de génotype différent pour la caséine αs₁. L'un était caractérisé par une caséine exempte de caséine αs₁, l'autre par une teneur très élevée en caséine αs₁ (4,7 g kg⁻¹) alors que le lait de chèvre de mélange en contenait 3,3 g kg⁻¹ environ. Le diamètre moyen des micelles dans le lait exempt de caséine αs₁ était de 280 nm et celui du lait riche en caséine αs₁ de 199 nm, soit une valeur inférieure de 29% à la première. La mesure des diamètres micellaires a été faite par spectrométrie de corrélation de photons et par microscopie électronique à transmission après coloration négative. L'histogramme de répartition du volume micellaire selon la taille des particules montre que le lait exempt de caséine αs₁ a toutes les caractéristiques des laits de chèvre moyens : large dispersion des volumes micellaires de 20 à 270 nm, pas de maximum de fréquence très marqué, une grande proportion de la caséine à l'état de grosses micelles. Au contraire, le lait à forte teneur en caséine αs₁ présente une répartition unimodale nette, qui se démarque fortement de l'autre lait. L'analyse chimique des laits (caséine
INTRODUCTION

Casein micelles are present in all mammalian milks, but the published studies concern primarily cow milk. Micelles are spherical particles composed of casein associated with minerals (White and Davies, 1958; Holt and Jenness, 1984). Micelle size in cow milk varies from 20 to 600 nm in diameter, as determined either by electron microscopy (Schmidt et al, 1973), or by photon correlation spectroscopy (PCS) (Holt et al, 1978). Number frequencies show 80% of the micelles smaller than 20 nm. The diameter corresponding to the median volume is about 120 nm. A bimodal distribution of the micelles, with a second maximum, has also been described (Holt and Baird, 1978; Holt et al, 1978; Kimber et al, 1980; Anderson et al, 1984; Donnelly et al, 1984). The second maximum, when found, is for diameter values of about 255 nm (Holt, 1985). In addition, giant micelles have often been described (Buchheim and Welsh, 1973; Brooker and Holt, 1978, 1979). Variations in the mean diameter have been observed and related to the stage of lactation (Holt and Baird, 1978), to the variation between individual animals or to other environmental factors. Nevertheless, the mean size of micelles in milk remains a characteristic of the species.

Mechanisms involved in micelle size regulation have not yet been explained. More information is needed on casein aggregation and on micelle structure, which both remain largely unknown (Rollemia, 1992). Many schemes have been proposed, each explaining some of the properties (Payans, 1966; Rose and Colvin, 1966; Schmidt, 1982), but none of them is entirely satisfactory. The ability to form micelles in vitro from a solution containing the whole constituents (Schmidt et al, 1977) shows that the only mechanisms required depend on chemical affinity without any particular specific enzymatic step. Studies on in vivo formation of micelles in mammary cells have also provided information on casein aggregation (Beery et al, 1971; Farrell, 1973). A theory for the casein association has been proposed (Bloomfield, 1979) for a micelle model involving subunits and to explain an unimodal distribution of sizes. This theory is, however, not able to explain all the experimental observations; in particular, it remains difficult to explain the limiting process for the aggregation of casein during synthesis, and consequently the regulation of micellar size.

Some relations have been established between the size of micelles and their composition in individual caseins and colloidal minerals. In cow milk, small micelles have a lower content of βCN than the largest, balanced by a higher level of κCN (Dalgleish et al, 1989). Small micelles have also a lower mineral content (Dalgleish et al, 1989). The limiting effect of high levels of κCN on micelle size has also been demonstrated by Schmidt et al (1977) during the in vitro formation of artificial micelles.

A micelle model other than the cow milk micelle seemed to us useful to obtain new concepts on micelle structure and size. In fact, there are few data available on milks other than cow milk and they are not sufficient to undertake a comparative study, even with the important contribution of Holt

Some goats with different genotypes for milk casein have been bred at the institute: goats with A genotype, having a high level of $\alpha_{s1}$ casein ($\approx 5$ g kg$^{-1}$), and goats with O genotype having no $\alpha_{s1}$ casein in their whole casein. These levels are extreme compositions compared to the mean value of about 3 g kg$^{-1}$ found in normal goat milk.

We investigated whether such a difference in composition affected the micelle size and structure in the milks.

**MATERIALS AND METHODS**

Milks A and O, of selected composition for $\alpha_{s1}$ casein, were produced each by 3 animals. The animals originated from the INRA Animal Crossing Center of Bourges (France) where they have been genetically typed and characterized as homozygous at the $\alpha_{s1}$CN loci (AA and OO variant, respectively). During our experimentation, the animals were bred at the INRA Center of Le Rheu (France). The milk used in the experiments was fresh raw milk, either individual milk or mixed milk from 3 animals of the same specific genotype. Reference milk was fresh goat bulk milk of a neighbouring plant.

Determination of total nitrogen matter (TNM) and of pH 4.2 soluble nitrogen matter (SN), extracted by the Rowland procedure (1938) were made by Kjeldahl analysis ($N \times 6.38$). Casein (CN) yield was calculated as: $CN = TNM - SN$. The pH was chosen at 4.2 for the separation of SN, because it corresponds to the isoelectric pH of goat casein in milk, as determined in preliminary experiments.

Atomic absorption spectrophotometry (Spectra A 300 - Varian, Palo Alto, CA, USA) was used to determine Ca and Mg mineral levels, on the milks for total minerals, and on their ultrafiltrates (CF 25 Centricon, Amicon, Paris, France) for the soluble minerals.

High performance liquid chromatography (HPLC) (Varian 5000, Palo Alto, CA, USA) allowed the separation of individual components in total casein. Reverse phase separation on a C4 column -4.6 mm diameter, 150 mm length (Vydac Interchim, Montluçon, France) - was achieved using as eluents, (A) 0.1% trifluoroacetic acid (TFA) in water, and (B) 0.096% TFA in acetonitrile/water (80/20, v/v). Separation was obtained in 30 min on the column at 40°C, with eluent B increasing from 37 to 53% with a flow rate of 1 ml min$^{-1}$ (Jaubert and Martin, 1992). Sample preparation involved separation of casein from milk at 30°C by precipitation at pH 4.2. To 10 ml of milk was added 10 ml water, 0.6 ml 10% (v/v) acetic acid, 0.4 ml 1 mol l$^{-1}$ sodium acetate and 10 ml water. A centrifuging step (1 000 g, 15 min) was used to separate the total casein fraction in a pellet. The precipitate was washed once in water and centrifuged again to obtain the washed casein. It was dissolved in 10 ml of a pH 7 buffer (8 mol l$^{-1}$ urea, 1.3% Na$_3$ citrate, 0.1 mol l$^{-1}$ trishydroxy-methyl-amino-methane/HCl, 10 mmol l$^{-1}$ dithiothreitol), then diluted 1/5 in the same buffer. Next, a dilution of 1/6 in eluent A was made, and the pH was adjusted to 2.2 by the addition of 10% TFA. An aliquote of 50 µl was injected on the column. Proportions of individual caseins in total casein were obtained from the peak areas on the HPLC profiles, corrected by the specific relative absorbance of individual caseins calculated from the results of Jaubert (1992): $\kappa$CN, 0.934; $\delta$CN, 0.877; $\alpha_{s1}$CN, 0.810; $\beta$CN, 1.0; $\kappa$, 1.00. Fraction x of casein corresponded to an hydrophobic component which eluted after the $\beta$CN peak. The content of individual caseins in milk was calculated from the measured proportions combined with the total casein content of milk estimated by N analysis.

Photon correlation spectroscopy (PCS) was used to determine mean size of micelles in skimmed milks. Measurements were performed with a Coulter N4MD apparatus (Coultronics, Hialeah, FL, USA), equipped with a He Ne laser light (632.8 nm), with the following experimental conditions: scattering angle 90°, temperature 20°C, viscosity 1.002 cP, refractive index of the solvent 1.333, measure time 600 s and sample time 4.5 µs. From the measured autocorrelation function of the scattered light was calculated an intensity-weighted average diffusion coefficient. It allowed the calculation of a mean diameter ($O_s$, nm) corresponding to the size of a spherical particle with an equivalent diffusion coefficient. Owing to the relative size of micelles and the wavelength of the light and also to the polydispersity, $D_0$ would not correspond to a simple average of the size distribution. A measure of the breadth of the particle distribution was also obtained, expressed
as a standard deviation (SD). Milks were skimmed by 2 centrifuging steps (1 000 g, 15 min), then diluted 1/300 in a pH 6.6 buffer (Dalgleish, 1984) and filtered on a 0.8 μm membrane (Home, 1984). During PCS measurements, we also checked that there was no interference from fat globules, by testing micelle suspensions with added EDTA (Holt et al, 1978).

Observations on milk micelles by transmission electron microscopy were made on a Philips CM12 apparatus (Philips Industrie, Bobigny, France), on a transmission mode, with a 80 kV voltage. Two analyses were performed on mid-lactation milks, 1 in June (VI), the other in September (IX). Bulk milk was analysed once in June. Milk samples (from mixed milks) were skimmed as already described, then diluted 1/250 in 0.2 mol l⁻¹ sodium cacodylate buffer at pH 7.4. An aliquot was dropped on a collodion-treated carbon grid (300 mesh, 3 mm diameter). After 30 s, excess sample was removed on a filter paper and the grid was immersed in a 2% (w/v) uranylacetate solution for 2 min. The grid was then drained and air dried. From the plates obtained, individual diameters of about 1 000 particles were measured: for each type of milk. The whole size data were separated into 14 classes of diameter, of 20 nm width. Results were treated as described by Schmidt et al (1977) to obtain the number mean diameter, Dₙ, and the volume moment average diameter, Dᵥm.

**RESULTS AND DISCUSSION**

The mean size of casein micelles in individuals and bulk milks was measured in duplicate using PCS (table I). Bulk milk micelles had a mean diameter value of 237 nm with a SD of 0 nm. This value is in the same range as those published by Remeuf et al (1989) for a lot of individual milks, 255 (SD = 17) nm, also obtained by PCS, and that published by Buchheim et al (1989), 282 nm, obtained by electron microscopy. The O milks had a higher mean diameter than the bulk milk, 280 (SD = 12) nm, while the A milks showed a lower mean diameter, 199 (SD = 11) nm. The spread of the results was low for animals of the same genotype, as shown by the low values of the standard deviation. Consequently, the mean diameter value of each milk is significantly different (P = 0.01) from the other 2. The mean diameter of casein micelles in A milks was lower by 29% than in O milks.

Electron microscopy provided details on the distribution of micelles by size class. A high difference in the size distribution in A

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**Table I. Mean diameter (Dₙ, nm) of casein micelles in individual milks, obtained by photon correlation spectroscopy (standard deviation).**

*Diamètre micellaire moyen (Dₙ, nm) mesuré par spectrométrie de corrélation de photons dans les laits individuels (écart type).*

<table>
<thead>
<tr>
<th>Milk type</th>
<th>Animal reference</th>
<th>Dₙ</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>a</td>
<td>187 (1)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>b</td>
<td>189 (35)</td>
<td>199 (11)</td>
</tr>
<tr>
<td></td>
<td>c</td>
<td>221 (0)</td>
<td></td>
</tr>
<tr>
<td>O</td>
<td>1</td>
<td>261 (1)</td>
<td>280 (12)</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>301 (3)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>279 (1)</td>
<td></td>
</tr>
<tr>
<td>Bulk milk</td>
<td></td>
<td></td>
<td>237 (0)</td>
</tr>
</tbody>
</table>
Micelle size in goat milk

and O milks appeared as well on the histogram by number (fig 1) as on the volume distribution (fig 2).

Histogram of A milk micelles by number showed more micelles in the interval 40–140 nm than in O milk (43% instead of 14%). However, A milk had less small-micelles (< 40 nm) and less large micelles (> 140 nm).

These differences had consequences on the relative distribution of micellar volume. In A milk, 70% of the total volume is contained in the classes 40–140 nm, with a mean value of 88 nm, and the other 26% is contained in > 140 nm micelles, with a mean value of 195 nm. In O milk, almost all the volume (73%) is in micelles > 140 nm in size with a mean value of 207 nm, the 40–140 nm classes containing only 24% of the volume (mean 96 nm).

It appears that the wide distribution of micelle size in O milks might result from the presence of several different micelle populations, each adjusted around different mean values. For example, from figure 2, the distribution may be taken to be bimodal, with 2 parts centred on means of 96 and 207 nm. In A milk, a tendency for preferential synthesis of small micelles arises.

The bulk milk (fig 3) had an intermediate histogram of volume distribution. It was similar to that already described by Buchheim et al (1989) for goat casein: a large spread of micellar sizes, with a smooth frequency maximum at a high mean diameter, many micelles of low size and a high proportion of the volume in large-sized micelles. The bulk milk is in fact a mixed milk from goats with a variety of casein genotypes and showed an intermediary distribution situ-

Fig 1. Micelles number distribution according to their size in A and O milks (from microscopy data). $D_{vm}$: volume moment average diameter.

Répartition du nombre de micelles selon leur taille dans les laits A et O (d'après les mesures de microscopie).
Fig 2. Total micellar volume distribution according to micelle size in A and O milks (from microscopy data). $D_{vm}$: volume moment average diameter.

Répartition du volume total micellaire selon la taille dans les laits A et O (d'après les mesures de microscopie).

Table II. Mean micelle diameter (nm) obtained by photon correlation spectroscopy (PCS) and by electron microscopy on mixed milks.

Comparaison des diamètres micellaires moyens (nm) obtenus par microscopie ou par spectrométrie de corrélation de photons (PCS).

<table>
<thead>
<tr>
<th>Milk type</th>
<th>Month</th>
<th>PCS $D_s$ 1</th>
<th>Microscopy $D_{vm}$ 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>VI</td>
<td>186</td>
<td>132</td>
</tr>
<tr>
<td></td>
<td>IX</td>
<td>179</td>
<td>101</td>
</tr>
<tr>
<td></td>
<td>m</td>
<td>182</td>
<td>116</td>
</tr>
<tr>
<td>O</td>
<td>VI</td>
<td>248</td>
<td>192</td>
</tr>
<tr>
<td></td>
<td>IX</td>
<td>260</td>
<td>169</td>
</tr>
<tr>
<td></td>
<td>m</td>
<td>254</td>
<td>180</td>
</tr>
<tr>
<td>Bulk milk</td>
<td>VI</td>
<td>237</td>
<td>125</td>
</tr>
</tbody>
</table>

$D_s$: PCS mean diameter; $D_n$: number mean diameter; $D_{vm}$: volume moment average diameter; m: mean of the 2 successive determinations.

$D_s$: diamètre moyen PCS ; $D_n$: diamètre moyen en nombre ; $D_{vm}$: diamètre moyen de partition du volume. Laits de mélange ; m : moyenne des 2 déterminations.
Micelle size in goat milk

Fig 3. Micelles number and total micellar volume distributions according to micelle size in bulk milk (from microscopy data). $D_{vm}$: volume moment average diameter.

Répartition du nombre des micelles et du volume selon la taille dans le lait de mélange (d'après les mesures de microscopie).

...ated between the other 2 extremes, A and O, initially described. The mixing of A and O milks produced a very flat distribution with a mean value not well defined.

Micelle mean diameters, calculated from the data of electron microscopy, are given in table II. $D_n$ values are nearly the same for the 3 milks. Conversely, $D_{vm}$ is lower by 35% for A milk, compared to O milk. The difference is nearly the same as that already obtained by PCS, and this, although the diameter values themselves are lower with electron microscopy.

These lower diameter values with electron microscopy may arise from the sample preparation we employed, involving air drying which could lead to micellar shrinkage by dehydration. Cryofracture preparation might not have this disadvantage and might allow determination of diameter values similar to PCS (Holt et al, 1978). Nevertheless, we chose direct spreading on grid, combined with negative staining, to obtain a high resolution and, incidentally, more data on the micellar structure. The true values of diameter for native micelles seems likely to be closer to those given by PCS measurements in our experiments.

The chemical composition of the milks and of their colloidal material was determined.

The casein fraction of the A and O milks (mixed milks of each 3 goats of a genotype) was separated by HPLC into its individual...
constituents (fig 4). Both milks contain κCN, αs2-CN and β-CN. The A milk has also some αs1-CN which is totally lacking in the O milk.

The total casein content of milks is given in table III, as are their individual casein contents. There are few differences in κCN, αs2-CN and β-CN content between A and O milks, but αs1-CN was present only in the A milk, at the level 4.7 g kg⁻¹. The proportions of individual caseins in the totals are therefore different for the 2 milks.

Total and soluble minerals were determined in the individual milks. The results in table IV present the mean value of the 3 milks of each type. Values were in the range of those of the bulk milk and of others already published for goat milk (Richardson et al., 1974; Holt and Jenness, 1984). The standard deviations of some results were high, particularly the total phosphorus and total calcium of O milks. On the other hand, soluble calcium in O milk was higher than in A milk while the pH was slightly higher in O milk. An opposite result would have been expected because generally higher pH is associated with a lower level of Ca (White and Davies, 1958). The results show that equilibria of the other soluble constituents,

<table>
<thead>
<tr>
<th>Milk type</th>
<th>κCN</th>
<th>αs2-CN</th>
<th>αs1-CN</th>
<th>β-CN</th>
<th>x</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>3.3</td>
<td>2.4</td>
<td>4.7</td>
<td>10.4</td>
<td>1.0</td>
<td>21.8</td>
</tr>
<tr>
<td></td>
<td>(15)</td>
<td>(11)</td>
<td>(21)</td>
<td>(48)</td>
<td>(5)</td>
<td>(100)</td>
</tr>
<tr>
<td>O</td>
<td>3.4</td>
<td>2.4</td>
<td>0</td>
<td>10.4</td>
<td>1.1</td>
<td>17.3</td>
</tr>
<tr>
<td></td>
<td>(20)</td>
<td>(14)</td>
<td>(0)</td>
<td>(60)</td>
<td>(6)</td>
<td>(100)</td>
</tr>
<tr>
<td>Bulk milk</td>
<td>3.2</td>
<td>3.6</td>
<td>3.3</td>
<td>15</td>
<td>-</td>
<td>25.1</td>
</tr>
<tr>
<td></td>
<td>(13)</td>
<td>(14)</td>
<td>(13)</td>
<td>(60)</td>
<td>-</td>
<td>(100)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Milk type</th>
<th>pH</th>
<th>Ca</th>
<th>P</th>
<th>Mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>6.54</td>
<td>27.5</td>
<td>29.2</td>
<td>5.1</td>
</tr>
<tr>
<td></td>
<td>(0.07)</td>
<td>(2.3)</td>
<td>(2.8)</td>
<td>(0)</td>
</tr>
<tr>
<td>O</td>
<td>6.62</td>
<td>26.9</td>
<td>28.0</td>
<td>4.6</td>
</tr>
<tr>
<td></td>
<td>(0.02)</td>
<td>(1.0)</td>
<td>(5.3)</td>
<td>(0.5)</td>
</tr>
<tr>
<td>Bulk milk</td>
<td>6.60</td>
<td>31.7</td>
<td>32.2</td>
<td>5.2</td>
</tr>
</tbody>
</table>

Table III. Individual casein contents in total casein of mixed milks: g kg⁻¹ and (%)

Table IV. Total and soluble mineral content in individual A and O milks. Mean value of the 3 individual milks of the same genotype and value of the bulk milk: mmol kg⁻¹ (standard deviation).
Micelle size in goat milk

The composition of the colloidal fraction of milks is presented in table V. Micelles in A and O milks have a similar colloidal calcium content, relative to casein: 21.5 and 22.9 mol mol\(^{-1}\) CN, respectively, but total colloidal phosphate is relatively higher in O milk.

The A and O milks are very close in the composition of their minerals and most of their individual caseins. Only \(\alpha_sCN\) content is different, giving a higher total casein content and different relative proportions of the caseins. It is tempting to conclude from these results that \(\alpha_sCN\) might be implicated in the adjustment of micelle size to a lower value.

The appearance of goat milk micelles, as observed by electron microscopy, was quite different in respect to the density for electron beams. In the bulk milk, 2 types of micelles were present, large micelles with a high density and small micelles of low density. Richardson et al (1974) have already noted these 2 types of particles in goat milk, with sizes of 80 and 200 nm and a difference in density. It is possible that the different appearance is only due to the size: Holt (1975) has demonstrated that in cow milk, large micelles have a higher density of matter than small micelles, which have, on the contrary, a higher hydration. Micelles from A milk were frequently of low density (fig 5). In O milk, 2 types of micelles were present, large micelles with a high density (fig 6) and small micelles of low density, as in A milk.

The milk sample preparation, without cutting or fractures, allowed the study of the micellar surface. The external boundary was either a sharp outline or a rough surface, from 1 micelle to another. The same micelle may have a clean outline on one part and a rough one on another part of its surface. Similar observations on cow micelles were previously reported by Brooker and Holt (1978).

The surfaces of the micelles seem to be similar in A and O milks. Micelles look as if they were covered by small spherical units of low density, either wrapped and surrounded by an amorphous matrix of high density, in micelles with a sharp outline, or extruding into the external volume, in rough micelles. This gives the micelles the appear-

<table>
<thead>
<tr>
<th>Milk type</th>
<th>Casein (CN) content g kg(^{-1}) milk</th>
<th>CN/MAT mmol kg(^{-1})</th>
<th>Coll Ca mmol kg(^{-1})</th>
<th>Coll P mmol mol(^{-1})</th>
<th>Coll Ca/CN mol mol(^{-1})</th>
<th>Coll P/CN mol mol(^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>21.8 (2.2)</td>
<td>0.70 (0.03)</td>
<td>19.6 (2.4)</td>
<td>16.2 (1.1)</td>
<td>21.5 (2.1)</td>
<td>17.8 (1.6)</td>
</tr>
<tr>
<td>O</td>
<td>17.3 (3.0)</td>
<td>0.66 (0.04)</td>
<td>16.0 (2.5)</td>
<td>15.4 (2.6)</td>
<td>22.9 (7.9)</td>
<td>22.2 (8.1)</td>
</tr>
<tr>
<td>Bulk milk</td>
<td>25.1</td>
<td>0.76</td>
<td>23.5</td>
<td>18.6</td>
<td>23.5</td>
<td>18.6</td>
</tr>
</tbody>
</table>
ance of a “blackberry”. In the rough-surfaced micelles, the structure of these low density areas appears to be a rather spongy cotton-wool-like one, with material extruding from the particle in stringy shapes. It is not possible to say if these 2 micellar aspects correspond to different native micelles or are instead induced by the sample preparation. In any case, they demonstrate the existence in the micelles of a filamentous structure for the caseins, analogous to that already seen in the Golgi vesicles (Brooker and Holt, 1979). The internal parts of the micelles look homogeneous with a continuous electron-dense structure.

Some other plates show micelles as clear “blackberries”, with the dark matrix spread around them (fig 5, pl 4 and fig 6, pl 6). These last figures agree well with the micelles generally described when negative staining is used. The preceding ones corresponded rather to a positive staining of the micelles. These clear areas might contain some of the protein material, i.e caseins, while surrounding high density material would be formed of a gel of colloidal calcium phosphate, able to react with uranylacetate and to combine with the heavy metal with a high affinity (Hayat, 1989). Thus, it would be this phosphate gel matrix which would give to micelles their well-defined surface aspect, when positive staining is obtained. The fixation of an excess of reagent would lead to a change in the gel properties. It would lose its capacity to adhere to the micelle (maybe due to the breakage of Pser-Ca-coll Pi linkages in the presence of uranylacetate) and would precipitate around the micelle, releasing the proteic “blackberry”. Thus, the negative staining would show more often, shattered micelles, dissociated from their mineral matrix (fig 5, pl 4; fig 6, pl 6).

In conclusion, the appearance of micelles in goat milk seems not to be affected by the presence or not of αs2-CN among the caseins. A difference in electron density was
Fig 5. Micelles in A milk.
Aspect des micelles dans le lait A.
Fig 6. Micelles in O milk.

Aspect des micelles dans le lait O.
observed only in relation to the size. On the other hand, the micelle size itself was found much smaller in micelles with $\alpha_{s1}CN$. It is not possible to say if the 2 are related, but, if they were, the $\alpha_{s1}CN$ might be involved in a mechanism limiting the micelle size.

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