

R. M. Parry, Jr.†

## Milk Coagulation and Protein Denaturation\*

## INTRODUCTION

This chapter is concerned with the complex interactions which occur among the many milk components and proteins, and the resulting effect on the properties of milk in the fluid, concentrated and dried state. In several cases these effects are cumulative, but no quantitative method of estimating the contribution of each component to observed changes has been forthcoming. Empirical observations have given some indication of the path of these changes leading to destabilization. These changes will be discussed in three sections: first, the equilibrium (or nonequilibrium) nature of fluid milk; second, the changes induced by heating this system; and third, the changes manifested by concentrating the solids of milk.

## COLLOIDAL STABILITY OF MILK

Milk is a polyphasic secretion containing emulsified fat, colloidal casein micelles, and dissolved protein, lactose and salts, and it undergoes continual change in the mammary gland lumen prior to milking. Most of these changes are attributable to reactions on the enzymatic level. Subsequent handling procedures, such as cooling, heating, homogenization, and concentration can disturb the colloidal-dissolved state equilibrium. Therefore, the age and treatment history of milk is very important to understanding and predicting its properties.

Coagulation of the milk system is often attributed to destabilization of the casein micelles. However, study of this phenomenon reveals that the end result may be a summation of many minute changes in the colloid system. The interactions of caseins and salts that are responsible for these changes will be discussed in this section.

\* Revised from chapter 11 in the 1st edition by Leon Tumerman and Byron H. Webb.

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## Casein Dispersion

Chapter 3 deals with the various casein components and their genetic variability. Our concern here is with the behavior of the three caseins which constitute over 90% of those present in normal milk, namely,  $\alpha_{s1}$ -,  $\beta$ - and  $\kappa$ -casein. Chapter 9 discusses the various proposed models of how these proteins interact in milk to form micelles. This discussion assembles observations on the behavior of these proteins in milk, and those made on the purified components, to elucidate the factors influencing changes occurring in the milk system.

The casein micelle population is estimated to be in the order of  $10^{12}$  particles per cubic centimeter of milk with an average free path of approximately 3,600 Å between particles.<sup>254</sup> The micelles are in constant kinetic motion, and because they are closely packed in milk, the entire colloidal dispersion may be immobilized by cohesion of a relatively small number of the particles. Unrestrained growth of the casein micelles, therefore, leads to gelation of milk. However, in normal milk the micelles, as a separate entity, are markedly stable to extremes of temperature and concentration. The structural stability and relative inertness of the micelles can be demonstrated by centrifuging milk to sediment the micelles into a pellet. This dense mass can be redispersed and the micelles will be identical with the natural system in size distribution when examined by electron microscopy. This property of reversibility after close approach (concentration) and dilution attests to the internal rigidity of the colloidal particle, which has been attributed to its protein-protein and protein-salt interactions.<sup>142,193</sup>

Figure 11.1 is an electron microscope photograph of skimmilk. The casein micelles are recognized as the rough-surfaced spherically shaped particles. These colloidal casein particles range in size from 500 to 2,500 Å and show a high degree of light scattering, which gives rise to the characteristic "milky" appearance to the fluid.

Numerous studies have been made of micelle population in milk by the electron microscope.<sup>20,23,117,164,196,202,214</sup> Figure 11.2 is a micelle size distribution nomograph of glutaraldehyde-fixed skimmilk. Some disagreement as to the size distribution of casein micelles is reported in the literature; the disagreement arises from the method(s) of specimen preparation. The glutaraldehyde procedure of Carroll *et al.*<sup>20</sup> gives good fixation with minimal shrinkage of the particles. It is in reasonable agreement with workers who have separated micelles into size classes by differential centrifugation and who have made size measurements by light-scattering techniques.<sup>35,52,134</sup> Figure 11.2 shows that the most frequently occurring casein micelle size is around 1,300 Å.

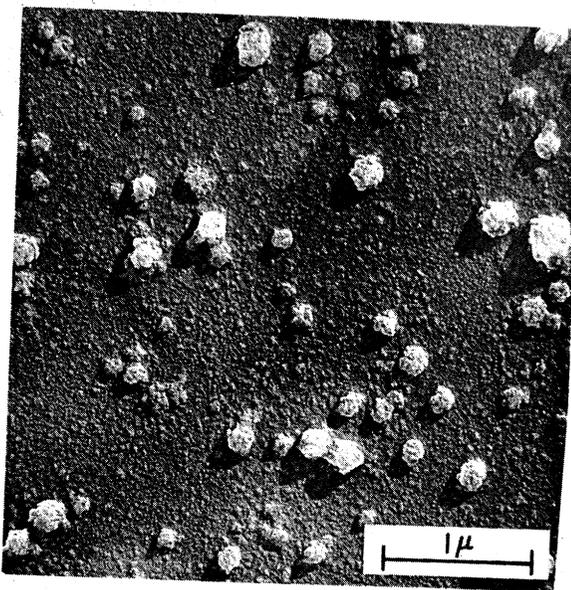
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## MILK COAGULATION AND PROTEIN DENATURATION

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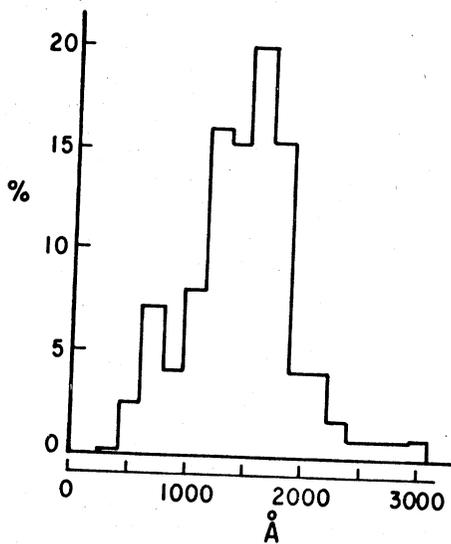
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From Parry and Carroll<sup>172</sup>

FIG. 11.1. ELECTRON MICROSCOPE PICTURE OF SKIMMILK MICELLES, GLUTARALDEHYDE FIXED AND SHADOWED WITH PLATINUM



From Carroll *et al.*<sup>19</sup>

FIG. 11.2. RELATIVE FREQUENCY OF OCCURRENCE OF CASEIN MICELLES VERSUS SIZE IN RAW MILK

several workers to suggest that the aggregate is composed of sub-units.<sup>150,158,209,214,244</sup> Although this could explain the size distribution and rough-surfaced appearance of the micelle, studies of casein composition as a function of size indicate that the composition of the micelles is far from constant.<sup>134,193,223</sup>

Considerable variation can be found in the literature on the quantitation of  $\alpha_{s1}$ -,  $\beta$ - and  $\kappa$ -casein present in the micelle. The problem seems to center on the difficulty of accurately determining  $\kappa$ -casein. It has been found that the dye-binding capacity of this protein is considerably less than that of  $\alpha_{s1}$ - or  $\beta$ -casein, which are approximately equal in this respect.<sup>144</sup> This unequal binding of dye to the caseins yields very low values for  $\kappa$ -casein when quantitated by polyacrylamide gel electrophoresis. Quantitation of sialic acid, a carbohydrate component of  $\kappa$ -casein, has been shown to be unreliable, since the carbohydrate content varies from 0 to 10%.<sup>139,194,255</sup> The use of the known sulfur content of  $\kappa$  as an analytical determinant has been recently questioned by the report that the minor  $\alpha_s$  components ( $\alpha_{s3}$ ,  $\alpha_{s4}$  and  $\alpha_{s5}$ ) also contain sulfur;<sup>82</sup> the reader is referred to Chapter 3 for further discussion of these proteins. Casein quantitation has been reported also on the basis of the carboxyl end-terminal amino acid released by carboxypeptidase A.<sup>187,197</sup>

A detailed study of three methods available for casein quantitation has been made by Rose *et al.*<sup>197</sup> This is the best study available to date and compares the sialic acid and sulfhydryl tests to a new method using analytical anion-exchange cellulose chromatography (DEAE). This technique would appear to give the best quantitative results and should be the least affected by compositional variations that are prominent in other techniques.

Table 11.1 shows the results of the above three casein analyses on a sample of whole isoelectric casein and various centrifugal cuts used for size separation of the micelles. It is apparent from this table that specific identification of the procedure used to separate casein is necessary, because of large compositional variations between pH 4.6 precipitated casein and the micellar protein obtained by centrifugation. This is due to the partial solubility of  $\kappa$ -casein at pH 4.6. Reported values for the isoelectric point of  $\kappa$  are 3.7<sup>262</sup> and 4.1<sup>194</sup> as determined by free-boundary electrophoresis. However, its behavior in urea-polyacrylamide gel electrophoresis, where it moves slower than either  $\alpha_s$  or  $\beta$ , would indicate a charge more in line with its isoionic point of pH 5.2.<sup>262</sup> The solubilization of  $\kappa$  can be seen when "washing" acid casein at pH 4.0, a preparative treatment often used to remove a proteolytic enzyme associated with casein. If the reported lower isoelectric points were those of the native protein, solubilization and hence loss

of the protein would probably not occur to such an extent. The results of column gradient isoelectric focusing on the caseins<sup>104</sup> in 7M urea and mercaptoethanol have shown the isoelectric point of  $\kappa$  to be 5.95 to 6.53, which would explain the high solubility at pH 4 in dilute acetic acid.

Table 11.1 also shows the differences in casein composition among different micelle sizes. This unusual characteristic of an aggregated system was first observed by Sullivan *et al.*<sup>223</sup> Such an inverse relationship between micelle size and  $\kappa$  content holds true regardless of the method chosen for determination of the  $\kappa$ ; that is, when  $\kappa$ -casein is examined by methods previously mentioned, they all indicate less  $\kappa$  in the biggest micelles than in the smallest, even though the methods vary considerably in the absolute amount determined.

An often neglected casein fraction, which may play an important role in milk stability, is serum casein. This nonmicellar casein does not sediment with the micelles (100,000  $\times g$  for 60 min) and has been reported to be 3 to 10% of the total casein in uncooled milk.<sup>188,193</sup> The noncentrifugable or serum casein increases in cooled milk (stored overnight at 4°C) to approximately 20% of the total casein.<sup>193</sup> The composition of this fraction, shown in Table 11.1, indicates that the content of  $\kappa$  rises to a third of the total casein in fresh noncooled milk. It has also been reported that micelles, which were centrifuged and redispersed 7 times in a milk salt-simulating buffer, lose their ability to be clotted by rennin.<sup>172</sup> This would suggest that fluid milk instability problems may be attributable to the serum  $\kappa$ -casein destabilization acting as an initiator of clot formation.

Table 11.1

Centrifugation Time (min.) <sup>b</sup>	CASEIN COMPOSITION <sup>a</sup>				Sulfhydryl <sup>d</sup> per 25,000 gm	Sialic Acid (%) <sup>d</sup>
	Proportion					
	$\alpha_{s1}$	$\beta$	$\kappa + \gamma$	Minor		
0 (Isoelectric Casein)	50.00	31.8	14.8	2.5	—	—
0-7½	47	34	16	3	0.4	0.28
7½-15	46	32	18	4	0.45	0.35
15-30	45	31	20	4	0.52	0.46
30-60	42	29	26	3	0.68	0.65
Serum <sup>c</sup>	39	23	33	5	0.98	0.88

<sup>a</sup> Data obtained Rose *et al.*<sup>197</sup>

<sup>b</sup> All centrifugations were done in a Spinco No. 30 rotor, 30,000 rpm, 100,000  $\times g$  max.

<sup>c</sup> Casein not sedimented after 60 min centrifugation.

<sup>d</sup> SH-groups and sialic acid are reported for freeze-dried material, uncorrected for moisture.

Effects of temperature on the casein distribution between the micelles and serum phase have been examined by Rose,<sup>193</sup> Murphy *et al.*,<sup>158</sup> and Downey and Murphy.<sup>31</sup> These authors have reported that dissociation (and hence solubilization of  $\beta$ -casein into the serum phase) occurs readily upon cooling of milk to 5°C. This is in agreement with Sullivan *et al.*<sup>224</sup> and Payens *et al.*,<sup>173,174</sup> who have studied the temperature-dependent association of purified  $\beta$ -casein. They found that  $\beta$ -casein has a monomer molecular weight of 26,000 daltons at 5°C; increasing the temperature to 25°C causes rapid aggregation and eventual precipitation.

$\beta$ -Casein accounts for approximately 50% of the casein released upon cooling, the remainder being evenly divided between  $\alpha_s$  and  $\kappa$ . The total percentage of casein solubilized shows variation between cows; i.e., ranges are reported from 10 to 21%<sup>31</sup> and from 14 to 44%<sup>193</sup> by different investigators. Downey and Murphy found very high serum or nonmicellar casein contents in late lactation cows and levels up to 55% of total casein from a cow having clinical mastitis. The fact that migration of casein from the "micellar state" to the serum phase does not occur in the same casein ratios as found in the micelle would appear to be significant in understanding the manner by which these proteins associate inside the micelle. Interestingly, the micelles, when repeatedly "washed" by centrifugation and redispersion in a milk salt-simulating buffer,<sup>101</sup> do not show any size change when examined in an electron microscope.<sup>172</sup> No work has described the properties of micelles which have been rewarmed to 30°C although serum casein does return to its previous low level of about 6%. One could imagine that micelles, after such a treatment (similar to that found in fluid milk processing), might have some alteration in their surface properties due to the large amount of previously solubilized  $\beta$ -casein that may well precipitate on or near the micelle surface.

### Salt Dispersion

The nonprotein components play a critical role in the physical stability of milk. The inorganic components are a complex mixture, dispersed in both the micelles and the serum phases. The work of Davies and White<sup>28</sup> first measured the salt content of both phases and studied the changes which occur upon cooling. They found serum phase increase of all salts except chloride ion when milk cooled from 20 to 3°C. The serum or filtrate obtained at 3°C becomes "cloudy" on rewarming to 37°C. This indicates equilibrium changes in the salt balance by formation of complex colloidal salts of sufficient particle size to scatter visible light. The ions most frequently thought to undergo complexation are

calcium,  $\text{HPO}_4^-$ ,  $\text{H}_2\text{PO}_4^-$  and citrate; the resultant insoluble salts of calcium phosphate probably have structures resembling apatite.<sup>12</sup> This rigid salt structure, also referred to as colloidal calcium phosphate, maintains micelle structure in spite of the loss of a significant percentage of casein when milk is cooled. This was demonstrated by Jenness *et al.*,<sup>102</sup> who found complete micelle disruption upon removal of the colloidal calcium phosphate.

The soluble (diffusible) calcium has been estimated by means of phase separation, including renneting, ultrafiltration and dialysis. The total calcium concentration is approximately 30 mM per liter, of which 10mM is diffusible and 2 to 3 mM is ionized.<sup>22,212,243</sup> Calculating from the dissociation constants of calcium citrate and calcium phosphate, Smeets<sup>215</sup> verified the average ionic calcium concentration of 2.75 mM per liter by using the murexide method.

It is well known that variations in free  $\text{Ca}^{2+}$  can alter the heat stability of milk. The increase in serum calcium found on cooling could adversely affect heat stability. The formation of colloidal calcium salts, in treatments such as forewarming, might reverse this destabilizing influence.

About two-thirds of the calcium in milk is located in the micelle. It occurs covalently bound to the phosphate esters of serine (or possibly threonine) complexed with  $\text{HPO}_4^-$ ,  $\text{H}_2\text{PO}_4^-$  and citrate,<sup>195</sup> and also is associated with acidic groups on the proteins.<sup>30</sup> From the work of McGann and Pyne<sup>142</sup> and Jenness *et al.*,<sup>102</sup> we know that when chelating agents are added to milk (e.g., EDTA or citrate), disruption of micelles occurs, apparently into small units of casein measuring 100 to 125 Å in diameter.<sup>17,39,158,209,214</sup> The occurrence of these subunits and their relationship to micelle structure are discussed in more detail in Chapter 9. Micelle disruption into subunits also has been found when isoelectric casein (pH 4.6 precipitated) is redispersed at neutral pH.<sup>166</sup> These treatments, which totally remove colloidal calcium phosphate, indicate that the most important role of this salt is in maintaining micelle integrity. Hence, when examining the effects of milk manufacturing on product instability, it is important to consider not only protein dispersion, but also the role and the ratio of serum to colloidal salt.

Boulet *et al.*<sup>13</sup> have reported an unusual approach to describe micelle salt dispersion. Using Sephadex G-150, they separated whole milk into three fractions: (1) micelles, (2) whey protein, and (3) serum salts, lactose and low molecular weight components. Analysis of the calcium, phosphate and citrate showed that "fraction one" micelles contained no citrate and had lost 22% of the calcium, 50% of the magnesium, and 47% of the phosphate contained in "whole" micelles of milk. These authors concluded that the loss of these salts

on gel filtration is due to the removal of labile ions adsorbed in the micelle, probably in the form of a diffuse double layer. The removal of this layer of ions does not destroy the integrity of the micelle, since rechromatography of micelles gives the same salt content. The "fraction one" micelles have a calcium-to-inorganic-phosphate ratio between 2.52 and 4.12, which is much higher than the ratio of any known calcium phosphate salt. This indicates that more than one type of salt form must be responsible for the internal structure of the micelle. Boulet *et al.*<sup>13</sup> suggest that approximately half the  $\text{Ca}^{++}$  is in apatite form, with the remainder in primary salt interactions between casein carboxyls and serine phosphate.

Yamauchi *et al.*<sup>257</sup> studied the behavior of  $^{45}\text{Ca}^{++}$  added as the chloride salt to milk, to determine the distribution and exchange of this cation between soluble and colloidal phases of milk. They found that of the calcium in the colloidal phase about 40% was not exchanged after 48 hr, terming it "hard-to-exchange" calcium. This calcium was absent, or nearly so, in colloidal phosphate-free milk and calcium caseinate phosphate dispersion. The speed of exchange in the latter system was faster than in milk. They suggest that hard-to-exchange calcium is present in a part of the colloidal phosphate portion of casein micelles. This nonexchangeable property is typical of hydroxyapatite and is in reasonable agreement with Boulet's suggestion of 50% apatite.

#### EFFECT OF HEAT ON FLUID MILK

The preceding section summarizes some of the critical observations necessary to understanding the colloidal milk dispersion, particularly those factors involving casein and salt. The introduction of irreversible changes in the milk system, such as processing treatments, may decisively affect the stability of the milk colloids. Heat sterilization of concentrated milk, for example, may simultaneously cause partial dephosphorylation of casein, denaturation of the serum proteins, interactions among the lactose, casein and serum constituents, an increase in acidity derived from multiple sources, and some irreversible changes in salt equilibria. All have a bearing on the coagulation process in varying degrees. Such induced changes, superimposed on the natural variation in milk composition, contribute to some of the instability of the colloidal dispersion. These factors frequently obscure the underlying cause of coagulation, and anomalous results are not uncommon. Hence, no satisfactory correlation has yet been made between the heat stability of milk and its analytical composition; nor is it possible on the basis of available data to predict with accuracy the heat stability of a concentrate from the original fluid milk. Although preheat treat-

ment of fluid milk imparts resistance to clotting by enzymes, as well as greater heat stability on subsequent concentration, it lowers the stability of both fluid and concentrated milks to freezing. Similarly, various forms of phosphate that act as calcium sequestrants effectively retard heat coagulation and are, therefore, indispensable to the manufacture of evaporated milk.

### **Protein Denaturation**

The term denaturation as applied to proteins has been subject to various connotations in milk literature. Native protein molecules are known to be folded into well-defined, more or less rigid, three-dimensional structures. For most proteins this structure is compact and globular, as exemplified by lysozyme,  $\alpha$ -lactalbumin and  $\beta$ -lactoglobulin. In some proteins the native structure is rod-like; or it is a rod with globular appendages as in the case of myosin. The caseins, however, are known to be essentially random coils in comparison with other protein secondary structures.<sup>80,165,175,207,210,225</sup>

The native structure of a protein remains stable over a fairly wide range of external conditions, but its internal organization into  $\alpha$ -helical or  $\beta$ -structures and/or disulfide bonds can be permanently disrupted by changes in physical or chemical environment. This process is irreversible denaturation. In many instances however, denaturation is a reversible process, showing that certain structural changes reflect changes in the stabilities of various possible conformations of the protein molecule. Examples of denaturing agents important to the dairy industry are heat and acidity.

Denaturation is complicated by the fact that not all proteins behave similarly in the presence of the same disruptive agent (conversely, widely different effects can be found with different agents<sup>228</sup>) and many possible molecular configurations may occur between the native and irreversibly denatured forms.<sup>99</sup> Furthermore, as the protein approaches this denatured state, its association behavior towards other proteins is markedly altered. This has been observed in  $\beta$ -lactoglobulin- $\kappa$ -casein systems when they are heated.

Casein, while considered to be "denatured" in the chemical sense of having very little, if any,  $\alpha$ -helix or  $\beta$ -structures, does have a "native" structure, which is primarily due to self-association with other caseins. This structure, therefore, is mostly of the quaternary type arising from hydrophobic, electrostatic and, to a limited extent, hydrogen bonding between protein molecules.<sup>207,210</sup> Thus, the effect of temperature and pH can drastically affect casein association and result in micelle alteration, to an even greater extent than observed with compact globular proteins.

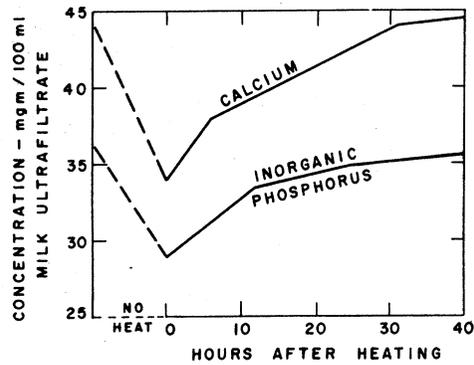
The serious voids in our understanding of the equilibria and compositional changes in milk at high temperatures hardly allow for speculation on the mechanism of heat coagulation. This mechanism is further obscured by variations in composition and heat stability of different milks, and even of milks drawn from different quarters of the udder of a single cow. Consequently, no satisfactory correlations have yet been established between heat stability and the analytical composition of normal fluid milks or their concentrates.<sup>5</sup>

In the strictest sense, casein is not a heat-coagulable protein. Its dispersion in normal fluid milk is very stable to heat and may resist coagulation for as long as 14 hr at boiling temperatures, and 1 hr at 130°C. The heat coagulation of casein in milks of normal stability occurs largely as a result of compositional changes in the milk, caused by sustained exposure to high temperatures. Of major significance among these effects are the increase of acidity, the conversion of soluble calcium and phosphates to colloidal forms, and interactions (denaturation, hydrolytic cleavages) between the protein components. The impact of such heat effects on colloidal stability is amplified in concentrated milks where coagulation tends to increase logarithmically with milk solids concentration.

### Salt and pH

Salt balance and acidity are regarded as two of the most important factors in the heat stability of milk. The low heat stability of colostrum milks is ascribed to a higher level of ionic calcium.<sup>199,251</sup> Noncolostrum milks of inordinately low heat stability (Utrecht abnormality) are of normal composition with respect to total calcium and acidity, but evidence a notably higher ionic calcium activity.<sup>211</sup> Milks of normal heat stability have a calcium-ion concentration ranging from 2.0 to 4.0 millimolar in the ultrafiltrate, whereas the ionic calcium values in milks exhibiting the Utrecht abnormality range from 4 to 7 millimolar.<sup>11</sup> This defect can be simulated in milks of normal heat stability by the addition of calcium salt ( $\text{CaCl}_2$ ) to elevate the ionic calcium concentration above 4 millimolar.<sup>185</sup> Adjustment to a more alkaline pH, or addition of calcium sequestrants, constitute effective corrective measures for this particular heat-stability defect.<sup>211,212</sup>

Heat treatment is known to cause reduction of both the total soluble and ionic calcium. Under pasteurizing conditions, the reduction is slight, but significant losses of soluble calcium and phosphorus occur above 76°C.<sup>7,81,231</sup> Values reported for changes in salt distribution in heated milk show large discrepancies, which may be caused by inadequate precautions against subsequent shifts in equilibrium and



From data by Hilgeman and Jenness<sup>61</sup>

FIG. 11.3. ULTRAFILTERABLE CALCIUM AND PHOSPHORUS IN MILK HEATED AT 78°C FOR 30 MIN, MEASURED INITIALLY AND AFTER COOL-AGING AT 5°C

by variations in the time lapse between heat treatment and analysis. Figure 11.3 shows the initial loss of about 25% of the soluble calcium in milk heated to 78°C for 30 minutes.<sup>61</sup> When the milk is aged at 5°C, gradual reversion toward the original soluble calcium level occurs over a period of 24 to 48 hr. Soluble phosphorus undergoes a similar change. This decrease in soluble calcium and phosphorus upon heat treatment and its reversion on cool aging has also been confirmed by analysis of calcium and phosphorus in centrifuged milk<sup>38</sup> as well as in ultrafiltration.<sup>198</sup>

The concentration of both soluble calcium and magnesium is increased in evaporated milk, but less than that of potassium and sodium, which implies precipitation of some of the  $\text{Ca}^{++}$  and  $\text{Mg}^{++}$  cations, possibly as the phosphate salts. This departure from the anticipated activity of calcium and magnesium ion becomes even more pronounced with increasing milk solids concentration. The combined effect of added disodium phosphate stabilizer and heat sterilization may lower the ionic calcium concentration in evaporated milk by 20 to 40%. The addition of 0.15% disodium phosphate to raw skimmilk lowers the ratio serum  $\text{Ca}^{++}/\text{total Ca}^{++}$  from 0.317 to 0.216, which is further reduced to 0.116 following heat treatment at 88°C for 15 min.<sup>38</sup>

Since changes in the electrolyte composition of heated milk are undoubtedly involved in the mechanism of heat coagulation, their measurement at elevated temperatures is critical. This is particularly true in view of the evidence that heat-induced changes in the salt balance revert on cooling, and may escape detection. Analyses by

Rose<sup>198</sup> of ultrafiltrate separated from heated milk samples establish that the changes in the salt balance are much more extensive than had been anticipated. Total ultrafilterable calcium and phosphorus, separated from milk at 94°C, are reduced by 50 and 18%, respectively, over raw milk controls, while calcium-ion concentration shows a 60% reduction. These changes, whose extent is a function of temperature level, generally attain equilibrium within 5 min. Reversion of the salt balance to 75 to 90% of the value of the original unheated milk requires cool aging at 5°C for 22 hr.

Reduction of the soluble and ionic calcium concentration in heated milk is partially attributable to a conversion of soluble calcium phosphate to the colloidal state, as discussed above. Evenhuis and Vries<sup>45,46</sup> suggest that crystallization of calcium phosphate to hydroxyapatite occurs in heated milk, a process that is greatly accelerated if a large surface area for nucleation is provided, for example, in the form of inactive yeast cells.<sup>15</sup> The precipitation and crystallization process begins at 60°C increasing rapidly at higher temperatures. If the phosphate in milk does crystallize to hydroxyapatite on heating, the Ca/P ratio of the precipitate would be 1.67, a value in good agreement with the analysis of van der Burg.<sup>15</sup> Approximately 48% of the casein-bound calcium and magnesium is precipitated with phosphate in milk treated with yeast cells for 20 min at 20°C. This precipitation of half the cations from casein is in accord with the conclusion of Boulet *et al.*<sup>13</sup> and Yamauchi *et al.*,<sup>257</sup> who similarly noted that approximately half the calcium is "exchangeable." The calcium in raw rennet whey is, however, readily precipitated with the denatured serum proteins, and does not require the use of a yeast cell surface. This precipitated calcium phosphate is highly resistant to dissolution on cooling. The precipitation of soluble calcium as the apatite crystal would leave the milk unsaturated with respect to calcium and thus enhance its heat stability. The beneficial effects of forewarming have similarly been related to heat modification of the salt equilibrium. It has also been suggested<sup>15,115,183</sup> that the advantage of strong preheat may originate with detachment of some of the colloidal phosphate from its complex with casein, a change noted in the polarograms of barium caseinate-barium phosphate complexes.<sup>181</sup>

Jeness and Parkash<sup>103</sup> have noted that part of the differences in heat stability and pH stability curves between individual milks could be eliminated by dialysis against bulk milk. This dialysis did not equalize the concentration of ultrafilterable calcium, magnesium or phosphorus of the test samples and the bulk milk. However, removal of colloidal calcium phosphate by acidification and then neutralization usually resulted in increased heat stability of both unconcentrated

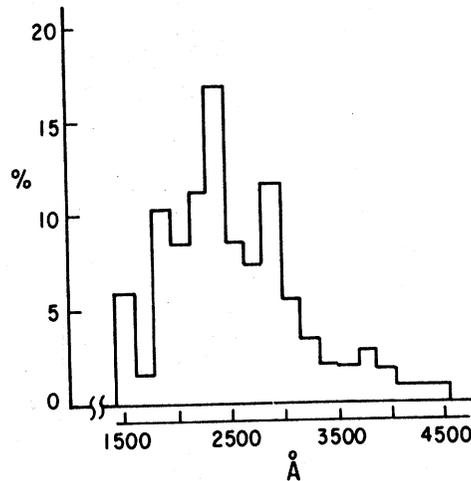
and concentrated (2:1) skimmilk, but the pH stability curve was the same type as that of the original skimmilk.

The influence of titratable acidity or pH upon heat coagulation has long been a recognized correlation. The general relationship between adjusted pH and spontaneous coagulation temperatures of raw skimmilk, as defined by Miller and Sommer,<sup>149</sup> is shown in Figure 1. A high order of sensitivity to pH is evident for values between 6.0 and 6.4. At pH values below 6.4, added phosphate tends to displace the curve toward higher stability, while calcium has the reverse effect. Such heat stability curves have been found to exhibit a maximum at a pH that appears to be a specific characteristic for each milk.

Thus the colloidal and serum salt levels are critical in maintaining casein integrity, and it has been pointed out that this delicate equilibrium can be disturbed by lowering or raising the temperature. The stability of milk after exposure to a processing treatment is similar to that sensitive to pH.

The acidity of milk increases with temperature, partially as a result of changes in the buffer capacity of the milk salts and the expulsion of  $\text{CO}_2$  on heating. Miller and Sommer<sup>149</sup> observed that the pH of skimmilk decreases approximately 0.1 pH for each  $10^\circ\text{C}$  temperature rise. Rose *et al.*<sup>198</sup> reported that the hydrogen-ion concentration of 94°C milk ultrafiltrate is at least twice that of a comparable 25°C ultrafiltrate. Concentration of milk is accompanied by a significant decrease in pH, which may contribute to the greater heat-susceptibility of condensed milks. Under prolonged heat treatment at elevated temperatures additional acidity is developed as a result of further changes in the milk. This acidity may be derived from thermal decomposition of the lactose to organic acids, interaction of lactose with the milk proteins, hydrolytic dephosphorylation of casein and displacement of the calcium-phosphate equilibrium.

Of the total acidity developed in milk heated at  $120^\circ\text{C}$  for 90 minutes to the point of coagulation, Pyne and McHenry<sup>183</sup> attribute one-half to lactose decomposition, one-third to casein dephosphorylation and the residual acidity to phosphate equilibria. Such heat-developed acidity contributes materially to the heat coagulation of milk.<sup>182,183</sup> In a study of 26 samples of fluid skimmilk it was noted that the pH at the time of coagulation ranged from 5.5 to 6.0, the more rapidly coagulating samples generally developing the most acidity.<sup>183</sup> The level of acidity developing before the heated milk coagulates appears to be inversely related to the calcium-ion activity, suggesting that the developed acidity may enhance the coagulating effect of ionic calcium. On the average, fluid skimmilks with an "effective calcium-ion concentration" in excess of 4.8 mM per l coagulated within 20 min at  $130^\circ\text{C}$ .

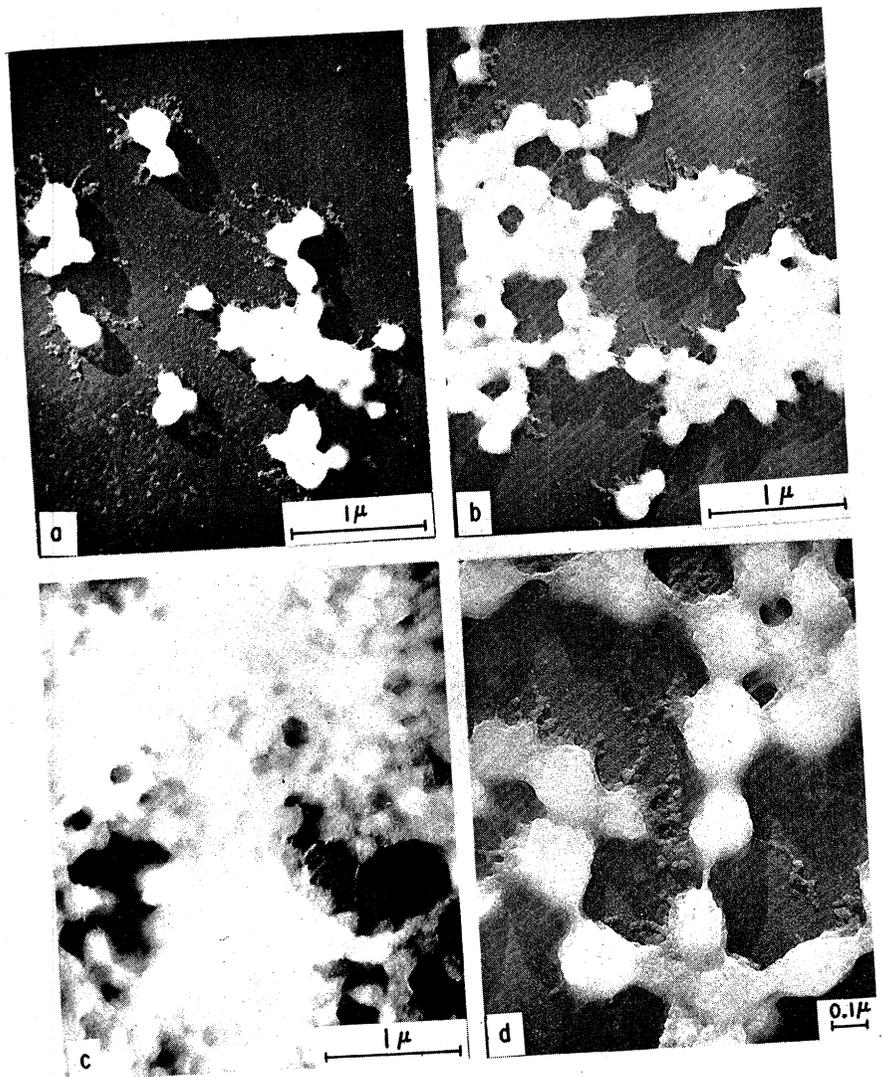


From Carroll et al.<sup>19</sup>

FIG. 11.10. RELATIVE FREQUENCY OF OCCURRENCE OF CASEIN MICELLES VERSUS SIZE IN HTST 3:1 CONCENTRATED SKIMMILK

was complete. Fig. 11.11c shows the tight packing of the casein micelles in the gel and the areas of bridging between the micelles. At higher magnification (Fig. 11.11d), the bridging of micelles is depicted more vividly. It appears that the particulate material, also present in ungelled samples, may comprise the bridging material in the gelled samples. It is important to note that the micelles have not lost their individuality, but their surfaces are much more textured than those observed at the beginning of the storage period.

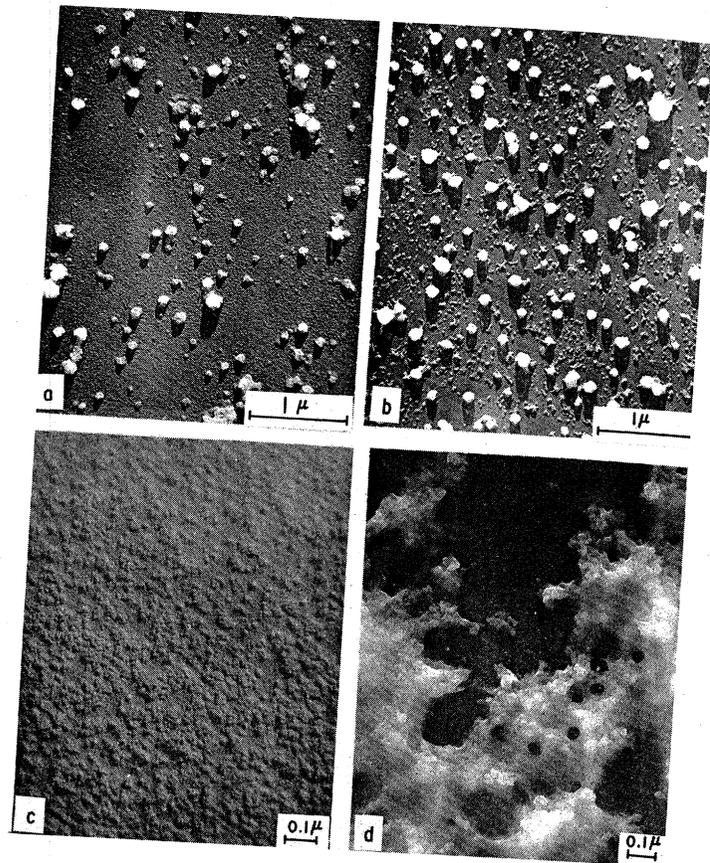
The importance of the particulate material in the gelation phenomenon can be seen in the electron micrographs of Fig. 11.12. Skimmilk was concentrated 2:1 at low temperature (Fig. 11.12a) and then heated at 100°C for 15 min (Fig. 11.12b). An increase in the amount of particulate material surrounding the micelles is seen after heat treatment; and close inspection shows that chain-like aggregates have formed; also, an increase in both average size and electron density of micelles resulted from heat treatment. In Figs. 11.12c and 11.12d the samples received the same treatment, except that the casein micelles were removed by sedimentation. Before heating, the supernatant consists of discrete particles 150 to 200 Å in diameter. These particles have been observed by several investigators, and it has been suggested that they represent small micellar units,<sup>19</sup> although, as was discussed earlier (page 608), they are known to have an abnormally high  $\kappa$ -casein content compared to the casein composition of the micelle. When this superna-



*From Carroll et al.<sup>19</sup>*

FIG. 11.11. EFFECT OF STORAGE ON HTST 3:1 STERILE CONCENTRATED SKIMMILK  
 a—9 weeks. b—13 weeks. c—17 weeks. d—15 weeks at higher magnification.

tant was heated at 100°C, a flocculant precipitate was formed, which appeared (Fig. 11.12d) as large masses similar to the bridging material connecting the micelles in gelled concentrated milk (Fig. 11.11d).  
 Electron microscopy of micelles of concentrated skimmilk shortly



From Carroll *et al.*<sup>19</sup>

FIG. 11.12. ELECTRON MICROGRAPHS OF SKIMMILK

a—2:1 concentrated skim milk prepared in laboratory. b—Skim milk (a) heated at 100°C for 15 minutes. c—Supernatant from skim milk (a) after casein micelles removed by centrifugation. d—Skim milk (c) heated at 100°C for 15 minutes.

after sterilization has shown them to be smooth-surfaced and electron-dense. The micelles lose this appearance during prolonged storage, and the micelles in the gel are more textured than those in the fresh concentrate. This could be explained by the interaction of the "κ-casein rich" supernatant with β-1g by the thiol-disulfide mechanism, forming a protein particle which could associate with the micelle itself or form the chain-like aggregates seen in the electron microscope. These highly coalescent particles associating through hydrophobic and charge interactions could be stabilized by the serum calcium, thus forming

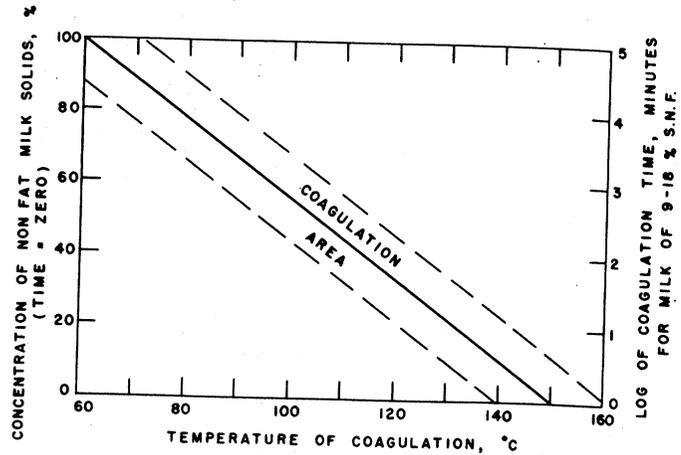
electron-dense micelles. Fox *et al.*<sup>54</sup> have reported that concentrated milks prepared by conventional evaporation procedures and by HTST developed a sediment of calcium phosphate which increased with time. This would indicate that the complex of calcium- $\kappa$ -casein  $\beta$ -lg casein micelle loses its calcium during storage causing: (1) formation of an insoluble salt sediment, (2) less opacity or electron density of the micelles, and (3) promotion of micelle aggregation or gelation mediated through the highly coalescent surface proteins which are now unsatisfied ionically because of the loss of cations.

### Effect of Concentration on Heat Coagulation

The coagulation of milk by heat is a function of the milk solids concentration, as well as of the temperature and time of heating. The way in which increases in concentration of nonfat milk solids cause the product to coagulate at successively lower temperatures is shown in Fig. 11.13. The relationship is generally linear with respect to temperature and logarithmic with respect to heating time.<sup>23,162,247,251</sup> Cole and Tarassuk<sup>23</sup> reported finding some deviation from this straight-line relationship when they studied milks heated in the temperature range 110 to 160°C. About 12 hr of heating at 100°C is necessary to coagulate fresh milk. At 130°C coagulation occurs in approximately 1 hr, while at 150°C the reaction occurs in about 3 min. Though there are wide variations among milks, the time of coagulation of an average evaporated milk (total solids 26%), prepared from milk of good quality, may increase from 10 min at 131°C to 60 min at 114.5°C and to 7,500 minutes at 80°C.

The time of coagulation at a definite temperature varies with the forewarming treatment to which the milk is subjected before concentration. Preliminary heating or forewarming applied to a milk not subsequently concentrated lowers its heat stability. Forewarming continues to cause lowering in heat stability of milks that are concentrated up to a 13% nonfat-solids level. If a milk is further concentrated to 14% or more solids-not-fat, its heat stability is markedly increased by a proper forewarming treatment.<sup>247</sup> Thus there is a critical solids level, about 13.5%, below which forewarming of raw milk decreases stability and above which it raises stability. Hence, forewarming is an important manufacturing step in the evaporated milk industry, where a milk of at least 18% solids-not-fat is to be produced.

No heat stability test is known which, when used on raw milk, will accurately predict the stability of its concentrated product. It is, therefore, not possible to predetermine the forewarming to apply to a fresh milk, so that after concentration it will respond to a sterilization process



Drawn from data of Webb and Holm<sup>247</sup>

FIG. 11.13. THE GENERAL RELATIONSHIP BETWEEN THE HEAT COAGULATION AREA OF MILKS, THEIR CONCENTRATIONS, AND THE TIME AND TEMPERATURE OF HEATING

by producing a required body. The evaporated milk industry determines heat stability and regulates the body of the finished product by making pilot-batch tests and by addition of stabilizing salts. Tests to determine approximate heat stability, such as alcohol,<sup>27</sup> phosphate,<sup>207</sup> or protein stability test,<sup>221,252</sup> are sometimes used on raw milk. A decision as to the optimum forewarming conditions is usually made on the basis of the behavior of the milk received the previous day.

Marked variations in stability occur with variations in forewarming temperature and time. Rapid improvement in resistance of the concentrate to heat coagulation is brought about by increasing forewarming temperature. Milk to be concentrated and sterilized as evaporated milk is usually forewarmed in a hot well by direct steam injection to raise it to 95°C, where it is held for 10 to 20 min. The stability thus imparted to the milk will usually enable it to withstand heat sterilization as a 26% solids evaporated milk. Greater heat stability can be obtained by the use of high-temperature forewarming carried out by injecting steam directly into the milk, or by forcing the milk through a pressure-heating system, usually in the form of a tubular heater. As the temperature of forewarming is raised, shorter holding times are necessary to attain maximum heat stability. For the manufacture of a 3:1 sterilized milk of 35% solids content, a high-temperature forewarming treatment within the range of 115°C for 2 min to 138°C for 15 sec will usually be required. The forewarming of sweetened condensed milk, discussed later, must be conducted differently from that of evaporated milk.

The rapid decrease in the heat stability of milk with increasing concentrations makes it impractical to attempt to sterilize a product which has been concentrated to a ratio substantially higher than 3:1 (7% nonfat solids). In commercial practice evaporated milk concentrated 2:1 can be sterilized under several temperature-time combinations, such as 115°C for 16 min, 130°C for 2 min, and 140°C for 3 sec. Milks concentrated to 3:1 can sometimes be adjusted by forewarming and salt-balance procedures so that they will withstand the heat necessary for sterilization.

Statistical analysis has shown that the addition of carrageenan to sterilized milk concentrate, and the interaction between carrageenan and forewarming, were highly significant in maintaining storage stability and flavor in the product.<sup>226</sup> The daily processing of such a concentrate by the usual sequence of processing steps would be difficult, and with some milk supplies, almost impossible. The relatively low heat stability of concentrated milks may be avoided in high-temperature sterilization processes by sterilizing the milk prior to aseptic homogenization and canning.<sup>40,220</sup>

### **Viscosity of Sterilized Milk Concentrates**

Viscosity control during processing and storage is an important consideration in dairy-product manufacture. Pre-coagulation thickening occurs in the manufacture and storage of evaporated, plain and sweetened condensed milks, ice-cream mix, and various specialty products. Thickening of milk precedes its coagulation by heat, and increases rapidly with increasing concentration. The actual process of thickening occurs in a relatively short time, just prior to separation of the curd. Coagulation is easily determined by visual appearance of curd aggregates, but thickening is a less perceptible change. Since thickening is a transient state, it is often difficult to stabilize a milk concentrate at any desired viscosity. Evaporated milk must be treated so that the sterilization heat brings it almost to, but never beyond, the point of coagulation. Here the thickening reaction produces a desirable creamy body, although some thinning occurs during storage. The rate of thickening during sterilization in the can at 116°C although variable, becomes greatest in the 10 min preceding coagulation.<sup>29</sup> Milks of high heat stability, which reach the end of the sterilization period before entering the thickening phase, do not develop the high viscosity shown by milks of lower heat stability.

Evaporated milk is subjected to conditions of agitation that vary considerably in different sterilization processes. A heavy-bodied milk will not form under the severe agitation sometimes used to attain

rapid heat exchange. However, Gammack and Weckel,<sup>56</sup> using an intermittent hold, Ball,<sup>3</sup> by addition of lactic acid to raw milk, and Tarassuk *et al.*,<sup>227,229</sup> by a proteolytic enzyme treatment before concentration, produced evaporated milks which (after sterilization) had higher viscosities than untreated control samples.

In the case of sweetened condensed milk, excessive thickening developed during manufacture will lead to objectionable gelation in storage. Thickening may be caused by improper forewarming, high acidity in the milk, high concentration of solids, high storage temperature, and other factors.

Sweetened condensed milk should be forewarmed at a lower temperature than evaporated milk to avoid storage thickening. The effect of time and temperature of forewarming of milk on the viscosity of its sweetened condensed product has been studied by several investigators.<sup>178,218,248,250</sup> Representative values are shown in Table 11.5. Although forewarming at 71°C gives a low-viscosity product, this temperature does not effectively destroy many bacteria, yeasts, molds, and enzymes, the presence of which often cause body and flavor defects during storage. It is commercial practice to forewarm the milk at about 82°C. This yields a product that has sufficient viscosity to retard separation of fat and crystallized lactose during storage, but will not gel when held at room temperature or lower for several weeks. When high-temperature short-time heating equipment is available, forewarming at 115°C for less than 1 min may be used to produce low-viscosity sweetened condensed milk for shipment to warm climates. Thickening is usually measured and expressed as relative viscosity; generally no attempt is made to determine plasticity.

Table 11.5

EFFECT OF TIME AND TEMPERATURE OF FOREWARMING ON THE VISCOSITY OF SWEETENED CONDENSED SKIMMILK<sup>a</sup>

Forewarming Conditions		Viscosity after Storage at 15.5°C in Poises		
°C	Min	1 Day	24 Days	58 Days
71	10	25	40	95
82	10	360	534	853
95	10	570	846	—
115.5	0.5	20	30	69

The data are those of Webb and Hufnagle<sup>248</sup> except that the values at 71°C for 10 min were estimated from references cited in the text.

Completion of the sterilizing process does not mark the end of the effects of time and temperature on the stability of the concentrated milk system. Changes continue, but at a rate dependent not only on previous heat treatments, but also on time and temperature of storage. Most products thicken with age, but evaporated milk made by the usual longhold sterilization method thins to a basic viscosity during the first few days of storage.<sup>29</sup> In contrast, high-temperature short-time (HTST)-sterilized milk (135°C for 30 sec) thickens until it gels in storage. The rate of thickening decreases as the processing heat increases.<sup>8</sup> The viscosity of sweetened condensed milk increases logarithmically with the temperature of storage and arithmetically with storage time.<sup>249</sup> For conditions of constant viscosity, time varies logarithmically with storage temperature. Whether the gelation of HTST-sterilized milks in storage represents an extension, at a slow rate, of the heat-coagulation reaction is uncertain.<sup>230</sup> Thickening during the storage of HTST-concentrated milks proceeds rapidly after an initial increase in viscosity. The more severe the heat treatment to which samples are subjected, the more stable are the milks against thickening. Samples of 2:1 milks processed in a tubular heater at 135°C for 30 sec and subsequently heated in cans at 115°C for 2, 6, 12 and 18 min, started to thicken at 32, 36, 41, and 51 weeks, respectively.<sup>8</sup>

Many workers<sup>3,18,77,230,250</sup> have shown in various ways that the onset of gelation is hastened by lessening exposure of the milk to heat during the sterilization process, as in HTST proceeding. Milks sterilized by irradiation without heat gel quickly in storage.<sup>83,84,85</sup> This gel-forming property of irradiated milk is probably related to the same mechanism that causes gel formation in milks processed with a minimum of heat during sterilization. There is no correlation between long-continuing fluidity and high heat stability in evaporated milk.<sup>8</sup> Heat appears to stabilize milk against gelation in storage more effectively when applied to milks of high solids content (26 to 34%) than to milk of normal concentration.<sup>230,250</sup> Heat applied to milk concentrates at a solids level of 32 to 45%, followed by dilution to 26%, imparts to the diluted milk a greater resistance toward age-thickening.<sup>220</sup> Thickening is retarded by low-temperature storage.<sup>56</sup>

There has been intensive effort to devise a sterilization process for preparation of a beverage-quality concentrated milk at the 2:1 or 3:1 level by manipulation of time and temperature of heating. The objective has been the production of a concentrate with a minimum of cooked flavor, discoloration, fat separation, thickening and staling in flavor during storage.<sup>9,14,18,40,161,220,226,250</sup> Of these defects, thickening and gelation have been the most obvious and objectionable. An acceptable sterile concentrate can be prepared by the use of optimum processing

conditions, followed by storage at 10 to 15°C. A typical process sequence would include forewarming at 115°C for 2 min, concentration not to exceed 3:1, sterilization at 135°C for 30 sec, cooling to at least 98°C, aseptic homogenization at 4,000 psi, cooling to 20°C, and aseptic canning. Passing the uncooled canned milk through a hot-water bath at 72°C for 10 min further delays gelation but increases cooked flavor. Both flavor and physical stability are benefited by refrigerated storage. The proposal has also been made that raw milk be sterilized, then aseptically homogenized, concentrated, and packaged.<sup>14</sup> This procedure avoids coagulation problems during processing but does not delay storage thickening.

One process for retarding gel formation adjusts the calcium-sodium ion ratio of the milk by use of an ion-exchange resin.<sup>219</sup> Calbert and co-workers<sup>18,60</sup> have sought to stabilize HTST-processed milk by holding it after sterilization at 94°C to permit critical viscosity development. The incipient coagulum is next destroyed by homogenization and it is then not expected to reform during storage. One problem in the use of this procedure is to so adjust the degree of thickening that, when the product is finally smoothed out by homogenization, neither sediment formation nor gelation will occur in storage.

Polyphosphates effectively retard age thickening and gelation in HTST-sterilized milk concentrates and sweetened condensed milk.<sup>132</sup> Early work indicated these salts had a rather uncertain stabilizing effect, but optimum percentages of polyphosphate glass, having an average of 4.8 phosphorus atoms per chain, now appear to afford effective stabilization.<sup>131</sup>

The polyphosphates appear to slowly hydrolyze to orthophosphate, which has a thickening effect, thus retarding lactose crystallization. Much of the apparent stabilization may be due to the protein-peptizing effect of the polyphosphates. Tumerman and Guth<sup>238</sup> found that by the addition of 0.25 to 1.0% NaCl to milk before or after concentration, the freezing point was lowered and lactose crystallization was suppressed, giving increased stability to frozen concentrates. Samel and Muers<sup>203</sup> found that polyvalent anions, including phosphate, destabilize sweetened condensed milk because of the formation of insoluble calcium salts, which increase viscosity on aging. Leviton *et al.*<sup>132</sup> places an optimum concentration for a sterilized concentrate at 0.6 lb polyphosphate per 100 lb milk solids, thereby extending storage life of a 3:1 concentrate at 21°C from 50 to 347 days. The kind of phosphate salt used as a stabilizer is important, since the common  $\text{Na}_2\text{HPO}_4$  and some other phosphates increase gel formation during storage.<sup>36,132</sup>

Kaleb *et al.*<sup>106,107,108</sup> have investigated the properties of milk gels obtained by heating 50% solutions of nonfat dry milk as a possible

new food product. They reported that maximum gel firmness is obtained by heating the solutions to 100°C for 10 min; other factors, such as protein concentration and temperature of testing, are important in determining firmness. Compared to fibrin, the concentration of milk proteins must be much higher to form gels, and unlike gelatin, milk gels are irreversible. The nutritional value of thermally induced milk gels apparently changes little when heating at 100°C for 10 min, but higher temperatures and longer times will dramatically decrease quality.<sup>106</sup> Similarly, the nutritional quality of milk decreases considerably on storage of concentrated or dry milk or by heating.<sup>42,43,167</sup>

### Salt Balance and Heat Stability of Concentrated Milks

Sommer and Hart<sup>216,217</sup> first showed that a critical balance between the natural acidic and basic salt components of milk appeared necessary to provide maximum stability to heat coagulation. In most cases inadequate resistance to coagulation is related to the presence of an excess of calcium and magnesium. Addition of phosphates, citrates, or carbonates to such milks was shown to improve their heat stability appreciably. It is generally assumed that the salt balance directly affects the heat stability of the casein, but this balance may operate indirectly on the casein through its effect on denaturation and interaction with the serum proteins.<sup>163</sup> The relation between the mineral composition of milk and its heat stability has been studied intensively, but the distribution and role of milk-serum electrolytes at high temperatures remains obscure.<sup>44,183,198,251</sup>

When milk is preheated and concentrated, the effect of acid and stabilizing salts may be the same or opposite to that observed in the original raw milk. Evaporated milk may respond to small changes in pH in the same manner as fluid milk, but its pH sensitivity in the presence of added  $\beta$ -lacto-globulin is much less than that of fluid milk.

Three types of concentrated milks are shown in the lower curves of Fig. 11.14. One kind of milk is stabilized by addition of orthophosphate or citrate; another is stabilized by addition of calcium or magnesium; and a third is destabilized by salt additions.<sup>87,247</sup> The three types of milk are usually derived from a raw milk, of which the curve shown in upper Fig. 11.14 is typical.

Concentrated milk III, stabilized by small percentages of  $\text{CaCl}_2$  or other chloride including hydrochloric acid, is comparatively rare, apparently being secreted by from 10 to 20% of the cows of a normal herd. If a Type III raw milk is placed in storage, there will be a gradual shift in the heat stability curve of its evaporated product through Type

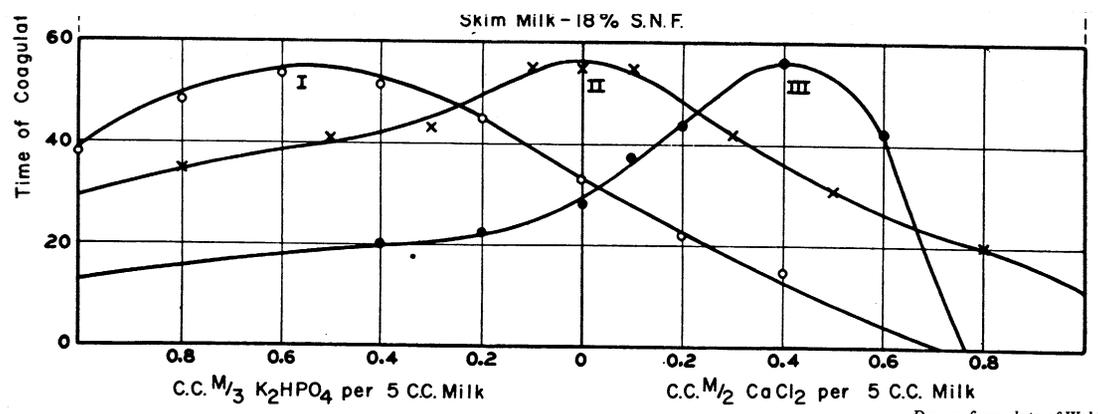
II to Type I, although little change may be noted in the stability curve of the unevaporated sample. The shift from Type III to Type I will be accelerated by the development of lactic acid during storage,<sup>245</sup> but it may also proceed without the accompaniment of a measurable change in pH.

There is no clear division among the three types of milk, since they merge into one another, and numerous curves can be obtained showing a difference in degree of variation. There may be a shift from type to type as a result of a change in forewarming temperature, as shown in Fig. 11.15.<sup>246</sup> Thus a milk forewarmed at 95°C was heat-stabilized by calcium after concentration. When higher forewarming temperatures were used, both calcium and phosphate destabilized the milk, but some stability was obtained at certain critical levels of phosphate.

Many different salts may be used with substantially the same results as are obtained with  $\text{CaCl}_2$  and  $\text{Na}_2\text{HPO}_4$ .<sup>247</sup> Differences in the valency of the ions concerned generally account for the differences in ionic concentration found necessary to produce a given result. The chlorides of H, Na, K, Ca, Mg, Ba, Al and Th furnish a source of strong cations, while the sodium and potassium citrates and orthophosphates may be used when strong anions are required. In general, calcium has a greater impact than phosphate on heat stability. The addition of small percentages of phosphate to a milk to increase heat stability is useful if a phosphate-stabilized milk is encountered (curve I, Fig. 11.14). However, if the milk is stabilized by calcium (curve III), the addition of phosphate does not increase stability, but at the same time it effects no marked lowering of stability. This fact accounts for the success of phosphate salts in evaporated milk manufacture.

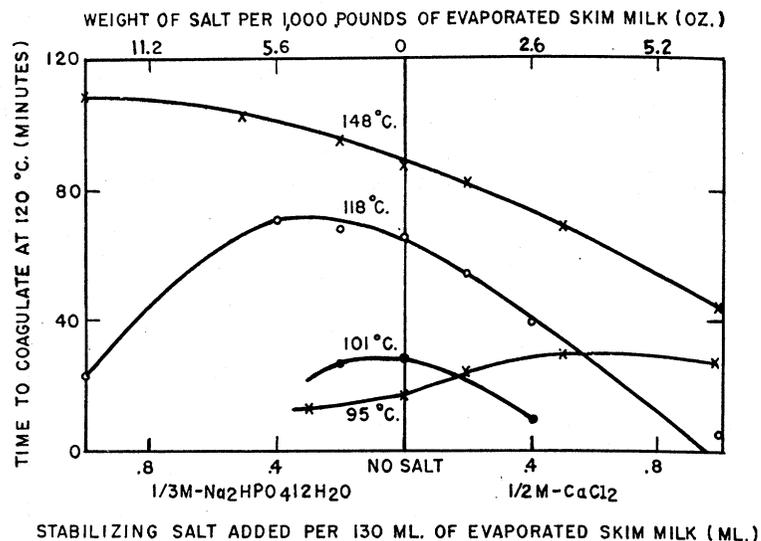
The addition of salts to milk before forewarming and condensing modifies the heat stability curve of evaporated milk. If a small proportion of calcium chloride is added to a fresh milk which normally has a stability curve of a type II milk, Fig. 11.14, the resulting curve is shifted toward curve I. If a phosphate salt is added to the fresh milk, the heat stability curve is shifted toward curve III. This behavior is to be expected, since it is the normal adjustment arising from an excess of either a strongly positive or negative ion for this particular milk.

From the foregoing discussion it can be seen that the effect of salts on the heat stability of milk is largely empirical but of paramount importance in the manufacture of evaporated milk. The stabilizing salts that have been approved by the Food and Drug Administration<sup>51</sup> are calcium chloride, sodium citrate, and disodium phosphate. The hearing record shows that these salts are needed in evaporated milk manufacture to control heat coagulation in certain types of milk and



*Drawn from data of Webb and Holm<sup>247</sup>*

FIG. 11.14. THE EFFECT OF THE ADDITION OF VARYING AMOUNTS OF DIFFERENT ELECTROLYTES UPON THE HEAT STABILITY OF NORMAL AND EVAPORATED SAMPLES OF THE THREE DIFFERENT TYPES OF MILK



From Webb and Bell<sup>246</sup>

FIG. 11.15. THE EFFECT OF STABILIZING SALTS AND FOREWARMING TREATMENTS UPON THE HEAT STABILITY OF EVAPORATED SKIMMILKS

at certain seasons of the year. Their use is permitted to the extent of 0.1% of the weight of the evaporated product.

An exception to the stabilizing influence of orthophosphate has been noted. Stewart *et al.*<sup>220</sup> advocate use of forewarming rather than phosphates to obtain stabilization in HTST sterilization. The principal defect in this product, gel formation in storage, can be minimized if phosphate addition is avoided in processing.

While the addition of salts to milk is a simple method of changing its heat stability, this can also be done by removing ions from the system. Skimmilk treated to remove 60% of its calcium may be added in an amount equal to 0.5 to 2.5% of the batch of original milk, and the mixture processed into evaporated milk.<sup>168,169</sup> Stabilization by this means is equal to the use of from 2 to 7.3 oz of disodium phosphate. Evaporated milk of 40% solids can be stabilized against heat coagulation by the use of a mineral ion-exchanged milk as a 10% replacement for normal solids.<sup>110</sup>

### Effect of Nonionic Substances

Nonionic and inert materials, such as fats, starches, sugars, and vegetable pulps, usually promote protein coagulation during heating

be dispersed. Suspended particles appear to have a tendency to concentrate the protein on the surfaces.

This accumulation of protein on the surfaces tends to stabilize the protein stability by encouraging local adsorption, which in turn stabilizes the entire system. Ground charcoal, activated carbon, and ground filter paper have been shown to lower heat stability. Lactose, sucrose, and dextrose, usually in the form of dextrose under certain conditions, have been shown to lower heat stability.<sup>253</sup> Lactose removal from concentrated milk by ultrafiltration and centrifugation, or hydrolysis has been shown to lower heat stability and prevent age-thickening.<sup>41</sup>

Starches and gums on the heat stability of milk are of great importance in the manufacture of certain foods. Sterilized cream-style soups, sauces, and milkshakes must have a smooth body, free from lumps. Starches such as corn or potato starch are common thickeners. They tend to adsorb protein, causing precipitation of the protein. Since coagulation cannot be avoided, the material must be of a type that produces a soft gel type of coagulum. Milkfat also affects the heat stability of the system. Homogenization, however, increases the dispersion of fat globules, thus providing more surfaces for adsorption, simultaneously concentrating protein on these surfaces, forming a fat-protein complex. This complexing of fat globules often occurs, and this further stabilizes the protein. It follows, therefore, that the degree of fat clumping will also modify the effect of homogenization on heat stability. The effect of homogenization on heat stability is not appreciable at low pressures, but becomes appreciable with increases in pressure.

Homogenization has an important bearing on the stability of milk products. Preheating temperature, homogenization pressure, and solid-not-fat concentrations, and salt content all affect the fat clumping and coagulation. The effect of homogenization is related to the pressure applied.<sup>246A</sup> The general effect of homogenization is shown in Fig. 11.16. Rehomogenization, which is done in a two-stage valve, wherein the second pressure is higher than the first, breaks up the larger clumps and consequently results in a more stable product than that observed after a single homogenization. Higher homogenization temperatures favor the stability of milk products. Homogenized milks when homogenization is practiced at higher pressures are more stable during storage.<sup>141</sup>

Homogenized creams when added to coffee is a

form of heat coagulation.<sup>246A</sup> Coffee cream is usually homogenized to retard fat separation and to impart a smooth, creamy body. The effect of homogenization on the heat stability of 20 and 30% cream is shown in Fig. 11.16. The salt content of the cream or of the coffee, chiefly the presence of relatively high levels of calcium in either, is an important factor.<sup>236</sup> Acid development before or after processing quickly renders cream susceptible to feathering. King<sup>114</sup> lowered the feathering value of cream by adding to it 5% of skimmilk treated with an ion-exchange resin to lower its calcium content.

In the processing of milk, coffee cream, and evaporated milk, the destabilizing effect of homogenization is incidental to the primary objective of retarding fat separation in storage. The interrelationships of

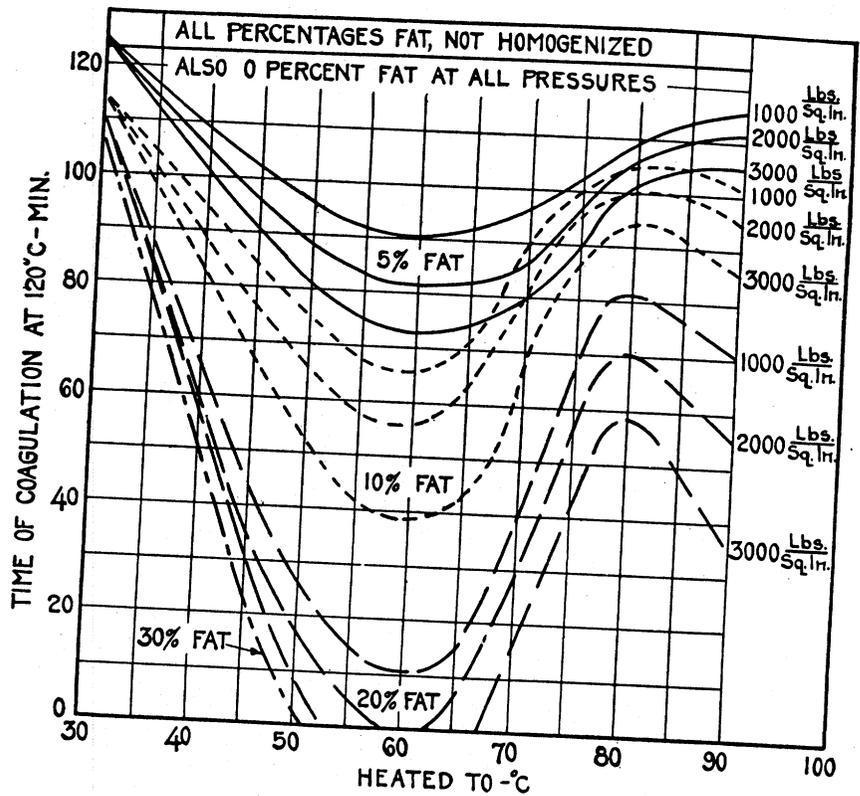


FIG. 11.16. VARIATIONS OF COAGULATION TIME OF MILK OF NORMAL SOLIDS-NOT-FAT CONTENT WITH CHANGES IN FOREWARMING TEMPERATURE, FAT CONTENT, AND HOMOGENIZATION PRESSURES

No homogenization below 50°C.

viscosity, heat stability, conditions of homogenization, and storage on fat separation in evaporated milk have been studied. Trout has reviewed work on the homogenization of milk from all aspects.<sup>237</sup>

### Solubility of Dried Milks

**Effect of Heat.**—The native properties of milk components are substantially unmodified by moderate milk-drying conditions. In freeze-dried milk, the equilibrium ratio of  $\alpha$ - and  $\beta$ -lactose and the salt distribution remain essentially intact. The normal size dispersion of the caseinate phase and its clottability by rennin are substantially recovered on reconstitution of the dried product. Depending on the preheat conditions, dryer design and temperature of operation, the properties of spray-dried powder may vary significantly. The initial temperature of an evaporating milk droplet, in a spray dryer with parallel air flow, does not appreciably exceed the unit bulk temperature, and can be effectively held to temperatures below 60°C.<sup>24,140</sup> As the falling rate period is approached in the course of further evaporation, the temperature rises to a final value determined by the final temperature of the drying gas and the residence time in the dryer. Under properly controlled spray-drying conditions, the changes in milk protein structure and solubility are minor. Spray drying does not denature the serum protein significantly, and the level of serum-protein denaturation in dry milk is substantially equal to that of the condensed milk from which it is processed.<sup>68</sup>

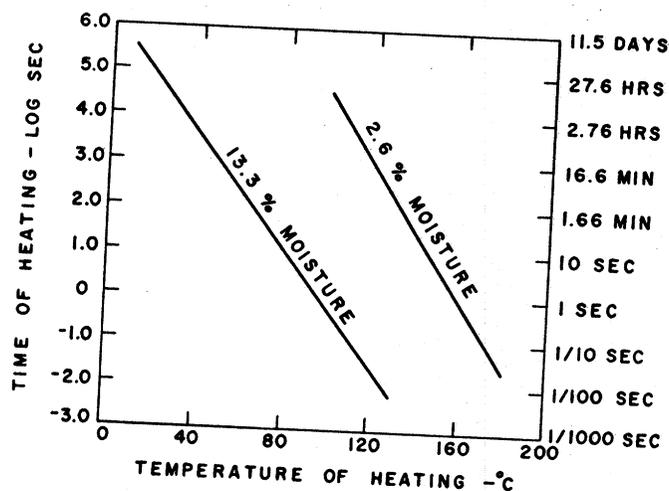
Loss of solubility of dry milk during processing or storage is largely a manifestation of changes in the stability of the caseinate-phosphate complex. The solubility of spray-dried powder is unaffected by preheat treatments of the fluid milk that cause extensive serum-protein denaturation.<sup>138</sup> Exposure of dry milk to a dry-heat treatment sufficient to cause total insolubilization of the caseinate phase may have negligible effect on the serum protein. In a maximally insolubilized nonfat dry milk, the total casein, comprising 35% of the solids, may be rendered insoluble, while the solubility of the serum components remains intact. Jenness and Coulter<sup>100</sup> suggest that preheat may exert a stabilizing influence against coagulation during drying, similar to the benefit of preheat in evaporated-milk sterilization.

While preheat conditions do not significantly affect the initial solubility of dried whole milk, powders processed from high-preheat milk tend to develop insolubility, during subsequent storage at 37°C, more rapidly than low-preheat powders. The free fat in dry whole milk decreases somewhat on storage and appears to be related to solubility decrease and film formation in the reconstituted milk.<sup>135</sup>

Heat damage in nonfat dry milk can be reduced in the spray-drying step. Atomizing equipment which gives the narrowest possible particle size distribution is superior, since in sprays having a broad size distribution the solids from smaller droplets tend to burn long before sufficient water is removed from the larger drops. Also, condensing equipment that can only operate efficiently with one or more stages held at 85°C or higher is undesirable, unless it can be demonstrated that the holding time of the milk is short.<sup>170</sup>

Under normal spray-drying conditions the casein solubility is spared. At excessively high exit-air temperatures, the casein is spray-dried milk powders may insolubilize at a rate that is approximately logarithmic with the temperature.<sup>235</sup> The conditions that prevail during roller drying of milk alter the solubility of the casein extensively. The film of milk on the heated drum has a residence time of several seconds, during which it attains progressively increasing solids concentration upon continuous evaporation. At solids level above 60%, the casein is particularly vulnerable to the high temperature of the drum surface. Coagulation is essentially instantaneous at solids concentrations exceeding 80%. Wright<sup>256</sup> determined that each increase of 1% in milk solids lowered by 1°C the temperature required to effect a constant degree of insolubilization for a fixed heating period. The maximum quantity of protein rendered insoluble by any moist heat treatment approximates 75% of the total. At a fixed moisture level, the time required to produce a constant degree of insolubility is a logarithmic function of the temperature. This relationship is shown in Fig. 11.17. The rate of protein insolubilization increases nearly fivefold for each 10°C rise in temperature. The slope of this curve is similar to that derived from measurements on the heat stability of evaporated milk. Similarly, the coagulation time for milk powder at 86.7% solids is extrapolable from the heat-stability curves of concentrated milks of 28 to 38% solids levels, suggesting that the heat coagulation of evaporated milk and the insolubility of dried milk may have a common origin. At moisture levels below 13%, the rate of insolubilization of heated milk is sharply reduced. Nevertheless, considerable solubility loss is sustained by milk powder with a residual moisture below 3%, when subjected to dry-heat treatment (see Fig. 11.17).

The "solubility value" of the protein in milk powder is a function of the temperature and energy applied to effect its redispersion in water to colloidal dimensions.<sup>91</sup> The solubility of dry-heated powder varies with the temperature of reconstitution, whereas the protein in high-moisture powders is irreversibly insolubilized.<sup>92,256</sup> With increasing temperature of reconstitution, full solubility of the protein



From data by N. C. Wright<sup>256</sup>

FIG 11.17. THE TIME-TEMPERATURE RELATIONSHIP FOR THE DEVELOPMENT OF 50% PROTEIN INSOLUBILITY IN NONFAT MILK POWDER HEATED AT 2.6 and 13.3% MOISTURE

Solubility measured at 20°C.

in dry-heated milk can be recovered. The solubility of milk powder in 20 and 50°C water is therefore a useful criterion for differentiating dry heat from moist heat insolubility. Howatt and Wright<sup>91,92</sup> demonstrated that protein insolubility in roller-dried milk is partially reversed at elevated temperatures of reconstitution. The momentary final contact of the dry-milk film with the hot surface of the drum during roller drying, therefore, accounts for a significant proportion of the total insolubility of roller-dried milk powder. Wright<sup>256</sup> considers dry-heat insolubility a physical, rather than a chemical, change in the casein, since the rate of insolubilization is directly proportional to the time of heating, and is independent of the quantity of unchanged casein. The casein in milk powder treated with absolute alcohol undergoes similar insolubilization, that is also reversible at higher temperatures of reconstitution. Dehydration of critical water, such as loss of water of imbibition, is therefore suggested as a possible cause of dry-heat insolubility.

The solubility changes in dry-heated milk powder are wholly related to destabilization of the caseinate complex, with no detectable alteration in the serum protein and nonprotein nitrogen components.<sup>256</sup> The insolubles of dry milk manufactured by different processes are generally of similar composition and comprise casein together with calcium

and phosphorus in a ratio suggesting tricalcium phosphate.<sup>246</sup> In roller-dried whole milk, some fat appears to be associated with the insoluble caseinate complex.

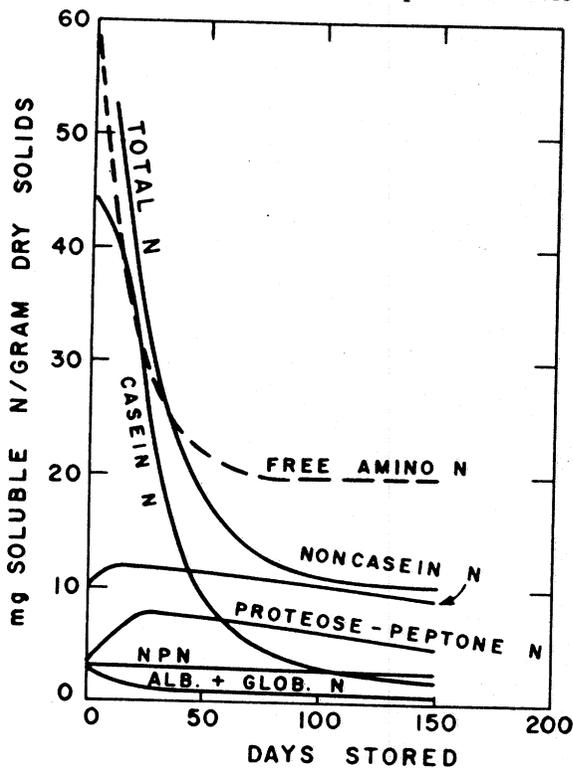
**Effect of Storage Conditions on Stability of Dried Milks.**—The stability of dry milk during storage is critically affected by moisture content and storage temperature. High moisture levels, due to inadequate dehydration or reabsorption of atmospheric moisture, promote insolubilization at relatively mild storage temperatures. The rate of solubility loss is a function of both moisture concentration and temperature.<sup>25,26,75,76</sup> Below 5% moisture, solubility changes are relatively insignificant at normal storage temperatures. In a study of the solubility changes in hydrated nonfat dry milk, Henry *et al.*<sup>75,76</sup> found that the temperature coefficient of the reaction leading to insolubility exceeds a value of 5 at temperatures above 20°C. Consequently, the solubility of moist powders may remain unchanged for long storage periods at 20°C, but falls rapidly at 37°C. The solubility of nonfat dry milk falls as the humidity, time or temperature is increased. Similarly, the nutritional value of nonfat dry milk has been shown to decrease on storage.<sup>42,43,167</sup> The insolubility is reversible on reconstitution in 50°C water, resembling the differential solubility of dry heated milk powder or roller-dried powder. The initial solubility of nonfat dry milk can be retained for 700 days at 37°C storage if the moisture level does not exceed 4.7%.

The occurrence of solubility loss in powder of 7.6% moisture is coincident with crystallization of the lactose, as a result of which the equilibrium relative humidity increases from 42 to 55%. The change in activity of the water due to lactose crystallization may contribute to the overall reactions leading to solubility loss. The change in stability is nonbacterial, and the rate appears to be unrelated to the gas atmosphere in the container. The insoluble component is predominantly casein, which can be totally insolubilized during prolonged storage at 37°C, in powder hydrated to 7.6% moisture. Solubility of the lactalbumin and lactoglobulin components is similarly impaired. Changes in the distribution of soluble nitrogen in hydrated milk powder are shown in Fig. 11.18.<sup>76</sup>

A number of significant changes occur in high-moisture milk powder, concurrent with the loss of solubility. The lactose is gradually bound by the protein, accompanied by a parallel reduction of free amino nitrogen. The pH decreases steadily, and the characteristic changes associated with the Maillard reaction between sugars and amino nitrogen become evident, including development of brown discoloration and production of carbon dioxide, reducing substances, and fluorescing

compounds. Stale and caramelized flavors also develop rapidly in milk powder under conditions of high humidity and elevated storage temperatures, and a significant loss in biological value of the protein is incurred. Changes in nonfat dry milk hydrated to 7.6% moisture and aged 100 days at 37°C include a nearly total loss of protein solubility, a pH decrease of 0.4 unit in the reconstituted milk, crystallization of 80% of the lactose, and destruction of 70% of the original amino nitrogen and approximately 6% of the lactose.<sup>76</sup>

Most of the deteriorative changes in moist milk powder are attributed to a 1 : 1 interaction between the free amino groups of the milk proteins, largely the ε-amino groups of the lysine residues and the potential aldehyde group of lactose.<sup>76</sup> The initial complex is soluble and colorless,



*Data from Henry et al.<sup>76</sup>*

FIG. 11.18. CHANGES IN THE DISTRIBUTION OF THE SOLUBLE NITROGEN IN NONFAT MILK POWDER CONTAINING 7.3% MOISTURE, STORED AT 37°C

Simultaneous changes in free amino nitrogen, determined by the Van Slyke method and expressed as mgm per gm of protein nitrogen, are shown in the dashed curve.

and as much as 65 to 75% of the reactive amino nitrogen may be bound before appreciable solubility or color change becomes evident.<sup>76,126</sup> The reaction rate is largely determined by the activity of the moisture. The temperature coefficient exceeds 6 for milk powders of 7.6% moisture, decreasing to a value of 2 at moisture levels below 5%. The progressive loss of amino nitrogen during storage is paralleled by a decrease in lactose and an increase in the weight of the nondialyzable fraction. The complexed sugar becomes irreversibly bound and, through a series of undefined degradative changes, yields the brown pigments characteristic of the Maillard reaction. The reaction in moist powder consumes a maximum of 70% of the total free amino nitrogen as determined by Van Slyke analysis.

While the loss of protein solubility in dry milk is generally attributed to the sugar-protein interaction, the mechanism is obscure. Henry *et al.*<sup>76</sup> speculate that insolubility may finally arise from induced denaturation of the protein molecule as a consequence of its complex formation with lactose, or subsequent degradation reactions.

On the basis of amino nitrogen binding capacity, the relative order of reactivity of the various sugars toward casein is xylose > arabinose > glucose > maltose > lactose > fructose.<sup>133</sup> Lea *et al.*<sup>125,127,128</sup> established that the  $\epsilon$ -amino group of lysine is the primary reaction site in the casein-glucose system. The decrease in amino nitrogen is at a maximum in the 65 to 70% relative humidity range, and the temperature coefficient at 15 to 25°C is 5.4. The greater reactivity of glucose induces more rapid insolubilization of the caseinate complex in addition to intense discoloration. In freeze-dried preparations of various sugar-milk protein mixtures stored at 55% R.H., sucrose is unreactive, but glucose inactivates 70 to 80% of the available amino groups, although at a concentration of only 1/6 equivalents.<sup>126</sup>

The interaction of lactose and casein is a contributing but not essential factor in the development of insolubility in high-moisture milk powder. The already high acidity in concentrated milk solids is further increased as a consequence of sugar-protein interaction, and this secondary change may have an additional important influence on solubility. Insufficient attention has been directed toward the observation that the caseinate complex, in the total absence of sugar, will insolubilize at 55% R.H.<sup>59,74</sup> While glucose appears to accelerate the development of insolubility, casein in the absence of sugar may insolubilize more rapidly than casein in the presence of lactose. Furthermore, sucrose protects the solubility of the casein, and the insolubilizing effect of glucose in the presence of sucrose or lactose is largely suppressed.

From studies on water absorption by milk powders and their components, Berlin *et al.*<sup>10</sup> have shown that the first water absorbed at low

relative humidity is by the milk proteins. As the vapor pressure increases, lactose becomes the principal water-absorption site until sufficient is acquired to convert it into the nonhygroscopic  $\alpha$ -hydrate form. At relative humidities above 50%, the lactose binds little water, but rapid hydration of the salts occurs. It may be during this stage that the concentration of the phosphates, particularly the potassium form, in the salts becomes high enough to destabilize the casein micelles and render them insoluble.

Lactose is actually essential to the stability of the caseinate complex under many conditions, and its crystallization in milk products during frozen storage is highly detrimental to solubility. Furthermore, Gerlsma<sup>59</sup> observed that the caseinate complex, substantially freed from lactose by centrifugal separation, loses approximately half of its capacity to redisperse on spray drying. Restoration of lactose, or addition of glucose, sucrose, or sorbitol, effectively protects the solubility of the caseinate during spray drying. Therefore, crystallization of lactose in high-moisture milk powder must exert a more substantial influence on the deterioration of protein solubility than that attributed merely to its effect on the activity of the water in the system. As a protective factor, lactose conceivably moderates the destabilizing influence of calcium ion, either by direct complex formation<sup>78</sup> or by lowering the ionizing power of the calcium salts in its solutions.<sup>239</sup> The concentrated, amorphous lactose matrix in moist milk powder, prior to its crystallization, may further act as a physical barrier against micellar aggregation, as in frozen concentrated milk, thereby preserving the ability of colloid to redisperse on reconstitution. Interaction between lactose and casein, particularly under conditions involving extensive degradation of the sugar-protein complex, would inevitably supplement the deterioration of casein solubility.

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