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THE FAT-GLOBULE MEMBRANE OF HOMOGENIZED MILK AND SOME RELATED FACTORS

By

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INTRODUCTION

Certain characteristic properties of milk are altered by homogenization. When milk is homogenized by recommended procedures, however, the majority of these changes enhance its desirability as a beverage. If the homogenization process is not carefully controlled, undesirable qualities, such as high viscosity, various defects in flavor or curdling when used for cooking purposes, are manifest. Earlier research established the fact that the homogenization of milk at relatively low temperatures (80° F.) caused a rather sharp increase in the viscosity of the freshly homogenized milk, which continued to increase up to 72 hours of storage at 45° F. Some of the other factors believed to be associated with the occurrence of a high viscosity were variations in the processing of the milk and variations in the composition of the milk which contributed to the formation of clumps or clusters of fat globules due, possibly, to the presence of proteinaceous material.

The extent to which homogenization modifies the nature of the fat-globule membrane is not clearly understood, because the available information consists primarily of conjectures and

postulations proposed to aid in the explanation of some of the changes that are known to occur as the result of homogenization. There is no clear-cut experimental evidence concerning the composition or the nature of the changes which occur in the fat-membrane material due to homogenization. A few of the characteristic properties of homogenized milk which have served to focus the attention of investigators on the fundamental nature of the fat-membrane material include: (a) the reduced stability of homogenized milk to high temperatures, (b) the reduction in the curd tension of milk that has been efficiently homogenized, (c) the increased susceptibility of homogenized milk to develop solar-activated flavors and (d) the retardation of the copper-induced type of oxidation in homogenized milk. These are only a few of the reported observations which point to the importance of obtaining fundamental information concerning the composition and properties of the fat-globule membrane surrounding milk fat and especially homogenized milk fat.

The object of this investigation has been to study various factors related to the development of high viscosity in milk and to determine, insofar as possible, the nature of the normal fat-globule membrane and the influence of homogenization on

the fat-globule membrane. For the sake of clarity and continuity in the presentation, the investigation is presented in three independent sections. Section I is a study of some of the factors which influence the viscosity of homogenized milk (homogenization efficiency) when homogenization procedures and the composition of the milk are varied. The experimental work reported in Section II makes use of a calorimetric technique to obtain information on the physical state of globular fat at various homogenization temperatures and its influence on the development of high viscosities in homogenized milk. Section III presents a method for the isolation and subsequent characterization of the fat-membrane proteins and makes a comparison of some of the chemical and physical properties of creams obtained from nonhomogenized and homogenized milk.

A general summary of the over-all investigation and some of the pertinent conclusions are included in the last section.

SECTION I

THE EFFECT OF VARIATIONS IN THE HOMOGENIZATION PROCEDURE AND IN THE COMPOSITION OF MILK ON THE EFFICIENCY OF HOMOGENIZATION AND ON THE DEVELOPMENT OF HIGH VISCOSITY

REVIEW OF LITERATURE

Viscosity

Definition of Viscosity

Davies (1939) defined the viscosity of a liquid as follows: "The viscosity of a liquid—its internal friction or its resistance to shear, agitation or flow—is measurable in absolute units—the poise—which may be defined as the force required to produce a difference in the velocity of flow of a liquid of 1 cm. per second when this force is exerted on 1 sq. cm. between two parallel planes each 1 sq. cm. in area and 1 cm. apart. . . . The viscosity is usually expressed in centipoises, the standard being that of water at 20° C. (= 1.005 centipoises)."

Viscosity of Milk, Homogenized Milk, Skimmilk and Whey

Soxhlet (1876) was the first to investigate the changes in the viscosity of milk due to alterations in temperature. He reported values obtained with an Ostwalt-type tube viscometer

that ranged from 4.25 to 1.64 centipoises at temperatures varying from 0° to 30° C. Kobler (1908) worked at a temperature from 15° to 18° C., Taylor (1913) made viscosity determinations at 20° C., and Cavazzani (1904) conducted experiments at 37° C. and all obtained data supporting Soxhlet's findings.

The report of Buglia (1908) is the first reference known to show the effect of homogenization on the viscosity of milk. His data showed that the homogenization of raw milk resulted in an increase in viscosity. Bishop and Murphy (1911) were the first in America to demonstrate that homogenization increased the viscosity of whole milk by showing a decrease in the rate of flow of the homogenized milk on an inclined glass plate and that the viscosity of skimmilk was unaffected by homogenization. Wiegner (1914) and Quagliariello (1917) also reported that homogenization increased the viscosity of milk. Evenson and Ferris (1924) reported that homogenization of whole milk increased the viscosity and also, the viscosity increased with increasing homogenization pressures.

The study of milk viscosity reported by Bateman and Sharp (1928) was outstanding in that some of the more fundamental principles for determining viscosity were investigated.

They used a Bingham-type viscometer with which it was possible to vary the shearing pressure on the liquid under examination. In this manner, it was possible to study the influence of shearing pressure on viscosity. They found that the viscosity of milk varied with shearing force, indicating, according to the theorization of Hatschek (1912), that milk is not a true viscous liquid and, that a single viscosity measurement at an unknown shearing gradient had little theoretical value. At a shearing pressure of about 200 grams per square centimeter, a value approximating the shearing pressure of an ordinary gravity-flow, Ostwald-type viscometer, they reported values for the relative viscosity of skimmilk at about 1.48, raw whole milk at 1.55, pasteurized whole milk at 1.56 and homogenized whole milk at about 1.67 centipoises. Although some of their individual values varied, it was generally noted that homogenization at 50° C. and 4,000 pounds pressure per square inch caused a rise in the viscosity of whole milk, but was without effect on the viscosity of skimmilk. In addition, it was observed that the viscosities of homogenized milk and skimmilk remained constant when repeatedly run through a capillary, whereas the viscosity of whole milk was reduced by this treatment. They

attributed this difference to the lack of fat-clumping and to the regularity in the size of the fat globules in homogenized milk.

Trout, Halloran and Gould (1935) noted that raw milk homogenized at 90° F. was increased in viscosity, but that pasteurized milk homogenized at 145° F. was reduced in viscosity; a factor which they attributed to the effect of heat on milk. From 12 trials, they reported an average value for raw milk of 2.152 centipoises before homogenization and 2.315 centipoises after homogenization at 2,500 pounds pressure. In a similar set of trials with pasteurized milk, 2.142 was the average viscosity value obtained before homogenization and 1.814 centipoises following homogenization.

Whitaker, Sherman and Sharp (1927), employing the Ostwald-type viscometers immersed in a temperature-controlled water bath, found that the specific viscosities for skimmilk for temperatures between 5° and 80° C., varied from 2.96 to 6.57 centipoises, respectively. After conversion to relative-viscosity with the aid of Bingham and Jackson's (1918) specific-viscosity values for water, these values then ranged from 1.95 to 1.59 centipoises for temperatures varying between 5° and 80° C. with a minimum of 1.52 between 60° and 70° C. Similar results

were obtained for skimmilk by Eilers and Korff (1945), who reported relative-viscosity values for whey at 5° and 80° C., as 1.27 centipoises with a minimum value of 1.19 between 50° and 70° C. More recently, Tapernoux and Vuillaume (1934) reported the viscosity (specific) of milk and skimmilk over the temperature range from 0° to 40° C. as 3.44 to 1.23 and 2.84 to 1.08 centipoises, respectively. Eilers, Saal and van der Waarden (1947) converted the values for milk to relative viscosity and found values of 1.59 at 0° C. and 1.64 centipoises at 40° C. These calculated values are not in agreement with the relative viscosity values referred to previously; because, in general, the relative viscosity of fluid milk and closely related products increase as the temperature is decreased.

Some Factors Affecting the Viscosity of Milk and Related Products

Pasteurization and heat treatment. Woll (1895) reported that pasteurization at 67° C. for 20 minutes causes a slight reduction in the viscosity of milk and cream and an increase in the viscosity of whey. Babcock and Russell (1896), Evenson and Ferris (1924), Dahlberg and Hening (1925) and others also reported a reduction in viscosity following the pasteurization of

whole milk and cream. A noteworthy exception to this general observation was reported by Achard and Stassano (1925), who noted an increase in the viscosity of pasteurized milk.

Whitaker et al. (1927) recorded a decrease in the viscosities (measured at 24° C.) of skimmilk and whey pasteurized at temperatures up to 60° C., whereas higher temperatures (60° to 100° C.) resulted in an increase in viscosity. At temperatures above 100° C. the viscosity of skimmilk continued to increase. Under the same conditions, they noted a marked reduction in the viscosity of whey. From these results they postulated that the presence of casein in the skimmilk was responsible for the increase in viscosity and that it served to sustain the coagulated serum proteins; since their coagulation by heat in the absence of casein (rennet whey) resulted in a decrease in viscosity. Similarly, Bateman and Sharp (1928) reported a decrease in the viscosity of skimmilk pasteurized at 62° C. for 30 minutes. After a more careful consideration of some of the inherent properties of viscosity, they concluded that the decrease in the viscosity of skimmilk after pasteurization probably resulted from a slight alteration or denaturation of the protein materials.

Homogenization procedures. The general effect of homogenization on the viscosity of milk has already been reviewed, however it seems appropriate to review in some detail the effects of certain variations in homogenizing procedures on the viscosity of milk.

Wiegner (1914) calculated from viscosity measurements made prior to homogenization that 2.27 per cent of the casein of normal milk was adsorbed on the fat globules, whereas 25.20 per cent was adsorbed after homogenization. He assumed that casein was the only substance adsorbed and that its density was about 1.46. The foregoing postulation is in accord with the studies of Odén (1912) who emphasized the importance of the particle size of the dispersed phase on the viscosity of the overall system, and with Hatschek (1912) who concluded that the increased viscosity resulting from homogenization was due to the adsorption of a film around the dispersed particles. Trout et al. (1935) reported, however, that a reduction occurred in the viscosity of pasteurized milk when it was homogenized with increasing pressures.

Evenson and Ferris (1924) noticed that high homogenization pressures increased the viscosity of "remade" milks.

Tretsven (1939) concluded that the viscosity of homogenized milk was a function of the fat content and of the homogenization temperature; low temperatures being most conducive to high viscosities. Farrell (1942) recommended the use of high homogenizing temperatures to avoid fat-clustering and the excessive viscosities which are induced at low homogenizing temperatures. Bateman and Sharp (1928) observed that raw whole milk which had been warmed to 50° C. and homogenized at 4,000 pounds pressure increased in viscosity and that the higher value remained constant on repeated viscosity measurements. In this respect homogenized milk is similar to skimmilk, in that the viscosity does not change on repeated measurements, but is unlike nonhomogenized milk, in which the viscosity decreases gradually on successive measurements. This uniformity in the viscosity of homogenized milk was also noted by Caffyn (1951), who used homogenized milk to study certain inherent factors peculiar to milk viscosity.

Whitaker and Hilker (1937) studied the effect of homogenization at 3,000 pounds pressure on various lots of 4.0 per cent milk and 20 per cent cream which had been handled differently with respect to heat treatment. The average size of

the fat globules, their tendency to cluster as the result of heat treatment and homogenization were of primary consideration, but no viscosity determinations were reported. Milk samples which had been preheated to 145° F. and then homogenized at various lower temperatures and raw milk which had been held overnight at 40° F. and then warmed prior to homogenization showed no fat-clumping when homogenization temperatures were below 100° F. All of the samples were pasteurized at 145° F. for 30 minutes and then stored for 18 hours at 60° F. prior to examination; a factor which may or may not have influenced clumping. The cream samples which had been treated in a similar manner, however, showed definite clumping of the fat globules when homogenized at temperatures as low as 50° F. following preheating. Homogenization at 80° F. was found to be conducive to fat-clumping in cream samples which were warmed to the homogenizing temperature after a preliminary holding period at 40° F.

Trout and Scheid (1941) studied the efficiency of homogenization of raw milk at various temperatures after a 24-hour storage period at 40° F. Some homogenization effects were noted when the milk was homogenized at 80° F., but satisfactory

dispersion of the fat globules did not occur until the homogenization temperature reached 100° F. or above. Since it was assumed that pasteurization would normally follow homogenization, no viscosity studies were made on these samples. More recently, Moore and Trout (1947) reported on the occurrence of a progressive thickening in homogenized, pasteurized milk. Further investigation revealed that the viscosity of a 4.6 per cent pasteurized milk, homogenized at 80° F. and 2,500 pounds pressure, increased markedly and continued to increase up to the fourth day of storage at 45° F. They also noted that a characteristic "chalky" flavor and the presence of protein aggregates accompanied the progressive thickening.

Variations in the composition of milk. Bogdan (1905) believed that the viscosity of milk was proportional to the total solids content, but variations were observed in isotonic samples. Taylor (1913) utilized viscosity determinations to estimate the solids-not-fat content of milk. Bateman and Sharp (1928) presented data to show the fallacies of the above concepts by showing that the viscosity of skimmilk varied with the treatment and the physical state of the components.

Oertel (1908) reported that viscosity did not change with a rise or fall in solids, but that it depended on the size and nature of the fat globules. At the same time, Bishop and Murphy (1911) observed an increase in the viscosity of milk and cream following pasteurization, but could not produce a change in the viscosity of skimmilk. They attributed the increased viscosity to the fat content of the milk. However, Spottel and Gneist (1945) concluded that the fat content of milk could not account for the difference in the viscosity of morning and evening milk. Tretsven (1939) stated that the viscosity of homogenized milk was partially dependent on the fat content.

The data of Whitaker et al. (1927) indicated that lactose played a minor role in the over-all viscosity of skimmilk, but that the protein components were of prime importance. Numerous investigators, notably among them Wiegner (1914), Buglia (1908), Hatschek (1912), and Bateman and Sharp (1928) attributed the increase in the viscosity of homogenized milk to the adsorption of the dispersed phase (probably proteins) on the increased fat surface as the result of homogenization. Palmer and Dahle (1922) and later Palmer and Anderson (1926) concluded that the viscosity of milk resulted primarily from the

adsorption of colloidal proteins on the surface of the fat globule and not from the percentage of fat in the milk.

Fat-globule clustering. As has been indicated previously, mechanical manipulations are conducive to the clumping of fat globules and they play an important role in determining the viscosity of fat-containing milk products. Babcock and Russell (1896) were the first to observe that a diminution in the clusters of fat globule in pasteurized milk and cream was accompanied by a reduction in the viscosity. Bishop and Murphy (1911) observed that the homogenization of cream at 140° F. produced a higher viscosity than homogenization at 70° F., but that repasteurization tended to decrease the viscosity. Sommer (1946) explained that the increase in viscosity resulting from homogenization depended not only on the reduction in the size of the fat globule, but also on the extent of fat-clustering. Abundant clustering would favor higher viscosity.

Doan made a series of noteworthy contributions to the information concerning the effect of homogenization on the viscosity of whole milk and cream. He (1927) speculated that homogenized milk would cream if the fat globules could be induced to clusters, but later observations showed that the fat

globules of homogenized milk had little tendency to cluster even after prolonged storage (1929a). He believed that the heightened Brownian movement of the small globules, which served to keep the fat globules in motion, was greater than the force of interfacial tension tending to draw the globules together in clusters. Excessive clumping in 4.0 per cent milk homogenized at 100° F. at 3,500 pounds pressure was encountered only when the serum-solids to fat ratio was less than 0.40 to 0.50. If, however, the fat content were increased to 6 per cent, the critical ratio became 0.50 to 0.60; whereas an increase in fat content between 8 and 18 per cent raised the critical ratio to 0.60 to 0.85.

Doan (1929b, 1929c) noted that in addition to an adequate concentration of serum-solids, the calcium-ion concentration was also a contributing factor to both fat-globule clustering and protein stability in homogenized milk. Nevertheless, he (1932b) concluded that neither the electrostatic charge nor the agglutinin theories of clustering adequately explained why the fat in homogenized milk failed to cluster. Doan (1928) and Doan and Minster (1933) observed clusters of fat globules in milk of unusually high-fat content which had been homogenized at low

temperatures (100° F. and lower) and abnormally high pressures. In most instances, fat-clustering in homogenized milk could be eliminated by double-stage homogenization. Wittig (1949) reported that homogenization at low temperatures resulted in fat-clumping, which was attributed to a change in the film-forming material rather than to the fluidity of the fat. The film material varied with the temperature, thus its dispersion was increased by the use of homogenization temperatures between 60° and 65° C. and was retarded between 37° and 42° C.

Whitaker and Hilker (1937) observed some clustering in homogenized milk which had been processed above 100° F., pasteurized at 145° F. for 30 minutes and held for 18 hours at 45° F. Dahle and Jack (1937) recorded some clumping in homogenized milk processed under normal conditions with a single-stage valve, but no clumping was evident when milk was homogenized with a double-stage valve. Moore and Trout (1947) reported an excessive viscosity in milk homogenized at 80° F. and 2,500 pounds pressure. They were also able to detect a copious amount of clumping which was believed to be proteinaceous in nature. Dunkley and Sommer (1944) reinvestigated the theories used to explain fat-clustering and the creaming of

milk and postulated that: "homogenization prevents the formation of a definite cream line by denaturation of euglobulin rather than by subdivision of the fat globules."

Sharp (1940) and later Sommer (1946) stated that fat-globule clumping in homogenized milk could be induced by the addition of agglutinin (euglobulin). The work of Doan (1929a) showed that high preheating temperatures on any fraction or combination of milk serum-solids had a tendency to reduce fat-clustering in homogenized milk.

Doan (1927) and Theophilus (1941) observed that the enhanced creaming ability of "viscolized" milk and milk to which a quantity of homogenized milk had been added was due to the formation of numerous fat clusters. Trout (1950) noted that: "marked clustering does occur in homogenized 10- or 12-per cent milk, commonly known as 'half-and-half,' and in homogenized cream." Babcock (1931) reported that the fat-clumping which occurred in homogenized cream was increased by high homogenization pressures as well as by low homogenization temperatures. Doan (1931) recognized that single-stage homogenization of cream frequently caused the formation of fat-globule clusters and a decrease in heat-stability. He found that the

addition of high-quality serum-solids or, in some instances, a stabilizing salt such as sodium citrate, frequently resulted in an increase in heat-stability and a decrease in the fat-clustering tendency. He concluded that double-stage homogenization was the most efficient and practical means of preventing fat-clustering. These observations are in agreement with those of Webb (1931), who likewise found that the use of double-stage homogenization (2,000 pounds total pressure and 500 pounds on second stage) was beneficial in lessening the detrimental effects of homogenization on the heat-stability of cream. To insure maximum dispersion and a minimum of separation of fat in evaporated milk, Webb and Holm (1939) recommended the use of high homogenizing pressures and a double-stage valve.

In somewhat similar studies involving ice cream mixes, Mortenson (1918), Sherwood and Smallfield (1926), Martin and Dahle (1925), Reid and Moseley (1926), Hening (1928) and others have reported the occurrence of excessive fat-clustering and protein instability in the mix. In most instances these defects were induced by single-stage homogenization at high pressures. Generally, they reported that agitation after homogenization, rehomogenization at a lower pressure, or double-stage

homogenization eliminated fat-clumping. Reid (1927) stated that fat-clumping in single-stage homogenized cream was so pronounced that variations in homogenization pressures had little effect.

Apparently, excessive fat-clustering in homogenized milk and other dairy products play an important part in determining the ultimate physical properties of these products. The fat-clustering which occurs in some homogenized milk is probably closely associated with the normal fat-clustering tendencies in nonhomogenized milk. Therefore, it seems appropriate to review briefly some of the theories which have been advanced to explain this phenomenon in nonhomogenized milk.

Theories Proposed to Explain Clustering

Gravitation of fat globules. Both McKay and Larsen (1906) and Bancroft (1926) conjectured that the difference in the rates of rise of fat globules of different sizes provided the necessary opportunity for globule contact and the formation of clusters. The greater the opportunity for cluster-formation, the greater would be the tendency to rise. Sommer (1946) emphasized the importance of the absolute size and the variations in

the size of fat globules as factors influencing clumping. The large globule with its greater buoyancy would overtake the smaller globule in the upward sweep, thus providing an opportunity for contact and cluster-formation. This fact was observed by Doan (1927) and Theophilius (1941) as a factor responsible for the deep creaming of "viscolized" milk. Dahlberg and Marquardt (1929) concluded that the size of the fat globule was not the most important factor responsible for the difference in creaming properties.

Electrostatic charge on fat globules. Sirks (1923) reported that the electrophoretic mobility of the fat globule, measured in a microelectrophoretic cell, varied and therefore had no relationship to the extent of fat-clustering in milk. Dahlberg and Marquardt (1929) attempted to explain fat-clustering on the proposition that the fat globules in milk carry opposite charges and that clustering results from mutual attraction of oppositely charged globules. However, such a theory is not supported by experimental data, because it has been shown by several investigators (Sirks, 1923; Jack and Dahle, 1937a) that the fat globules in milk carry a negative charge and there is

no reason to suppose that both positively and negatively charged globules would be present in the same system.

Schneck and Brandt (1931) and North, Courtney and Sommer (1935) found some relationship between the electric charge on the fat globule and its tendency to clump. They attributed the decreased fat-clustering tendency in heated milk to an increased charge on the globules. Sirks (1923) presented data to show that a decreased fat-clustering tendency was accompanied by an increase in the electrokinetic mobility of the fat globules. Furthermore, Dahle and Jack (1937) presented data from which they concluded that the change in the mobility of the fat globules as a result of heat treatment was not related to the fat-clustering tendency.

Interfacial tension. McKay and Larsen (1906) suggested that the improved fat-clustering and creaming observed in milk that had been stored at low temperatures might be due to an increase in the surface tension of milk. Lower surface tension in cold milk has also been reported by Sharp and Krukovsky (1939). Doan (1929a) postulated that fat-clumping in homogenized milk might be explained on the basis of the interfacial tension between the fat and serum. Van Dam and Sirks (1922)

and North et al. (1935) observed no important relationship between the interfacial tension at the fat/serum interface and the fat-clumping tendency.

Stickiness. Rahn (1921, 1922) and van Dam and Sirks (1922) noted increased fat-clustering and improved creaming properties in milk to which a hydrophilic colloid, such as gelatin, gum tragacanth or gum arabic, had been added. The enhanced clustering property was explained on the basis of an increase in the stickiness of the membrane adsorbed on the fat globule. Rahn believed that the colloids adsorbed on the surface of the fat globules formed an adhesive membrane capable of binding the globules together when they came in contact with one another. Van Dam and Sirks (1922) emphasized the part played by plasma in the formation of fat-globule clusters. The observations of Schneck and Muth (1930) lend support to the foregoing statements.

Brunner and Jack (1950) believed that the fat aggregation which occurs during the churning process was due in part to the physical state of the fat. The necessary condition being that the fat be neither solid nor liquid, but that it should be in a semi-soft or "sticky" condition. Wittig (1949) was more

concerned with the nature of the fat-film which influenced fat-clumping than with the physical state of the fat.

Agglutination. Whether the term "agglutination," as applied to the clustering of fat globules, is used in its proper connotation is questionable. This expression has generally been reserved to express bacterial agglutination and, according to Dunkley and Sommer (1944), its use to describe the fat-clustering phenomenon: "can only be justified on the basis of convenience."

Babcock (1889a) believed that the clustering of fat globules in milk was made possible by the presence of fibrin. Hekma (1922) reported that fibrin was not a constituent of milk and, therefore, the "fibrin" theory of Babcock was untenable. Van Dam, Hekma and Sirks (1922) believed that the clustering of fat globules could be accounted for by an agglutinin present in milk. Further studies by Hekma and Sirks (1923) demonstrated that the creaming substance in milk was thermolabile at the same temperature as bacterial agglutinins. By the fractionation of milk serum, it was found that the constituent primarily involved in agglutination was identified with the globulin fraction. This

observation has also been reported by Palmer, Hening and Anderson (1926).

Brouwer (1924) fractionated globulin which had been salted out by half-saturation with ammonium sulfate into euglobulin and pseudoglobulin and demonstrated that the euglobulin fraction was beneficial to fat-clustering and creaming properties. Rowland (1937) noted that the reduction in creaming ability, probably accompanied by a corresponding loss in fat-clustering, was proportional to the amount of albumin and globulin that was denatured by heat.

Sharp and Krukovsky (1939) considered the clustering of fat globules to be an agglutination process and concluded that the agglutinin in milk is normally adsorbed on the surface of the solid fat globules and is released into the serum upon liquefaction of the fat. These data were verified by Dunkley and Sommer (1944) who isolated the agglutinin material and characterized it as euglobulin. They concluded, "that the clustering of fat globules in milk takes place by the same mechanism as that involved in the agglutination of bacteria." They also observed that in addition to agglutinin, complementary materials

in milk, such as low-salt concentration, were required to produce fat-clumping.

Hening and Dahlberg (1932), Weise, Nair and Fleming (1939), Burgwald (1940), Skelton and Herreid (1941), Smith and Doan (1948) and others have reported favorably on their ability to promote fat-clustering and increase the viscosity of fluid cream by a "rebodying" or "reseparation" technique which involved careful temperature manipulations.

Homogenization Efficiency

Expression of Homogenization Efficiency

Efficiently homogenized milk is usually expressed in terms of the degree of dispersion of fat, thus relating to the efficiency with which the process was accomplished. The definition of homogenized milk which has been generally accepted was furnished by the United States Public Health Service (1947) and is stated as follows: "Homogenized milk is milk which has been treated in such a manner as to insure break-up of the fat globules to such an extent that after 48 hours quiescent storage no visible cream separation occurs on the milk and the fat-percentage of the milk in the top 100 ml. of milk in a quart

bottle, or of proportionate volumes in containers of other sizes, does not differ by more than 10 percent of itself from the fat percentage of the remaining milk as determined after thorough mixing."

Other definitions (Tracy, 1941; Babcock, 1947; Doan, 1946) have been offered to describe properly homogenized milk and, in general, they imply the same conditions as prescribed by the definition quoted above.

Reported Values and Methods of Measurement

Fat-globule size. Van Slyke (1891) measured the size of fat globules and reported values for Jersey and Guernsey milks ranging from less than 2.4 to more than 12 microns in diameter; 37.5 per cent were less than 7.2 microns. The diameters of Holstein fat globules ranged from less than 2.4 to 9.6 microns, and 88.7 per cent were smaller than 7.2 microns. More recently, Campbell (1932) reported that the mean fat-globule volume for Guernsey milk was 18.8 per cubic micron and that 65 to 80 per cent of the globules were less than 3.5 microns in diameter. The largest number of fat globules was in the 2-micron class. He also gave the mean fat-globule

volume for Holstein milk as 10.4 per cubic micron and that 20 to 94 per cent of the fat globules were less than 3.5 microns in diameter, whereas 1.5 to 3.5 times as many globules were in the 1-micron class as were in the 3-micron class.

Wiegner (1914) observed that the average size fat globule was subdivided into approximately 1,200 small globules by homogenization. He recorded the mean diameter of fat globules in homogenized milk as 0.27 micron, measured with the aid of a Siedentopf-Zsigmond microscope. On the other hand, Sommer (1935) calculated that if the average fat globule were 6 microns in diameter, then only 216 globules, 1 micron in diameter, would be obtained after homogenization.

Rahn and Sharp (1928) stated that roughly 85 per cent of the fat globules in homogenized milk are smaller than 2 microns in diameter and that all are under 3 microns. Halloran (1932) believed that the fat globules in homogenized milk would have to be less than 2 microns in diameter in order not to cream. Wittig (1949) was even more definite and claimed that in properly homogenized milk, which he labeled "micronized," the fat globules should be less than 1 micron in diameter.

Homogenization efficiency tests. Parfitt (1938) recommended the use of a high-powered microscope equipped with an ocular micrometer to measure the size of fat globules in homogenized milk. Properly homogenized milk should contain uniformly dispersed fat globules about 1 micron in diameter. Farrall, Walts and Hanson (1941) utilized the microscopic measurement of the sizes of fat globules in homogenized milk to calculate an index of homogenization efficiency. Doan and Mykleby (1943) felt that the Farrall Index was as well adapted to the estimation of homogenization efficiency as was the United States Public Health Service's gravity separation method, but the opportunities for error, due to faulty technique, were about the same. They recommend a Farrall Index of about 12 as being indicative of properly homogenized milk, in comparison to the United States Public Health Service Index of about 8 per cent.

According to Trout (1950), von Sobbe (1914) and Burr and Weise (1914) were the first investigators to advocate the estimation of homogenization efficiency by testing various layers of the milk after quiescent storage. They recommended that 250 milliliters of preserved milk be set aside in special graduated cylinders for 72 hours at room temperatures. Finally, two

50-milliliter portions should be drawn off from the top and the remaining lower portion should be tested for fat. If the original fat content were equal to 100, then the fat content of the bottom portion, after being converted to a percentage of the original fat content, was calculated to express the homogenization efficiency.

Procedures for estimating the degree of homogenization based on the fat separation principle were recommended by the United States Public Health Service (1939, 1947). As adopted in 1947, the test consists of testing the top 100 milliliters and the remainder of the quart after 48 hours of quiescent storage at 45^{o} F. From these two measurements, the percentage difference was calculated using the value obtained from the top 100 milliliters as the base. The percentage difference should not exceed 10 per cent (United States Public Health Service, 1947). Various investigators (Trout and Scheid, 1942; Doan and Mykleby, 1943) have made critical evaluations of the earlier United States Public Health Service (1939) procedure.

Snyder and Sommer (1943) reported a method in which centrifugal force was utilized to hasten the separation of fat in tests involving homogenization efficiency.

Mayer (1917) thought that the even distribution of fat in homogenized milk should make it possible to utilize optical density measurements as a means of determining the degree of homogenization. More recently, Ashworth (1949) applied this principle in perfecting a turbidity test for determining the efficiency of homogenization.

Factors Influencing the Efficiency of Homogenization

Doan and Minster (1933), Farrall (1941) and Judkins (1943) stressed the importance of the mechanical condition of the homogenizer and the valve system in the efficient production of homogenized milk. Josephson, Doan and Adams (1941) were not able to obtain as efficient homogenization by means of rotary-type homogenizers as could be obtained with the conventional piston-valve machines. Doan and Mykleby (1943) were able to obtain more efficient homogenization per pound of pressure by a wire-core type valve than with the solid valve.

Trout (1950), after reviewing the literature on homogenization, concluded that 2,500 pounds pressure was adequate to produce a homogenized milk which would meet the United States Public Health Service standard, provided the homogenizer

and valves were in good mechanical condition. Pressures in excess of 2,500 pounds were of little value, even though a finer dispersion of the fat might be achieved.

Jones (1929), Doan (1932a, 1937) and others recommended that milk should be homogenized at or about the pasteurization temperature to insure that the fat is in a liquid state. Trout and Scheid (1941) were able to demonstrate limited homogenization effects in milk homogenized at 80° F. but no noticeable fat dispersion was recorded. Good fat dispersion was obtained in milk homogenized at temperatures above the melting point of milk fat. These observations were essentially the same as those reported by Whitaker and Hilker (1937).

Doan and Mykleby (1943) reported that high-temperature pasteurization lowered the United States Public Health Service Index on milks that had been improperly homogenized, but had no effect on efficiently homogenized samples. On the other hand, Wittig (1949) believed that alteration of the film-material could account for a decrease in homogenization efficiency when high-heat-treated milk was homogenized at 300 atmospheres (ca 4,500 pounds). Maximum homogenization efficiency was obtained with 100 atmospheres (ca 1,500 pounds).

Summary of the Review of Literature

The viscosity of milk, usually expressed as relative viscosity, has been reported by numerous investigators at about 1.56 centipoises. This value decreases slightly after pasteurization at normal pasteurization temperatures, but increases if the pasteurization temperature is high enough to affect the serum proteins. Generally, homogenization has been found to cause an increase in the viscosity of milk which was enhanced by high homogenization pressures, high fat content and low homogenizing temperatures. The basic viscosity of milk, as well as the increase in the viscosity of milk following homogenization, has been attributed to both the quantity and nature of the constituent fat and plasma proteins, but most generally to the physical state of the milk fat.

An unusually high degree of fat-globule clumping generally has been associated with increasing viscosities in dairy products containing fat. Fat-clumping has been explained by many theories, most of which are as follows: gravitational or fat sweeping, electrostatic charge, interfacial tension, stickiness and the agglutinin theory. Of these, only the agglutinin theory is believed to be applicable in explaining the normal

fat-clumping in milk. However, some investigators have elected to explain the aggregation of fat globules, as found when milk is violently agitated, on the basis of the physical state of the globular fat.

Efficient homogenization reduces the average size of the fat globules in milk from about six microns to about one micron in diameter and increases the area of the fat surface approximately fivefold. Reputedly, it is the increased surface area of homogenized fat which adsorbs plasma proteins and causes an increase in the viscosity of homogenized milk. The efficiency of homogenization has been tested by numerous techniques. The two most important ones are designated as the United States Public Health Service Index, which measures the separation of fat after 48 hours of quiescent storage, and the Farrall Index, which measures the range in the size of the fat globules. The factors recognized as influencing the efficiency of homogenization have been reported as: homogenization temperature and pressure, pasteurization temperature, the mechanical condition of the homogenizer and the composition of the milk.

In view of the above reports in the literature concerning the various factors which influence the efficiency of homogenization

and the development of a high viscosity, experiments were conducted with milks of varying fat and solids-not-fat content to study the effect of variations in temperature and pressure on the development of a high viscosity in homogenized milk.

EXPERIMENTAL PROCEDURE

The milk used in this study was herd milk selected from patrons of the Michigan State College Creamery. The milk was filtered upon receipt and immediately holder-pasteurized at approximately 143° F. for 30 minutes, or as otherwise noted. Samples saved for subsequent analysis ranged from 2.82 to 6.60 per cent fat and from 11.12 to 15.62 total solids as determined by the Mojonnier procedure.

After pasteurization the milk was vat-cooled and portions were withdrawn at 140°, 100°, 90°, 80°, 70° and 60° F. to be homogenized at 1,500, 2,500 and 3,500 pounds pressure. Both single-stage and double-stage (500 pounds pressure on second stage) homogenization were employed. A 25-gallon per hour, laboratory model, homogenizer was used during the first phase of the study, but because of certain inconsistencies, all of the final data were obtained with a piston-type, 500-gallon per hour homogenizer. In one series of trials the milk was cooled, following pasteurization, to 45° F. and stored for approximately 24 hours, after which it was warmed to the indicated homogenization temperatures.

In order to evaluate the effect of these procedures on the efficiency of homogenization and the viscosity of the finished milk, it was necessary to save the following three samples for subsequent examination: one 1-quart sample, one 100-milliliter sample in a graduated cylinder and one 1-pint sample. All of the samples were cooled immediately in ice-water and stored at 45° F. until examined.

Cream volumes were determined by measuring the volume of the cream layer in the 100-milliliter sample stored in the graduated cylinder after 24-hour storage. The size of the fat globules and the degree of dispersion were determined after one milliliter of the well-mixed milk was diluted with 25 milliliters of distilled water by examining a hanging drop under the oil-immersion lens of a Spencer microscope which was equipped with a calibrated ocular micrometer. Homogenization efficiency was further ascertained by means of the United States Public Health Service (1947) test which expresses the homogenization efficiency in terms of the percentage difference in the fat content between the top 100 milliliters and that remaining in the 1-quart sample held at 45° F. for 48 hours. Viscosity determinations were made on the 1-pint samples with the aid

of a Brookfield Synchro-Lectric viscometer, which expresses the viscosity of a fluid product as "relative viscosity" when measured at 20° C. In order to obtain the maximum viscosity in the processed milk, all samples were held at 45° F. for 72 hours.

Although the milk samples were probably representative of the variations that occur in normal milk, it seemed desirable to prepare a series of recombined milks in which the ratio of solids-not-fat to fat varied over a wider range. Fresh cream (32-per cent fat), skimmilk, distilled water and low-heat nonfat milk solids were used to prepare synthetic milks of the following composition:

Series A. Eight per cent solids-not-fat and fat ranging from 3 to 6 per cent.

Series B. Eleven per cent solids-not-fat and fat ranging from 3 to 6 per cent.

Series C. Three per cent fat and solids-not-fat ranging from 8 to 11 per cent.

Series D. Six per cent fat and ranging from 8 to 11 per cent.

These milks were processed and examined in a manner similar to that described for the normal milk samples.

RESULTS

Influence of Homogenization Procedures

The data presented in Table 1 show the influence of various temperatures on the efficiency of homogenization and viscosities of low-test and high-test milk homogenized at 2,500 pounds pressure with a single-stage valve. These data are representative of that obtained from three different trials with low-fat milk (2.8 to 4.0 per cent fat) and three different trials with high-test milk (4.4 to 6.6 per cent fat). The balance of the experimental data are recorded in Tables 4 to 7 inclusive.

An inspection of the data in Table 1 shows that the efficiency of homogenization decreased as the homogenizing temperature was lowered. Homogenization in the temperature range of 80° to 70° F. was accompanied by slight clumping in the low-test milk, whereas in the high-test milk the clumping tendency was classified as moderate to profuse. An increase in the viscosity of high-fat milk was also recorded at these temperatures. Homogenization at temperatures lower than 70° F. causes a partial churning of the fat which was manifest as

an aggregate fat mass or "cream plug" at the top of the sample bottles. Homogenization is not complete at the lower temperatures since there was an increase in the number of large fat globules.

The behavior of high-test pasteurized milk is demonstrated by the data presented in Table 2 by an increase in the viscosity and by the tendency of the fat to clump when the milk was homogenized at 80° F. When the homogenization pressure was raised from 1,500 to 3,500 pounds (single-stage), a pronounced increase occurred in the tendency for the fat globules to form clusters and by an increase in the United States Public Health Service Index, indicating a decrease in homogenization efficiency. At 3,500 pounds pressure, the fat clusters were so profuse that they were practically indistinguishable one from another when observed microscopically. A sharp increase also occurred in the viscosity after the 72-hour storage period.

The data compiled in Table 3 show the influence of two methods of preparing homogenized milk on homogenization efficiencies, fat-clumping tendencies, and viscosities in high-fat, pasteurized milk homogenized at 3,500 pounds pressure.

Single-stage, high-pressure homogenization of high-fat milk was conducive to fat-globule clumping and an increase in the viscosity when the milk had been stored at 45° F. for 24 hours prior to warming to the homogenization temperature, and also in that which has been cooled from 145° F. to the various homogenization temperatures. The critical temperature at which this phenomenon occurred was lower for the cooled samples (80° to 70° F.) and higher for the warmed samples (90° F.).

Double-stage Homogenization

Data illustrating the influence of single-stage and double-stage homogenization are shown in Figure 1. Double-stage homogenization reduced or entirely eliminated excessive fat-clumping and prevented the occurrence of high viscosity in the high-fat milk which had been homogenized at temperatures and pressures conducive to the development of this characteristic.

Composition

The literature citations concerning the influence of certain naturally occurring variations in the composition of herd milk on the efficiency of homogenization and the production of

high viscosities have been considered. Further trials with representative samples of prepared milks of controlled composition are presented in Table 8 and in Figures 2 and 3.

The data in Figure 2 illustrate the effects of homogenization at a given temperature and the effects produced by varying the pressures on the viscosity of milks compounded to contain fat at levels ranging from 3 to 6 per cent while the level of solids-not-fat was maintained at 11 per cent (Series A and B). Regardless of the composition, homogenization at normal pressures (2,500 pounds and below) had little effect on the development of excessive viscosity, but the employment of high homogenization pressures (3,500 pounds and above) caused a sharp increase in the viscosity of milks testing 5 per cent or more of fat when the milk was homogenized within the critical temperature range (70° to 80° F.).

Milk samples compounded to keep the fat content constant at 3 and 6 per cent, while the levels of solids-not-fat varied between 8 and 11 per cent were treated in a similar manner (Series C and D). Representative data obtained from these trials are presented in Figure 3. Little change was noted in the viscosity of low-fat milk when the solids-not-fat content

varied from 8 to 11 per cent and the homogenization temperature was maintained at 80° F. (Series C). However, the viscosity of the high-test milks represented by Series D showed an increase in viscosity as a result of high-pressure homogenization at 80° F. An increase in viscosity was apparent at 2,500 pounds and pronounced at 3,500 pounds pressure. Apparently, the solids-not-fat content, within the range studied, had little effect in inhibiting or promoting fat-clustering and the development of an excessive viscosity. A slight increase in viscosity was noted, however, when the concentrations of both the fat and solids-not-fat contents were increased.

TABLE 1

The influence of various temperatures on the efficiency of homogenization and on the viscosity of milk homogenized at 2,500 pounds pressure after pasteurization

Temperature of Homogenization (^{o} F.)	Homogenization Efficiency as Shown by: Range in the Diameter of Fat Globules (μ)	Homogenization Efficiency as Shown by: USPHS Index (%)	Viscosity After 72-hour Storage at 45^{o} F. (centipoises)
Low-fat milk (3.51% fat and 12.18% total solids)			
140	1-2	8.1	2.4
100	1-2	7.4	2.4
90	1-2	9.0	2.4
80	1-4*[1]	12.1	2.4
70	2-6*	46.2	2.4
60	2-6*	——++[2]	2.2
High-fat milk (6.6% fat and 15.62% total solids)			
140	1-2	9.8	2.8
100	1-2	8.3	2.8
90	1-3	10.1	2.8
80	2-4***[1]	14.0	3.2
70	2-6**	68.0+[2]	3.2
60	2-8*	——++++	2.8

[1] Degree of fat-clumping

[2] Cream-layer formation

TABLE 2

The influence of various pressures on the efficiency of homogenization and on the viscosity of milk homogenized at 80° F. after pasteurization

Homogenizing Pressure	Homogenization Efficiency as Shown by		Viscosity After 72-hour Storage at 45° F.
	Range in the Diameter of Fat Globules	USPHS Index	
(lb./sq. in.)	(μ)	(%)	(centipoises)
Low-fat milk (3.5% fat and 12.18% total solids)			
0	2-9	——	2.4
1,500	2-4	15.2	2.4
2,500	1-4*[1]	12.1	2.4
3,500	1-3*	10.2	2.4
High-fat milk (6.6% fat and 15.62 total solids)			
0	3-12	——	2.8
1,500	2-5*	15.0	2.8
2,500	2-4***	14.0	3.2
3,500	2-4****	21.4	6.2

[1] Degree of fat-clumping

TABLE 3

The influence of various temperatures on the efficiency of homogenization and on the viscosity of milk containing 6.6 per cent fat homogenized at 3,500 pounds pressure following pasteurization at 145° F. and after a 20-hour storage at 45° F.

Temperature of Homogenization (° F.)		Homogenization Efficiency as Shown by: Range in the Diameter of Fat Globules (μ)	Homogenization Efficiency as Shown by: USPHS Index (%)	Viscosity After 72-hour Storage at 45° F. (centipoises)
140	(C)[1]	1-2	9.1	2.8
	(W)[2]	1-2	8.6	2.8
100	(C)	1-2	9.3	2.8
	(W)	2-4	11.2	2.8
90	(C)	1-3	10.4	2.8
	(W)	3-6***[3]	20.0	4.2
80	(C)	2-4***	21.4	6.2
	(W)	3-8	Cream plug	2.6
70	(C)	3-5**	36.0	3.0
	(W)	3-9	Cream layer	2.8
60	(C)	3-8	Cream plug	2.8
	(W)	3-9	Cream layer	2.8

[1] (C) - Milk pasteurized at 145° F. for 30 minutes and cooled to homogenization temperature.

[2] (W) - Pasteurized milk, stored 20 hours at 45° F., warmed to homogenization temperature.

[3] Degree of fat-clumping.

TABLE 4

The influence of various temperatures and pressures of homogenization on the efficiency of homogenization and the viscosity of milk (2.8 per cent fat and 11.01 per cent total solids)

Homogenization Temperature	Homogenization Pressure (lb./sq. in.)								
	1,500			2,500			3,500		
	Homogenization Efficiency as Shown by		Viscosity After	Homogenization Efficiency as Shown by		Viscosity After	Homogenization Efficiency as Shown by		Viscosity After
	Range in Diameter of Fat Globules	USPHS Index	72-hour Storage at 45° F.	Range in Diameter of Fat Globules	USPHS Index	72-hour Storage at 45° F.	Range in Diameter of Fat Globules	USPHS Index	72-hour Storage at 45° F.
(° F.)	(μ)	(%)	(centipoises)	(μ)	(%)	(centipoises)	(μ)	(%)	(centipoises)
140	1-2	10.0	2.2	1-2	6.5	2.2	1-2	6.5	2.2
100	1-2	10.0	2.2	1-2	6.5	2.2	1-2	6.5	2.2
90	1-2	11.0	2.2	1-2	7.0	2.2	1-2	6.5	2.2
80	1-4	14.0	2.2	1-3	8.4	2.2	1-3* [1]	8.0	2.2
70	2-5	20.0	2.2	1-4	12.0	2.2	1-4*	11.0	2.2
60	2-6	——	2.1	2-6	——++ [2]	2.1	2-6	——+++ [2]	2.0

[1] Degree of fat-clumping

[2] Cream-layer formation

TABLE 5

The influence of various temperatures and pressures of homogenization on the efficiency of homogenization and the viscosity of milk (4.0 per cent fat and 12.56 per cent total solids)

Homogenization Temperature	Homogenization Pressure (lb./sq. in.)								
	1,500			2,500			3,500		
	Homogenization Efficiency as Shown by		Viscosity After 72-hour Storage at 45° F.	Homogenization Efficiency as Shown by		Viscosity After 72-hour Storage at 45° F.	Homogenization Efficiency as Shown by		Viscosity After 72-hour Storage at 45° F.
	Range in Diameter of Fat Globules	USPHS Index		Range in Diameter of Fat Globules	USPHS Index		Range in Diameter of Fat Globules	USPHS Index	
(° F.)	(μ)	(%)	(centipoises)	(μ)	(%)	(centipoises)	(μ)	(%)	(centipoises)
140	1-2	13.0	2.5	1-2	12.0	2.5	1-2	7.5	2.5
100	1-2	13.0	2.5	1-2	12.2	2.5	1-2	7.5	2.5
90	no samples collected								
80	1-3	20.2	2.5	1-3	14.4	2.5	1-3	10.0	2.7
70	2-4	52.0	2.5	1-4*[1]	36.5	2.5	1-4*[1]	15.2	2.6
60	2-6	——++[2]	2.3	2-6*	——++[2]	2.3	2-6*	——+++[2]	2.3

[1] Degree of fat-clumping

[2] Cream-layer formation

TABLE 6

The influence of various temperatures and pressures of homogenization on the efficiency of homogenization and the viscosity of milk (4.4 per cent fat and 13.10 per cent total solids)

Homogenization Temperature	Homogenization Pressure (lb./sq. in.)								
	1,500			2,500			3,500		
	Homogenization Efficiency as Shown by		Viscosity After 72-hour Storage at 45° F.	Homogenization Efficiency as Shown by		Viscosity After 72-hour Storage at 45° F.	Homogenization Efficiency as Shown by		Viscosity After 72-hour Storage at 45° F.
	Range in Diameter of Fat Globules	USPHS Index		Range in Diameter of Fat Globules	USPHS Index		Range in Diameter of Fat Globules	USPHS Index	
(° F.)	(μ)	(%)	(centipoises)	(μ)	(%)	(centipoises)	(μ)	(%)	(centipoises)
140	1-2	8.6	2.6	1-2	8.0	2.6	1-2	7.6	2.6
100	1-2	9.0	2.6	1-2	8.0	2.6	1-2	7.6	2.6
90	1-3	10.1	2.6	1-2	10.0	2.6	1-2	8.2	2.6
80	2-5	20.4	2.6	2-4	15.2	2.6	2-4** [1]	14.1	2.8
70	2-5	68.2	2.6	2-5	50.0	2.6	2-4	45.6	2.6
60	2-6	——++ [2]	2.6	2-6	——++ [2]	2.5	2-6	——++ [2]	2.5

[1] Degree of fat-clumping

[2] Cream-layer formation

TABLE 7

The influence of various temperatures and pressures of homogenization on the efficiency of homogenization and the viscosity of milk (6.2 per cent fat and 15.50 per cent total solids)

Homogenization Temperature	Homogenization Pressure (lb./sq. in.)								
	1,500			2,500			3,500		
	Homogenization Efficiency as Shown by		Viscosity After 72-hour Storage at 45° F.	Homogenization Efficiency as Shown by		Viscosity After 72-hour Storage at 45° F.	Homogenization Efficiency as Shown by		Viscosity After 72-hour Storage at 45° F.
	Range in Diameter of Fat Globules	USPHS Index		Range in Diameter of Fat Globules	USPHS Index		Range in Diameter of Fat Globules	USPHS Index	
(° F.)	(μ)	(%)	(centipoises)	(μ)	(%)	(centipoises)	(μ)	(%)	(centipoises)
140	1-3	12.0	2.8	1-2	10.0	2.8	1-2	9.2	2.8
100	1-3	12.0	2.8	1-2	10.1	2.8	1-2	9.2	2.8
90	1-3	15.0	2.8	1-3	12.2	2.8	1-3	11.0	2.8
80	2-5*[1]	—[3]	2.8	2-4***[1]	16.0	7.8	2-4****[1]	16.2	22.0
70	2-5*	91.5	2.8	2-4**	48.0	3.0	2-6**	70.0+[2]	7.0
60	2-8*	——++[2]	2.6	2-8**	——+++[2]	2.6	2-8**	——+++	2.6

[1] Degree of fat-clumping [2] Cream-layer formation [3] Sample was lost

TABLE 8

The influence of variations in fat and solids-not-fat on the viscosity of adjusted milk homogenized at various pressures

Adjusted Milk	Fat (%)	Solids-not-fat (%)	Viscosity of Homogenized Milk (lb./sq. in.) 0 (centipoises)	1,500 (centipoises)	2,500 (centipoises)	3,500 (centipoises)
	3.10	8.00	2.5	2.5	2.5	2.5
Series	4.15	8.00	2.5	2.5	2.5	2.7
A	5.00	8.00	2.5	2.5	2.7	3.2
	5.95	8.00	2.7	2.8	3.2	5.9
	3.15	11.00	2.8	2.8	2.8	2.8
Series	4.00	11.00	2.8	2.8	2.8	2.8
B	4.90	11.00	2.9	2.9	3.0	3.2
	5.95	11.00	3.0	3.3	3.4	6.5
	3.00	8.30	2.5	2.5	2.5	2.5
Series	3.00	7.10	2.5	2.5	2.5	2.5
C	3.00	9.90	2.6	2.6	2.6	2.6
	3.00	10.95	2.8	2.8	2.8	2.8
	6.00	8.10	2.9	3.0	3.0	3.2
Series	6.00	9.25	3.0	3.0	3.2	3.4
D	6.00	10.00	3.3	3.3	3.4	3.5
	6.00	10.95	5.0	4.8	6.0	6.2

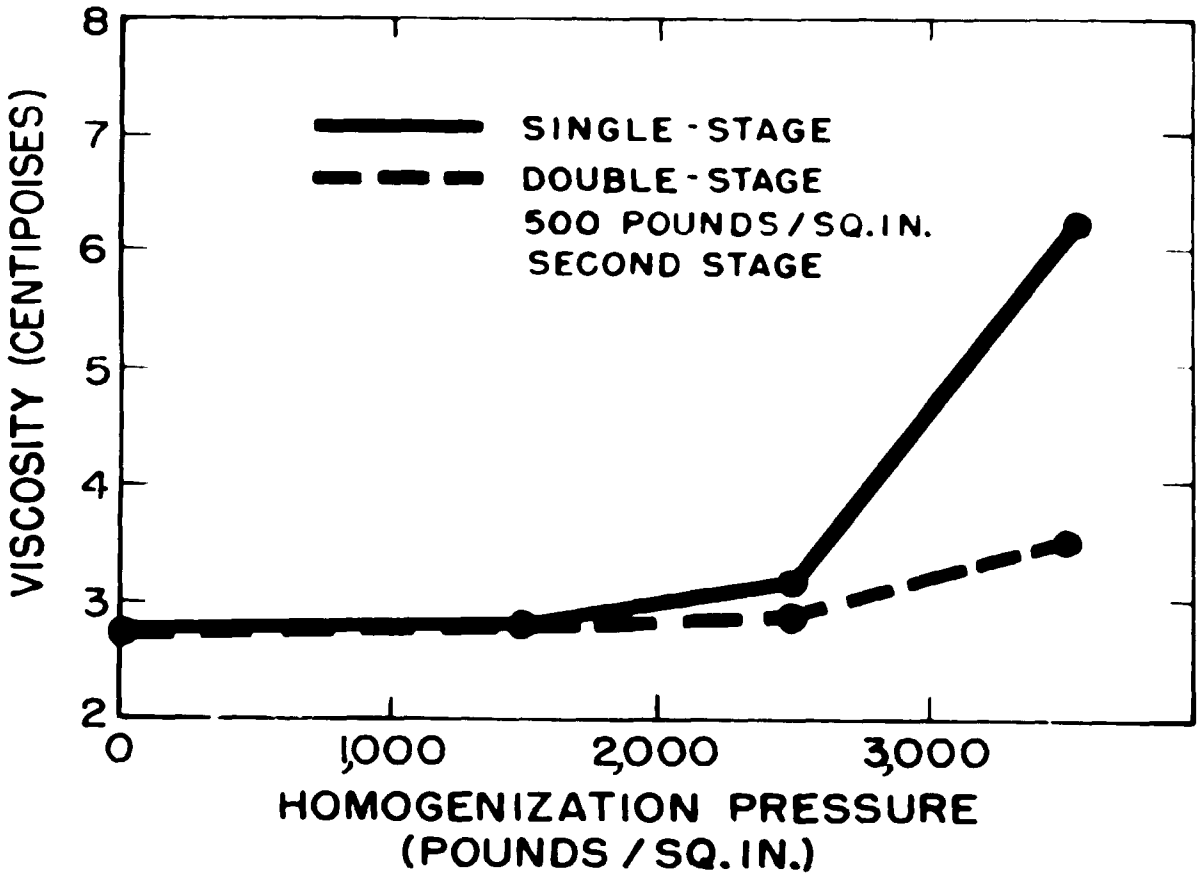

Figure 1. The effect of single- and double-stage homogenization on the viscosity of high-fat milk (6.6 per cent) homogenized at 80° F.

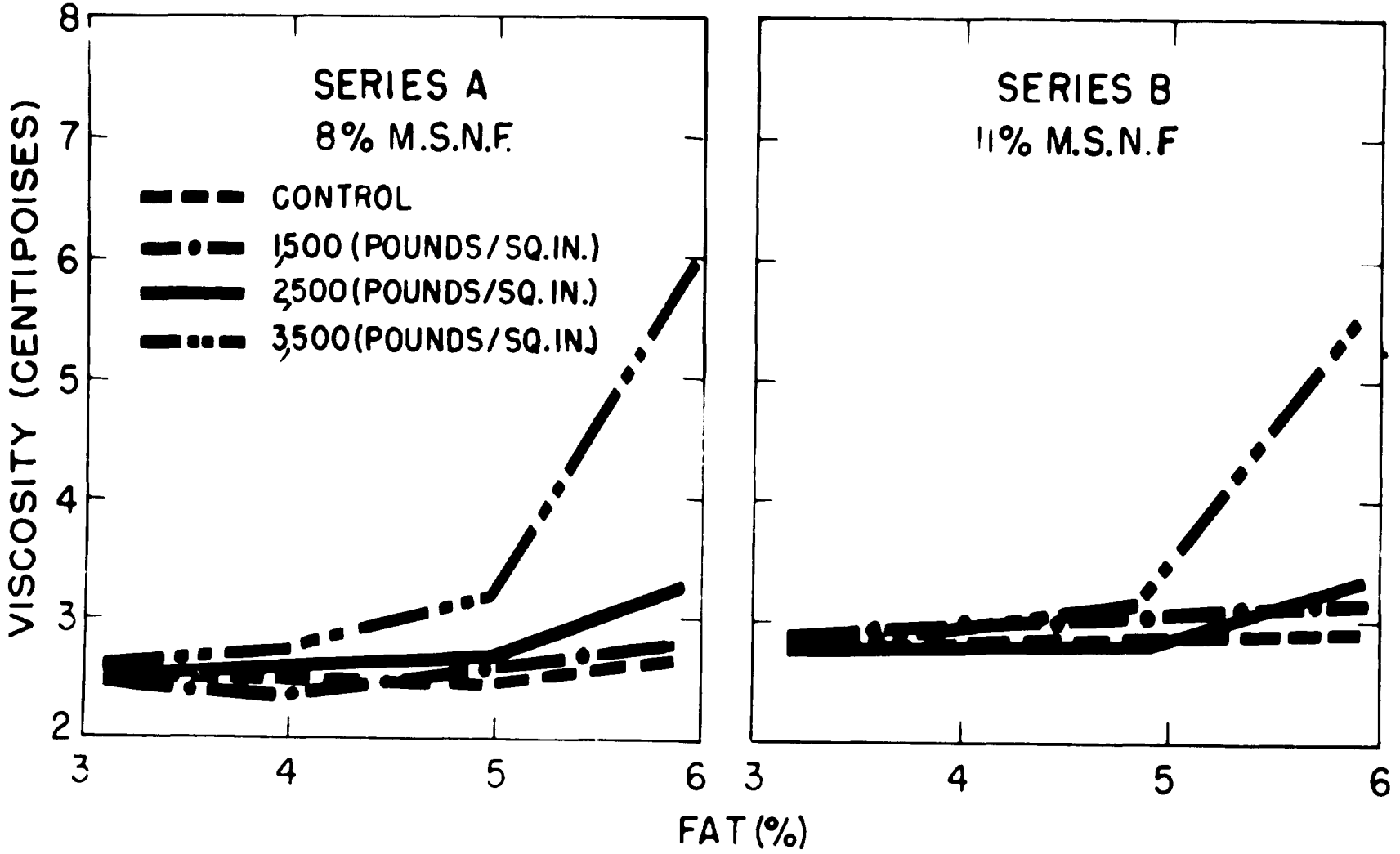

Figure 2. The influence of different homogenizing pressures maintained at 80° F. on the viscosity of milk containing 8 per cent solids-not-fat plus various amounts of fat (SERIES A) and corresponding data on milk containing 11 per cent solids-not-fat (SERIES B).

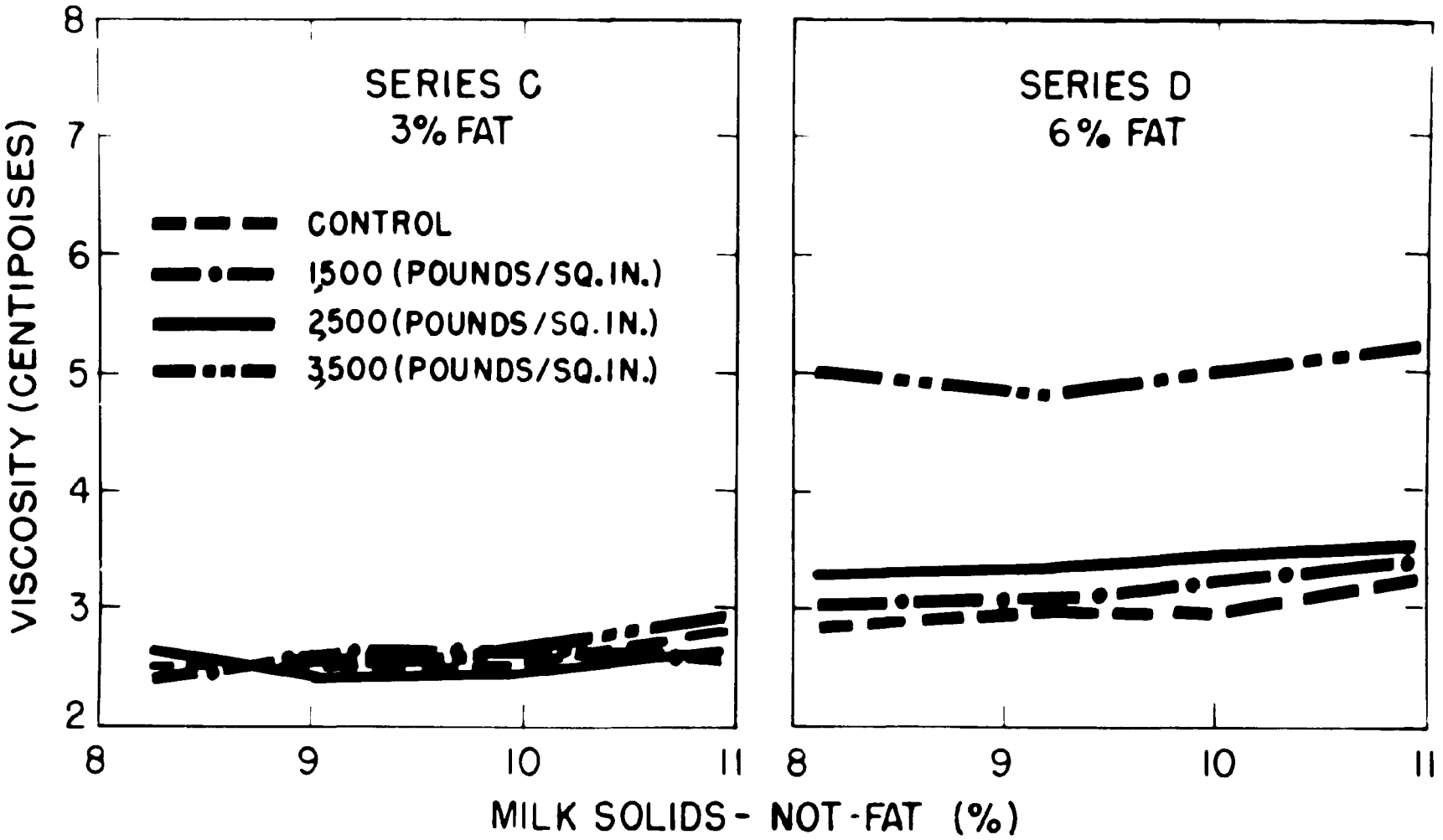

Figure 3. The influence of different homogenizing pressures maintained at 80° F. on the viscosity of milk containing 3 per cent fat plus various amounts of solids-not-fat (SERIES C) and corresponding data on milk containing 6 per cent fat (SERIES D).

DISCUSSION

The results obtained from this investigation indicate that the fat content of milk, the temperature and pressure of homogenization and the type of homogenization employed (single-stage or double-stage valve systems) are important factors in controlling the efficiency of homogenization and on the development of an excessive viscosity in homogenized milk. The physical state of the globular fat at different homogenization temperatures is apparently responsible for some of the defects noted in homogenized milk as well as other undetermined phenomena closely associated with the fat when it is in this critical condition.

Viscosity

High homogenization pressures (greater than 2,500 pounds) and low homogenization temperatures (ca 80° F.) augment inefficient homogenization and high viscosities in high-fat milk (5.0 per cent or more), but have little or no influence on the development of excessive viscosities in low-fat milk (less than 4.0 per cent). Such an observation would lead one to suspect that

the ratio of solids-not-fat to fat was involved, but homogenization trials with prepared milks of known composition indicate that moderate variations in the solids-not-fat content of milk exert little influence on fat-clumping and the development of an excessive viscosity in milk homogenized at 80° F. (Figures 2 and 3). A marked increase in viscosity was recorded, however, for milk containing a high-fat content provided the homogenization pressures exceeded 2,500 pounds pressure. Doan (1929a) reported no clumping in milk homogenized at 100° F. at 3,500 pounds pressure, provided the plasma-solids to fat ratio remained below 0.3 to 0.5, however, clumping and increased viscosities were reported when the solids-not-fat to fat ratio varied from 2.2 to 1.3. These differences might be accounted for on the basis of the results obtained in the present investigation which was carried out at a temperature at which fat was in a state of shifting equilibria (80° F.), whereas Doan's data represented milk that had been homogenized while the fat was in the liquid state (100° F.).

Doan (1931) also showed that the degree of clumping could be reduced by increasing the amount of plasma-solids in homogenized cream. Although the data presented in Figure

3 show some reduction in the tendency of fat to clump at high levels of solids-not-fat, the effect is so inconspicuous that it can be disregarded. Apparently, there was enough of the fat-surfacing material present in the lowest level of solids-not-fat to cover all of the fat surfaces, thus any variation in the level of solids-not-fat in normal milk has little effect in retarding fat-clumping (Doan, 1929a; Wittig, 1949).

Some of the data presented in this paper relative to the development of high viscosities are similar to the progressive thickening of homogenized milk observed by Moore and Trout (1947). Why such a phenomenon as an increase in viscosity should occur under the conditions of this experiment furnishes material for interesting speculation.

Unreported data show that the homogenization of skimmilk under conditions similar to those reported for whole milk, has no effect on the immediate or the 72-hour viscosity values, but when the skimmilk was supplemented with additional nonfat dry milk solids, the basic viscosity was raised slightly. Therefore, any potential for a marked increase in viscosity must be closely associated with the fat content of the milk and/or alterations in protein stability.

Although the sensitivity of the viscosity method used in this study was not sufficiently delicate to detect slight differences in viscosity, it is generally believed that the homogenization of whole milk results in a slight increase in viscosity (Bateman and Sharp, 1928). Under normal homogenization pressures and at temperatures above 100° F., it was not possible to measure a change in the viscosity of pasteurized whole milk. The increase in the viscosity of homogenized milk has been attributed to an increase in the volume of the dispersed phase resulting from an increase in the adsorption of serum proteins on the newly formed and numerously dispersed fat globules (Hatschek, 1912). Under these conditions, high pressures of homogenization should disperse the fat to a greater degree and increase the viscosity of the homogenized milk, provided the fat is in a liquid state. On theoretical grounds, this phenomenon could not account for the sharp increases in viscosity reported in this study. Any further increase in the viscosity of homogenized milk must be due to an additional increase in the volume of the dispersed phase and an accompanying decrease in the volume of the dispersion medium. Profuse clumping of the fat globules in homogenized milk could cause such a change.

The clumps of fat globules would act as individual globules, since some of the dispersion medium would be trapped in the clusters. Figure 4 has been prepared to illustrate how the dispersion and clumping of homogenized fat could account for an increase in the viscosity of homogenized milk. Microscopic examinations made on samples of homogenized milk of high viscosity revealed extensive clumping of the fat globule. Such an observation lends support to the above postulation.

Since the occurrence of clusters of fat globules has been associated with the high viscosities reported in this study, the clumping tendency should be explored. Under normal conditions of homogenization, fat is in the liquid state and the degree of dispersion is a function of the applied pressure and the mechanical efficiency of the homogenizer. When the fat globule is homogenized and broken up into numerous smaller globules, a layer of protein is assumed to surround the new fat surfaces. Whether this surface layer is a specific surface-active material, as suggested by Rahn and Sharp (1928), Wittig (1949) and others, or one of the serum proteins, is a matter of considerable controversy and speculation. At any rate, whole milk which has not been over-heated or subjected to extremes in processing

treatment should contain adequate quantities of these materials to supply the requirements of the new surface (Doan, 1929a; 1931). Wittig (1949) has reported inferior homogenization efficiencies at high pressures when excessive pasteurization temperatures were employed. Doan and Mykleby (1943), on the other hand, observed an increase in the homogenization efficiency when high-heat treated milk was homogenized at normal pressures, as indicated by the United States Public Health Service Index.

Homogenization Efficiency

When temperatures below 70° and 60° F. are utilized, homogenization is incomplete regardless of the pressure used. Such evidence is furnished by the relatively large cream volume which results from incomplete dispersion of the fat globules in the homogenized milk. These data are in agreement with the observations of Trout and Scheid (1941) and Whitaker and Hilker (1937) who recorded only slight or no fat dispersion when milk was homogenized at 80° F. or lower. In fact, Trout and Scheid (1941) demonstrated that when raw milk was rehomogenized five times at 40° F. at 5,000 pounds pressure, the fat failed to

disperse. Their experiments showed that a definite cream plug of coalesced or churned fat was formed when low-temperature homogenization was employed.

In the homogenizing temperature range between 80° down to 70° F., also between 90° up to 100° F. when the milk has been warmed from a preliminary cold-storage period at 45° F., no cream layer was noted in cream volume studies. However, a nonuniform dispersion and profuse fat-clumping were observed. In high-fat milks, these characteristics were always accompanied by a marked increase in viscosity. Moore and Trout (1947) attributed progressive thickening to a protein-like cluster. Such a phenomenon could be assumed to be a denaturation of the protein as the result of homogenization, although this fact has not been definitely established from the existing evidence (Dunkley and Sommer, 1944; Doan, 1946; Menefee, Overman and Tracy, 1941; Shahani and Sommer, 1951b). At the above temperatures, it is possible that there is a partial solidification of the globular fat (Brunner and Jack, 1950; King, 1951) which would promote a selective adsorption of protein (Sharp and Krukovsky, 1939; Dunkley and Sommer, 1944) at the fat/serum interface and thereby enhances fat-clustering.

Or, according to Hunziker (1940), Bird and Derby (1937), Brunner and Jack (1950), King (1951) and others, fat-gathering results when the physical state of the surface of the fat globule is conducive to "sticking." The necessary requirement for fat-globule contact would be furnished by the mechanical action of the homogenizer. King (1951) has also attributed fat-clumping to the presence of free fat which could conceivably act as a cementing agent to hold the fat globules together.

When an increase in viscosity occurred, it developed to a maximum value within 72 hours. This behavior probably resulted from a solidification of the fat in the fat globule and a maximum value developed for a given storage temperature. According to van Dam (1923) and Rishoi and Sharp (1938a, 1938b), globular fat requires several hours to reach a condition of physical equilibrium at 45° F.

In most instances when an increase in viscosity accompanied specific processing procedures, it was possible to retard or inhibit the development of high viscosity by using a second, low-pressure homogenization either as a separate process or in a second-stage valve system.

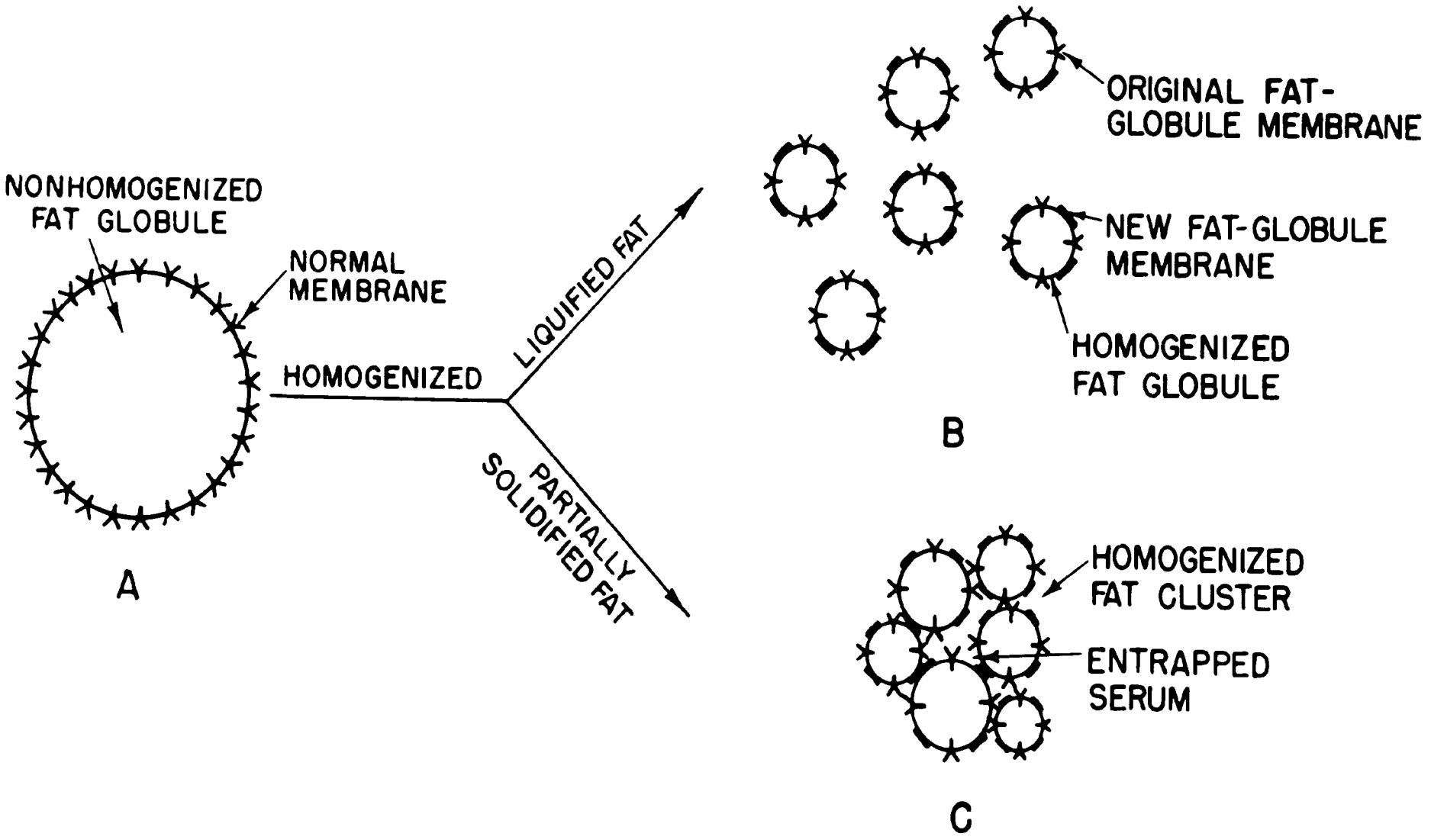

Figure 4. Schematic drawing showing how the dispersion and clustering of homogenized fat could, in part, account for an increase in the viscosity of homogenized milk.

A - Normal fat globule with its adsorbed membrane-like coverings.

B - well-dispersed globules resulting from efficient homogenization.

C - Fat-globule clusters formed by inefficient homogenization.

SUMMARY OF SECTION I

High-fat milk (greater than 5.0 per cent), low homogenization temperatures (80° to 70° F.) and high, single-stage homogenization pressures (greater than 2,500 pounds per square inch) are conditions which contribute to the clustering of fat globules and to the development of high viscosity in homogenized milk.

Normal variations in the solids-not-fat content of milk produced insignificant changes in the viscosity of homogenized milk.

When the processing procedures were conducive to the development of high viscosity, the excessive viscosity could be reduced or completely eliminated by rehomogenization at 500 pounds pressure or by second-stage homogenization.

SECTION II

THE RELATIONSHIP BETWEEN THE EQUILIBRIA OF GLOBULAR FAT EXISTING AT HOMOGENIZATION TEMPERATURES AND THE TENDENCY TOWARD THE DEVELOPMENT OF HIGH VISCOSITY IN HOMOGENIZED MILK

REVIEW OF LITERATURE

Wittig (1949) reported that homogenization at low temperatures resulted in the clumping of fat, not because of changes in the fluidity of the fat, but because of changes in the film-forming substances whose adhesive properties varied with the temperature. The dispersion of fat increased when the temperature was raised to 60° to 65° C. and decreased when the temperature was lowered to 37° to 42° C. Sharp and Krukovsky (1939) believed that the material adsorbed on the liquid fat globules was different from that adsorbed on the solid globules. The latter material was described by them as an agglutinin whose presence on the solidified fat-globule increased the tendency to clump. This observation was later confirmed by Dunkley and Sommer (1944). Skelton (1941) believed that some specific substance was adsorbed on solid fat, but was released when the fat was melted. He concluded that it could be lecithin. Moore and Trout (1947) were of the opinion that the homogenization of milk at 80° F. was accompanied by considerable adsorption on the fat surface and that rapid crystallization within

the fat resulted from the vigorous agitation accompanying homogenization.

According to Hunziker (1940), Bird, et al. (1937), van Dam and Burgers (1935) and as discussed later by Brunner and Jack (1950), the coalescence of fat globules into butter granules during the churning of cream was not as dependent on the nature of the material adsorbed on the surface of the fat as it was on the physical state of the fat at the surface of the globule. At optimum churning conditions, both solid and liquid fat exist at the fat-globule surface and under these conditions, coalescence was thought to occur because of the sticking together of fat globules at these semisoft areas. Churning studies made by Rishoi and Sharp (1938a) and Sharp (1940) led them to form a somewhat similar concept concerning the physical state of the fat at the churning temperature. Skelton and Herreid (1941) believed that a certain amount of solidification within the fat globule would have to occur to produce maximum viscosity in cream.

King (1950) estimated the thickness of the fat globule membrane as 5 to 10 millimicrons. By the use of a polyphase microscope, he found that the surface layers of high-melting

fats were oriented systematically on the surface of the globule as the result of pressure exerted on fat globules by clumping. He pointed out later (King, 1951) that fat-clumping and coalescence occurred most satisfactorily at temperatures between 15° and 25° C., a temperature at which the fat is in a transitional state.

Two practical methods for determining the physical state of globular fat are available: (1) a dilatometric technique which measures small differences in the specific volume of fat as it changes from a liquid to a solid or from a solid to a liquid, and (2) a direct calorimetric technique which measures the specific heat of the fat, from which the quantities of solid and liquid fat can be calculated. Van Dam (1922) and Rishoi and Sharp (1938b) employed the dilatometer method and Rishoi and Sharp (1938a) made use of the calorimetric method in studying the physical equilibria in milk fat at different temperatures. In the present study, a direct calorimetric technique described by Jack and Brunner (1943) has been employed.

The purpose of the experimental work reported in this section was to determine the amount of solidified fat that is present in globular fat when milk is homogenized efficiently

and to indicate the variations that occur when milk is homogenized at temperatures which are conducive to the development of a high viscosity.

EXPERIMENTAL PROCEDURE

Samples of pasteurized milk, which had been prepared according to the techniques described in Section I, were used for the calorimetric studies because of its susceptibility to increase in viscosity when homogenized at low temperatures (70° to 90° F.) and high pressures (greater than 2,500 pounds). The samples were divided into two 1-quart lots. One quart was used as the control and was placed in ice water for approximately 20 hours prior to calorimetric examination. The other sample was warmed up to 140° F. and then cooled slowly in cold running water to the desired homogenization temperature.

Figure 5 shows the calorimeter assembly. The calorimeter vessel is a wide-mouth vacuum flask of 1-liter capacity which had been wrapped with sheet asbestos and tape to increase the insulation. The heating element is a 30-watt, immersion type, chromalax heater which operates from a 115-volt alternating current. The temperature was measured to 0.02° C. by means of a thermometer calibrated from -10° to 60° C. The voltage was measured to the nearest 0.1 volt with a cabinet-type

voltmeter. The input of heat generated was calculated by the use of Joule's equation:

$$H = \frac{E\ I\ t}{4.186}$$

where H = calories generated,

E = the potential difference between terminals of the heating element,

I = current passing through the heating element,

t = time in seconds, and

4.186 = Joule's constant.

The calorimeter was calibrated with water over a temperature range from 0^{o} to 60^{o} C., to determine the experimental errors due to thermal leakage, heat losses due to evaporation, the effect of agitational friction and the thermal capacity of the calorimeter. Figure 6 shows the correction data obtained for water. These values were used to adjust each set of data to compensate for environmental influences and the heat requirements of the calorimeter (Table 9, Column H^{e}).

A 500-gram sample was weighed into the calorimeter which was then placed in the operating position. A 15-minute temperature adjustment period was allowed before starting a determination, after which the temperature was recorded and

the heating current was applied until the desired temperature range was covered. The voltage was recorded every five minutes after the heating period was started and the temperature was read at the end of the holding period. The rate of heating was approximately 1.5° C. per minute.

The values for the specific heat of skimmilk at 0° and 60° C. have been reported by Hammer and Johnson (1913) as 0.940 and 0.963, respectively. The values used in this study for calculation purposes were reported by Jack and Brunner (1943) and ranged from 0.943 at 0° C. to 0.966 at 60° C. The specific heat of liquid and solid fat was reported to be 0.5 and the value for the heat of fusion was 19.5 calories per gram (Jack and Brunner, 1943).

The degree of fat solidification was calculated from the following equation:

$$\text{Percentage of solid fat} = \frac{H^f - (0.5 \times t_2 - t_1)}{19.5} \times 100$$

where H^f = heat input per gram of fat or the total heat required to raise the temperature of one gram of fat from t_1 to t_2,

$(0.5 \times t_2 - t_1)$ = amount of heat necessary to raise the temperature from t_1 to t_2, provided no heat is used for melting fat, and

19.5 = calories of heat necessary to melt 1 gram of solidified fat.

The viscosity values were determined at 20° C. with the aid of a Brookfield Synchro-Lectric viscometer.

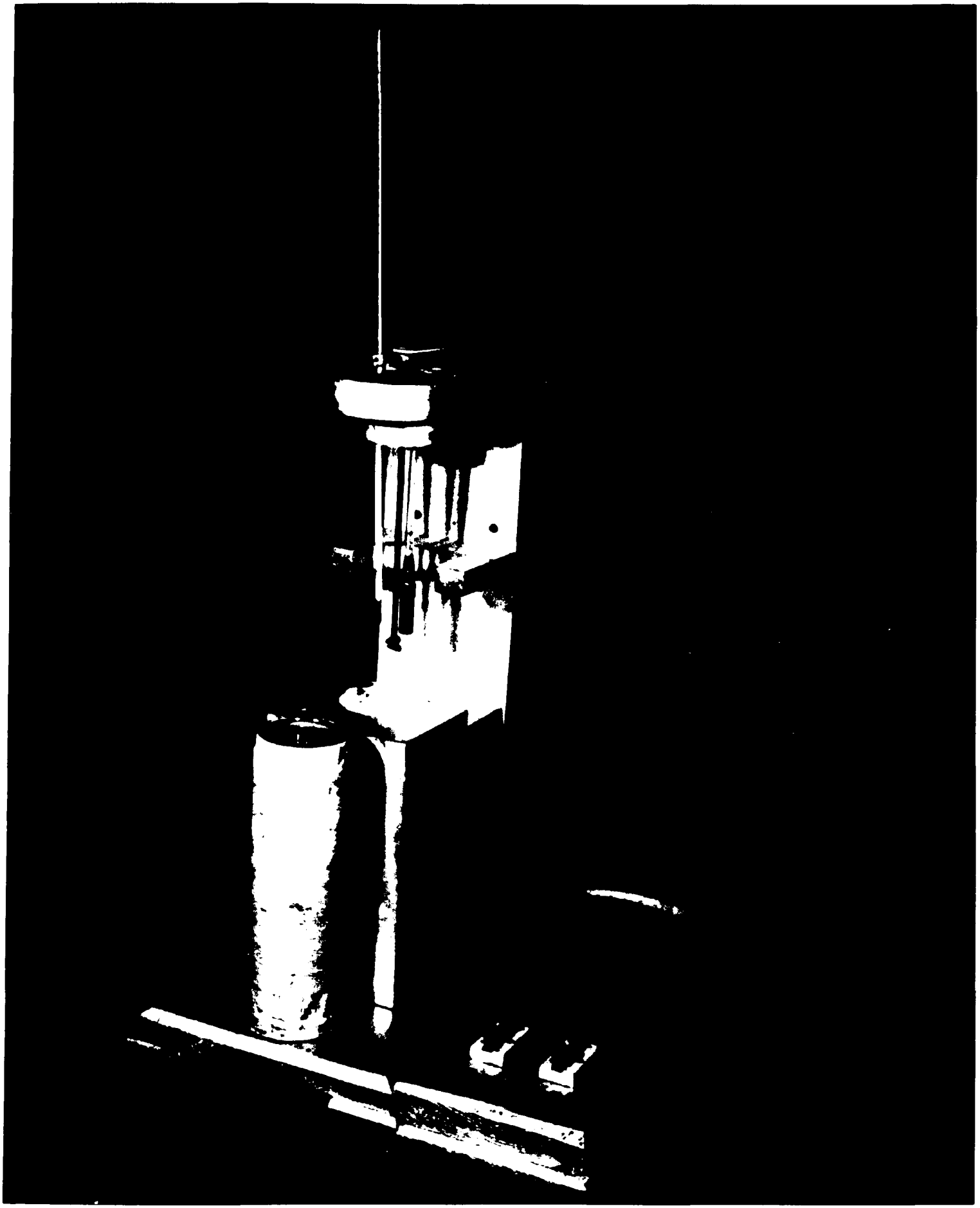

Figure 5. Calorimetric assembly used in this study.

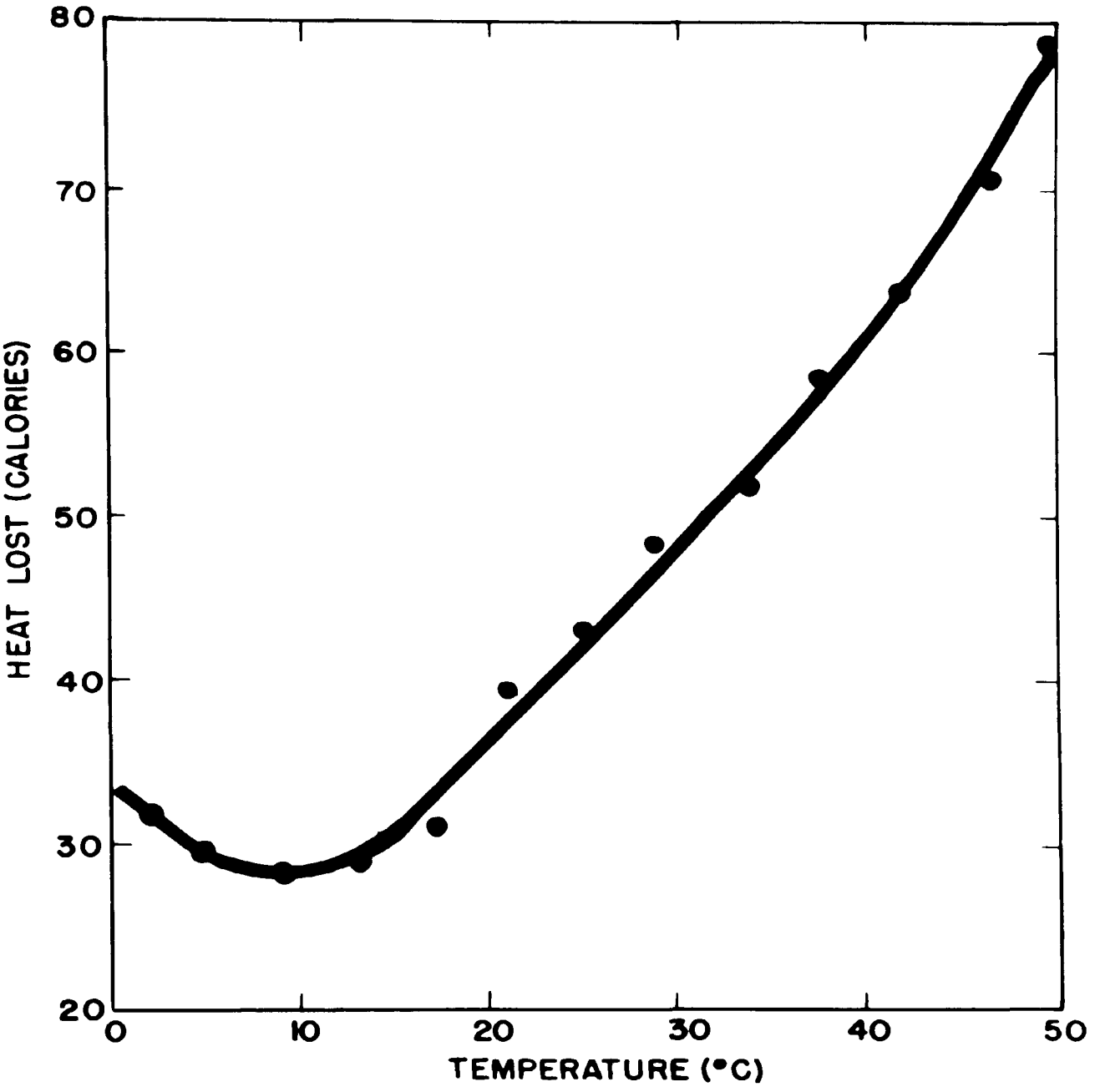

Figure 6. Thermal-correction curve for the calorimetric assembly used in this study.

RESULTS

Typical data taken from Section I showing the effects of previous temperature treatments and homogenization temperatures on the development of viscosity in homogenized milk are presented graphically in Figure 7. The experimental values for the percentages of solidified fat are plotted against viscosity values. When the milk was homogenized immediately following pasteurization, maximum viscosity was obtained at a temperature of about 80° F., but when the pasteurized milk was cooled to 45° F., held for 20 hours and then warmed to various homogenization temperatures, maximum viscosity was obtained at about 90° F.

The percentage of solidified fat at the homogenization temperature was determined from the calorimetric data obtained for each of the samples represented in Figure 7. These data are tabulated in Table 9 and are shown graphically in Figure 8. The degree of solidification of the milk fat in any one sample depended principally on the temperature and the previous heat-treatment of the milk. In milk samples which had been pasteurized, cooled and held at 45° F. for 20 hours

prior to homogenization, the maximum viscosity developed when the homogenization temperature was about 80° and 70° F., at which temperature the degree of solidification ranged from 0.5 to 2.0 per cent. Similarly, the samples of pasteurized milk which had been warmed to the homogenization temperature, following a 20-hour storage period at 45° F., exhibited a maximum viscosity at about 80° to 90° F., at which temperature the degree of solidification decreased from 18.0 to 7.0 per cent, respectively.

The relationship between viscosity and the percentages of solidified fat resulting from different homogenization pressures are shown graphically in Figures 9 and 10. These two figures show essentially the same information as has been deduced already from Figures 7 and 8, namely, that the percentage of solidified fat accompanying maximum viscosity ranged between 0.5 and 2.0 per cent in milk homogenized following pasteurization in contrast to 18.0 and 7.0 per cent in milk which had been cooled and then warmed to the homogenization temperatures. At lower homogenization pressures (less than 2,500 pounds) the degree of solidification remained the same, the only difference being a reduction in the viscosity which

accompanied the lower homogenization pressure. When homogenization pressures below 2,500 pounds were used, no viscosity was developed even though the degree of solidification of the fat remained the same.

TABLE 9

The percentage of solidified fat in a 500-gram sample of milk (6.6 per cent fat) at different processing temperatures

Sample Number	Heating Period (sec.)	Temp. at Beginning of Heating Period (^{o} C.)	Temp. Rise During Period (^{o} C.)	Voltage	Heat Added From Circuit (H) (cal.)	Heat Corrections (cal.)	Effective Heat (H^{e}) (cal.)
		Homogenized[1] immediately following pasteurization					
1	450	38.00	10.05	116.0	5,330.3	670.2	4,660.1
2	960	26.74	21.90	116.0	11,358.0	1,268.0	10,090.0
3	1,080	21.01	25.05	116.5	12,885.5	1,319.2	11,566.3
4	1,300	15.52	30.08	116.0	15,430.7	1,468.5	13,962.2
		Homogenized following a 20-hour storage period at 45^{o} F.					
5	1,320	15.72	29.98	116.0	15,601.0	1,454.0	14,147.0
6	1,120	21.00	25.20	115.8	13,195.7	1,350.0	11,845.7
7	970	26.41	21.79	116.0	11,464.4	1,272.0	10,192.4
8	620	32.00	14.21	116.8	7,430.2	830.0	6,600.2
9	446	38.06	10.04	116.4	5,308.0	659.7	4,648.3

[1] Samples 1, 2, 3 and 4 were homogenized at 100^{o}, 80^{o}, 70^{o} and 60^{o} F., respectively.

[2] Samples 5, 6, 7, 8 and 9 were homogenized at 60^{o}, 70^{o}, 80^{o}, 90^{o} and 100^{o} F., respectively.

TABLE 9 (Continued)

Sample Number	Heat Requirements of Skimmilk (cal.)	Heat Available for Fat (cal.)	Heat Available per Gram of Fat (H^f) (cal.)	Sensible Heat Required per Gram of Fat (cal.)	Heat Used to Melt 1 g. of Fat (cal.)	Heat Required to Melt 1 g. Solid Fat (cal.)	Solidified Fat (%)
1	4,494.1	166.0	5.0	5.0	0.0	19.5	0.0
2	9,725.4	364.6	11.1	11.0	0.1	19.5	0.5
3	11,140.0	426.3	12.9	12.5	0.4	19.5	2.1
4	13,338.2	624.0	18.9	15.4	3.5	19.5	18.0
5	13,266.7	880.3	26.7	15.0	11.7	19.5	60.0
6	11,198.1	647.6	19.6	12.6	7.0	19.5	35.9
7	9,715.5	476.9	14.5	10.9	3.6	19.5	18.5
8	6,320.7	279.5	8.5	7.1	1.4	19.5	7.2
9	4,482.0	166.3	5.0	5.0	0.0	19.5	0.0

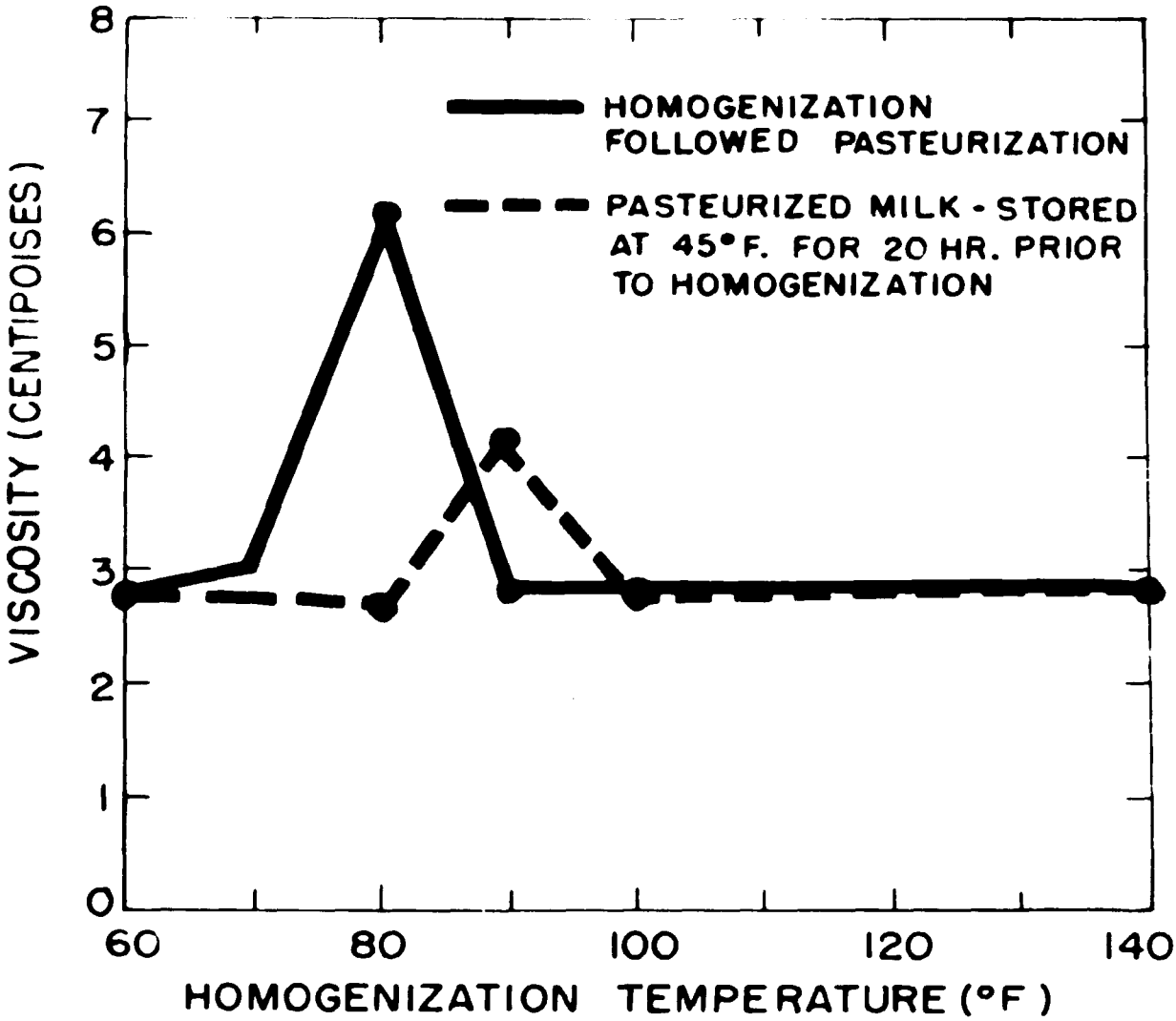

Figure 7. Shows the effect of the temperature of homogenization on the development of viscosity when 6.6 per cent milk was homogenized at 3,500 pounds pressure.

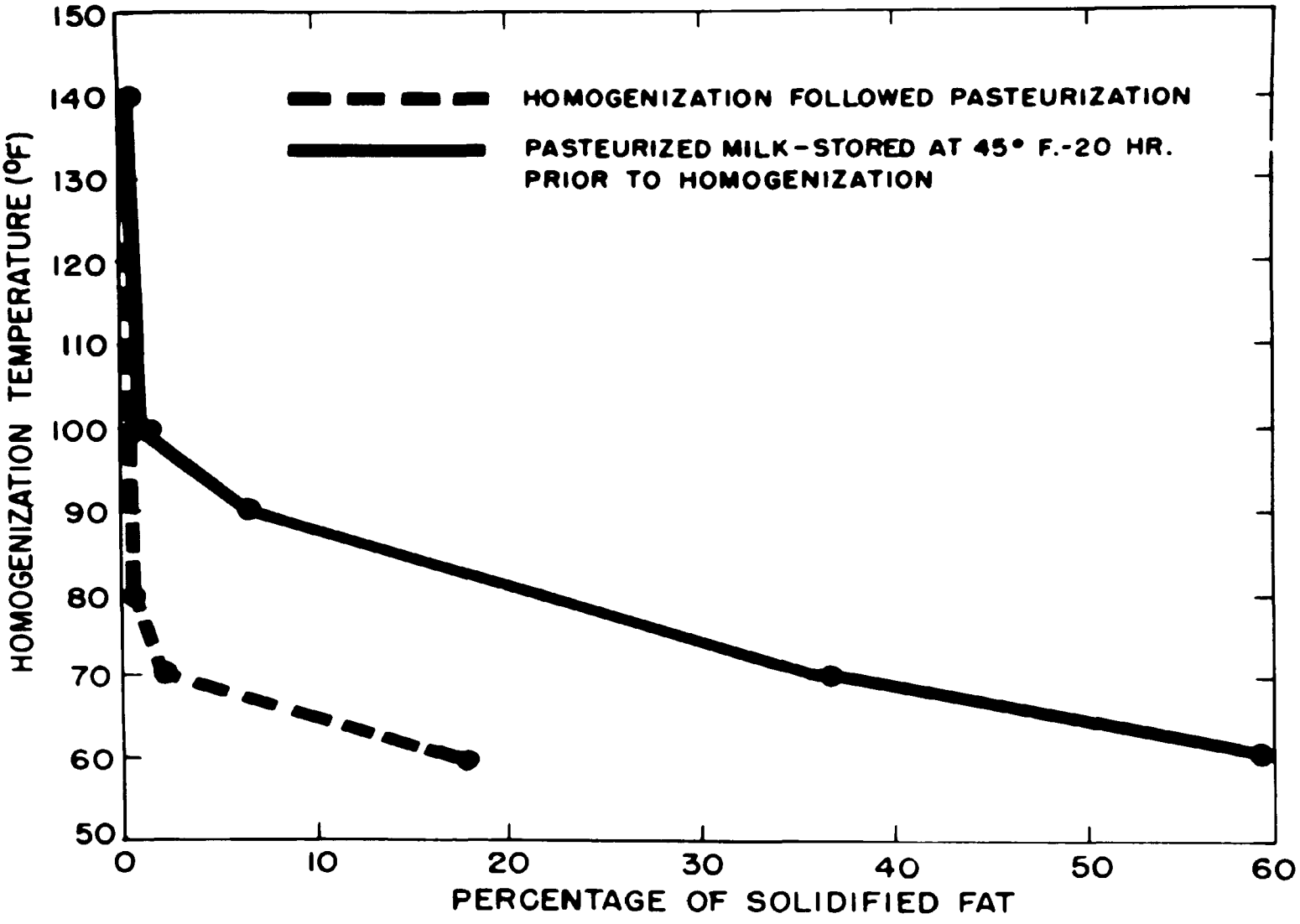

Figure 8. Shows the percentage of solidified fat obtained at various homogenizing temperatures (6.6 per cent fat in the milk).

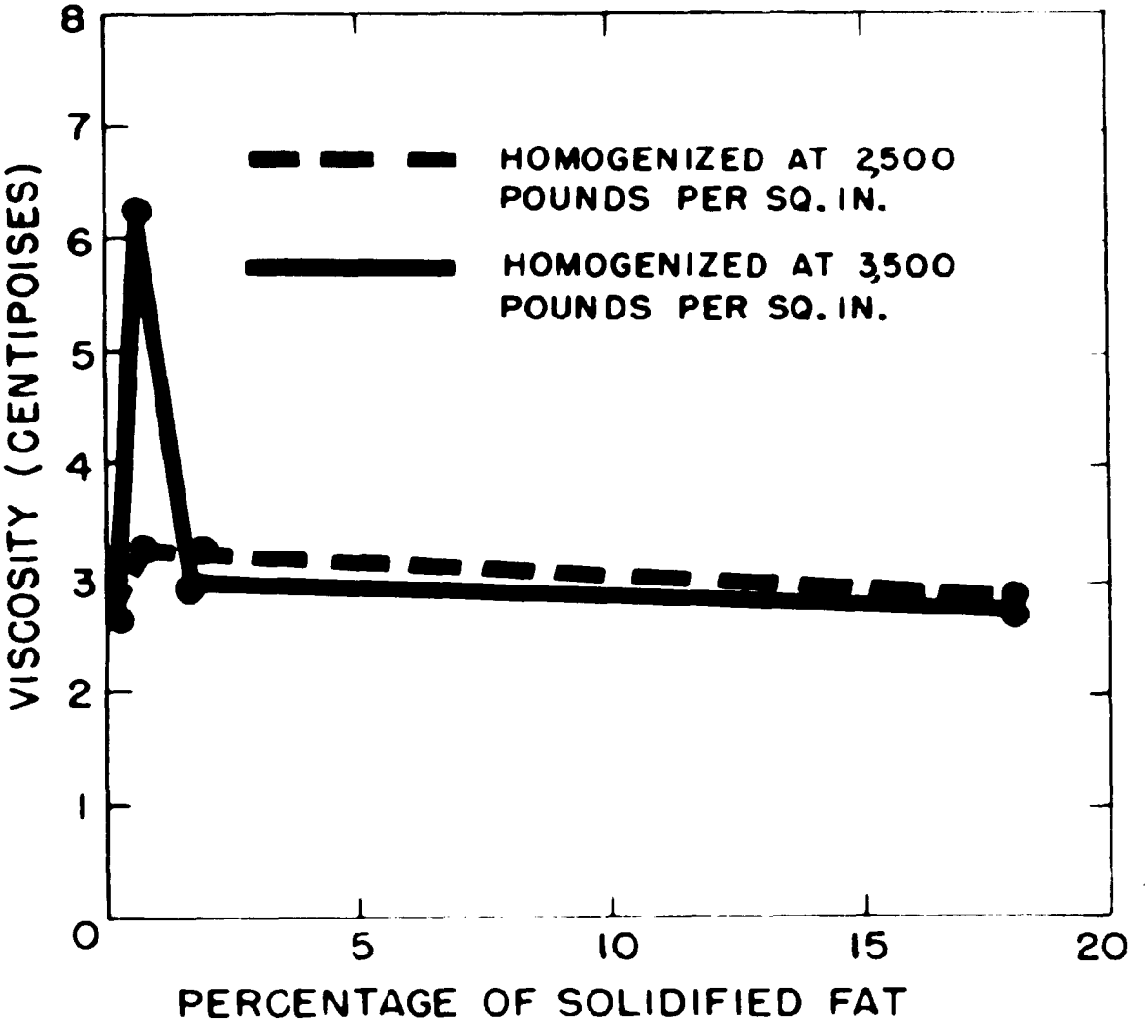

Figure 9. The relation between the degree of solidification of fat and the development of viscosity in high-fat milk (6.6 per cent) homogenized following pasteurization at temperatures ranging from 140° to 60° F.

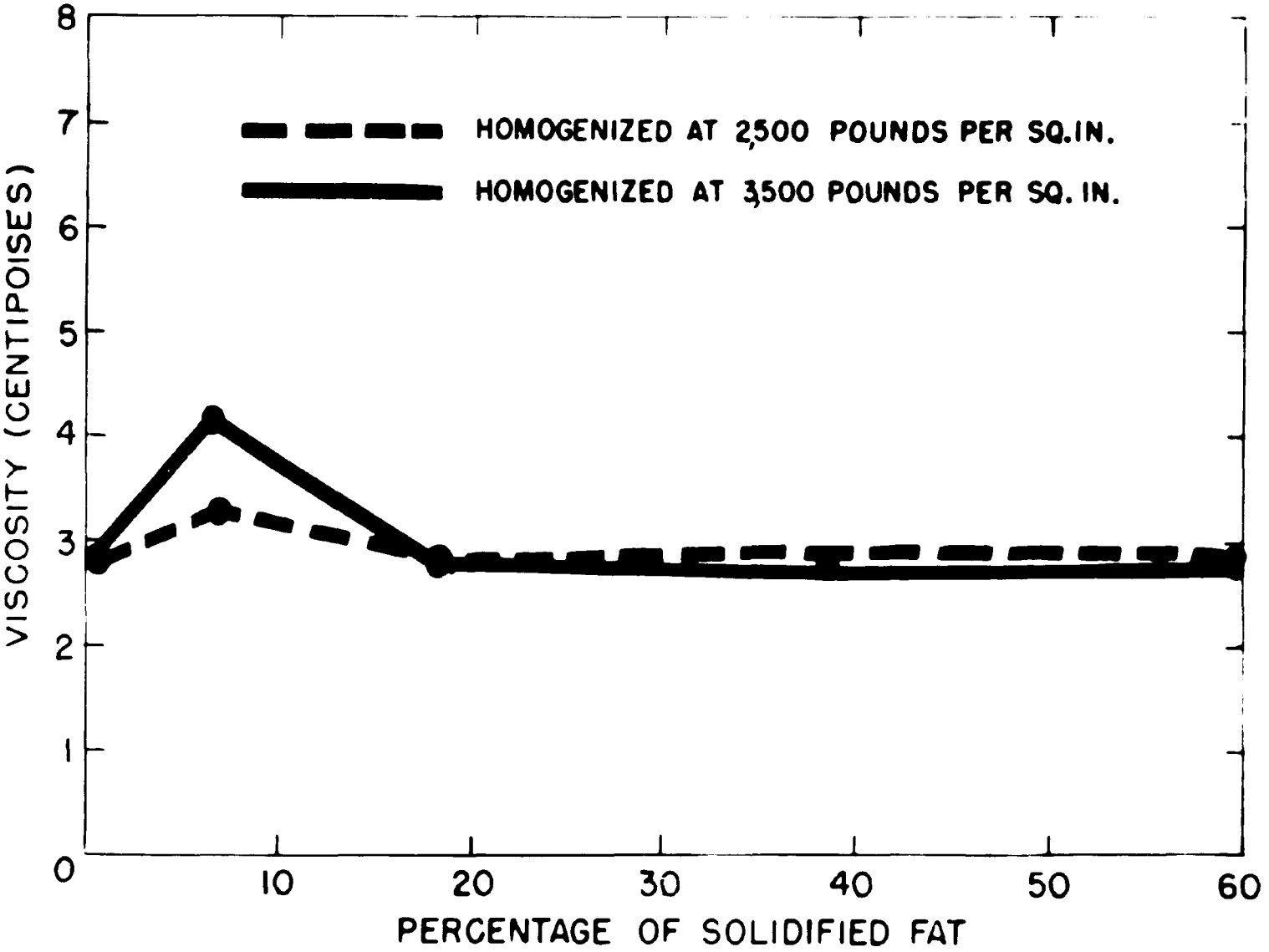

Figure 10. The relation between the degree of solidification of fat and the development of viscosity in high-fat milk (6.6 per cent) homogenized at temperatures ranging from 60° to 140° F. following a 20-hour storage period at 45° F.

DISCUSSION

A review of the general homogenization-viscosity data obtained and discussed in Section I and the viscosity and physical-state data reported in this section reveal significant information. Whenever the experimental processing conditions were conducive to fat-clumping and an increase in viscosity, the temperatures of homogenization varied between 80° and 70° F. for pasteurized milk which was cooled to the homogenizing temperature following pasteurization and between 80° and 90° F. for pasteurized milk which had been stored for 20 hours at 45° F. and then warmed to the various homogenizing temperatures. At these temperatures, the globular fat was neither totally solid nor totally liquid, but it existed as a mixture of solid and liquid fat. Apparently, the physical state of the fat in this temperature range is critical, since various chemical and physical properties of milk are influenced to a great extent by the state of the fat. Most of the concepts, ideas and theories on fat-globule clumping are concerned principally with the role played by a specific clumping material—agglutinin—which is adsorbed on the fat-globule surface during the

solidification process. Since milk fat exists in the form of globules when it is in a liquid state, other emulsion-stabilizing materials are possibly adsorbed on the fat surface. The fact that fat-globule clumping or coalescence can be induced after the fat-clustering factor has been inactivated by heat is common knowledge.

Conceivably, certain of these concepts might be utilized to explain, partially, the high viscosities of homogenized milk recorded in this study. Certainly, in addition to the homogenization temperature and the corresponding degree of fat solidification, the pressure of homogenization exerts an important influence on the development of viscosity. Data in Figures 9 and 10 show that at identical degrees of fat solidification (same homogenization temperature), viscosity was a function of the pressure; the higher the pressure the greater the viscosity. The higher homogenization pressures produce a greater degree of fat dispersion which, in turn, furnish an increased opportunity for fat-globule contact and an increase in the fat-cluster structure. The abnormal viscosities obtained in this investigation were encountered only during the homogenization of milk high in fat (higher than 5.0 per cent).

The progressive thickening of homogenized milk described by Moore and Trout (1947) is similar to the increases in viscosity observed in this study, which attained a maximum viscosity after 3 to 4 days of storage at 45° F. The slight increase in viscosity from the time of homogenization to the third or fourth day might be explained on the supposition that the globular fat in the cluster-structure continued to solidify until a state of equilibrium was approached (van Dam, 1923; Rishoi and Sharp, 1938a, 1938b). Thus, the fat-clusters would tend to stiffen into a firm network and gradually increase in viscosity.

Data presented in Figures 8, 9 and 10 show that a large portion of the fat is in a solidified state at homogenization temperatures lower than 70° F. Such an over-all change in the physical state of the fat conceivably could explain the coalescence of fat globules and the subsequent cream plug formation which resulted from the churning-effect produced by homogenization. The internal resistance of fat globules at 60° or 50° F. is probably sufficient to prevent their dispersion on homogenization, but not great enough to destroy their tendency to coalesce or form independent clusters of fat, evident as a cream plug.

Homogenization at temperatures above 100° F., when the fat was in a liquid state, produced a uniform dispersion of the fat which was proportional to the increase in the homogenization pressure.

Postulations to explain why pasteurized milk which had been homogenized within a rather narrow critical-temperature range (70° to 90° F., depending on the heat treatment) developed an unusually high viscosity are offered: (1) the fat globules are held together by an agglutinin, which, according to the more recent theories, is adsorbed selectively on the surface of the partially solidified fat globule, and (2) the surface layer of the solid fat globules may be surrounded by semiliquid fat, a condition which would tend to make them stick together.

Whatever the mode of clustering, the probability is that the finely dispersed fat globules form an extensive and highly integrated cluster structure. Such a structure would cause an increase in the viscosity of homogenized milk over and above that accounted for on the basis of fat dispersion and the resulting increase in surface adsorption. As the fat in the individual globules solidifies in cold storage, the cluster structure stiffens and produces an additional increase in the viscosity.

SUMMARY OF SECTION II

Fat is partially solidified at homogenization temperatures (70° to 80° F., depending on the heat treatment) which are conducive to the formation of an extensive fat-globule cluster structure and an abnormally high viscosity. The amount of solidification of fat in milk which was homogenized immediately after pasteurization increased from 0.5 to 2.0 per cent when the homogenization temperature was reduced from 80° to 70° F. and decreased from 18.0 to 7.0 per cent in milk which had been stored at 45° F. for 20 hours prior to homogenization when the temperature was increased from 80° to 90° F. There was a distinct difference in the degree of solidification in the fat of the milk processed by these two methods of heat treatment, but the physical state of the peripheral layers of the fat globules were probably similar in both cases. To a large extent, the physical state of the fat governs the amount of fat-clustering at any given homogenization temperatures.

SECTION III

A PRELIMINARY REPORT ON SOME OF THE CHANGES MANIFESTED IN THE NATURE OF THE PROTEINACEOUS MEMBRANE MATERIALS CLOSELY ASSOCIATED WITH THE SURFACE OF THE FAT GLOBULE AS A RESULT OF HOMOGENIZATION

REVIEW OF LITERATURE

Nature of the Normal Fat-globule Membrane

Photographic Characteristics of the Fat-globule Membrane

The fact that a membrane surrounding the fat globule does exist was vividly demonstrated by Hansson (1949), who made electron photomicrographs of materials isolated from the surface of fat globules. As shown by these photographs, the membrane is not a continuous film which envelops the fat, but is a net-like structure probably attracted to the fat surface through its colloidal properties. Trout (1950) showed an electron photomicrograph of homogenized milk in which material associated with the fat-globule can be recognized. Prior to these data, which definitely established the existence of a fat-globule membrane, considerable research was accomplished in an attempt to establish the presence of the membrane, and more specifically, to characterize the nature of the substance or substances comprising the fat-globule membrane. Some of these data have been obtained by direct experimentation with

isolated fat-membrane and some were obtained indirectly by the determination of certain physical and chemical properties.

Composition of the Fat-globule Membrane of Nonhomogenized Milk

Lacto-fibrin and haptogenic membrane. More than 100 years ago, Ascherson (1840) postulated that the fat particles in milk were surrounded and stabilized by a "haptogenic" membrane. He attributed the formation of this haptogenic material to the capillary condensation of albumin and its subsequent aggregation on the fat surface. According to his report, he had observed this material under an ordinary microscope; a statement which has been severely criticized by Babcock (1885) and others. In spite of the criticism directed at Ascherson's hypothesis, the fundamental concepts which he advanced at a time when very little information was available concerning colloidal solutions and their physical properties have proved to be astonishingly sound.

Babcock (1885) pointed out that fat globules were considered to be either (a) "particles of free fat in the form of an emulsion with the serum of the milk," or (b) "surrounded by a thin membrane and therefore cells filled with fat," or

(c) "that the albuminous matter of the milk is attracted and in some way condensed upon their surface, forming what is called haptogenic membrane." He believed in the second condition; namely, that the fat was surrounded by a thin membrane, but he did not state how he considered this condition to be different from the haptogenic membrane postulated by Ascherson (1840). In 1889, Babcock (1889a, 1889b) reported the presence of traces of fibrin in milk which he believed to be the fat-globule stabilizing material normally found in milk. He postulated that the lacto-fibrin clots had the same effect as a true fat-globule membrane and that it had to be removed before churning could occur. Interestingly, Babcock attempted to explain the natural agglutination of fat globules by this theory. Sharp and Krukovsky (1939), some 50 years later, showed that the agglutination of fat globules exerts a major influence in the clustering of fat and subsequent creaming of normal milk. Hekma (1922) found that Babcock's lacto-fibrin was an artefact, thus making his hypothesis untenable.

Proteins. Evidence was presented by Béchamp (1896) which indicated that the fat-globule membrane consisted primarily of albumin, while Storch (1897) believed it to be a

mucin-like protein; neither casein nor lactalbumin. Abderhalden and Völtz (1909) concluded from their analysis of hydrolyzed portions of the fat-membrane material that it probably consisted of a mixture of proteins, other than casein. Van Dam and Sirks (1922) found that separated cream contained more nitrogen than was theoretically possible on the basis of its fat-free serum and that this difference probably represented the nitrogen found in the fat-globule membrane. They reported values varying from 0.08 to 0.215 per cent of the protein in milk. Hattori (1925) presented data to demonstrate that the fat-membrane material was a different protein than any of the existing known milk proteins, which he named "haptein." Haptein contained about 12 per cent nitrogen and was recovered from milk of undetermined composition at the rate of 0.01 to 0.028 per cent.

Rahn and Sharp (1928) placed considerable emphasis on a "schaumstoff" present in milk, which they believed to be responsible for the foaming properties of dairy products and probably the material adsorbed on the fat-globule surface. Titus, Sommer and Hart (1928) isolated "fat-hulls" from milk by the gravity separation method of Abderhalden and Völtz (1909) and reported a nitrogen value of 15.2 per cent for this

material. Other analyses showed that the fat-hulls and casein have nearly identical phosphorus, sulfur and tryptophan contents. From these data, it was concluded that the membrane material was probably casein. However, they were aware that the hull material could have been contaminated with other proteins since the solubility of the hull substance in sodium hydroxide was much lower than was the solubility of casein. On the basis of cataphoretic studies with normal and artificial emulsions, Prieger (1930) concluded that emulsions of natural fat have protein adsorbed at the fat/liquid interface, since their isoelectric points were lower than those of artificially emulsified fat.

Phospholipid-protein complex. In 1924, Palmer (Palmer and Samuelsson, 1924) began a very extensive investigation relating to the chemical and physical properties of the fat-globule membrane. The "membrane" was obtained from washed-cream buttermilk by an isolation procedure introduced by Storch (1897) in his studies with the fat-membrane. From their early studies, Palmer and Samuelsson (1924) concluded that the fat-membrane was composed of a mixture of globulin-like proteins and phospholipids. Later, Wiese and Palmer (1932), working with emulsions of butter oil emulsified in the various

known protein fractions of milk, concluded that normal, whole buttermilk contained a substance which had emulsifying properties far superior to the other milk fractions. Palmer and Wiese (1933) determined the isoelectric point of the washed-cream buttermilk sol to be about pH 3.9 to 4.0, and that it could be isolated free of calcium. They obtained yields of about 0.028 per cent from milk containing 3.5 per cent fat. The crude fat-membrane consisted primarily of protein, phospholipids and some high-melting glycerides. Wiese and Palmer (1934) dialyzed and analyzed a lipid-free substance from the crude membrane material and the results convinced them that they were dealing with a milk protein new to the family of milk proteins. It was especially characterized by an unaccountably low nitrogen content; about 12 per cent. Schwarz and Fischer (1936) isolated a fat-film material from washed cream and upon subsequent purification and analysis concluded that at least 30 per cent of the fat-film consisted of nonnitrogenous components. The proteinaceous component also differed in composition from the membrane protein of Wiese and Palmer (1934), the haptein of Hattori (1925) and it did not resemble any known milk protein.

Lundstedt (1934) postulated that lecithin released from the surface of the fat globule as a result of agitation was adsorbed on the calcium caseinate surface, thus causing a reduction in the curd tension in agitated milk. Palmer and Tarassuk (1936) could not lower the curd tension in milk by adding Lecithin and, therefore, concluded that the reduction of the curd tension in agitated milk was due to the release of membrane-protein material. Subsequent investigations (Tarassuk and Palmer, 1939; Palmer and Tarassuk, 1940) led them to conclude that the reduction in curd tension which accompanied the agitation of milk was the result of two different phonomena; namely, (a) the partial destruction of the fat-globule membrane protein, and (b) the liberation of natural esterases which prevented normal rennet clotting.

Rimpila and Palmer (1935) analyzed the fat-membrane material isolated from the washed-cream buttermilks from mixtures of re-emulsified butter oil and milk protein. They concluded that the artificially prepared membranes were not similar to the fat membrane found in normal milk. They obtained nitrogen values for the purified, adsorbed proteins that were somewhat lower (12.35 to 13.75 per cent) than the nitrogen

values found to be present in the milk protein fraction which served as the emulsifying medium. They could not account for this difference on the basis of their analytical data, because the preparations were free of cholesterol, carbohydrates and large amounts of inorganic material. All of the membrane preparations contained proteins, phospholipids and ether extractable nonphospholipid materials. They emphasized the significance of the latter component in that it comprised over 50 per cent of the crude membrane.

Jack and Dahle (1937b) measured the electrokinetic mobilities of fat globules in milk diluted 1 part to 200 parts with a buffer and from these values derived an isoelectric point of pH 4.3 for normal fat globules. They concluded that since the isoelectric point of the known milk proteins was in the range of pH 4.5 to 4.6, the major component of the surface layer of the fat must be protein, but that some other material with a lower isoelectric point, i.e., phospholipid with an isoelectric point of pH 2.0, was also present. They demonstrated that the isoelectric point, as determined by electrokinetic mobility measurements, was shifted from a normal of pH 3.3 to 4.6 when fat was dispersed in a casein sol after the addition of

phospholipids, but that a value similar to that for normal fat globules (pH 4.3) was obtained when the casein sol was added to the fat-phospholipid emulsion. From these results, it was concluded that a double-layer membrane—phospholipid/protein—probably existed on the surface of the fat globule. Electrokinetic mobility determinations made by Moyer (1940) on fat globules in unwashed and washed cream showed the isoelectric points to be pH 4.6 and 3.7 to 3.8, respectively. These observations, as well as those of Jack and Dahle (1937b) support Palmer's theory on the composition of the fat-membrane, in which he believed it to be a phospholipid-protein complex.

Phospholipid-protein-glyceride complex. Jenness and Palmer (1945a, 1945b) reported data which confirmed the published data of Rimpila and Palmer (1935) in that protein and phospholipids accounted for only 40 to 65 per cent of the membrane material. They found that the normal fat-membrane contained from 0.46 to 0.86 grams of protein for every 100 grams of fat, or 34 to 49 milligrams of protein for every 100 square centimeters of fat surface, whereas cream which had been washed six times contained from 8.9 to 16.3 milligrams of protein per 100 grams of fat, or 0.57 to 0.86 milligrams of protein

per 100 square centimeters of fat surface. Their data showed that the major portion of the phospholipids was retained in the butter after churning the washed-cream. They also isolated a high-melting glyceride (52° to 53° C.), strongly attached to the protein-phospholipid complex, which accounted for about one-half of the membrane material. The significance of this finding was emphasized in a recent report by King (1950) who showed that a layer of liquid fat, presumably the higher melting fractions of the butterfat, orients itself at the surface of the fat globule. Hilditch and Maddison (1941) identified the major fat acids of phosphatides as oleic and palmitic. The association of these acids may well explain the orientation of phosphatides on the fat surfaces. Palmer (1944) summarized his work on the fat-membrane material with the following statement: "The fat globules in cows' milk are wholly or partially surrounded by a special group of substances whose origin may be due, in part, to their greater capillary activity. The other surface-active substances occurring in major concentration in milk plasma evidently constitute the outer layer of the fat globule surfaces if indeed they are normally concentrated there at all.

The latter are readily removed when cream is washed by dilution with water."

Serum proteins. Townley and Gould (1943) reported that thrice-washed cream was nearly as high in volatile sulfur as the original cream, although the total sulfur content was reduced to about one-half and the albumin nitrogen content was reduced to about one-ninth. From these data, they concluded that the protein material intimately associated with the fat globule contained volatile sulfide-containing proteins. King (1947) has shown that about 60 per cent of the fat in butter remains in the form of globular fat which in all probability is still covered with its natural surface material.

Various minor constituents in milk have been associated with the fat-globule surface. Evidently most of these materials are either highly surface-active or have a specific attraction or function in connection with the fat globule.

Flavoprotein. Toyama (1932) reported that a flavin-like xanthine oxidase was part of the crude membrane of the fat globule. Sharp and Hand (1940) found 70 milligrams of xanthine oxidase per 100 grams of fat and that about one-half of it

remained attached to the fat after six washings. They concluded that the flavoprotein was heat-labile at about 176° F., since none could be isolated from fat pasteurized at this temperature.

Copper-containing proteins. Davies (1933) found that dissolved copper and iron readily combined with the fat membrane. The significance of this finding in relation to the development of the lipid-type oxidized flavor in dairy products is evident. Perhaps the copper-protein isolated from milk by Dills and Nelson (1942) was associated with the fat membrane in some manner. In a unique approach, King (1951) pointed out that all of the fat globules in milk are not surrounded with a stabilizing hydrophilic membrane, but that some are hydrophobic.

Agglutinin. Sharp and Krukovsky (1939) showed that the agglutinin-type material, which Dunkley and Sommer (1944) identified as euglobulin, was selectively adsorbed on the surface of solidified fat globules and released into the plasma as the fat was liquified.

Carotenoids. Kon, Mawson and Thompson (1944) believed that milk carotenoids were adsorbed on the surface of the fat

globule. This was concluded from the observation that the fat obtained from buttermilk was richer in carotenoids than was the original milk fat. The average size of the fat globules obtained from buttermilk was much less than the average size of milk fat globules, hence the concentration of carotenoids in buttermilk fat was thought to be due to a greater adsorption area per unit of mass.

Leucocytes. Peters and Trout (1945a, 1945b) published a series of papers in which they showed how leucocytes can attach themselves to the fat in milk. They attributed such a phenomenon to an electrostatic attraction resulting from a difference in the charge on the fat globule and the leucocyte.

Phosphatases and lipases. Kay and Graham (1933) were aware that the milk phosphatases were closely associated with the fat. Rimpila and Palmer (1935) found that 50 per cent of Kay and Graham's phosphatase remained in cream which had been washed six times, probably strongly attached to the fat membrane. Krukovsky and Herrington (1939) observed that lipase activity depended to a large extent on the crystalline state of the globular fat and that milk could be activated or

deactivated by suitable heat treatment. Later, Krukovsky and Sharp (1940) demonstrated that resurfacing the fat globules, as a result of homogenization, enhanced lipolysis.

General comments. In view of the above reports, Sommer's (1951) statement: "it is rather startling that the efforts generally have been to identify the protein associated with the fat globules as a single protein," seems most appropriate. Mulder (1949) stated: "The surface layer must not be regarded as having been adsorbed from milk plasma; it consists of components of protoplasma of the cells of the lactiferous glands and a substance adsorbed from the milk plasma."

Some Changes in the Fat Globule Resulting from Homogenization

Changes in the Physical Dimensions

Wiegner (1914) presented data showing a 500- to 600-fold increase in the total number of fat globules as a result of homogenization and that each globule was divided into approximately 1,200 small globules. He calculated that the ratio of the surface area of the fat in homogenized milk to nonhomogenized milk was about 115 to 1. Sommer (1935) calculated

that a fat globule 6 microns in diameter would yield 216 smaller globules 1 micron in diameter on homogenization. Trout (1947) calculated the surface area of the fat globules in 1 milliliter of normal milk (average diameter of fat globule = 5 microns) to be about 377 square inches, which on homogenization increased to 1,885 square inches, or a 5-fold increase.

Milk Components Adsorbed on the Increased Surface of Homogenized Fat

Phospholipids. Sommer (1951) calculated, from unpublished data of El Rafey (1951), the amounts of phospholipid that theoretically could be adsorbed as a monolayer when the fat globules varied from 0.1 to 10.0 microns in diameter. He reported that a fat globule 1 micron in diameter would require 2.58 grams of phospholipids per 100 grams of fat. The data of Crane and Horrall (1943), however, showed a concentration of only 0.7 grams of phospholipids per 100 grams of fat in normal milk. Therefore, the major portion of the newly formed fat surface, created by homogenization, is left exposed to adsorb one or more of the ingredients found in milk plasma.

Proteins. Wiegner (1914) calculated the amount of casein adsorbed on the fat surface in nonhomogenized and homogenized milk to be about 2 and 25 per cent, respectively. He assumed that casein was the sole substance adsorbed. Doan (1938) believed that the decrease in the heat stability of homogenized milk resulted from an increase in the amount of casein adsorbed on the enlarged fat surface; that the adsorbed casein was fixed and, therefore, lost its stabilizing ability. Chambers (1936) reported that the reduction in curd tension accompanying homogenization resulted from an increase in the number of fat particles which served to weaken the curd matrix and provide an increased area upon which the plasma proteins become fixed.

Minor-protein fraction. Weinstein and Trout (1951) explained the increased susceptibility of homogenized milk to develop the solar-activated oxidized flavor on the basis that some of the oxidizable milk components were redispersed on homogenization and adsorbed on the newly formed surfaces.

Manifestations of the Adsorption of Various Milk Components on the Surface of Homogenized Fat

Lipolysis. Krukovsky and Sharp (1940) noted that re-emulsified fat (resurfaced) was attacked differently by milk lipase than was the normal fat emulsion. Lipolysis was enhanced and possessed a positive temperature coefficient, while lipolysis in the normal emulsion exhibited a negative temperature coefficient. Moore and Trout (1947) believed that the increase in viscosity of milk homogenized at lower temperatures was due to an increase in the adsorption of proteins on the fat globule.

Oxidation. Tracy, Ramsey and Ruehe (1933) discovered that homogenization retarded and inhibited the development of the copper-induced oxidized flavor, whereas Hood and White (1934) and others have shown that homogenization enhanced the development of the daylight-activated oxidized flavor. Thurston, Brown and Dustman (1935) have shown that the copper-induced oxidized flavor in milk originates with the phospholipids which are almost entirely oriented on the globule surface. Weinstein, Duncan and Trout (1951) demonstrated that the milk component most probably activated by daylight was a water-soluble,

surface-active protein. Therefore, homogenization must cause some rearrangement of the materials on the fat-globule membrane.

Electrokinetic mobility. Jack and Dahle (1937a) and Dahle and Jack (1937) reported electrokinetic mobility values for pasteurized, nonhomogenized and homogenized milk at 2.53 and 2.65 micra per second per volt per centimeter, respectively. Since their data also showed that the size of the fat globule exerted little influence on mobility, it can be assumed that the increased mobility of the homogenized fat globule is derived from the adsorption of additional proteins.

Surface tension. Numerous data are available which indicate indirectly that a change in the dispersion of protein accompanies homogenization. Webb (1933) reported that homogenization of pasteurized milk was accompanied by an increase in the surface tension of the milk. As the homogenization pressure was increased and the degree of fat dispersion became greater, a corresponding increase in surface tension was noted. Possibly the surface-active proteins comprising the milk plasma were being adsorbed or fixed on the fat surface, with a cor-

responding reduction in concentration in the serum. Trout, Halloran and Gould (1935) presented evidence to show that the foaming capacity of pasteurized milk was increased by homogenization which indicated that the newly dispersed fat was covered with materials which allowed it to enter the foam lamelae without interfering with the foam structure. According to the theory of King (1951), the amount of free fat (hydrophobic) was reduced by homogenization, thus alleviating its anti-foam characteristics.

Protein stability. Trout (1950) concluded, "from the effects of homogenization on some of the physical properties of milk, it may be presumed that a film of protein materials is adsorbed to the surfaces of the homogenized fat globules. Thus, the newly created fat globules are 'resurfaced.' The adsorbed layer appears to be different from that on normal fat globules." In addition to the more direct indications that the fat globule is resurfaced by homogenization, there is evidence to show that homogenization reduces the stability of homogenized milk to heat coagulation (Doan, 1938; Doan and Minster, 1933), alcohol (Halloran, 1932) and various casein precipitants (Sullam, 1942). The loss in the stability of the protein was thought

to be due to an increase in the calcium-ion concentration in the plasma, as well as to fat-clumping (Doan, 1929b; Doan and Minster, 1933; Doan, 1931). In all cases, the possibility of the adsorption of one or more protein components on the surface of homogenized fat has been discussed.

Considerable evidence is available in the literature relating to the processing of ice cream which shows that the fat globules are resurfaced by homogenization. A review of the literature in this field is beyond the scope of this study, but it suffices to say that efficient, permanent homogenization of ice cream mixes made from butter oil require the addition of emulsifiers which orient themselves at the fat/plasma interface, along with the serum solids of the mix to stabilize the fat emulsion.

Methods Used to Study the Protein Components of Nonhomogenized and Homogenized Milk and Their Respective Fat-globule Membrane Proteins

Nitrogen Distribution

Titus, Sommer and Hart (1928) characterized their "fat-hull" protein according to Van Slyke's system of nitrogen

distribution. Wiese and Palmer (1934) determined the nitrogen distribution in their fat-globule "membrane" protein by Morrow and Sandstrom's (1935) modification of the method of Van Slyke. They also compiled Van Slyke-nitrogen values for Hattori's (1925) "haptein," casein, lactalbumin, globulin and Osborne and Wakeman's (1918) and Linderström-Lang's (1929) values for alcohol-soluble proteins for comparison with their "membrane"-protein. Rimpila and Palmer (1935) reported similar data for the "membrane"-proteins of re-emulsified butterfat.

Rowland (1938) reported a method for determining the distribution of casein, albumin, globulin, proteose-peptone and nonprotein nitrogen in milk and studied the effect of heat on the various nitrogen fractions. Hetrick (1947) employed a modification of Rowland's procedure to study the effect of "mallorization" on the nitrogen distribution in milk. Menefee, Overman and Tracy (1941) followed Rowland's system of nitrogen distribution but employed their own (Menefee and Overman, 1940) semimicro-Kieldahl technique for determining nitrogen to study the effect of homogenization on the nitrogen distribution in milk. More recently, Shahani and Sommer (1951a, 1951b) determined the protein distribution in homogenized milk by

using a fractionation procedure based principally on the technique of Rowland (1938). Their procedure for determining globulin nitrogen differed from Rowland's (1938) method in that methanol was added to the noncasein filtrate to precipitate the globulin. They also made a detailed study of the influence of pasteurization and homogenization on the nonprotein nitrogen fractions. None of the above investigators was able to demonstrate any significant change in the nitrogen distribution in pasteurized milk, but Shahani and Sommer (1951b) showed a decrease in globulin nitrogen and an increase in alpha amino nitrogen in milk pasteurized at 155° F. for 30 minutes and homogenized at 2,000 pounds pressure.

Elementary Analysis

In general, research workers who have attempted to characterize proteins have analyzed for the percentage composition of nitrogen, carbon, hydrogen, phosphorus, sulfur, calcium, ash and oxygen. These data provide valuable keys in determining the relationship between proteins. Palmer and Wiese (1933) and Wiese and Palmer (1934) presented data of this nature for many of the proteins reputedly associated with the fat-

globule membrane derived from normal whole milk. Weinstein, Duncan and Trout (1951) presented similar data for a minor-protein fraction which was thought to be adsorbed on the surface of the fat in homogenized milk and compiled corresponding data from the literature for other known proteins of milk.

Amino Acids

The literature relating to the amino acid composition of the major and minor proteins isolated from milk is quite extensive. Microbiological, chromatographic and chemical techniques have been employed to the advantage of the respective investigators. Some of these data have been compiled by Weinstein, Duncan and Trout (1951). From data of this type it is possible to determine the minimum molecular weight for a protein or protein complex and draw conclusions concerning the characteristics of the protein. Fat-globule membrane proteins have never been characterized by this method.

Electrophoretic Characteristics

Several investigators have studied the cataphoretic characteristics of fat globules whose electrokinetic mobility is

largely determined by the nature of the material closely associated with the fat globule. Mohr and Brockman (1930) determined the isoelectric point of fat globules in an acetate buffer to be about pH 4.3. They were not able to detect any change in the isoelectric point as a result of pasteurization or homogenization, but the addition of casein raised the isoelectric point to pH 4.44. North and Sommer (1935), using streaming potential measurements, determined the isoelectric point of fat globules to be about pH 4.3. Jack and Dahle (1937a, 1937b) and Dahle and Jack (1937) determined the isoelectric point of normal fat globule in a 1 to 200 dilution to be pH 4.3. Their data indicated a slight increase in the electrokinetic mobility for homogenized milk fat—2.53 for normal fat and 2.65 micra per second per volt per centimeter for homogenized fat. They found that milk fat re-emulsified in a phospholipid sol had an isoelectric point of about pH 2.0—the isoelectric point of phospholipids—whereas the addition of a casein sol shifted the isoelectric point toward that of the isoelectric point of normal fat globules. From these data, they postulated the presence of a double-layer membrane on the surface of the fat globule, consisting of phospholipid and protein.

Moyer (1940) reported the isoelectric points of fat globules from washed cream and normal milk to be pH 3.8 and 4.6, respectively. This observation indicated that some of the membrane material had been lost in the washing process. Dunkley and Sommer (1944) determined the isoelectric point of normal fat globules to be pH 4.3 to 4.5.

No carefully controlled experimental evidence is available to show the specific effect of homogenization on the electrokinetic mobility of fat globules. Furthermore, there are no data available showing the electrokinetic characterization of isolated membrane-proteins and only a limited number of electrophoretic studies have been made on the various plasma and serum proteins of milk, skimmilk and whey.

Heyndrickx and De Vleeschauwer (1951) published electrophoretic patterns for total milk proteins which showed eight components when a sodium veronal buffer system was used at pH 8.0. Of these, two were identified as casein components with mobilities of 7.5×10^{-5} and 3.6×10^{-5} and another as globulin with a mobility of 1.9×10^{-5}. Deutsch (1947) reported the presence of six components in whey and that the patterns showed marked alterations following parturition in the cow.

Smith (1946a, 1946b) made an electrophoretic study of the immune globulins in bovine colostrum and whey and found that their concentration was highest in colostrum; diminishing rapidly following parturition. He also reported six whey components and believed that the component commonly referred to as lactalbumin was mostly Palmer's beta-lactoglobulin which comprised about 60 per cent of the total whey protein. Pedersen (1936) believed that Palmer's beta-lactoglobulin was homogeneous, however, Li (1946) studied the material electrophoretically in acetate buffers ranging from pH 5.3 to 5.6 and concluded that there were three distinct components present. He recorded the isoelectric point of the major component as pH 5.1.

Stanley, Andrew and Whitnah (1950, 1951) found six components in whey and identified three of them as euglobulin, pseudoglobulin and beta-lactoglobulin. Slatter and Van Winkle (1950) were able to identify four distinct components on the descending side of an electrophoretic pattern obtained at pH 6.6. They noted that ionic-strength values lower than 0.5 were conducive to better resolution. Kemp, Johnson and Swanson (1950) prepared protein fractions from whey by chemical procedures and studied their purity by electrophoretic analysis.

Weinstein, Lillevik, Duncan and Trout (1951) characterized a minor-protein fraction electrophoretically which had been isolated from a whey proteose-peptone fraction that was found to play a role in the solar-activation of homogenized milk.

The casein of milk has been studied electrophoretically by numerous workers (Mellander, 1939; Warner, 1944; Hipp, Groves, Custer and McMeekin, 1950; and others) who reported that casein is made up of three components; about 75 per cent alpha, 22 per cent beta and 3 per cent gamma. These percentages may be altered by enzyme activity, heat, iodination and other treatments (Warner and Polis, 1945; Kamal and Turner, 1951). Krejci (1942) obtained an alcohol-acid-soluble fraction from milk casein which was characterized electrophoretically as 75 per cent alpha, 15 per cent beta and 10 per cent gamma.

Summary of the Review of Literature

Nature of the Fat-globule Membrane

The early literature has classified the fat-membrane as lacto-fibrin, "haptogenic" material, "haptein," special-protein, mucin-like casein, albumin, a mixture of proteins other than casein, foam-material, phospholipid-protein complex

which was closely associated with a high-melting glyceride, agglutinin and a galaxy of minor constituents which include xanthine oxidase, phosphatase, carotenoids, cholesterol and possibly a copper-containing minor protein. These materials were believed to be specifically adsorbed on the surface of the fat as a part of the fat-membrane or were oriented there because of their high degree of capillarity. This adsorbed layer, which constitutes the fat-membrane, has been estimated to range from 4 to 12 millimicrons in thickness and comprised from 0.08 to 0.3 per cent of the total milk proteins.

Effect of Homogenization on Some of the Characteristics of Milk

Homogenization of milk results in approximately a 100-fold increase in the total number of fat globules and a corresponding 5- to 8-fold increase in the total surface area of the fat. Certain characteristics of homogenized milk, such as a decrease in its stability to heat and reduced curd tension, have encouraged investigators to postulate that one or more of the milk plasma-proteins are adsorbed on the newly formed fat surfaces of homogenized fat. Changes in the susceptibility of homogenized milk to copper-induced and solar-activated types

of oxidation also furnished additional evidence regarding the alteration of the surface of homogenized globular fat.

Methods Available for the Analysis of the Fat-globule Membrane

The majority of the direct evidence relating to the nature of the fat-membrane was obtained from elementary analyses and Van Slyke nitrogen values obtained from fat-membrane preparations which had been isolated from normal milk fat. Considerable information concerning the nature of the fat membrane was obtained by observing the electrokinetic properties of the fat globules dispersed in normal, washed and remade substrate.

In view of the paucity of information in the literature concerning the composition of the fat-globule membrane in non-homogenized milk and more specifically, in homogenized milk, experiments were conducted to isolate the membrane proteins in both homogenized and nonhomogenized milk, determine some of the amino acids and to observe also the patterns of the electrophorized proteins in various buffer solutions.

EXPERIMENTAL PROCEDURE

The milk used in this study was pooled herd milk which had been received and processed in the Michigan State College Creamery during the summer months. The average composition was 3.80 per cent fat and 12.30 per cent total solids.

Preparation and Determination of Nitrogen Fractions in Homogenized Milk

The milk was homogenized at various pressures ranging from 1,000 to 4,000 pounds per square inch immediately following pasteurization. The nitrogen fractions were separated as soon as possible after processing and stored at 45° F. in stoppered tubes until the nitrogen determinations could be made. Nitrogen was determined by a semi-micro Kjeldahl method similar to that reported by Menefee and Overman (1940). Each nitrogen value reported in the data represents the average of duplicate determinations that did not vary beyond experimental error.

Four nitrogen fractions were determined: total nitrogen, noncasein nitrogen, nonheat-coagulable nitrogen and nonprotein

nitrogen. Casein nitrogen was obtained by subtracting the non-casein nitrogen from the total nitrogen, the heat-coagulable nitrogen was obtained by subtracting the nonheat-coagulable nitrogen from the noncasein nitrogen and the proteose peptone nitrogen was obtained by subtracting nonprotein nitrogen from the nonheat-coagulable nitrogen. The various nitrogen fractions were prepared as follows:

(1) Total nitrogen. Pipette 5 milliliters of milk, brought to 20° C., into a 100-milliliter volumetric flask, dilute to volume with distilled water and mix thoroughly. A 10-milliliter aliquot, equivalent to 0.5 milliliter of milk, was used for the nitrogen determination.

(2) Noncasein nitrogen. Pipette 20 milliliters of milk into a 100-milliliter volumetric flask. Add 55 to 60 milliliters of distilled water, 3 milliliters of 1 N acetic acid, mix and allow to stand for 15 minutes. Adjust the pH to 4.67 ± 0.05 with 1 N sodium hydroxide, allow to stand for 45 minutes, mix and filter through a dry Whatman No. 42 filter paper. A 10-milliliter aliquot, equivalent to 2 milliliters of milk, was used for the nitrogen determination.

(3) Non-heat-coagulable nitrogen. Pipette 25 milliliters of the casein-free filtrate from (2) into a 50-milliliter volumetric flask, adjust the pH to 4.67 ± 0.05 and place the flask in a boiling water bath for 45 minutes. Cool to room temperature, transfer the contents to a 25-milliliter volumetric flask, make to volume and filter through a dry Whatman No. 42 filter paper. A 10-milliliter aliquot, equivalent to 2 milliliters of milk, was used for the nitrogen determination.

(4) Nonprotein nitrogen. Pipette 10 milliliters of milk into a 50-milliliter volumetric flask, make to volume with 15 per cent trichloroacetic acid, mix thoroughly, allow to stand until the precipitated proteins have settled (ca 15 to 20 minutes) and filter through a dry Whatman No. 42 filter paper. A 20-milliliter aliquot, equivalent to 4 milliliters of milk, was used for the nitrogen determination.

Preparation and Determination of Milk Solids, Nitrogen and Minerals in Skimmilk and Cream from Nonhomogenized and Homogenized Milk

Pasteurized milk was separated at 120° F. into cream and skimmilk by means of a semicommercial, mechanical separator. Part of the milk was homogenized at 130° F. at 2,500

pounds pressure (USPHS Index less than 10) and separated with the aid of a flow-reducing device placed in the intake to the separator bowl. The cream and skimmilk samples obtained from both milks were tested by standard Mojonnier procedures for total solids and fat. Ash was determined by standard procedures. Calcium was determined by Shohl's (1922) modification of the Kramer-Tisdall method. Phosphorus was determined by the Fiske and Subbarow method (1925). Phospholipid phosphorus was determined according to the method of Wiese, Nair and Fleming (1932). The nitrogen fractions for each sample were determined as outlined above.

Preparation and Determination of Milk Solids and Nitrogen in Washed Cream and Washed-cream Serum from Nonhomogenized and Homogenized Milk

The cream samples obtained from nonhomogenized and homogenized milk were diluted free of milk plasma by the cream-washing technique of Storch (1897), as modified by Palmer and Wiese (1933) and others. The separated cream was diluted with three volumes of distilled water, agitated gently for 30 seconds and then reseparated. This procedure was repeated six times in order to remove all of the plasma proteins,

as indicated by a faintly positive or negative biuret test in the wash water. Samples of cream from selected washings were saved for the determination of fat, total solids, the nitrogen fractions and lipoid phosphorus.

The washed creams were stored at 17° C. for 20 hours and then churned. The butter was melted in a water bath maintained at 45° C. to free the fat from the serum. The serum was then combined with the buttermilk-like serum to make up the washed-cream serum sample. Fat, total solids and the nitrogen fractions were determined on the washed cream.

Preparation of the Fat-globule Membrane Proteins for Electrophoretic, Microbiological and General Characterization

Fat-globule membrane proteins were prepared according to the flow diagram shown in Figure 11. Forty gallons of nonhomogenized milk and 70 gallons of homogenized milk were separated in order to provide an adequate working supply of fat-membrane proteins. The isolation procedure was somewhat similar to that used by Palmer and his colleagues (Palmer and Samuelsson, 1924; Palmer and Wiese, 1933; Rimpila and Palmer,

1935; Jenness and Palmer, 1945a). Palmer used a Sharples supercentrifuge to free the washed-cream serum from gross amounts of fat. The extraction of the fat was completed by treating the isoelectric, dialyzed membrane-proteins with successive portions of acetone, hot absolute ethanol and purified ethyl ether. In the present study, the lipid materials were extracted with a cold ethanol-ether extraction technique similar to that used by Hardy and Gardiner (1910) to separate the lipids from blood proteins. In two preparations of the fat-membrane proteins, a warm (40° C.) ethanol-ether treatment was substituted for the cold extraction to study the effect of this treatment on the characteristics of the protein. The ethanol-ether extraction technique used in this investigation was as follows:

(1) Three volumes of cold (0° to 3° C.) absolute ethanol were added to the cold (0° to 5° C.) dialyzed fat-membrane proteins. This mixture was agitated for 10 minutes by means of a mechanical stirrer and then filtered through a pleated Whatman No. 2 filter paper.

(2) The residue was washed three times with cold (0° to 3° C.) purified ethyl ether to remove the alcohol from the precipitated residue.

(3) Three volumes of ethyl ether, based on original volume of dialyzed membrane-proteins, were added. The temperature of mixture was adjusted to 40° C. and agitated for 15 minutes. The mixture was transferred to a separatory funnel to allow the precipitate to settle. The precipitate was drawn off and extracted two more times. The final separation of precipitated fat-membrane proteins and ether was accomplished by filtration through a No. 2 Whatman paper.

(4) The protein residue on the paper was washed several times with ether, redispersed with a small amount of distilled water and lyophilized.

The electrophoretic characteristics of the lyophilized fat-membrane proteins were studied with the aid of a Perkins-Elmer, Model 38, Tiselius electrophoresis apparatus as described by Moore and White (1948). The principle of its operation is based on Longsworth's (1942) scanning modification of the Toepler-Schlieren method. A Tiselius-type cell with a capacity of 2 milliliters and a cross-sectional area of 0.3 square centimeters was used to contain the protein solution and in which the moving boundaries of the various protein components are formed.

Electrophoretic solutions of the fat-membrane proteins were prepared by adding exactly 0.1 gram of the lyophilized sample to 10 milliliters of the desired buffer in a 15-milliliter centrifuge tube. The contents of the tube were mixed, allowed to stand for approximately 24 hours at 18° C. and then centrifuged at 2,000 revolutions per minute for one-half hour. The supernatant aquasol was dialyzed in a cellophane sac against 600 milliliters of buffer, maintained at 5° C. for 6 hours with continuous agitation, until constant conductivity was obtained on both sides of the cellophane membrane. A solution of the equilibrated fat-membrane proteins was used for electrophoresis at 1.2° C. The concentration was based on the micro-Kjeldahl determination for nitrogen.

The membrane samples were characterized electrophoretically in the following buffer systems: glycin-hydrochloric acid, pH 1.5; acetate, pH 3.0; phosphate, pH 6.5; veronal-citrate, pH 8.0; ammonia-hydrochloric acid, pH 9.0; and veronal-ethylamine, pH 10.8. The buffer solutions were prepared as follows:

(1) Glycine-hydrochloric acid buffer substrate. Dissolve 10.05 moles of glycine in 500 milliliters of distilled water,

adjust the pH to 1.5 with hydrochloric acid and dilute to one liter.

(2) Acetate buffer substrate. Dissolve 0.1 mole of sodium acetate in 500 milliliters of distilled water, adjust the pH to 3.0 with acetic acid and dilute to one liter.

(3) Phosphate buffer substrate. Dissolve 0.02 mole of monohydrated sodium phosphate and 0.011 mole of dibasic sodium phosphate in distilled water and dilute to one liter.

(4) Veronal-citrate buffer substrate. Dissolve 0.1 mole of sodium diethyl barbiturate and 2.25 grams of sodium citrate in 500 milliliters of distilled water, adjust to pH 8.0 with citric acid and dilute to one liter.

(5) Ammonia-hydrochloric acid buffer substrate. Add 0.1 molar hydrochloric acid solution to 0.2 molar ammonium hydroxide solution until the solution tests pH 9.

(6) Veronal-ethylamine buffer substrate. Dissolve 0.2 mole of ethylamine and 0.1 mole of diethylbarbituric acid in 500 milliliters of distilled water and dilute to one liter.

The buffers were calculated to be approximately 0.1 ionic strength, except the veronal-citrate, which was 0.09.

At any given pH, temperature and salt concentration, the migration of a given protein boundary per unit of time (t = sec.) depends upon the potential gradient (F). The potential gradient is calculated as follows:

$$F = i/ak$$

where F = the potential gradient,

i = current in amperes,

a = the cross-sectional area of the U-cell, and

k = the specific conductivity of the buffer protein solution.

The speed of migration, or the electrophoretic mobility (u), is defined as the distance moved in square centimeters per second under a potential gradient of one volt per centimeter in a specific buffer system and may be expressed by the following equation:

$$u(cm.^2/volt, sec.) = dak/it$$

where u = mobility in square centimeters per volt per second,

d = distance moved by the protein boundary,

a = cross-sectional area of the U-cell,

k = specific conductivity of protein-buffer solution (k = cell constant/resistance in ohms),

i = current in amperes, and

t = time in seconds.

Amino acid determinations were made by microbiological techniques by using Lactobacillus arabinosis, Streptococcus faecalis and Leuconostoc mesenteroides P-60. The media used in the various determinations were essentially the same as those described by Sauberlich and Baumann (1946) with the exception of those used for isoleucine and methionine, which were prepared according to the method of Kuiken et al. (1943) and Lyman et al. (1946), respectively. The hydrolyzates for the determination of isoleucine, leucine, lysine, methionine, phenylalanine and valine were prepared according to the method of Stokes et al. (1945). One gram of the fat-membrane proteins was dispersed in 25 milliliters of 6 N hydrochloric acid and autoclaved for 8 hours at 15 pounds pressure.

DL-Configurations of isoleucine, leucine, methionine, phenylalanine and valine were used in the preparation of standards for the amino acids, whereas the L-configuration was used for the preparation of a standard for lysine. All assays were run after the proper dilutions had been made.

Certain characteristic information such as the per cent of nitrogen, solubility in different buffer systems, physical appearance in the dry and hydrated forms, the Molisch, biuret and nitroprusside reactions of the fat-membrane proteins were also determined.

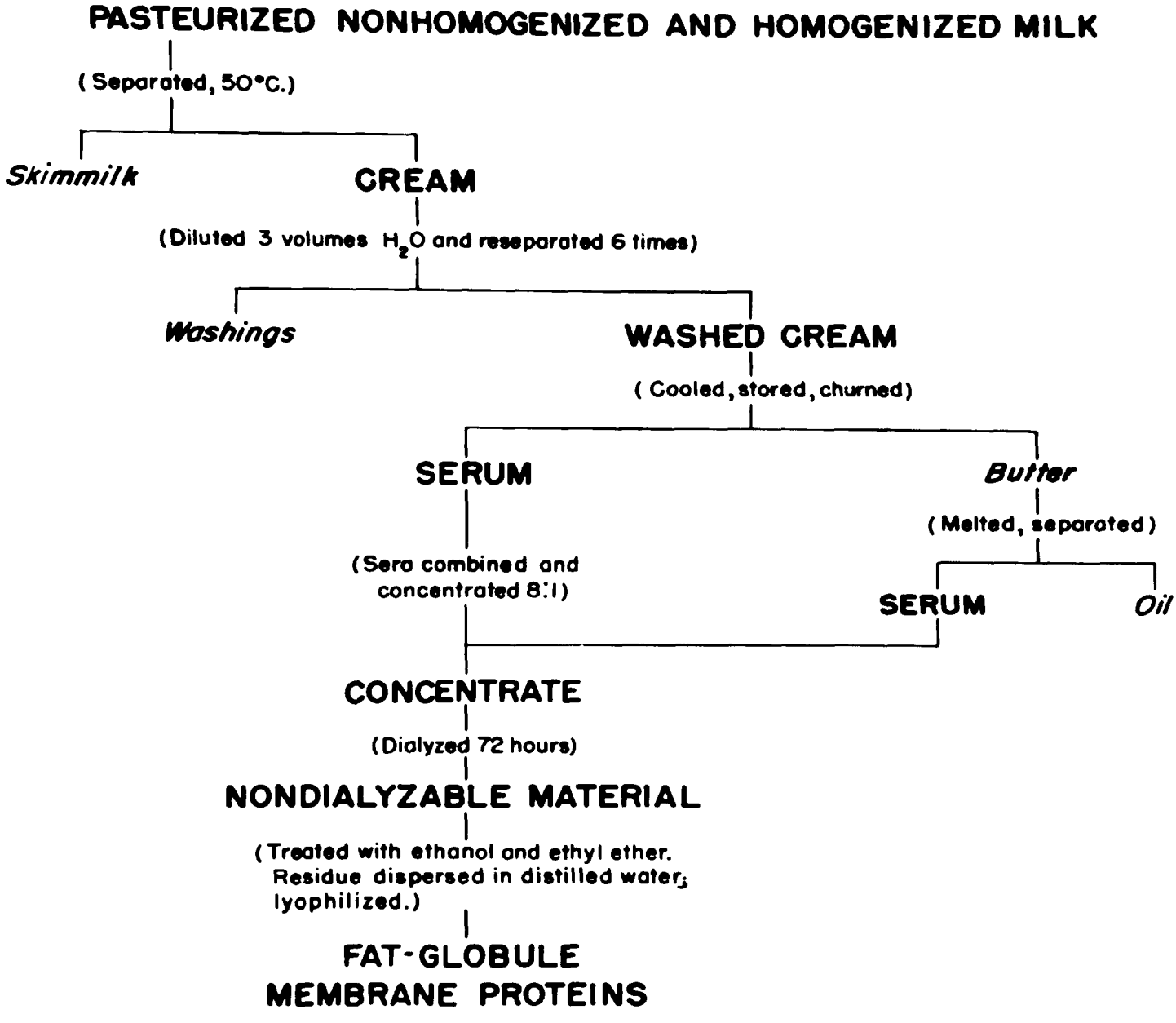

Figure 11. Flow sheet showing the procedures followed in the preparation of fat-globule membrane proteins.

RESULTS

Nitrogen Distribution in Homogenized Milk

The influence of homogenization on the distribution of nitrogen in various milk proteins in pasteurized milk is shown in Table 10. The results are expressed as milligrams of nitrogen per 100 milliliters of milk, but the values can be converted to the percentage of protein by multiplying by the factor 6.38, if desirable. The values for the amount of nitrogen in the various nitrogen fractions obtained from milk homogenized at 1,000 and 2,500 pounds pressure, show little variation from the corresponding values obtained for pasteurized milk when the values are expressed as percentage of the total nitrogen. The slight variations that were apparent in the homogenized milk, i.e., an increase in the amount of casein and nonprotein nitrogen accompanied by a decrease in the quantity of noncasein and heat-coagulable nitrogen, are in agreement with the results obtained by Menefee, Overman and Tracy (1941) and Shahani and Sommer (1951b).

Milk which had been homogenized at 4,000 pounds pressure, however, showed increases of 6.57 per cent in nonprotein nitrogen and 0.88 per cent in casein nitrogen and decreases of 3.96 per cent in noncasein nitrogen, 5.26 per cent in heat-coagulable nitrogen and 7.15 per cent in proteose-peptone nitrogen in comparison to milk pasteurized at 143° F. for 30 minutes. The temperature of the milk homogenized at 1,000, 2,500 and 4,000 pounds pressure was 145°, 149° and 153° F., respectively. Whether the increase in temperature contributed to the alteration in nitrogen distribution is not known. The data of Shahani and Sommer (1951b) show little difference between the nitrogen fractions of raw milk and milk pasteurized at 155° F. for 30 minutes. Yet, Rowland (1933-34) showed that the pasteurization of skimmilk at 62.5° C. for 30 minutes caused a 10 per cent decrease in the albumin-globulin nitrogen fraction.

Distribution of Milk Solids, Nitrogen and Minerals in Skimmilk and Cream from Nonhomogenized and Homogenized Milk

Data showing the percentage of total milk-solids, fat and solids-not-fat in cream and skimmilk which were separated from pasteurized milk before and following homogenization at

2,500 pounds pressure are recorded in Table 11. The contents of fat and total solids in skimmilk and cream separated from nonhomogenized milk are typical and indicate an efficient separation, whereas the amounts of fat and total solids in the skimmilk and cream separated from the homogenized milk show a different pattern.

In previous studies, Trout et al. (1935) showed that conventional separation of homogenized milk was not an efficient means of recovering the fat in the cream. In this study, a flow-reduction device was placed in the separator assembly ahead of the bowl to increase the efficiency of the centrifugal force of the separator. Such a procedure resulted in the separation of a relatively high-test cream (46.60 per cent fat) and a skimmilk testing 1.91 per cent fat. This represents, roughly, a 50 per cent recovery of the total available fat in the homogenized milk. From these data the theoretical percentage of solids-not-fat was determined for each of the two cream samples by the expression:

[100 - percentage of fat in cream]/100 x
percentage of solids-not-fat in skimmilk.

Nitrogen distribution determinations for the skimmilk and cream samples reported in Table 11 are tabulated in Table

12. The determined nitrogen values, in milligram per cent, are also expressed as "plasma-corrected" and "fat-free" nitrogen. The plasma-corrected values, which express the nitrogen concentrations in cream on a plasma-free basis, showed that a major difference exists between the total nitrogen values of the respective cream samples, which is manifested largely as casein nitrogen. Similarly, the fat-free nitrogen values, which express the nitrogen concentration in skimmilk on the basis of the nitrogen in fat-free plasma (skimmilk), showed a considerable decrease in the casein nitrogen, as well as in the noncasein nitrogen in skimmilk which was separated from homogenized milk as compared to the normal skimmilk.

The data in Table 13 emphasize further the difference between the distribution of milk components in nonhomogenized and homogenized milk and show that cream which was separated from homogenized milk contained higher concentrations of ash, copper and nonphospholipid phosphorus than the amounts found in cream separated from nonhomogenized milk. A definite compensating trend was noted in the skimmilk, similar to that already observed in the case of the nitrogen distribution data.

Distribution of Milk Solids and Nitrogen in Washed Cream and Washed-cream Serum from Nonhomogenized and Homogenized Milk

Up to the present time, the concentration and nature of the milk components supposedly associated with the surface of the fat globules have been derived from data which permit only indirect conclusions. A more direct approach to determine the nature of the fat-membrane substances was made in this investigation. By washing the cream six times with distilled water, it was possible to obtain a concentration of fat globules from which the unassociate plasma components were removed. Figure 12 shows the effect of successive dilutions on the fat content of the washed cream. The fat content of washed cream obtained from homogenized milk remained fairly uniform after six washings, whereas the fat content of cream from nonhomogenized milk increased at a greater rate with successive washings; the greatest increase occurred subsequent to the first two or three washings.

Data in Figure 13 illustrate the effect of successive washings on the concentrations of total nitrogen in the respective cream samples. The concentration of nitrogen, in grams per 100 grams of fat, dropped sharply with the first washing.

There was a gradual decrease in nitrogen content, in the case of nonhomogenized cream, up to the fourth or fifth washing, where it leveled off. The reduction in the concentration of nitrogen in homogenized cream was largely arrested after the first washing and remained fairly uniform up to the sixth washing.

In cream which had been separated from nonhomogenized milk, the concentration of phospholipid phosphorus (milligrams per 100 grams of fat) decreased slightly following the first washing and then remained fairly constant after four washings (Fig. 14). Further washings accelerated the decrease in phospholipid phosphorus. The concentration of phospholipid phosphorus in the washed, homogenized cream made a unique contrast. Subsequent to the fourth washing, the concentration of phospholipid phosphorus was higher than that found in the original cream sample.

The concentration of fat and solids-not-fat in the washed creams and washed-cream sera are recorded in Table 15. Attention should be directed to the fourth column where the concentration of solids-not-fat are expressed as grams per 100 grams of fat. The washed, homogenized cream contained

homogenized serum. This condition was not observed when serum from nonhomogenized milk was heated.

Some Characteristics of Fat-membrane Proteins Isolated from Nonhomogenized and Homogenized Milk

Some of the physical and chemical characteristics of the fat-membrane proteins isolated from nonhomogenized and homogenized milk are tabulated in Table 17. The nitrogen content of these materials, on a lipid-free basis, averaged 13.50 per cent.

Some Electrophoretic Characteristics of the Fat-membrane Proteins

Electrophoretic patterns of unit magnification for cold ethanol-ether extracted fat-membrane proteins in various pH and buffer media are illustrated in Figure 15 to 21, inclusive. The migrating protein components, represented by peaks in the electrophoretic patterns, were measured and the data tabulated in Table 18. The electrophoretic mobilities were calculated for each peak and recorded in Table 19. Where good electrophoretic resolution of the protein components occurred, two and sometimes three prominent and distinct peaks were observed in

the electrophoretic patterns of the fat-membrane proteins prepared from nonhomogenized milk. These data, then, are representative of the normal fat-membrane proteins. The patterns obtained from the homogenized fat-membrane proteins show three or four distinct peaks when good electrophoretic resolution was obtained. In most instances, however, there appeared to be a distinct difference in the basic characteristics of the electrophoretic patterns of fat-membrane proteins prepared from nonhomogenized and homogenized milk.

The effect of warm ethanol-ether treatment and cold extraction on the electrophoretic characteristics of the fat-membrane proteins in phosphate buffer is illustrated in Figure 22. Measurements of the component peaks are recorded in Table 18 and the corresponding electrophoretic mobilities are recorded in Table 20. Certain characteristic differences in the electrophoretic patterns indicate that the temperature of treatment had an effect on the nature of the resulting protein material. This difference was also substantiated by solubility studies. The proteins prepared by warm extraction were less soluble than those prepared by cold extraction.

The effects of variation in the concentration of fat-membrane proteins on the characteristics of the electrophoretic patterns obtained in similar buffer systems are shown in Figure 23. The peaks of the various membrane components were measured and the corresponding electrophoretic mobilities calculated. These data are tabulated in Tables 18 and 21. Although the potential gradients of the phosphate buffers used in this particular trial differed slightly, the electrophoretic mobilities of the respective components in 0.84 and 0.43 per cent protein concentrations differed enough to indicate that the concentration of the electrophorized material is a factor to consider when determining the electrophoretic mobility of a protein component.

Some Microbiological Characteristics of the Fat-membrane Proteins

The analytical results obtained from the microbiological assay of the cold ethanol-ether extracted fat-membrane proteins for six essential amino acids are given in Table 22. The amino acid composition of the minor-protein fraction, casein, lactalbumin, beta-lactoglobulin, pseudoglobulin and euglobulin are included for comparative purposes. The two fat-membrane proteins contained approximately the same concentrations of

methionine, valine, leucine, isoleucine and phenylalanine and none varied by more than 10 per cent. There was a 22 per cent difference in the amount of lysine in the two samples. The membrane-proteins obtained from the homogenized milk appear to contain more lysine than that obtained from the non-homogenized milk. The significance of this difference is subject to further investigation as well as the determination of additional amino acids. The preliminary data indicate, however, that the fat-membrane proteins differ markedly from the other characterized milk protein fractions in its amino acid content when all values are expressed as grams per 100 grams of anhydrous protein.

The fat-membrane proteins contain more methionine, leucine, isoleucine and phenylalanine; less valine and approximately the same amount of lysine as the minor-protein fraction reported by Weinstein, Duncan and Trout (1951). The lack of similarity between the amino acid content of the fat-membrane proteins and the other recognized milk proteins is evident. Hattori (1925) and Titus et al. (1928) reported values for lysine nitrogen in normal fat-membrane materials as 6.11 and 6.69 per cent, respectively. These values are in good agreement

with the value for lysine reported here as 5.92, whereas Wiese and Palmer (1934) found the lysine nitrogen concentration in "membrane" protein to be 11.01 per cent.

The minimum molecular weight (M min.) of the fat-membrane proteins was calculated by the equation:

$$(\text{M min.}) = (\text{Ra} \times \text{Ma} \times 100) \; / \; (\%)^{a}$$

where (M min.) = the minimum molecular weight,

Ra = is the integer representing the number of specific amino acid residues in the molecule,

Ma = is the molecular weight of the amino acid, and

$(\%)^{a}$ = is the per cent of amino acid in 100 grams of anhydrous protein.

Methionine, which represents the amino acid present in the smallest concentration, was used to make the adjustment to integers. The data used to make the (M min.) calculations are presented in Table 23.

In comparing these data with those reported for the minor-protein fraction, it is evident that the two preparations are not comparable. The minimum molecular weight of the fat-membrane proteins was calculated to be approximately 27,500 in comparison to 70,300 for the minor-protein fraction,

12,800 for casein and 42,020 for beta-lactoglobulin. The difference between the minimum molecular weight of the membrane proteins obtained from nonhomogenized (28,926) and homogenized milk (26,798) was approximately 6 per cent; a difference too small to suggest that there is an actual difference between the two. Further work is contemplated, however, on the isolation and the investigation of the properties of the fat-membrane proteins. Information on this type of protein is of fundamental interest and importance.

TABLE 10

The effect of homogenization at various pressures on the nitrogen distribution in milk pasteurized at 143° F. for 30 minutes

Nitrogen Fraction	Nonhomogenized milk (Control)		Homogenization Pressure (lb./sq. in.)								
			1,000			2,500			4,000		
	Nitrogen (mg./100 ml.)	Per cent of Total	Nitrogen (mg./100 ml.)	Per cent of Total	Per cent Variation from Control	Nitrogen (mg./100 ml.)	Per cent of Total	Per cent Variation from Control	Nitrogen (mg./100 ml.)	Per cent of Total	Per cent Variation from Control
Determined											
Total N	445.8	100.0	449.0	100.0	0.0	456.4	100.0	0.0	450.1	100.0	0.0
Noncasein N	90.1	20.2	91.8	20.4	0.2	91.4	20.0	0.2	88.0	19.6	-0.8
Nonheat-coagulable N	39.0	8.8	38.5	8.6	-0.2	40.2	8.8	0.0	39.1	8.7	-0.1
Nonprotein N	20.5	4.6	21.5	4.7	0.1	21.4	4.7	0.1	22.0	4.9	0.3
Calculated											
Casein N	355.7	79.8	357.2	79.6	-0.2	365.0	80.0	0.2	362.1	80.5	0.7
Heat-coagulable N	51.1	11.4	53.3	11.9	0.4	51.2	11.2	-0.2	48.9	10.8	-0.6
Proteose-peptone N	18.5	4.2	17.3	3.9	-0.3	18.8	4.1	-0.1	17.6	3.9	-0.3

TABLE 11

Distribution of fat and solids-not-fat in cream and skimmilk from nonhomogenized and homogenized milk

Product	Mojonnier Values Obtained for			Difference between the Theoretical[1] and Actual Percentage of Solids-not-fat in the Cream Samples	
	Total Solids (%)	Fat (%)	Solids-not-fat (%)	Theoretical (%)	Difference (%)
Pasteurized milk	12.25	3.76	8.49	. . .	. . .
Nonhomogenized					
skimmilk	8.85	0.10	8.75	. . .	. . .
cream	35.91	29.60	6.31	6.16	0.15
Homogenized					
skimmilk	10.63	1.91	8.72	. . .	. . .
cream	52.02	46.60	5.42	4.66	0.76

[1] Calculated on basis of formula:

Percentage of solids-not-fat in cream = [100 - percentage of fat in cream]/100 x percentage of solids-not-fat in skimmilk.

TABLE 12

Distribution of the nitrogen fractions in cream and skimmilk from nonhomogenized and homogenized milk

Nitrogen Fraction	Skimmilk		
	Non-homogenized (0.12% fat; 8.85% t.s.)	Homogenized (1.91% fat; 10.63% t.s.)	Difference Due to Homogenization
TOTAL N			
(mg./100 ml.)	470	442	. . .
Fat-free			
(mg./100 ml.)	470	451	-19
Plasma-corrected			
(mg./100 g. fat)	. . .	. . .	. . .
(mg./100 g. cream)	. . .	. . .	. . .
NONCASEIN N			
(mg./100 ml.)	106	100	. . .
Fat-free			
(mg./100 ml.)	106	101	- 5
Plasma-corrected			
(mg./100 g. fat)	. . .	. . .	. . .
NONHEAT-COAGULABLE N			
(mg./100 ml.)	47	49	. . .
Fat-free			
(mg./100 ml.)	47	50	3
Plasma-corrected			
(mg./100 g. fat)	. . .	. . .	. . .
NONPROTEIN N			
(mg./100 ml.)	30	26	. . .
Fat-free			
(mg./100 ml.)	30	27	- 3
Plasma-corrected			
(mg./100 g. fat)	. . .	. . .	. . .

[1] Fat-free N determined as follows: [(N determined) / (Vol. of plasma)] x 100.

TABLE 12 (Continued)

Cream			Pasteurized Whole Milk (3.76% fat; 12.25% t.s.)
Nonhomogenized (29.60% fat; 35.91% t.s.)	Homogenized (46.60% fat; 52.02% t.s.)	Difference Due to Homogenization	
336	295	. . .	456
. . .	. . .	. . .	. . .
54	150	96	. . .
16	67	51	. . .
75	63	. . .	98
. . .	. . .	. . .	. . .
10	13	3	. . .
34	29	. . .	40
. . .	. . .	. . .	. . .
7	9	2	. . .
22	15	. . .	28
. . .	. . .	. . .	. . .
7	2	- 5	. . .

[2] Plasma-corrected N determined as follows: [(100 - percentage of fat) / (100 x 1.034)] x skimmilk value.

TABLE 13

Distribution of ash, calcium and phosphorus in cream and skimmilk from nonhomogenized and homogenized milk

Component	Skimmilk		
	Non-homogenized (0.12% fat; 8.85% t.s.)	Homogenized (1.91% fat; 10.63% t.s.)	Difference Due to Homogenization
ASH			
(mg./100 ml.)	720	690	. . .
Fat-free (mg./100 ml.)	720	710	-10
Plasma-corrected (mg./100 g. fat)	. . .	. . .	. . .
TOTAL COPPER			
(mg./100 ml.)	118	114	. . .
Fat-free (mg./100 ml.)	118	116	- 2
Plasma-corrected (mg./100 g. fat)	. . .	. . .	. . .
TOTAL PHOSPHORUS			
(mg./100 ml.)	101	100	. . .
Fat-free (mg./100 ml.)	101	102	1
Plasma-corrected (mg./100 g. fat)	. . .	. . .	. . .
PHOSPHOLIPID PHOSPHORUS			
(mg./100 g. fat)	. . .	. . .	. . .

[1] Fat-free values determined as follows: [(total value) / (val. of plasma)] x 100.

[2] Plasma corrected values determined from following expression: [(100 - percentage of fat) / (100 x 1.034)] x skimmilk value.

TABLE 13 (Continued)

Cream			Pasteurized Whole Milk (3.76% fat; 12.25% t.s.)
Nonhomogenized (29.60% fat; 35.91% t.s.)	Homogenized (46.60% fat; 52.02% t.s.)	Difference Due to Homogenization	
490	392	. . .	710
. . .	. . .	. . .	. . .
0	77	77	. . .
81	72	. . .	112
. . .	. . .	. . .	. . .
-1.4	45	45	. . .
72	67	. . .	98
. . .	. . .	. . .	. . .
10	21	11	. . .
11	12	. . .	. . .

TABLE 14

The effect of successive dilutions and reseparations on the concentration of fat, nitrogen and lipoid phosphorus in cream from nonhomogenized and homogenized milk

	Sample No.	Number of Washings								
		0	1	2	3	4	5	6	7	8
		Fat (%)								
Nonhomogenized	1[1]	29.0	48.5	. . .	52.5	. . .	54.5	54.5	. . .	. . .
	2	27.4	48.9	. . .	52.3	. . .	54.1	55.0	. . .	. . .
	3	33.0	48.6	. . .	52.9	. . .	54.8	55.3	. . .	. . .
Homogenized	1[1]	52.5	52.5	. . .	53.0	. . .	53.5	54.5	. . .	. . .
	2	47.2	50.1	. . .	49.2	. . .	52.6	54.5	. . .	. . .
	3	54.2	54.6	. . .	55.2	. . .	55.8	56.4	. . .	. . .
		Nitrogen (g. per 100 g. of fat)								
Nonhomogenized	1[2]	1.16	0.19	. . .	0.08	. . .	0.05	0.04	. . .	. . .
	2	1.22	0.25	. . .	0.10	. . .	0.06	0.05	. . .	0.05
	3	1.10	0.20	. . .	0.08	. . .	0.05	0.04	. . .	0.04

TABLE 14 (Continued)

	Sample No.	Number of Washings								
		0	1	2	3	4	5	6	7	8
		Nitrogen (g. per 100 g. of fat) [Continued]								
	1[2]	0.64	0.19	. . .	0.18	. . .	0.17	0.17	. . .	. . .
Homogenized	2	0.70	0.20	. . .	0.18	. . .	0.15	0.16	. . .	0.16
	3	0.63	0.20	. . .	0.17	. . .	0.16	0.17	. . .	0.16
		Lipoid phosphorus (mg. per 100 g. of fat)								
	1[3]	20.8	19.0	. . .	19.0	. . .	18.5	. . .	17.6	17.0
Nonhomogenized	2	18.4	17.0	. . .	17.0	. . .	16.5	15.8	. . .	15.0
	3	19.4	17.2	. . .	17.0	. . .	16.9	16.2	. . .	15.1
	1[3]	21.1	20.8	. . .	20.7	. . .	20.5	. . .	21.2	21.8
Homogenized	2	19.2	18.8	. . .	18.6	. . .	18.4	18.8	. . .	19.4
	3	19.6	19.4	. . .	19.4	. . .	19.5	19.9	. . .	20.3

[1] Data used in Figure 12. [2] Data used in Figure 13. [3] Data used in Figure 14.

TABLE 15

Distribution of fat and solids-not-fat in washed cream and washed-cream serum of nonhomogenized and homogenized milk

Product	Mojonnier Values Obtained for			Solids-not-fat per 100 g. of Fat
	Total Solids (%)	Fat (%)	Solids-not-fat (%)	
Washed cream				
Nonhomogenized	54.30	54.10	0.18	0.33
Homogenized	55.03	54.40	0.63	1.16
Difference				0.83
Washed-cream serum				
Nonhomogenized	2.25	1.59	0.66	. . .
Homogenized	12.14	10.81	1.33	. . .

TABLE 16

Distribution of the nitrogen fractions in the washed cream and washed-cream serum from nonhomogenized and homogenized milk

Nitrogen Fraction	Washed Cream			Washed-cream Serum		
	Non-homogenized (0.18% SNF)	Homogenized (0.63% SNF)	Difference Due to Homogenization	Non-homogenized (0.18% SNF)	Homogenized (0.63% SNF)	Difference Due to Homogenization
TOTAL N						
(mg./100 ml.)	22	93	...	97	187	...
Fat-free[1]						
(mg./100 ml.)	...	...	...	99	210	111
Serum-corrected[2]						
(mg./100 g. fat)	41	171	130	...	...	...
NONCASEIN N						
(mg./100 ml.)	7	7	...	1	23	...
Fat-free						
(mg./100 ml.)	...	...	...	1	25	24

TABLE 16 (Continued)

Nitrogen Fraction	Washed Cream			Washed-cream Serum		
	Non-homogenized (0.18% SNF)	Homogenized (0.63% SNF)	Difference Due to Homogenization	Non-homogenized (0.18% SNF)	Homogenized (0.63% SNF)	Difference Due to Homogenization
Serum-corrected (mg./100 g. fat)	13	13	0	...	...	...
NONHEAT-COAGULABLE N (mg./100 ml.)	0	0	...	1	6	...
Fat-free (mg./100 ml.)	...	...	...	1	7	6

[1] Fat-free N determined as follows: [(N determined) / (Vol. of plasma)] x 100.

[2] Plasma-corrected N determined as follows: [(100 - percentage of fat) / (100 x 1.034)] x skimmilk value.

TABLE 17

Some characteristics of fat-membrane proteins isolated from nonhomogenized and homogenized milk

Property	Fat-membrane Proteins Isolated from			
	Nonhomogenized Milk		Homogenized Milk	
	Warm Ethanol Treatment (%)	Cold Ethanol Treatment (%)	Warm Ethanol Treatment (%)	Cold Ethanol Treatment (%)
Lipoid material	12.20	7.00	13.00	2.66
Nitrogen	12.50	12.20	11.80	13.30
Nitrogen (fat-free)	14.26	13.11	13.57	13.65
Molisch	+	+	+	+
Biuret	+	+	+	+
Nitroprusside				
Before heating	-	-	-	-
After heating	-	-	+	+
Appearance	fine, dull	fine, dull	crystalline, bright	crystalline, bright

TABLE 18

Distances migrated by the fat-membrane protein components in various buffers and the effects produced on migration by treatment with warm ethanol and by the concentration of membrane-proteins. (All measurements were made from the original electrophoretic negative.)

Buffer	pH	Peak No.	Nonhomogenized Milk		Homogenized Milk	
			Ascending (d_1) (cm.)	Descending (d_2) (cm.)	Ascending (d_1) (cm.)	Descending (d_2) (cm.)
Glycine-HCl	1.5	1	1.19	1.16	1.45	1.80
		2	0.77	0.67	1.16	1.58
		3	0.48	. . .	0.88	1.23
Acetate	3.0	1	1.69	1.59	3.65	3.60
		2	. . .	. . .	3.00	. . .
		3	. . .	. . .	1.32	1.50
		4	. . .	. . .	0.41	0.48
Phosphate	6.5	1	3.46	. . .	4.18	3.53
		2	3.30	3.20	3.00	2.46
		3	2.90	2.85	2.73	2.23
		4	0.82	0.95	0.38	0.56
Veronal-citrate	8.0	1	2.70	2.68	3.28	2.93
		2	2.39	2.42	2.67	2.20
		3	0.66	0.86	1.37	1.20
		4	. . .	. . .	0.26	0.53
Ammonia-HCl	8.0	1	2.91	. . .	3.14	2.70
		2	2.45	2.31	2.57	2.03
		3	2.26	. . .	2.23	. . .
		4	0.70	. . .	0.57	0.57

TABLE 18 (Continued)

Buffer	pH	Peak No.	Nonhomogenized Milk		Homogenized Milk	
			Ascending (d_1) (cm.)	Descending (d_2) (cm.)	Ascending (d_1) (cm.)	Descending (d_2) (cm.)
Veronal-ethyl-amine	8.0	1	3.25	3.02	3.58	2.82
		2	2.89	. . .	3.00	2.64
		3	1.53	1.68	2.13	1.90
		4	0.60	0.65	1.36	1.38
		5	. . .	. . .	0.62	0.84
Phosphate (Warm ethanol treatment)	6.5	1	3.36	. . .	. . .	. . .
		2	2.84	. . .	2.60	2.22
		3	2.64	2.62	2.07	1.67
		4	0.56	. . .	0.27	0.50
Phosphate (Effect of 0.43% concentration)	6.5	1	. . .	. . .	3.23	3.05
		2	. . .	. . .	2.47	2.37
		3	. . .	. . .	2.12	2.01
		4	. . .	. . .	0.70	0.85

TABLE 19

Mobility of peaks in the electrophoretic pattern of the fat-membrane protein components as shown in Figures 15 to 20. (Mobility [u] = $cm.^2$, $volt^{-1}$, $sec.^{-1}$ x 10^{-5})

Peak No.	Buffer System					
	Glycine-HCl pH 1.5 (Fig. 1) (+)	Acetate pH 3.0 (Fig. 2) (+)	Phosphate pH 6.5 (Fig. 3) (-)	Veronal-citrate pH 8.0 (Fig. 4) (-)	Ammonia-HCl pH 8.0 (Fig. 5) (-)	Veronal-ethylamine pH 10.8 (Fig. 6) (-)
	Nonhomogenized milk					
1 a	4.58	3.12	6.77	5.65	6.70	6.50
d	4.47	2.94	. . .	5.61	. . .	6.04
avg.	4.53A	3.03A+B	6.77?	5.63A	6.70A	6.27A
2 a	2.96		6.46	5.00	5.64	5.78
d	2.58		6.26	5.06	5.31	. . .
avg.	2.77B		6.36A	5.03B	5.48B	5.78B
3 a	1.90		5.78	1.38	5.20	3.06
d	. . .		5.58	1.80	. . .	3.36
avg.	1.90C		5.68B	1.59C	5.20?	3.21?
4 a			1.60		1.61	1.20
d			1.86		. . .	1.30
avg.			1.73C		1.61C	1.25C

TABLE 19 (Continued)

Peak No.	Buffer System: Glycine–HCl pH 1.5 (Fig. 1) (+)	Acetate pH 3.0 (Fig. 2) (+)	Phosphate pH 6.5 (Fig. 3) (–)	Veronal–citrate pH 8.0 (Fig. 4) (–)	Ammonia–HCl pH 8.0 (Fig. 5) (–)	Veronal–ethylamine pH 10.8 (Fig. 6) (–)
			Homogenized milk			
1 a	4.87	10.28	8.18	8.10	7.15	8.66
d	6.05	10.13	6.90	7.24	6.15	6.82
avg.	5.46A[1]	10.21A[1]	7.54A[1]	7.67A[1]	6.60A[1]	7.74A[1]
2 a	3.90	8.47	5.87	6.60	5.86	7.26
d	5.31	. . .	4.82	5.44	4.62	6.38
avg.	4.61B[1]	8.47B[1]	5.35B[1]	6.02B[1]	5.24B[1]	7.32 ?
3 a	2.95	3.72	5.34	3.39	5.08	5.16
d	4.27	4.25	4.36	2.96	. . .	4.60
avg.	3.61C[1]	3.99C[1]	4.85C[1]	3.18C[1]	5.08C[1]	4.88B[1]
4 a		1.15	0.74	0.64	1.30	3.29
d		1.35	1.04	1.31	1.30	3.34
avg.		1.25D[1]	0.89D[1]	0.88D[1]	1.30D[1]	3.32C[1]
5 a						1.50
d						2.02
avg.						1.76D[1]

a - Denotes ascending pattern. d - Denotes descending pattern.

A, B, C, and A^1, B^1, C^1, D^1 designate main components. ? - Doubtful component.

TABLE 20

The effect of temperature of ethanol treatment on the electrophoretic mobility of the fat-membrane protein components in phosphate buffer (pH 6.5). (Mobility [u] = $cm.^2$, $volt^{-1}$, $sec.^{-1}$, x 10^{-5}.)

Peak No.	Nonhomogenized Milk			Homogenized Milk		
	(cold)	(hot)	(diff.)	(cold)	(hot)	(diff.)
1 a	6.77	4.67	2.10	8.18	. . .	. . .
d	. . .	. . .	. . .	6.90	. . .	. . .
avg.	6.77	4.67	2.10	7.54A^1	. . .	. . .
2 a	6.46	3.95	2.51	5.87	7.51	-1.64
d	6.26	. . .	. . .	4.82	6.42	-1.60
avg.	6.36A	3.95A	2.41	5.35B^1	6.97B^1	-1.62
3 a	5.78	3.67	2.11	5.34	5.98	-0.64
d	5.58	3.65	1.93	4.36	4.83	-0.47
avg.	5.68B	3.66B	2.02	4.85C^1	5.41C	-0.56
4 a	1.60	0.78	0.82	0.74	0.78	-0.04
d	1.86	. . .	. . .	1.04	1.45	-0.41
avg.	1.73C	. . .	. . .	0.89D^1	1.12D^1	-0.23

a - Denotes ascending pattern.

d - Denotes descending pattern.

A, B, C, and A^1, B^1, C^1 and D^1 designate main components.

TABLE 21

The effect of concentration of the fat-membrane protein components in homogenized milk on the electrophoretic mobility in phosphate buffer (pH 6.5). (Mobility [u] = $cm.^2$, $volt^{-1}$, $sec.^{-1}$, x 10^{-5}.)

Peak No.	Concentration		
	(0.84%)	(0.43%)	(diff.)
1 a	8.18	10.04	1.86
d	6.90	9.47	2.57
avg.	$7.54A^1$	$9.76A^1$	$2.22A^1$
2 a	5.87	7.67	1.80
d	4.82	7.36	2.54
avg.	$5.35B^1$	$7.52B^1$	$2.17B^1$
3 a	5.34	6.58	1.24
d	4.36	6.24	1.88
avg.	$4.85C^1$	$6.41C^1$	$1.56C^1$
4 a	0.74	2.17	1.43
d	1.04	2.64	1.60
avg.	$0.89D^1$	$2.41D^1$	$1.52D^1$

a - Denotes ascending pattern.

d - Denotes descending pattern.

A^1, B^1, C^1 and D^1 designate main components.

TABLE 22

A comparison of some amino acid values obtained for the fat-membrane proteins with the minor-protein fraction, casein, lactalbumin, lactoglobulin, pseudoglobulin and euglobulin[1] (All values expressed as grams per 100 grams of anhydrous protein)

Constituent	Fat-membrane Protein from[2]		Minor-protein Fraction	Casein	Lactalbumin	Beta-lactoglobulin	Pseudoglobulin	Euglobulin
	Non-homogenized Milk	Homogenized Milk						
Isoleucine	5.66	6.00	3.66	6.1	....	8.40	3.1	3.1
Leucine	8.71	9.22	5.71	9.2	15.0	15.60	9.1	10.4
Lysine	5.92	7.54	5.74	8.2	9.0	11.40	7.2	6.3
Methionine	2.08	2.20	1.34	2.8	2.8	3.22	1.08	0.98
Phenylalanine	4.97	4.51	2.35	5.0	5.6	3.50	3.8	3.6
Valine	5.65	5.50	9.74	7.2	4.0	5.80	9.4	10.4
Total nitrogen (%)	15.67	15.67	15.67	15.63	....	15.60	....	....
Nitrogen (%)[3]	12.20	13.30	9.99	....	....	....	....	....

[1] Literature values reported by Weinstein, Duncan and Trout (1951).

[2] Samples prepared by cold ethanol-ether extraction.

[3] Actual nitrogen content of the anhydrous fat-membrane proteins.

TABLE 23

The number of amino acid residues in the fat-membrane proteins prepared by cold ethanol treatment

Constituent	Fat-membrane Proteins Obtained from					
	Nonhomogenized Milk			Homogenized Milk		
	Yield (%)	Moles per 10^5 g. Protein	Molar Ratio when Methionine = 4	Yield (%)	Moles per 10^5 g. Protein	Molar Ratio when Methionine = 4
Isoleucine	5.67	43.3	12	6.00	45.8	12
Leucine	8.71	66.4	19	9.22	70.3	19
Lysine	5.92	40.5	12	7.54	51.6	14
Methionine	2.08	14.0	4	2.20	14.8	4
Phenylalanine	4.97	30.1	9	4.51	27.3	7
Valine	5.65	48.3	14	5.50	47.0	13
(M min.)			28,926			26,798

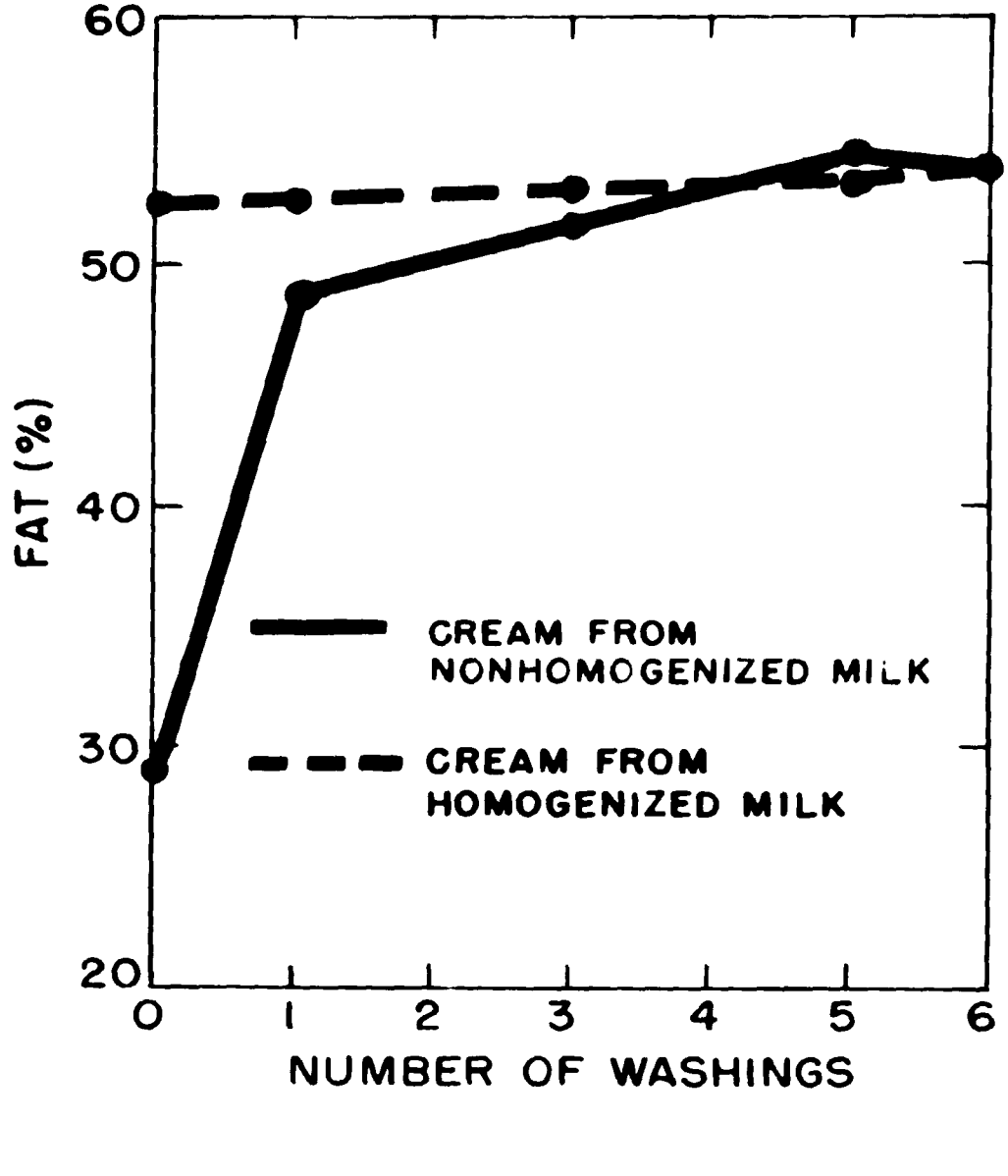

Figure 12. The effect of successive washings on the fat content of cream from nonhomogenized and homogenized milk.

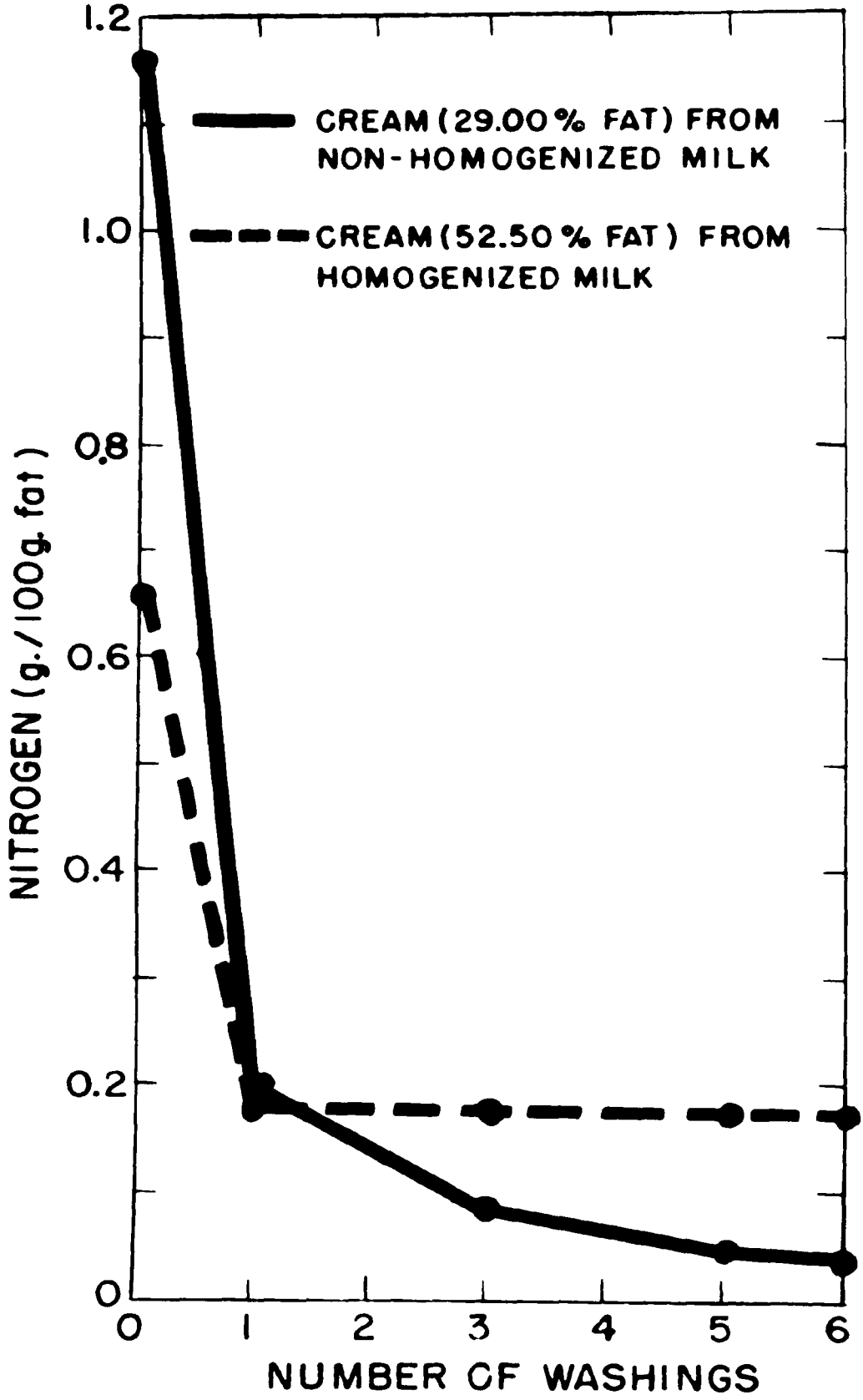

Figure 13. The effect of successive washings on the total nitrogen content of cream from nonhomogenized and homogenized milk.

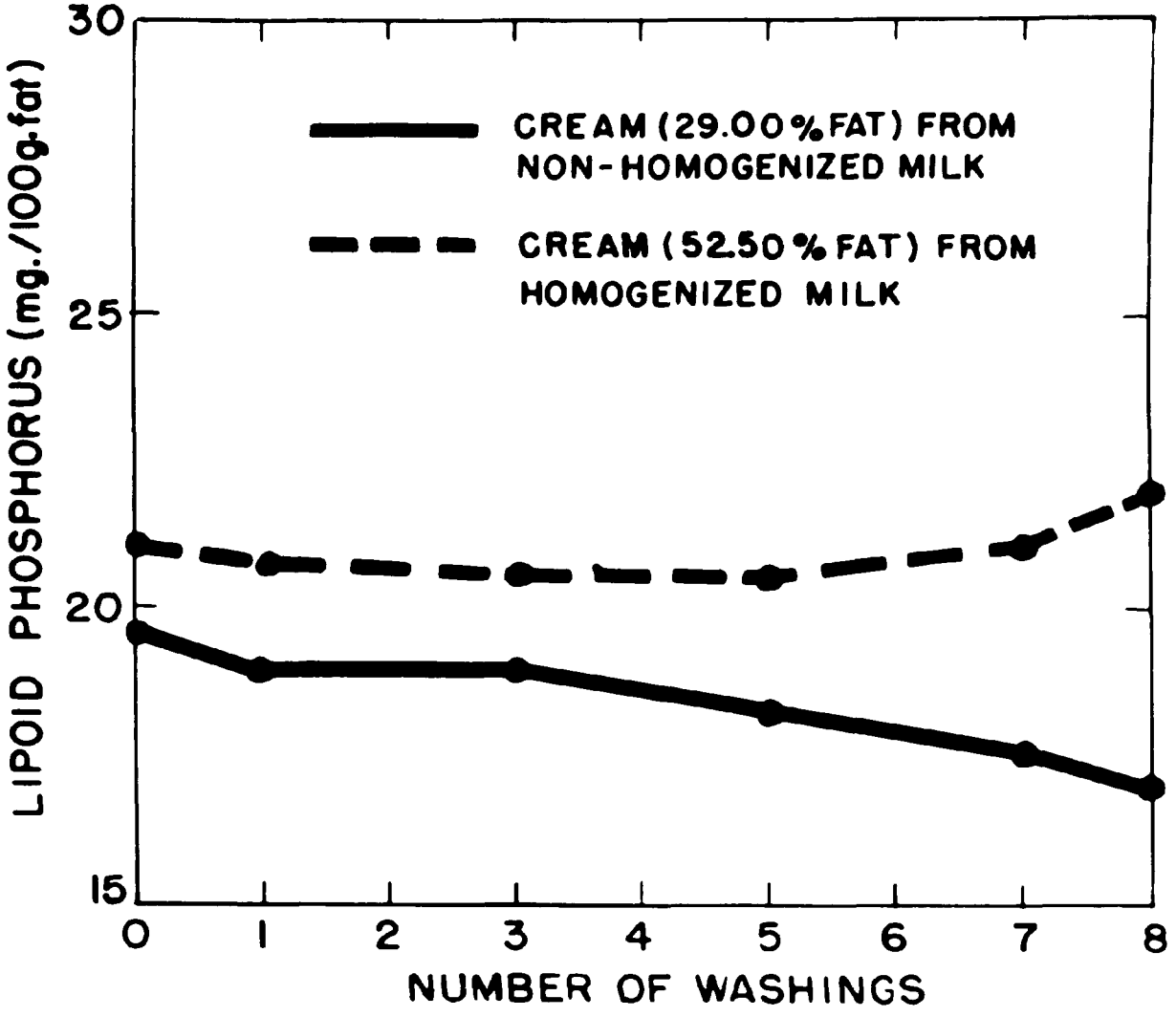

Figure 14. The effect of successive washings on the lipoid phosphorus content of cream from nonhomogenized and homogenized milk.

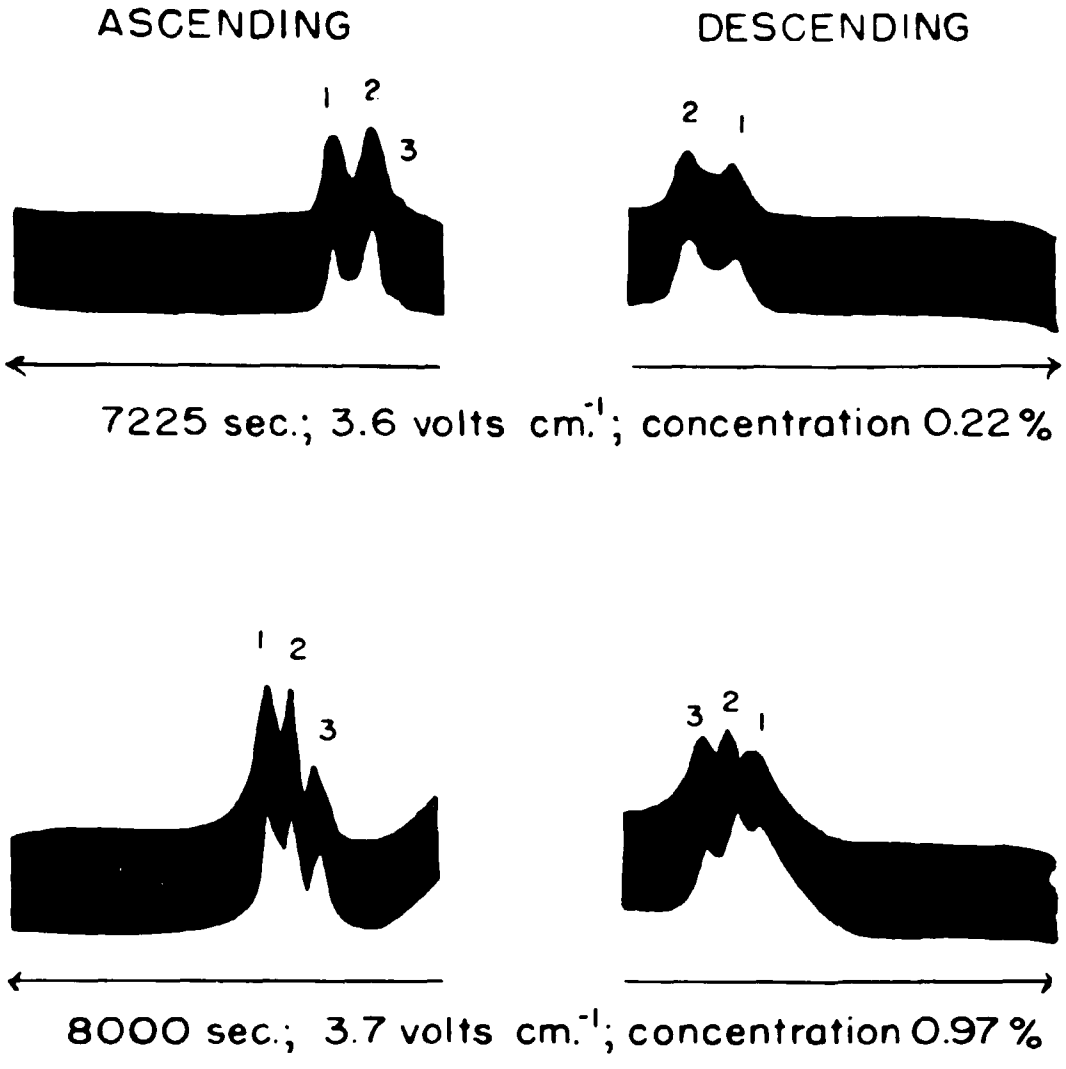

Figure 15. Electrophoretic patterns of fat-membrane proteins isolated from nonhomogenized milk (top) and homogenized milk (bottom) and dispersed in glycine-hydrochloric acid buffer at pH 1.5 and ionic strength of 0.1.

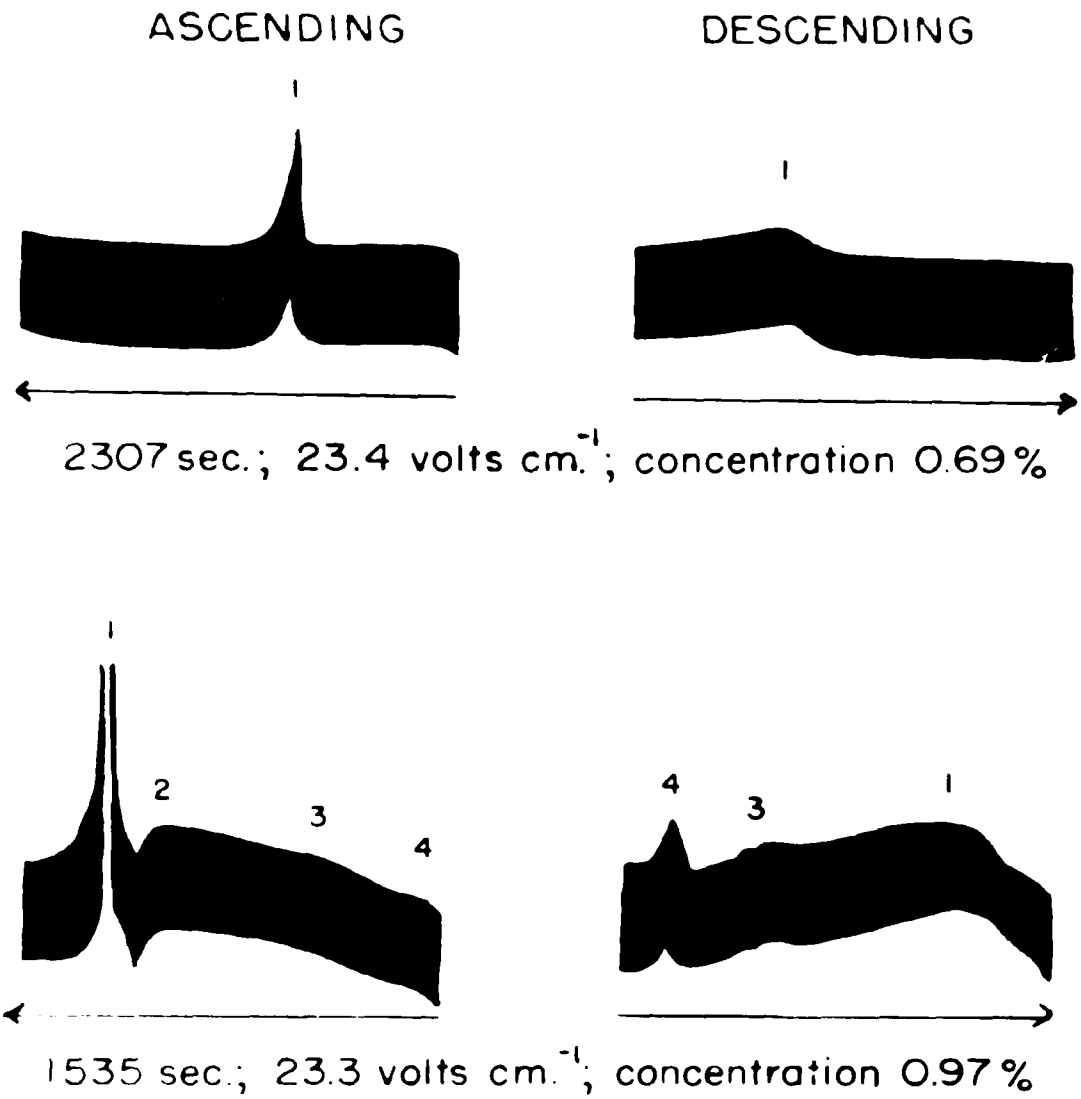

Figure 16. Electrophoretic patterns of fat-membrane proteins isolated from nonhomogenized milk (top) and homogenized milk (bottom) and dispersed in acetate buffer at pH 3.0 and ionic strength of 0.1.

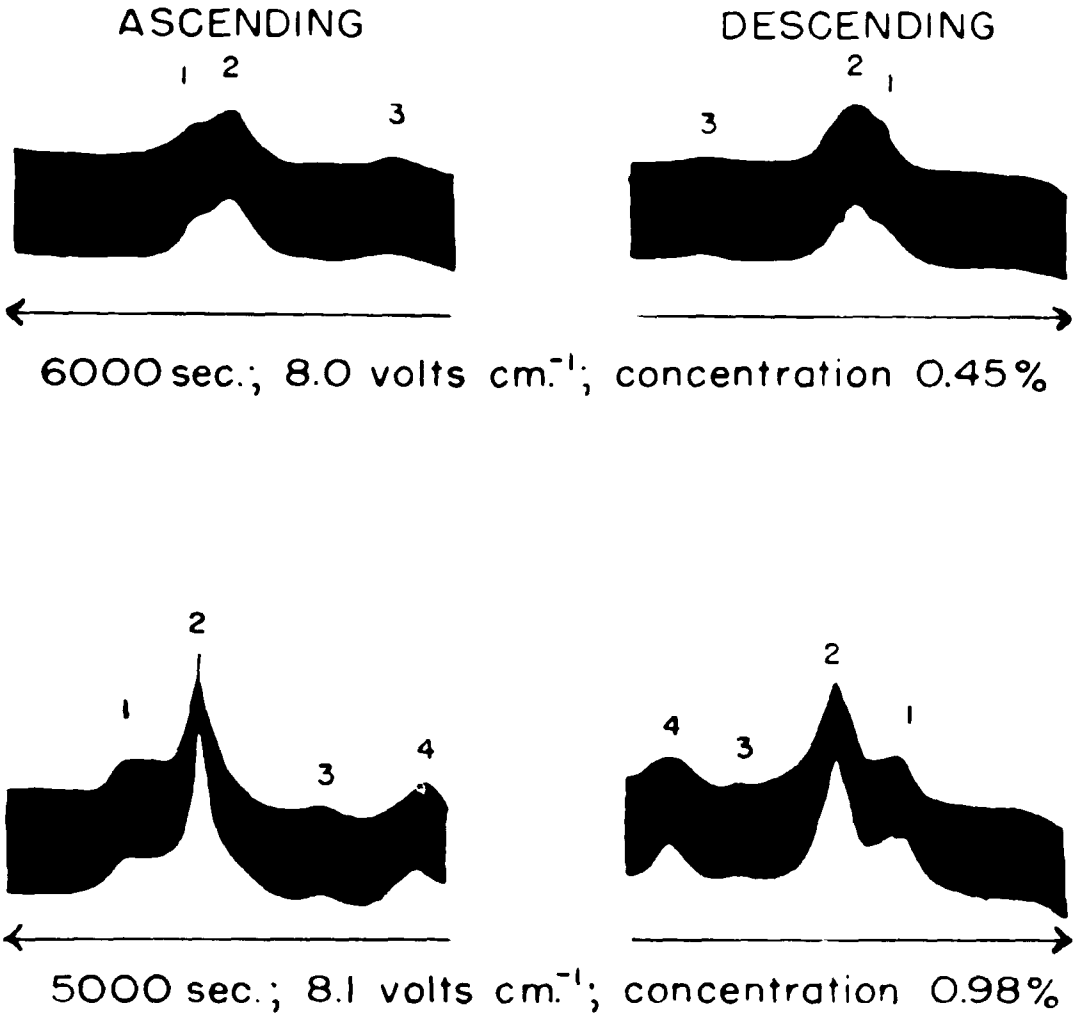

Figure 17. Electrophoretic patterns of fat-membrane proteins isolated from nonhomogenized milk (top) and homogenized milk (bottom) and dispersed in phosphate buffer at pH 6.5 and ionic strength of 0.1.

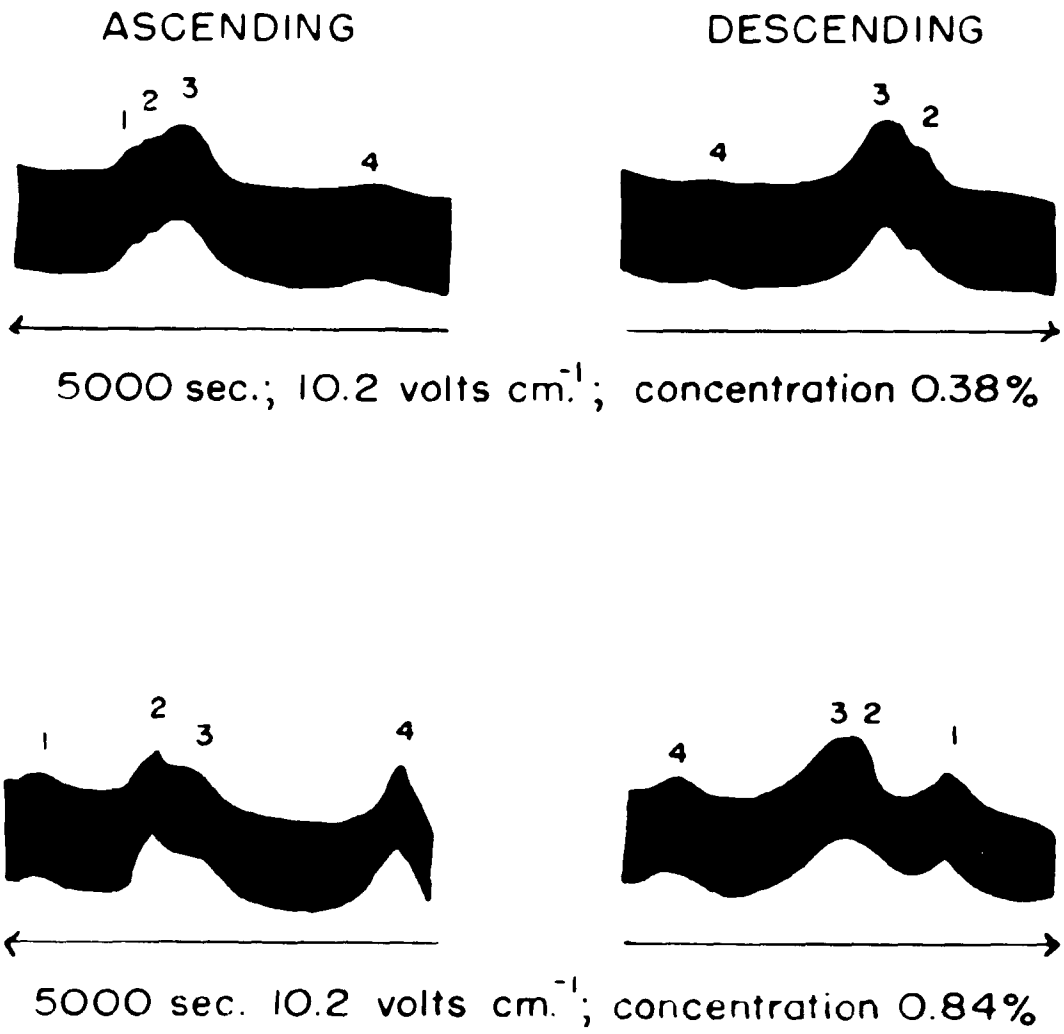

Figure 18. Electrophoretic patterns of fat-membrane proteins isolated from nonhomogenized milk (top) and homogenized milk (bottom) and dispersed in veronal-citrate buffer at pH 8.6 and ionic strength of 0.09.

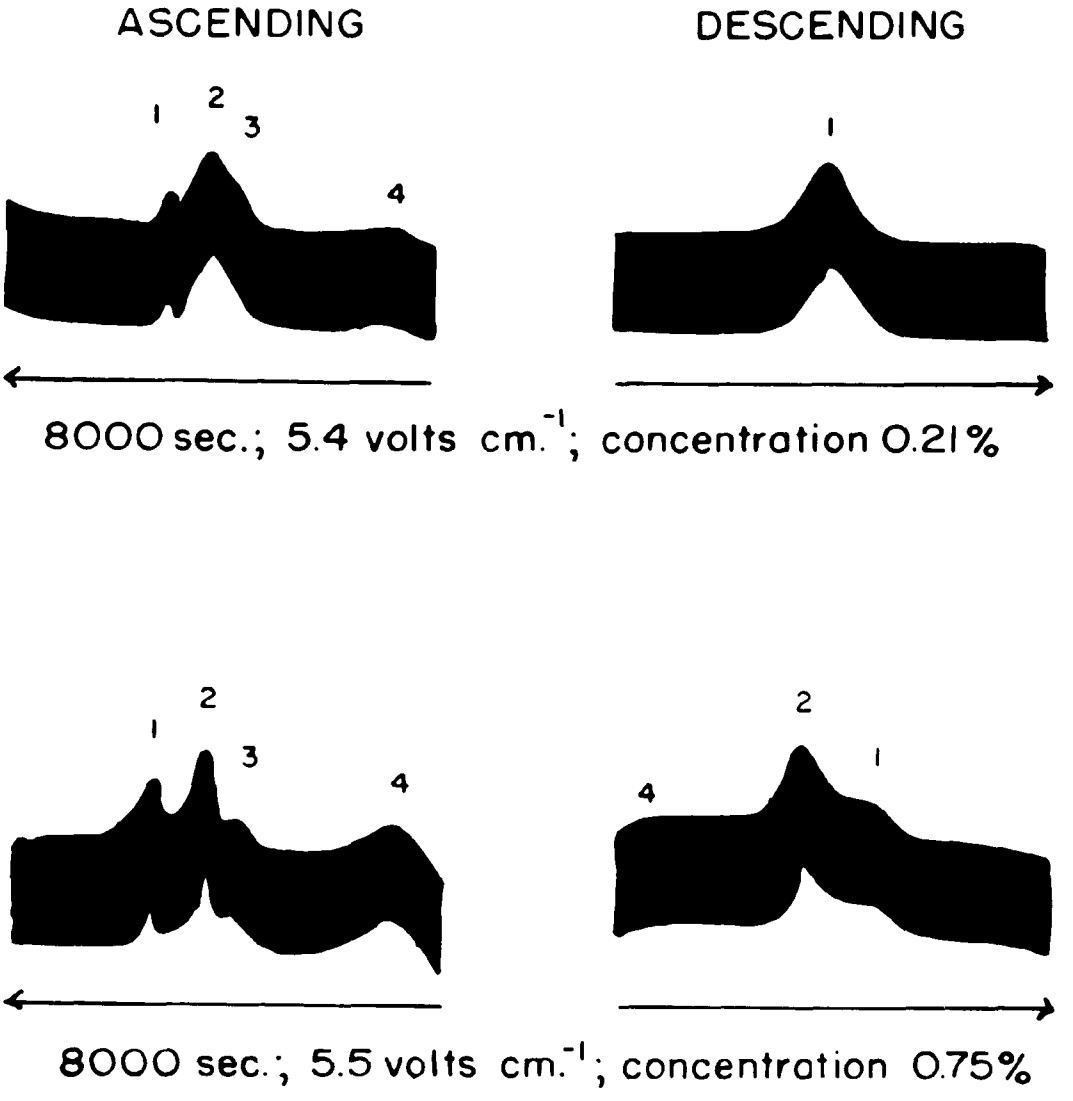

Figure 19. Electrophoretic patterns of fat-membrane proteins isolated from nonhomogenized milk (top) and homogenized milk (bottom) and dispersed in ammonia-hydrochloric acid buffer at pH 9.0 and ionic strength of 0.1.

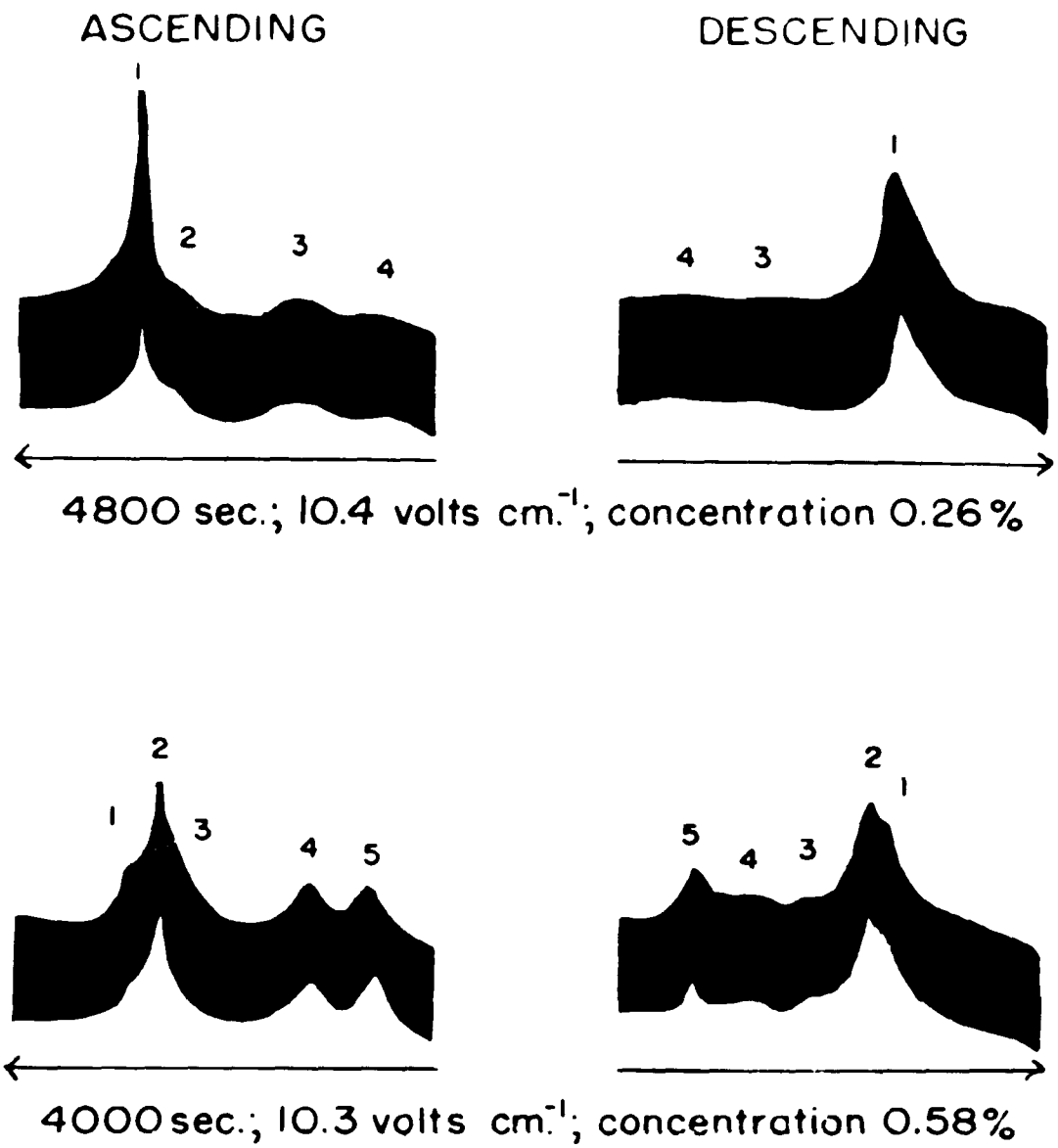

Figure 20. Electrophoretic patterns of fat-membrane proteins isolated from nonhomogenized milk (top) and homogenized milk (bottom) and dispersed in veronal-ethylamine buffer at pH 10.8 and ionic strength of 0.1.

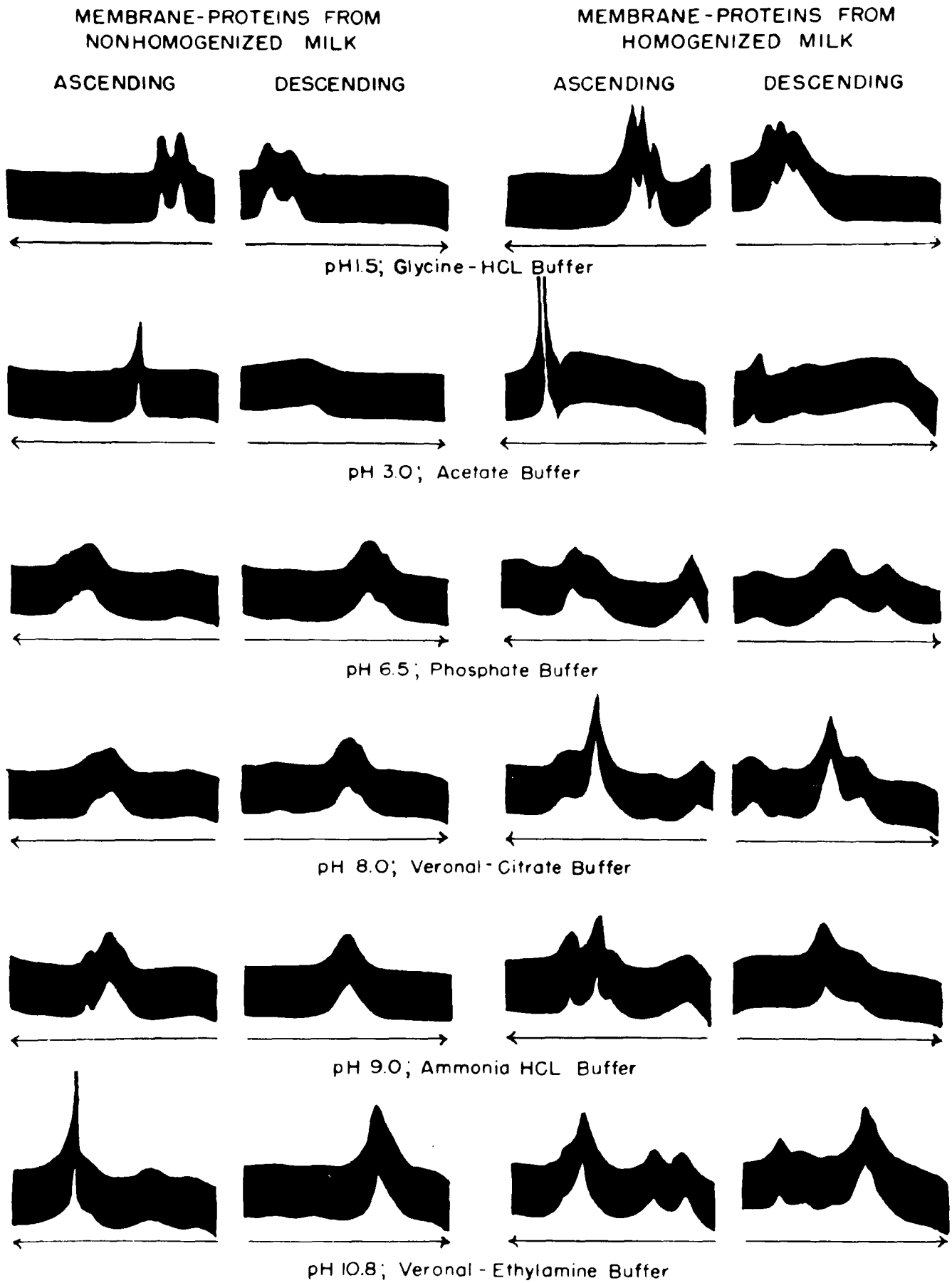

Figure 21. Composite of electrophoretic patterns of fat-membrane proteins in various buffer media.

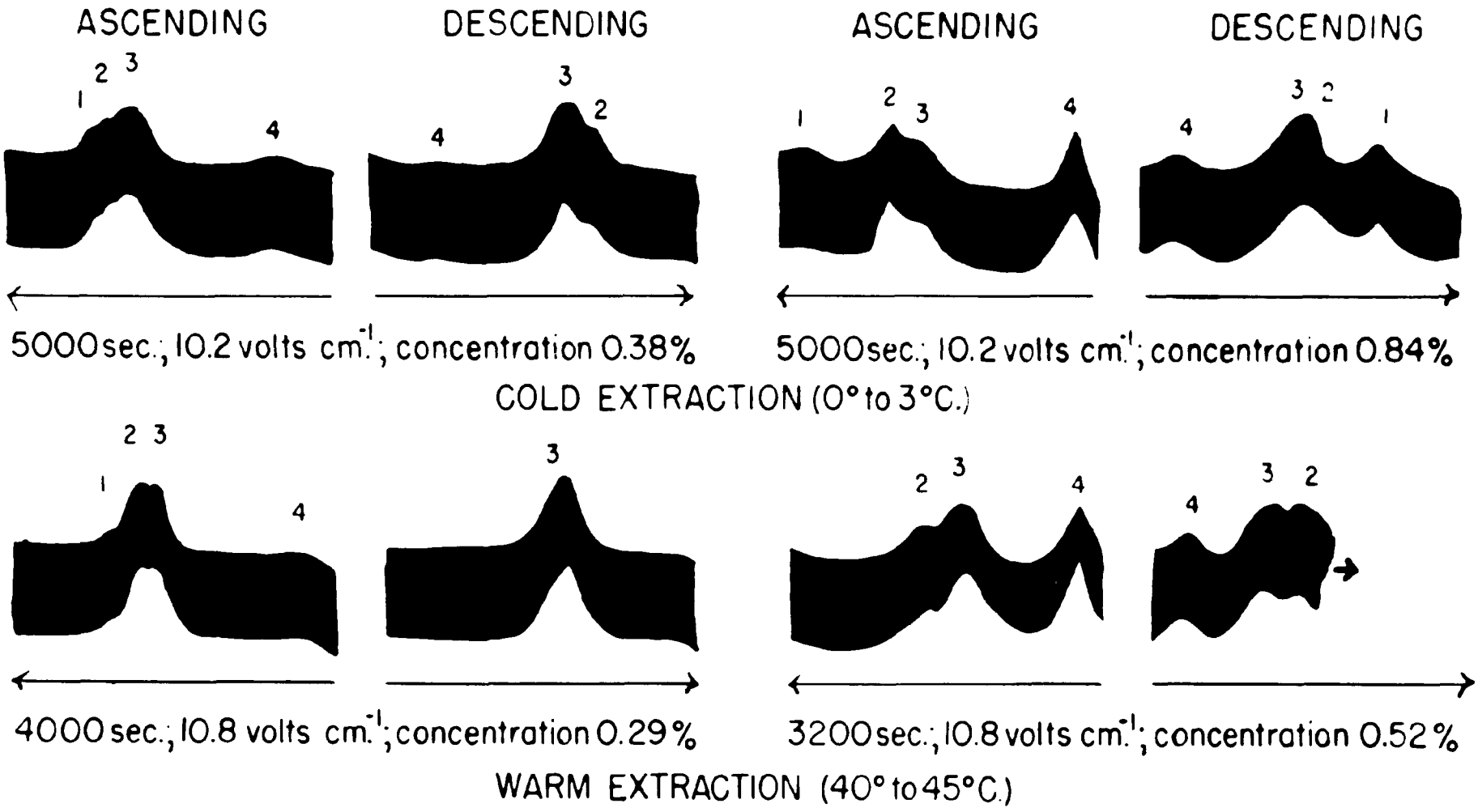

Figure 22. Electrophoretic patterns of fat-membrane proteins isolated from nonhomogenized milk (left) and homogenized milk (right) and showing the effect of treatment with both cold and warm ethanol.

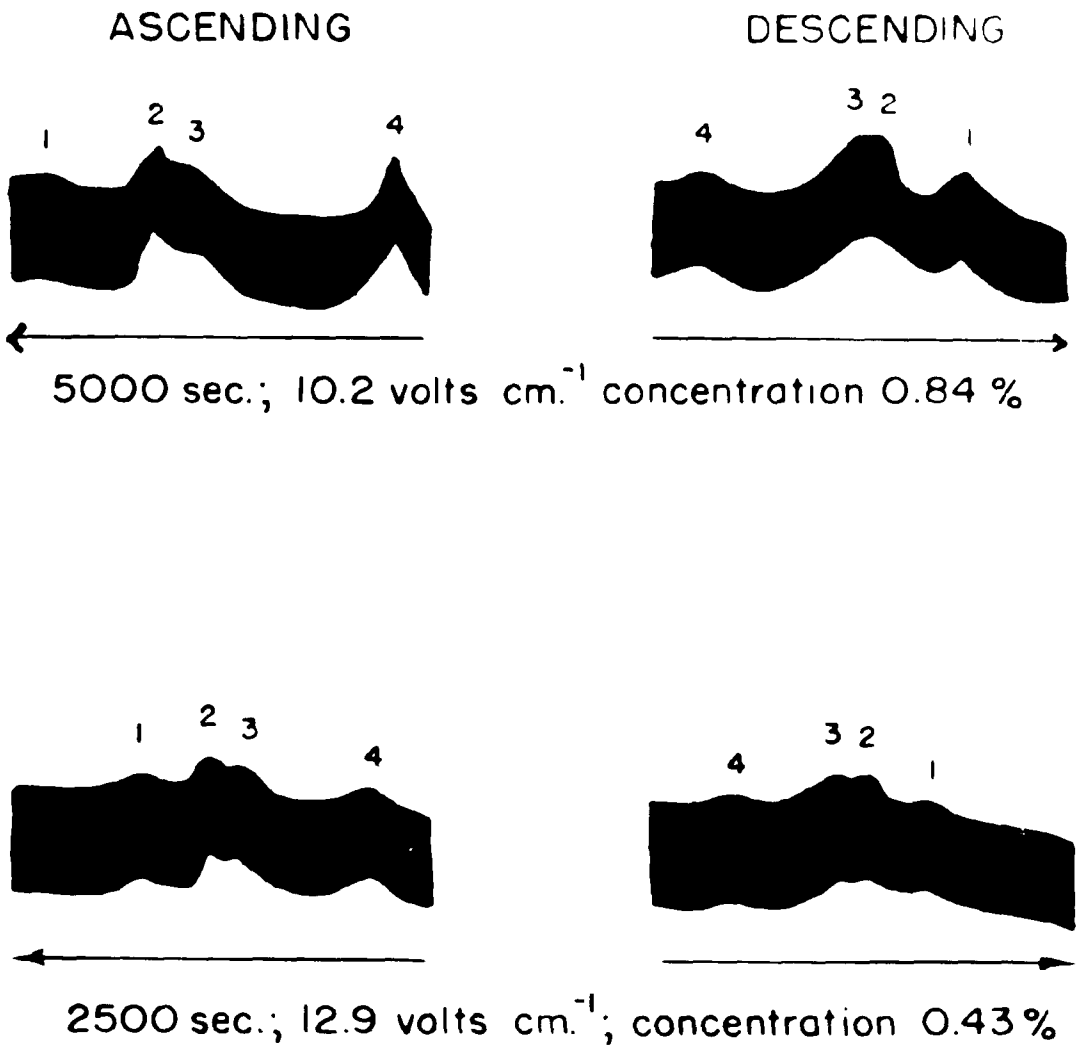

Figure 23. Electrophoretic patterns of fat-membrane proteins isolated from homogenized milk and showing the effect of protein concentration on the resulting patterns in phosphate buffer at pH 6.5 and ionic strength of 0.1.

DISCUSSION

General

The work reported in this section was undertaken to obtain more information concerning the effect of homogenization on the physical and chemical properties of proteins in milk and more specifically, the effect on the nature of the fat-globule membrane. The data presented is exploratory in nature and should not be construed or interpreted as final evidence. The difficulties which are experienced in isolating a biological component or group of components from a material as complex as milk are obvious. The investigator should always be aware of the fact that the material, as finally isolated, may not be entirely representative of its naturally occurring counterpart; and that resulting experimental data, although significant for the materials isolated, may not be entirely representative. On the other hand, one should not hesitate to invade the inner secrets of nature simply because of the lack of an absolute certainty of approach. The fat-membrane as it exists in nature, closely

associated with fat globules at the plasma/fat interface, offers such a challenge.

The membrane-materials of normal and remade milks have been investigated from time to time, notably among the more recent workers have been Palmer and his colleagues. However, the changes in the nature of the milk proteins and the fat-membrane wrought by homogenization have not been studied extensively. The available information is speculative in nature, since it has been recorded primarily from isolated observations on certain changes in the chemical and physical properties of milk following homogenization. Although the data presented in Section III are not conclusive, they supply additional fundamental information to the nature of the fat-membrane proteins occurring in both nonhomogenized and homogenized milk.

Discussion of Experimental Results

Influence of Homogenization on the Nitrogen Distribution in Milk

The fractionating technique used in this study to establish a definite series of nitrogen-containing fractions was adopted after careful preliminary trials with Rowland's (1933-34) procedure and certain of its modifications (Hetrick, 1947).

The four fractions isolated—total nitrogen, noncasein, nonheat-coagulable and nonprotein nitrogen—represent readily attainable fractions of milk proteins. No differentiation between the questionable albumin and globulin fractions was attempted because of certain irregularities in the separation procedures and in the characteristics of the fractions separated. If both of these components had been determined as a single fraction then any effect of processing would be manifested in the nitrogen value of the entire fraction. Such data would be suitable for comparative studies.

Both of Rowland's procedures for determining noncasein nitrogen were tried, but no noticeable difference was observed in the nitrogen values, so the method outlined in the experimental procedure was used because of its convenience.

The nitrogen distribution values obtained for milk which had been homogenized at different pressures showed a substantial increase in casein- and nonprotein nitrogen and a corresponding decrease in heat-coagulable and proteose-peptone nitrogen. These data reflected, in part at least, the heat effect of high-pressure homogenization (Rowland, 1933-1934), although the data of Shahani and Sommer (1951) do not seem to

be so pronounced in this respect. The significant changes in the nitrogen distribution have been assumed to be due to the adsorption of certain milk proteins on the increased fat surfaces when milk was homogenized at high pressures (Trout, 1950).

Influence of Homogenization on the Distribution of Milk Components in Cream and Skimmilk

Solids-not-fat. By making the assumption that the plasma portion of the separated creams (Table 11) is similar in composition to the skimmilk, it is possible to estimate the concentration of solids-not-fat in the cream samples (Table 11, Column 4). When the estimated value for the amount of solids-not-fat was subtracted from the determined value (Table 11, Column 3), the cream separated from homogenized milk contained a much larger amount of solids-not-fat per unit of fat than did the cream separated from nonhomogenized milk. This difference in the concentration of solids-not-fat was assumed to represent that portion of the solids-not-fat present in milk or cream which is closely associated with the surface of the fat globule, or possibly part of the fat-membrane proteins.

By an additional assumption; namely, that this specific value represents only protein-like materials, it is possible to draw some conclusion concerning the effect of homogenization on the nature and concentration of the fat-membrane material. At least it is not unreasonable to assume that the increase between the determined and the theoretical solids-not-fat value in the cream samples obtained from homogenized milk occurred as a direct result of homogenization, since other conditions remained equal. In this instance there was a 5-fold increase in the concentration of solids-not-fat which coincides with the 4- to 6-fold increase in the surface area of the fat due to homogenization (Trout, 1947). This difference in the concentration of solids-not-fat per unit of fat between creams from homogenized and nonhomogenized milk was even more significant when a similar difference also was found to exist between the plasma-free, washed creams from homogenized and nonhomogenized milk (Table 14).

Nitrogen distribution. Similar conclusions concerning the difference in concentration of solids-not-fat were evident from the data obtained from the distribution of nitrogen in the various fractions of cream and skimmilk (Table 12). These

data indicate that the increase in total nitrogen in the plasma-corrected cream from homogenized milk was determined as casein nitrogen even though lower values were recorded for total nitrogen and noncasein nitrogen. When the concentrations of nitrogen in the cream samples are expressed as protein, the creams from nonhomogenized milk contained 0.102 per cent protein, compared to 0.426 per cent protein in creams from homogenized milk (Table 11). Similar values were calculated by van Dam and Sirks (1922) for cream separated from nonhomogenized milk. The differences in the protein content between the fat-free skimmilk and fat-free creams were reported to be 0.08 and 0.215 per cent, respectively.

Minerals. Since some of the calcium nonphospholipid phosphorus and ash components in milk are conceivably associated with certain milk proteins, their concentration in each cream and skimmilk sample was determined (Table 13). When adequately expressed, these concentrations pointed to an increase in the concentration of protein materials per unit of fat in cream from homogenized milk.

Influence of Homogenization on the Distribution of Milk Components in Washed Cream and Washed-cream Serum

The data concerning the distribution of nitrogen in the various fractions of washed creams (Table 15) show that a higher concentration of nitrogen per unit of fat existed in cream from homogenized milk than in cream from nonhomogenized milk and that this increase was manifested as casein nitrogen. However, the washed-cream sera obtained from the homogenized samples yielded nitrogen values which indicated the presence of proteins other than casein, namely, heat-coagulable proteins and some proteoses or heat-stable proteins. Presumably, proteins that were adsorbed or associated with the fat-membrane, were determined as casein nitrogen by the fractionation procedure employed in this work. Such an observation seems logical, since all of the fat was retained in the protein precipitate formed at pH 4.67. However, in the case of the sera which were produced from washed creams as a result of churning, the colloidal proteins were free to function as in the natural state, consequently some of the protein was measured as heat-coagulable and some as proteose-peptone nitrogen. The largest concentration was measured as casein nitrogen.

Influence of Successive Washings on the Concentration of Fat, Total-protein Nitrogen and Lipoid Phosphorus in Cream from Homogenized and Nonhomogenized Milk

The data shown graphically in Figure 13 and 14 help to differentiate between the fat-membrane material of nonhomogenized and homogenized milk. The cream separated from nonhomogenized milk exhibited a gradual loss in total nitrogen after the second washing, either by (a) a continuation of the dilution effect, (b) the sloughing off of the membrane material or (c) a gradual loss of the smaller fat globules. Any of these possibilities would lower the concentration of nitrogen per unit of fat since the total surface area of the remaining fat globules would be smaller. Similar results were observed from the determination of the phospholipid phosphorus, but a more pronounced drop was observed after the fourth washing. Since the units differ for expressing nitrogen and phospholipid phosphorus, it was believed that the trend was more significant than the degree of change indicated. The data obtained for normal cream agree in a general way with those reported by Jack and Dahle (1937b).

The values obtained on the washings from the homogenized cream showed a different behavior. The nitrogen values

per unit of fat leveled off more quickly and at a higher value, which would indicate that more protein was tenaciously associated with the fat-globule surface. In general, the phospholipid phosphorus content of homogenized cream remained fairly constant per unit of fat on successive washings, but showed an unaccountable rise after the fifth washing. Nevertheless, the data serve to illustrate the increased stability of the fat emulsion in homogenized milk and a marked increase in the amount of protein adsorbed on the fat as a result of homogenization.

Some Chemical and Physical Characteristics of Fat-membrane Proteins

The 13 to 14 per cent of nitrogen in the fat-membrane proteins reported in this study is somewhat higher than that generally reported in the literature, in which the nitrogen concentration is approximately 12 per cent. Whether the methods of preparation and separation of the phospholipids from the protein used by various investigators had a degrading effect on some of the protein, thus causing the percentage of nitrogen to be lower, is not known and can only be conjectured. It should be noted, however, that the cold (0° to 3° C.) ethanol-ether extraction procedure resulted in the best fat-membrane

preparation, even to the extent of being more efficient in the removal of phospholipids from the crude membrane material. In these particular samples the percentage of nitrogen, on a lipid-free basis, was slightly lower (13.11 per cent) for the nonhomogenized milk, fat-membrane proteins than for the homogenized milk, membrane proteins (13.65 per cent). Since these data represent only one preparation, it is difficult to ascertain any definite relationships between the nitrogen content of the normal membrane-proteins and that isolated from homogenized milk. The two samples used in this study represent what was thought to be the best of eight previous preparations isolated by several different techniques, but were discarded for one or more technical reasons. The final isolation procedure represented the most satisfactory method of preparing the fat-membrane proteins for electrophoretic and microbiological analyses. In view of some divergent results obtained in this study, certain other modifications in the separation procedure remain to be studied.

The positive Molisch reaction furnished some clue regarding the nature of the membrane materials and indicated the presence of a carbohydrate residue on one or more of the

constituent proteins. The negative nitroprusside test indicated that the isolation procedure had no effect on certain sulfur-bearing proteins, if they were present. However, the nature of the results obtained with the nitroprusside test following the heating of the respective aquasols, strongly suggest the absence of proteins which contain heat-labile sulfur in the normal fat-membrane and the presence of such materials in the membrane-proteins prepared from homogenized milk. Hattori (1925) reported an absence of reducing groups in "haptein," a protein supposedly isolated from the fat-globule membrane. The normal fat-membrane protein preparations were lighter in weight per unit volume of powder than those prepared from homogenized milk and also lacked the clear crystalline structure and luster of the homogenized preparations.

Electrophoretic Characteristics of the Fat-membrane Proteins

Electrophoretic patterns in various buffer media. The electrophoretic patterns obtained with the membrane preparations in the glycine-hydrochloric acid, phosphate and veronal-citrate buffer systems showed the clearest electrophoretic resolution. In these particular patterns for normal fat-membrane

proteins, there were two main peaks, which indicated the presence of two different membrane-protein components of almost equal mobility. Occasionally a third and even a fourth component appeared in the electrophoretic pattern as a diffused peak of low concentration. These minor or diffused peaks usually occurred in the ascending side of the Tiselius U-tube and may represent certain abnormalities of electrophoresis, but whenever possible, the diffused peaks were measured and their corresponding electrophoretic mobilities calculated. The migration of protein components in the descending side of the cell takes place through the protein aquasol and are generally considered to be more representative of the protein components. However, because of unmeasurable changes in the conductivity and pH within the U-cell during electrophoresis, mobilities are usually determined for each component by averaging the mobilities in both the ascending and descending patterns.

An examination of the electrophoretic patterns obtained for the homogenized milk, fat-membrane proteins in the same buffer systems show two strong peaks of somewhat similar mobility as those appearing in comparable patterns for nonhomogenized membrane-proteins, but they show major differences in

the quality and quantity of the components. In addition to these principal components, a third peak of considerable prominence appeared, but it possessed a very low mobility. In fact, it occurs so near to the initial boundary that it was questionable as to whether it was a component-peak or a distortion of the initial boundary, but it was present in all of the ascending and descending patterns of the homogenized milk, fat-membrane proteins. A fourth peak of minor prominence and of diffused character also occasionally appeared. The appearance and characteristics of the electrophoretic patterns obtained by the electrophoresis of homogenized milk, fat-membrane proteins differed from the patterns obtained for normal membrane-proteins, therefore the assumption was made that the components adsorbed on the fat of normal milk differs from those associated with the fat of homogenized milk.

Mobility-pH relationships of various membrane-proteins. Because of the preliminary nature of these experiments, a direct comparison of the calculated peak-mobilities obtained in this study with the electrophoretic mobilities of the various milk protein components appearing in the literature would be misleading. Hence, the nature of the individual protein components

appearing in the electrophoretic patterns of nonhomogenized and homogenized fat-membrane proteins cannot be definitely ascertained until the various components have been separated in a homogeneous form and electrophoretically characterized in various buffer media at different pH values. These data could then be compared with similar data obtained from known milk protein components. In addition to these electrophoretic data, other chemical and physical tests also would be required to characterize the components. Such a study is contemplated.

Some information relative to the nature of the components can be obtained, however, by plotting the calculated mobilities against the pH values from which they were obtained. Figure 24, representing values obtained for nonhomogenized milk, membrane-proteins and Figure 25, representing the data for homogenized milk, membrane-proteins, show this relationship. The points of interception of the mobility curves and the pH line at zero mobility represents the area of no mobility, generally referred to as the isoelectric point of the protein component. Obviously, the data are too limited to establish an approximate isoelectric zone for the components involved, but in the case of the normal membrane-proteins, one

electrophoresis (unreported) was attempted in acetate buffer at pH 3.8 with no apparent migration of the protein components after two hours of exposure to the electric field. This observation suggests that the isoelectric zone of this particular sample was approximately pH 3.8. A similar trial was not made with the homogenized milk, membrane-proteins, therefore, the approximate location of an isoelectric zone was limited to the normal shape of the respective curves. More precise studies in this respect are contemplated. Within these limitations, it appears that the isoelectric range of normal membrane-proteins (ca pH 3.8) was approximately the same as that reported by Palmer and Wiese (1933) and Jack and Dahle (1937b), while that of the homogenized milk, membrane-proteins was somewhat higher.

Influence of temperature on the electrophoretic mobilities of membrane-proteins. The extraction of lipid material by the warm ethanol-ether procedure affected the electrophoretic characteristics of the fat-membrane materials, especially the homogenized milk, membrane-proteins. Whether the changes noted in the electrophoretic patterns shown in Figure 22 resulted from a loss of a protein component or a portion of a

component soluble in alcohol (Osborne and Wakeman, 1918; Krejci, 1942; and others), or from fundamental changes in the electrostatic properties of the proteins themselves could not be ascertained.

Influence of concentration on the electrophoretic mobility of membrane-proteins. The data show an increase in the mobility of the protein components of the homogenized milk, fat-membrane proteins and better electrophoretic resolution of the components when the concentration of membrane material in the electrophorized buffer-protein solution was reduced from 0.84 to 0.43 per cent. Smith (1946b) reported mobilities for whey protein components that demonstrated this behavior, however, Longsworth and MacInnes (1940) stated that mobilities vary only slightly with the concentration of the protein.

The membrane protein preparations were never totally soluble in any of the buffer solutions, but the degree of solubility varied with the pH of the buffer. The consistently greater solubility of the homogenized milk, membrane-material as compared to the normal fat-membrane material also suggests a difference in their respective compositions. Hattori (1925) found that "haptein" was insoluble in water, dilute acids and

bases, but was soluble in strong alkali. The question arises as to whether the electrophorized proteins in solution were representative of the whole membrane material, or only the hydrated portion. The electrophoretic patterns obtained at different concentration and in different buffer systems indicated that the hydrated portion was representative of the entire membrane preparations.

Amino Acid Assay of Fat-membrane Proteins

The differences that exist between the fat-membrane proteins of nonhomogenized and homogenized milk as shown by electrophoresis and other chemical and physical characteristics are not manifested to the same extent in the amino acid composition of the two membrane-protein preparations. Since the concentrations of only six of the essential amino acids were determined, no evidence is available concerning possible variations in the concentration of the four remaining essential amino acids. With the exception of lysine, the amino acid composition of the nonhomogenized, membrane-proteins are quite similar and the minimum molecular weights were of the same order.

The fact that the membrane-proteins are composed of a mixture of protein components, as shown in other parts of this study, permits several plausible explanations concerning the nature of these preparations. First, possibly the concentration of the major protein components in the membrane-proteins had not been altered materially by homogenization; secondly, perhaps the plasma proteins adsorbed on the fat surfaces as a result of homogenization were of essentially the same amino acid composition as the protein mixture that is normally associated with the fat-globule membrane; and thirdly, the rearrangement of proteins in the fat-membrane of homogenized milk may have been such that the over-all amino acid composition was by chance quite similar to the composition of the original membrane-proteins. On the other hand, since the membrane-proteins represent a mixture of proteins rather than a single homogeneous component, a reasonable assumption would be that the apparently slight variations in the over-all amino acid composition of these preparations denote very significant differences in their specific protein constitution.

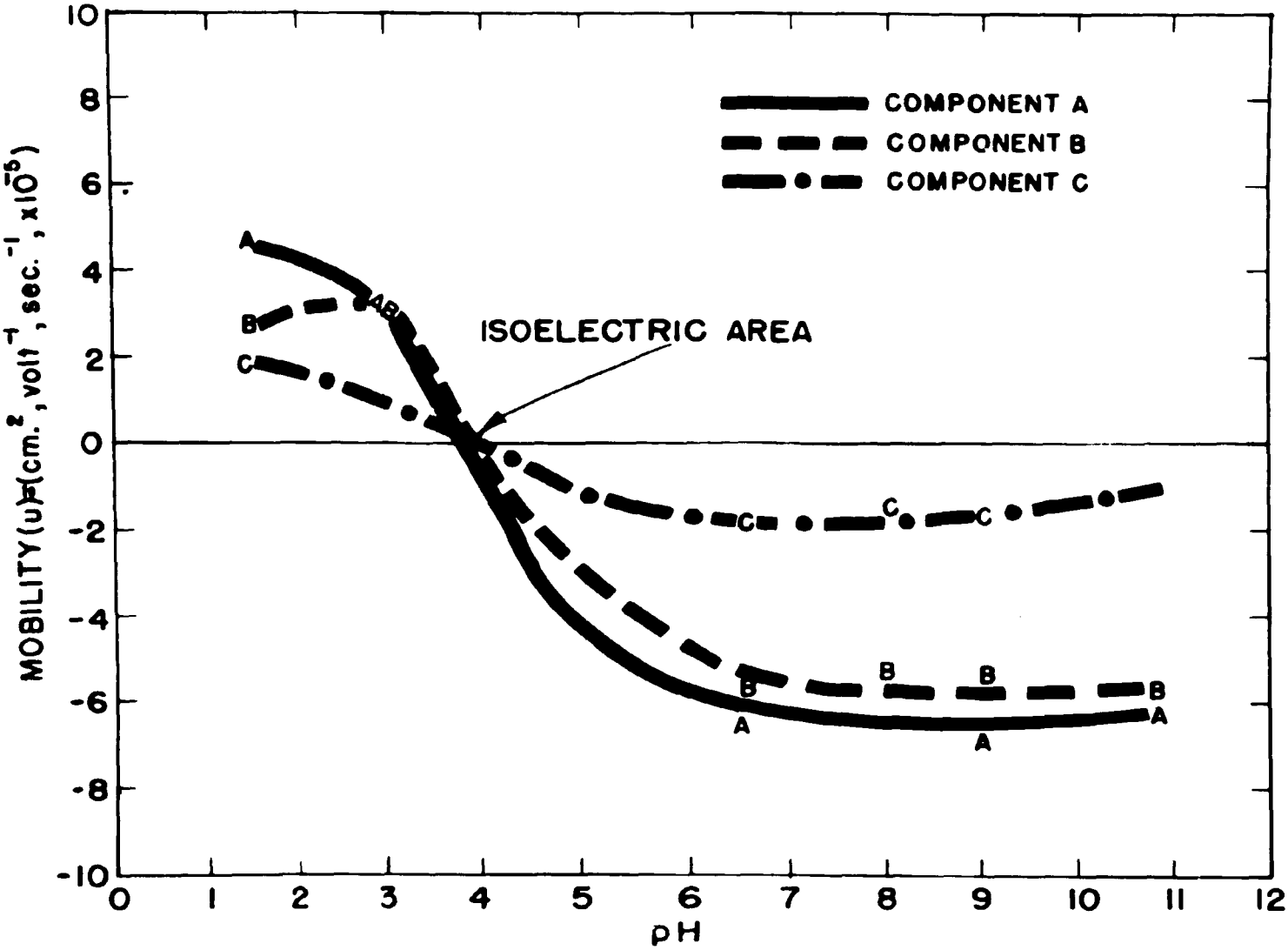

Figure 24. pH-mobility curves for the major components of the nonhomogenized-milk membrane-proteins.

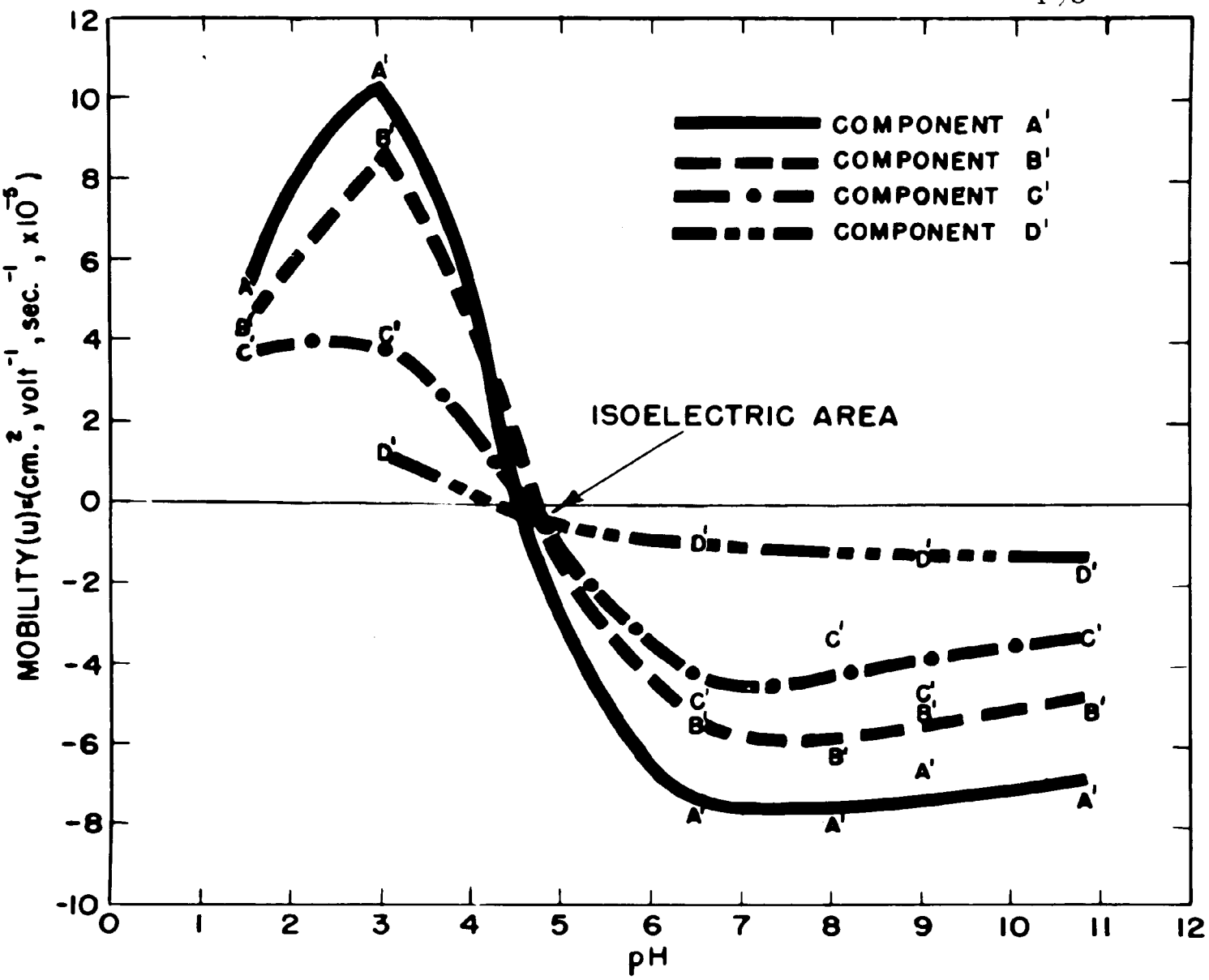

Figure 25. pH-mobility curves for the major components of the homogenized-milk membrane-proteins.

SUMMARY FOR SECTION III

Normal homogenizing pressures (2,500 pounds) had little effect on the distribution of nitrogen in various fractions of pasteurized milk, whereas high homogenizing pressures (4,000 pounds) caused a reduction in heat-coagulable and proteose-peptone nitrogen and an increase in the casein and nonprotein nitrogen.

The concentration of total solids in cream from homogenized milk showed a fivefold increase in the solids-not-fat content over that found in nonhomogenized milk. The distribution of nitrogen in the various protein fractions showed a similar increase. A threefold increase was obtained in total nitrogen per unit of fat in the cream separated from the homogenized milk and was determined as casein nitrogen. These observations also were supported by the mineral distribution data.

The determinations for total solids, fat and nitrogen in the washed cream samples from homogenized milk showed approximately a threefold increase in the concentration of solids and nitrogen per 100 grams of fat. The increase in total nitrogen was found in the casein-nitrogen fraction. The distribution

of nitrogen in the washed-cream sera showed that all of the nitrogen in the serum from the nonhomogenized milk was determined as casein-nitrogen while the nitrogen that was present in the sera from homogenized milk included casein-nitrogen, heat-coagulable nitrogen and proteose-peptone nitrogen.

The lyophilized fat-membrane proteins prepared from the washed-cream sera had a nitrogen content of approximately 13.5 per cent on the anhydrous, lipid-free basis. The preparation gave positive Molish and biuret reactions. Aquasols of the nonhomogenized milk, membrane-proteins gave a negative nitroprusside reaction, both before and after heating; whereas the aquasol of homogenized milk, membrane-proteins showed a positive nitroprusside reaction after heating.

Although no definite conclusions can be made from an examination of the electrophoretic patterns concerning the nature of the protein components in the fat-membrane preparations, it is evident that their characteristics are different. Generally, two or three components were observed in the nonhomogenized milk, membrane-proteins, whereas three to four components were observed in the homogenized milk, membrane preparation. The identification of the individual components has not been

achieved. The temperature of the ethanol-ether extraction and the concentration of protein in the electrophorized buffer solution influenced the mobilities of the protein components.

Microbiological analyses for isoleucine, leucine, methionine, phenylalanine and valine made on fat-membrane proteins from nonhomogenized and homogenized milk revealed only slight differences in the concentration of these amino acids between the two membrane-proteins, but a similar determination for lysine showed a rather significant increase in the concentration of lysine in the membrane material from homogenized milk. The amino acid composition of both of the membrane-proteins differed markedly from any of the recognized milk protein fractions.

GENERAL SUMMARY AND CONCLUSIONS

Experiments have been conducted with milks of varying fat and solids-not-fat content to study the effect of variations in temperature and pressure on the production of high viscosities in homogenized milk, to determine the amounts of solidified fat in globular fat at temperatures which are conducive to the development of high viscosities and to report results on the isolation, preliminary characterization and electrophoretic patterns of the fat-globule membrane proteins.

The Influence of Variations in the Homogenization Procedures and the Composition of Milk on the Efficiency of Homogenization and the Viscosity of Milk

Milk containing five per cent or more fat, low homogenization temperatures (80^{o} to 70^{o} F.) and high, single-stage homogenization pressures (2,500 pounds or higher) are conditions that contribute to the clustering of fat globules and to the development of a high viscosity in homogenized milk. The detrimental effects of these processing conditions on the viscosity of homogenized milk were reduced or completely

eliminated by double-stage homogenization or by maintaining the temperature of homogenization above 100° F.

Normal variations in the solids-not-fat content of milk produced insignificant variations in the viscosity of homogenized milk.

The Influence of the Physical State of the Fat on the Efficiency of Homogenization and the Viscosity of Milk

At homogenization temperatures (70° to 90° F.) conducive to the formation of an extensive fat-globule, cluster-like structure and a high viscosity, the milk fat was partially solidifed. The extent of fat solidification in milk homogenized immediately after pasteurization varied from approximately 0.5 to 2.0 per cent and from 7.0 to 18.0 per cent in pasteurized milk which had been stored at 45° F. for 20 hours prior to homogenization.

Milk that showed every indication of being efficiently homogenized was processed when the fat was in a liquid state and at temperatures above 100° F.

Some Characteristics of the Fat-membrane Proteins of Nonhomogenized and Homogenized Milk

Moderate homogenization pressures (2,500 pounds) had little effect on the nitrogen content of various fractions of milk proteins. High pressures (4,000 pounds), however, caused a reduction in heat-coagulable and proteose-peptone nitrogen and an increase in the casein and nonprotein nitrogen.

The distribution of fat and solids-not-fat in the skimmilk and cream separated from nonhomogenized and homogenized milk, as well as in the respective washed creams, showed that a greater proportion of the solids-not-fat were associated with the fat in homogenized milk, per unit of fat, than was associated with an equivalent amount of fat in nonhomogenized milk. This observation was substantiated further by data based on the distribution of protein nitrogen, total ash, total calcium and non-phospholipid phosphorus in the respective cream samples.

The distribution of nitrogen in the protein fractions obtained from the buttermilk-like sera of washed cream from nonhomogenized and homogenized milk showed that the major protein fractions in the washed-cream serum from homogenized

milk were made up of casein nitrogen, heat-coagulable nitrogen and proteose-peptone nitrogen.

Fat-membrane proteins were isolated from washed cream, buttermilk-like sera by extracting the lipoid materials by an ethanol-ether washing technique, after which the protein residue was dispersed in distilled water and lyophilized to an anhydrous powder.

The lyophilized membrane-proteins contained about 13.5 per cent nitrogen.

Aquasols of the nonhomogenized milk, membrane-proteins exhibited positive Molisch and biuret reactions, but were negative to the nitroprusside test in both the unheated and heated portions of the aquasols. Aquasols of homogenized milk, membrane-proteins gave positive Molisch and biuret reactions, but the heated portion of the aquasol showed a positive reaction to the nitroprusside test; indicating the presence of protein components that contain labile-sulfur (whey proteins).

The electrophoretic patterns obtained for the fat-membrane proteins in various buffer systems showed distinct differences in the characteristics of the protein components of the two membrane-protein preparations, indicating that the

fat-membrane material associated with normal milk fat is different than the fat-membrane material associated with the homogenized milk fat. Two and occasionally three distinct components were apparent in the electrophoretic patterns of nonhomogenized milk, membrane-proteins, whereas three and possibly four distinguishable components were present in the homogenized milk, membrane-proteins. Due to the preliminary nature of the work, it was not possible to isolate and characterize the individual components.

No definite conclusions can be formed from the results of the microbiological analyses for amino acids concerning any differences in the composition of the fat-membrane proteins of nonhomogenized and homogenized milk. Some minor differences in amino acid concentrations do exist, especially in the case of lysine which is present in a higher concentration in the homogenized milk, membrane-proteins than in the normal membrane-proteins. Both of the membrane-proteins differed in their amino acid compositions from any of the recognized milk protein fractions.

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