THE ROLE OF LEUCOCYTES IN THE SEDIMENT FORMATION IN HOMOGENIZED MILK

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by

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INTRODUCTION

In many instances the practice of homogenizing milk for fluid milk consumption has created the problem of sediment formation in the bottled product. This sediment appears often as a fine ring of silt or sediment at the bottom of the bottle, varying in intensity and color.

While the greyish and greyish-yellow colors are perhaps the most common ones, other colors such as whitish, brownish and reddish may be observed, depending on the main source of the sediment. Although this sediment may not lower the food value of the milk or impair the healthfulness of the product, from the esthetic point of view it is an important defect in the eyes of the consumer. The distributor is, therefore, confronted with the difficult problem of preventing or removing the suspended particles in the homogenized milk, which upon storage settle down and form the sediment.

Both, the selection of clean milk, according to the standards of the sediment test, and the filtration of milk prior to homogenization are not reliable measures to prevent sediment formation in every case. Power clarification, however, before or after homogenization will remove most of the suspended particles and thus render the milk sediment free.

Since the results of previous workers have shown that leucocytes, epithelial cells and cell debris make up a large part of the sediment in nearly every case, a study was undertaken to investigate more closely the behavior of leucocytes in milk. The problem was attacked from various angles, all with the purpose in mind, that after a more complete understanding of the behavior of the cells had been obtained, a simple and effective remedy might be suggested.

REVIEW OF LITERATURE

Reasons for the occurrence of sediment. The main reason for the occurrence of sediment in homogenized milk seems to be due indirectly to the inhibited or retarded fat arising in the processed milk. Homogenization decreases the size of the fat globules from 2.25-11.0 microns to about 1.2 microns, using 2,000 pounds pressure (Tracy, 1936). This in turn increases the number of fat globules and their total surface area. Each fat globule before and after homogenization is surrounded by a casein membrane (Titus et al, 1928). Wiegner (1914) found that the thickness of the absorbed membrane was the same both in the fat of nonhomogenized and homogenized milk. He showed that about 2.27 per cent of the total casein present in milk would surround the fat globules in normal milk, while in homogenized milk 25.2 per cent of the total casein would surround the fat globules. The increased proportion of casein to fat increases the specific gravity of the fat which coupled with the reduced size of the fat globule prevents them from rising, thus allowing for an even and fairly stable distribution of the fat. According to Breed (1912) and Wilcox (1912) leucocytes will adhere to the rising fat globules. Bechhold (1929) attributed this property to the opposite electric charges of the leucocytes and the fat globules. If no fat rising takes place, then the leucocytes due to their higher specific gravity (Babcock, 1934a, 1939, 1940) will settle to the bottom carrying some fat with them. Doan (1938) stated: "Homogenization tends to agglutinate particles of fine dirt, bacteria and cellular elements of milk, and to cause them to settle."

Factors affecting sediment formation. Several factors have been found to

affect sedimentation in homogenized milk. Since they vary widely they are being discussed separately.

1. Cellular constituents of milk. Doan (1938) attributed the amount of sediment produced in homogenized milk primarily to the number of cells present in the milk. Tracy (1935) also considered boy cells as a factor affecting sediment formation. Babcock (1940) claimed that if milk contained fewer than 100,000 leucocytes per cc. of milk no trouble with sediment would be encountered.

Plastridge et al (1939) found that out of 2,125 samples of milk from healthy cows, 80.2 per cent contained less than 100,000 leucocytes per cc. with an average count of 73,000. On the other hand, in milk containing non-hemolytic staphylococci the average count was 220,000 leucocytes per cc. with only 50 per cent of the samples below 100,000 leucocytes per cc.

Hucker (1942) analyzed 30,331 samples of milk from 8,000 cows and found that 68 per cent of the samples contained less than 500,000 leucocytes per cc. and 10 per cent more than 1,000,000 per cc. Ninety-eight per cent of the cows yielding milk with counts over 500,000 per cc. showed the presence of streptococci and 92 per cent of cows yielding milk with the same counts gave positive reaction with Brom Thymol Blue. Baker and Breed (1920) found that "decreasing hydrogen ion concentration of fresh milk bears a relationship to increase of number of leucocytes." Other workers, Burr et al (1908), Koning (1910), Hoyberg (1911), and Varrier-Jones and Camb (1924) all showed that pathological conditions of the udder resulted in increased leucocyte counts. Thus the use of milk from infected udders apparently would contain counts above 100,000 leucocytes per cc. and according to Babcock (1940) sediment formation would take place.

2. Stability of milk solids. Doan (1929) stated that homogenization destabilized proteins in the presence of fat. This effect increased with fat concentrations and with efficiency of homogenization. He claimed that part of the loss of protein stability was due to the increase in hydrogen-ion concentration during homogenization. A lowering of pH by 0.0445 was observed by homogenizing pasteurized milk at 2500 pounds pressure. Tracy (1935) attributed unstable proteins as partial cause of sediment formation. Rowland (1933) has shown that pasteurization will denature and coagulate some protein.

Charles and Sommer (1934, 1935) reported the white sediment in milk to be coagulated milk solids. The addition of sodium and calcium salts, as shown by Charles (1934) and Hahn and Tracy (1940) would affect the stability of proteins, the addition of calcium resulting in a less stable protein. Tracy (1941) stated that "by destabilizing the protein by the addition of calcium chloride it is possible to increase the degree of sedimentation, "and by stabilizing the protein with sodium citrate the amount of sediment can be reduced."

According to Bodansky (1934) proteins obtain their stability from their electric charge and the water of hydration. They are amphoteric in nature and thus may be either basic or acidic. At their isoelectric point which is pH 4.6 for casein, (Rogers, 1935), pH 4.7 for lactalbumin and pH 5.5 for globulin (Bechhold, 1929) they are only stabilized by the water of hydration. If water of hydration is removed by heat, coagulation of the micelles will take place (Bodansky, 1934). Salts affect the electric charge of the micelles and thus in turn affect the stability of the protein (Bechhold 1929). According to Davies (1936) the removal of the electric charge will result in agglutination, while dehydration will cause coagulation of the protein. Sommer (1938) claimed that while lactalbumin and lactoglobulin

retain their stability at the isoelectric point, casein was not sufficiently hydrolyzed to prevent agglutination taking place.

3. Foreign particles. Trout and Halloran (1933) found that part of the sediment was made up of very fine dirt which could not be removed by ordinary filtering. Charles and Sommer (1934) reported that the gray color of the sediment was due to foreign dirt particles. According to Doan (1938, 1940) agglutination would facilitate settling, thus producing a grey sediment. The foreign particles resemble leucocytes in their behavior in milk in that they are carried up with the rising fat, and settle if no fat rising action takes place.

4. Effect of heat on sedimentation. As pointed out earlier, heat has a destabilizing effect on the milk proteins since it tends to remove the water of hydration, thus causing coagulation. According to Bechhold (1929) heat changes milk protein from hydrophilic colloids to hydrophobic colloids. Bodansky (1934) mentioned heat as one of the factors responsible for denaturation of proteins. He claimed that denaturation of proteins might take place at hydrogen ion concentrations far removed from their isoelectric point. Precipitation or flocculation, however, occurred at the isoelectric point. Rowland (1933) found that holding milk at 63° C. for 30 minutes resulted in the coagulation of 10.4 per cent of the lactalbumin. He observed that, "for each rise of 1° C. between 63° C. and 75° C. the rate in increase of denaturation was constant, the temperature coefficient of the reaction being 1.5." Davies (1936) also mentioned the fact that precipitation of albumin and globulin by heat favors molecular association of these proteins. This would tend to increase sedimentation, since the larger particles would be less stable.

5. Effect of pressure on sediment formation. The degree of pressure to which milk is subjected in the process of homogenization also seems to

affect sediment formation. Sommer (1938) stated that a pressure of 2500 pounds would increase the temperature of milk from 5 to 10° F. This would tend to increase the coagulation of proteins if homogenization were carried out at pasteurizing temperature.

A lowering in pH of milk by 0.0445 as a result of homogenization was observed by Doan (1929). Dahle et al (1930) found that as the homogenization pressure increased the pH of the ice cream mix decreased. A lowering of the pH increases the hydrogen ion concentration, thus reducing the electric charge on the protein molecules and rendering them less stable. Sommer (1938) explained the increase in hydrogen ion concentration to be due to the precipitation of tricalcium phosphate thus liberating free hydrogen ions. However, Istaz and von Soest (1907) could not observe an increase in titratable acidity after homogenization.

Doan (1938) found that piston-type homogenization gave greater deposits of sediment than did the Bump Rotary type when run at the same pressure. Higher pressures increased the sediment content due to the greater destabilizing effect on the proteins. The most important function of the homogenizer, however, is the shearing action of the pressure valve which reduces the size of the fat globules, together with the increased proportion of the total casein adsorbed at the fat surface, tends to stabilize the fat, as was mentioned previously. Tracy (1941) reported that with increasing pressure the percentage of nitrogen increased which he believed was due to the destabilization of proteins.

6. Effect of agitation on sediment formation. The work of Hahn and Tracy (1941) showed that agitation of homogenized milk, especially at high temperatures tended to speed up or increase sedimentation. Tracy (1941) claimed that agitation would cause clumping of cells, and thus facilitate settling.

7. Effect of storage temperature on sediment formation. Trout et al (1935) observed that heat shocking of homogenized milk seemed to favor sedimentation. Tracy (1935) reported that sediment was more likely to form when milk was warmed. Hahn and Tracy (1940) also found that increased temperatures after bottling would increase sedimentation. Tracy (1941) pointed out that storage temperatures of 40° F. were more desirable than higher temperatures so far as freedome of sedimentation was concerned. Sommer (1938) stated that low temperatures allowed for fat clumping and an increase in viscosity of the milk by the swelling or hydration of proteins, which would seem in the case of homogenized milk to tend to inhibit sedimentation in part at least.

Sources of sediment. According to the results of sediment analyses made by various workers the sources of sediment may be divided into the following three main groups, each of which will be dealt with separately.

1. Cell constituents entering milk during secretion. Milk as drawn from the udder of the cow contains various numbers of leucocytes, as shown by Russell and Hoffman (1907, 1908), Campbell (1909), Plastridge et al (1939), and Hucker (1942). According to Babcock (1934a, 1934b, 1934c, 1939, 1940) large numbers of leucocytes are present in the sediment of bottled homogenized milk. Tracy (1941) and Trout (1942) also reported the presence of leucocytes in the sediment. Baker and Breed (1920) found that milk with a high leucocyte content showed also an invariably high number of epithelial cells and cell debris. Brudny (1914) mentioned the fact that large numbers of epithelial cells may be present in normal milk. Both Babcock (1934a, 1934b, 1939, 1940) and Tracy (1941) reported the presence of epithelial cells in the sediment. Tracy (1941) also included cell debris and erythrocytes as occasional constituents of the sediment. Brudny (1914) stated that the presence

of erythrocytes could be detected by the reddish color of the sediment, while leucocytes and epithelial cells will give a yellowish sediment. Thus, leucocytes, erythrocytes, epithelial cells and cell debris may be regarded as sediment constituents entering milk during secretion.

2. Denatured milk solids. The work of Rowland (1933) showed that pasteurization would coagulate some of the albumin. Trout and Halloran (1933) and Trout et al (1935) analyzed sediment by the Mojonnier method and found that it contained fat and solids-not-fat. Charles (1934) reported that the white sediment was made up of casein. Charles and Sommer (1935) found the presence of "substances related to milk solids" in the sediment of homogenized milk. Tracy (1935, 1941) attributed some of the sediment to unstable proteins or particles of milk solids.

3. Foreign particles. Foreign particles include any dust, spores or other insoluble particles entering the milk from the time it is drawn until bottling, which cannot be removed by straining or filtering. Brudny (1914) mentioned that dirty milk would give a grey sediment. Trout and Halloran (1933) found very fine dirt to be a partial cause for sediment. Similar results were obtained by Doan (1938, 1940). Charles and Sommer (1934, 1935) concluded that grey-colored sediment in homogenized milk was due to dirt entering milk during handling on the farm.

Control measures. The following control measures have been suggested by various workers:

1. Selection of milk. The selection of milk appeared to be the first step for controlling sediment in the processed product. This was advocated by Jones (1929). Trout and Halloran (1933) advised clean milk production, especially in dry weather. Babcock (1934b) suggested selection of low-cell count milk in order to prevent sediment formation by leucocytes. The re-

sults of many workers, previously listed, show that mastitic milk contains large numbers of blood cells, which invariably would show up in form of sediment.

2. Filtration. Trials conducted by Dahlberg and Marquardt (1924) in which they compared the removal of cells by filtration and clarification, showed that filtration removed about 28 per cent of the total number, while clarification lowered the count by 66 per cent. Trout (1933) showed that the sediment test did not serve as a reliable guide in the selection of milk for homogenization. Milk with sediment scores of 9.5 would show dark sediment, while milk with a sediment score of 8.5 would show less sediment having a light yellowish color. Other experiments conducted (Trout 1934) with cream showed that filtration did not have as great an effect on removal of sediment as did clarification.

3. Clarification. The most common measure used to prevent sediment formation is by the use of clarifiers. McInerney (1917) reported that about 99 per cent of the insoluble dirt in milk is removed by clarification. According to Nieder (1936) clarification reduced the cell count of milk by 67.55 per cent. Trout and Halloran (1933) recommended power clarification. Babcock (1934b) suggested the same method. Hood and White (1934) advised power clarification at 40° F. which will eliminate entirely or reduce to a negligible quantity sediment deposits in homogenized milk. Opinions as to the time and temperature for clarification vary. Babcock (1934b, 1939, 1940) recommended: clarifying, homogenizing, pasteurizing, while Charles and Sommer (1934), Tracy (1935), Hahn and Tracy (1940) advise clarification after homogenization.

With respect to clarifying temperature, Hood and White (1934) found that 40° F. would give satisfactory results. Charles and Sommer (1934)

recommended clarification after homogenization while the milk was still hot. Marshall and Hood (1918) found that using 55° F., 75° F. and 100° F. as clarifying temperatures the dry weight of slime increased with increase in temperature. Using 90° F., 110° F., 125° F. and 140° F. the same trend was observed. Jacobsen and Olson (1931) found also an increase in reduction of cells with an increase in clarifying temperatures. Hammer (1916) in his studies on the clarification of milk, stated that, "the percentage of cells eliminated appeared to bear no relation to the original number of cells present, the temperature of the milk or the percentage of fat." By using the Doan-Buckley method he found on 52 samples of milk the percentage of cells eliminated to vary from 7 to 73 per cent with an average of 39 per cent. Hammer and Hauser (1918) obtained similar results. Work done at the Idaho Station (1926) showed that the clarification temperature seemed to have no effect on the amount of visible dirt removed.

4. Protein stability. Doan and Minster (1930) recommended the method of double homogenization or the use of a two-stage valve in order to increase the protein stability. They claimed that double homogenization would increase the pH slightly as compared with the effect of single-stage homogenization. Charles (1934) found that by the addition of sodium citrate the white sediment could be eliminated. Hahn and Tracy (1940) observed the same results.

5. Storage temperature and agitation. Hahn and Tracy (1940) found that holding bottled homogenized milk at temperatures between 40-45° F. would help to prevent sediment formation. The same authors found that agitation would cause clumping of suspended particles and thus facilitate sediment formation.

EXPERIMENTAL PROCEDURE

The outline of the general procedure used in performing the experimental work in this particular research problem was as described below. Special mention is made in the particular experiments of any changes in procedure and the reasons for them.

Source of Milk. All milk used in this work was obtained from the College Creamery. The homogenized milk, both regular (3.8% fat) and "Vitamin D" (4.5% fat) were from the college herd where machine milking was practiced, and a high degree of cleanliness was observed. The pasteurized milk was a mixed milk from regular College Creamery shippers.

Method of handling. Milk used for pasteurization was filtered at the incoming temperature which ranged from 50 to 60° F. A rotary pump was used to force the milk through the a von Gonton filter on its upward movement to the pasteurizing vat. Small lots were gravity filtered, using a regular milk filter cloth.

The milk to be used for homogenization purposes was clarified at the receiving temperature, using a DeLaval power clarifier. For clarifying small lots of milk a laboratory size, DeLaval power clarifier was used. All milk was pasteurized at 142° to 144° F. for 30 minutes and cooled to 40° F. over a sweetwater surface cooler.

Homogenization was performed by either of two Union Steam Pump, Duo-Visco valve viscolizers. This was done after pasteurization and prior to cooling, at a pressure of between 2000 and 2500 pounds per square inch. Both units were of sanitary construction, were washed regularly after each day's use, and kept in good working condition.

The milk was bottled directly after leaving the cooler, into clean sterile quart bottles and stored at 40° F. usually for 48 hours before

examinations of any kind were made.

Source of leucocytes. The leucocytes were obtained from the separator or clarifier slime at the College Creamery and were used either in the form of slime, or as washed leucocytes. Care was taken to collect and use only that portion of slime which resembled the leucocytes in color, omitting the reddish deposits close to the interior wall of the separator or clarifier bowl.

In preparing the washed leucocytes, a method similar to that suggested by Schuppius (1907) and used by Strynadka and Thornton (1938) was employed. The fresh slime was suspended in a physiological salt solution (8.5 gms. of NaCl in one liter of distilled water), centrifuged in a high-speed centrifuge, and the process repeated until the liquid remained fairly clear and the sediment showed a light greyish or greyish-yellow color. Microscopic observations of such preparations showed leucocytes only, with no other particles present. This sediment contained approximately one-hundred million leucocytes per milliliter.

Method of adding leucocytes. In both cases, whether slime or washed leucocytes were used, portions were weighed out into clean beakers and milk was added to produce a suspension. A spatula or rubber policeman was used to break up any clumps, and in addition, if it were felt necessary, the mixture was passed through a hand homogenizer to insure homogeneity. The so-prepared suspension was added to the respective portions of milk and mixed well before any samples were taken. The bottled product was shaken 25 times before sampling as in making a bacteriological examination.

Measuring the various layers. In order to determine the volume in the layers produced upon the storage of milk a cardboard measuring ruler was made for each of the quart and the pint bottles. The cardboard was cut to

fit the shape of the bottle, marked in inches to ½" divisions. By filling an empty bottle with water using a graduated cylinder, the volume in each division was obtained. This ruled cardboard could thus be placed conveniently close to the bottle and readings made without disturbing the contents of the bottle. The volume was then recorded in inches or in milliliters.

Examination for intensity of sediment. Sediment examinations were made after holding the bottles for 48 hours from the time of bottling. The milk having a temperature of 40° F. was carried into the laboratory room and examinations were made immediately afterwards by one or two persons before any changes in temperature occurred. Any shaking or tilting was guarded against, so as not to disturb any sediment present. The intensity of sediment was recorded as follows:

0 = no sediment

1 = slight sediment

2 = distinct sediment

3 = pronounced sediment

4 = very pronounced sediment

No higher value than 4 was given in any case, although the amount of sediment may have been even greater than the standard established for this group.

Method of obtaining various portions of the liquid. A suction-siphon arrangement was employed in drawing off the various layers formed, or in removing one-third portions of milk from each bottle. Clean bottles were used to collect the siphoned liquid. Care was taken not to intermix the individual layers, or portions, if such danger existed. This was especially necessary with pasteurized milk in which the upper layer usually carried more leucocytes per milliliter than did the lower portions.

Microscopic examinations. Of the three methods for counting leucocytes as listed by Campbell (1909) and Salus (1912), the volumetric method using the counting chamber seemed to be the most preferred one. Russell and Hoffman (1907) who compared the Stokes-Stewart smear-stain method with the Doan-Buckley volumetric method found that the latter showed almost uniformly higher results than the former. They found that "so far as the technique is concerned the volumetric method (Doan-Buckley) is more accurate. It can be made as rapidly as the smear sediment and is less trying on the eyes." They used the counting chamber method (1908) and so did Campbell (1908) in his work on leucocytes in milk. Accordingly, the counting chamber method was employed in this study.

The leucocyte counts were made according to directions recommended by the Spencer Lens Company, using Toisson's Fluid as a stain and Bright-Line Improved Neubauer Counting Chamber with 0.1 mm. depth between the surface of the counting chamber and the cover glass. White-cell blood pipettes were used. Each sample was transferred to the counting chamber, filling the squares on both sides of the moat. The cells were counted in the four-corner, 1 mm. squares and in the central ruled area on both sides of the haemacytometer (10 square mm. in all) thus giving a factor of 20,000. In making counts on sediment, the sample had to be diluted and a correspondingly larger factor had to be used. Wright's stain was used in preparing smeared stains and a procedure followed as outlined in the "Atlas of Hematology" by Osgood and Ashworth (1937).

Photographic preparations. In some instances, where photographs were desired, suitable adjustments in the method of procedure were made. The method used by Hammer and Hauser (1914) was employed to show the cream layer. The milk was added to a dry pint bottle, containing 10 cc. of a saturated

alcohol (95%) solution of Sudan III. The mixture was then shaken until all the color was uniformly dispersed. Thus the cream was of a reddish color and no difficulty was encountered in making the photographs. In photographing the washed cream in heat-treated whey mixture the light was adjusted from behind the bottles, so as to show the clearness or transparency of the whey. The cream, being less transparent than the whey showed thus a deeper shade on the picture.

The slides used for microphotographs were prepared at the time the experiment was made, and stained with Wright's stain, as mentioned previously.

Treatment of data. All leucocyte counts of two or more trials were treated logarithmically. The figures are based on the logarithmical average values shown in the tables. In some cases the data of one trial are shown only, although additional trials had been run, showing the same trend of results.

EXPERIMENTAL RESULTS

1. Effect on the migration of leucocytes when various amounts of separator slime were added to nonhomogenized and to homogenized milk.

The purpose of this experiment was to show the migration of leucocytes in nonhomogenized and in homogenized milk. By adding known weights and numbers of leucocytes to each quart bottle it was felt that the limit of the "carrying capacity" of leucocytes in the homogenized milk could be found. The nonhomogenized milk was used as a control, showing the behavior of leucocyte migration when the fat globules were not reduced in size.

Five trials were conducted, using fresh clarified, pasteurized, nonhomogenized and regular homogenized milk. Fresh separator slime was added at the rate of 0.0, 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, 5.0 grams per quart to quart bottles of nonhomogenized and homogenized milk. The bottles were held for 48 hours at 40° F., after which time examination for sediment and leucocyte counts of the upper, middle and lower one-third portions of each bottle were made.

For each gram of fresh separator slime added per quart of milk, an increase of approximately 800,000 leucocytes per ml. was observed in both the nonhomogenized and in the homogenized samples. This is shown in table 1, column 1. The distribution of leucocytes after storage, in numbers per millimeter and in per cent of total is shown in table 1 and figure 1 respectively. The data show that while in the nonhomogenized milk from 78 to 98.4 per cent of the total number of leucocytes were carried in the upper one-third portion of the bottle, the same portion in the homogenized milk contained only from 30.8 to 11.7 per cent of the total leucocytes per bottle. The homogenized milk, however, showed large increasing numbers in the lower

one-third portions, with greater additions of slime per bottle. Thus, upon the addition of 5 gms. of slime per quart 70.4 per cent of the total leucocytes, or over 8,000,000 per ml. were found in that portion.

The difference in the distribution of leucocytes in the bottles to which no slime was added is attributed partially to the low initial count and partially to the fact that clarification had removed the larger leucocytes, thus leaving the smaller, more stable leucocytes in the milk. It can be seen that there exists a fairly uniform distribution of leucocytes throughout the bottle in the homogenized milk to which no slime was added. There was, however, a slight settling and it might be said that the limit of the "carrying capacity" of leucocytes by this particular milk had been reached.

Upon the addition of 0.5 gm. of separator slime, 59.8 per cent of the total number of leucocytes were found in the lower one-third portion and slight sedimentation was observed, as shown in table 2. The same intensity of sediment, namely 1.4, was not observed in the nonhomogenized milk until 3 gms. of slime were added to a quart of milk. This sediment, however, although given the same intensity rating, showed only a comparative small number of leucocytes, namely 73,500 per ml., as compared with 1,334,000 per ml. in the homogenized milk showing the same intensity of sediment.

The rising of the leucocytes with the rising fat, and the settling in the absence of fat rising is vividly shown in figure 2. These columns are based on the average per cent of total leucocytes in each one-third portion of the bottle after storage, upon the addition from 0.5 to 5.0 gms. of slime per quart of milk. The strong attraction between the fat and the leucocytes in the nonhomogenized milk is clearly shown. The uniform distribution of fat in the homogenized milk might be a factor in holding some of the leucocytes

in suspension, thus a relatively larger number is carried in the upper and middle portions, as compared with the middle and lower portions of the nonhomogenized milk, resulting in a lower column in the lower third portion of the homogenized milk.

The intensity of sediment in the homogenized milk increased in direct proportion to the number of leucocytes present in the lower portion. In the nonhomogenized milk, however, the leucocyte count in the lower third portion of the bottle showed no relation to the intensity of sediment produced, as shown in figure 3. The sediment in the nonhomogenized milk, therefore, was not due primarily to leucocytes, while the same cannot be said in the case of the sediment in the homogenized milk.

Table 1. The distribution of leucocytes in nonhomogenized and pasteurized homogenized milk (Average of five trials)

Separator slime added gms./quart	Leucocytes per ml. before storage	Leucocytes per ml. after storage at 40° F. for 48 hours in the Upper one-third of bottle		Middle one-third of bottle		Lower one-third of bottle	
			% of total		% of total		% of total
Nonhomogenized							
0.0	131,000	328,400	78.0	55,700	13.2	36,700	8.8
0.5	493,000	948,200	89.0	63,500	6.0	53,700	5.0
1.0	967,200	1,658,000	95.3	32,000	1.9	38,900	2.2
1.5	1,374,000	2,214,000	95.1	34,900	1.5	79,400	3.4
2.0	1,890,000	3,318,000	96.2	60,800	1.8	68,800	2.0
3.0	2,439,000	5,142,000	97.3	68,400	1.3	73,500	1.4
4.0	2,905,000	6,157,000	96.8	58,500	0.9	142,000	2.3
5.0	4,091,000	8,290,000	98.4	47,000	0.6	91,900	1.0
Homogenized							
0.0	260,000	236,400	30.8	241,000	31.4	289,300	37.8
0.5	632,000	424,500	19.0	472,000	21.2	1,334,000	59.8
1.0	980,000	475,800	15.3	691,000	22.2	1,942,000	62.5
1.5	1,402,000	729,500	17.0	885,000	20.6	2,676,000	62.4
2.0	1,813,000	820,000	15.7	1,083,000	20.8	3,308,000	63.5
3.0	2,650,000	1,234,000	16.6	1,383,000	18.6	4,823,000	64.8
4.0	3,580,000	1,191,000	11.7	2,024,000	19.8	6,980,000	68.5
5.0	4,438,000	1,601,000	13.5	1,919,000	16.1	8,380,000	70.4

Table 2. Sediment in nonhomogenized and in homogenized milk to which separator slime had been added (Average of five trials)

Separator slime added gms./quart	Intensity of sediment when the milk was	
	Nonhomogenized	Homogenized
0.0	0.0	0.0
0.5	0.2	1.4
1.0	0.6	2.2
1.5	0.8	2.4
2.0	0.8	3.2
3.0	1.4	3.6
4.0	1.6	4.0
5.0	1.4	4.0

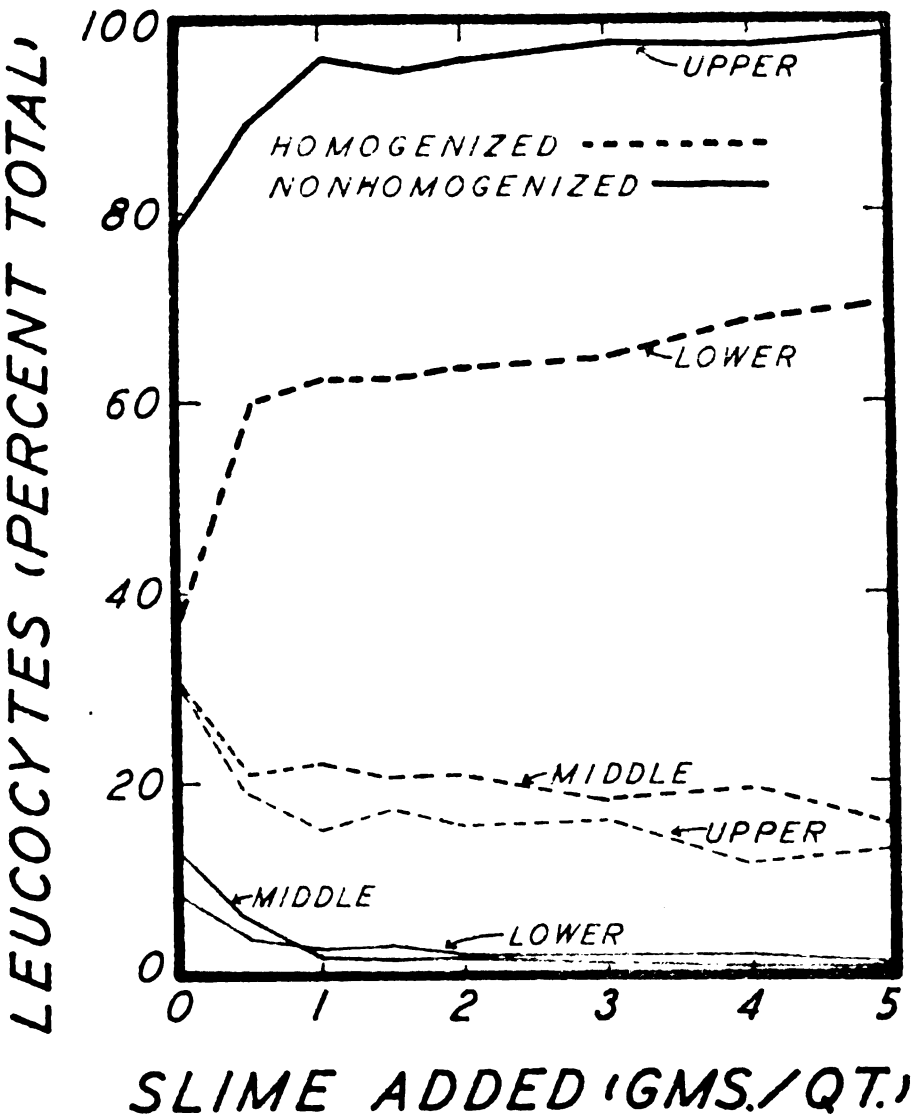


Figure 1. The distribution of leucocytes in the upper, middle and lower thirds of bottled nonhomogenized and homogenized milk having a wide variation in the number of leucocytes.

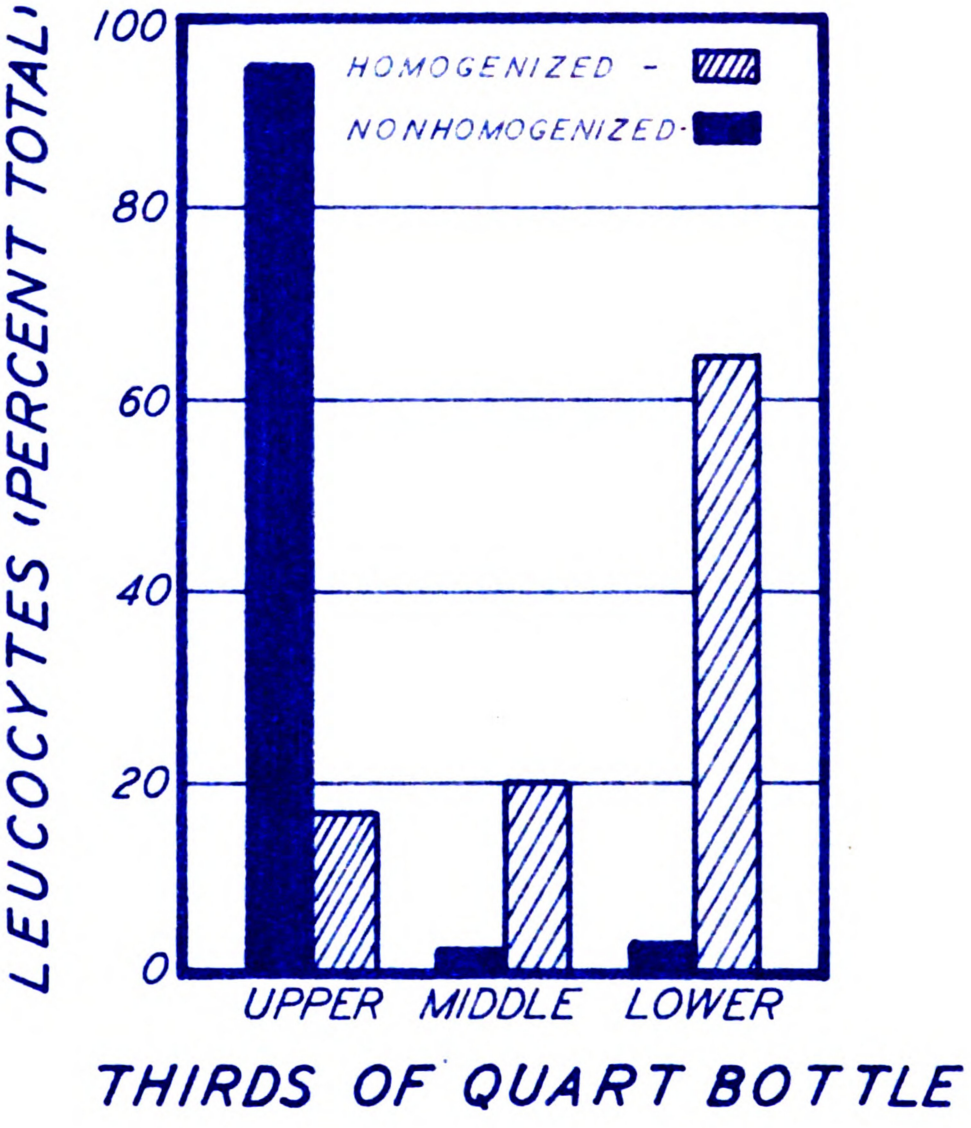


Figure 2. The percentage distribution of leucocytes in the upper, middle and lower thirds of bottled non-homogenized and homogenized milk.

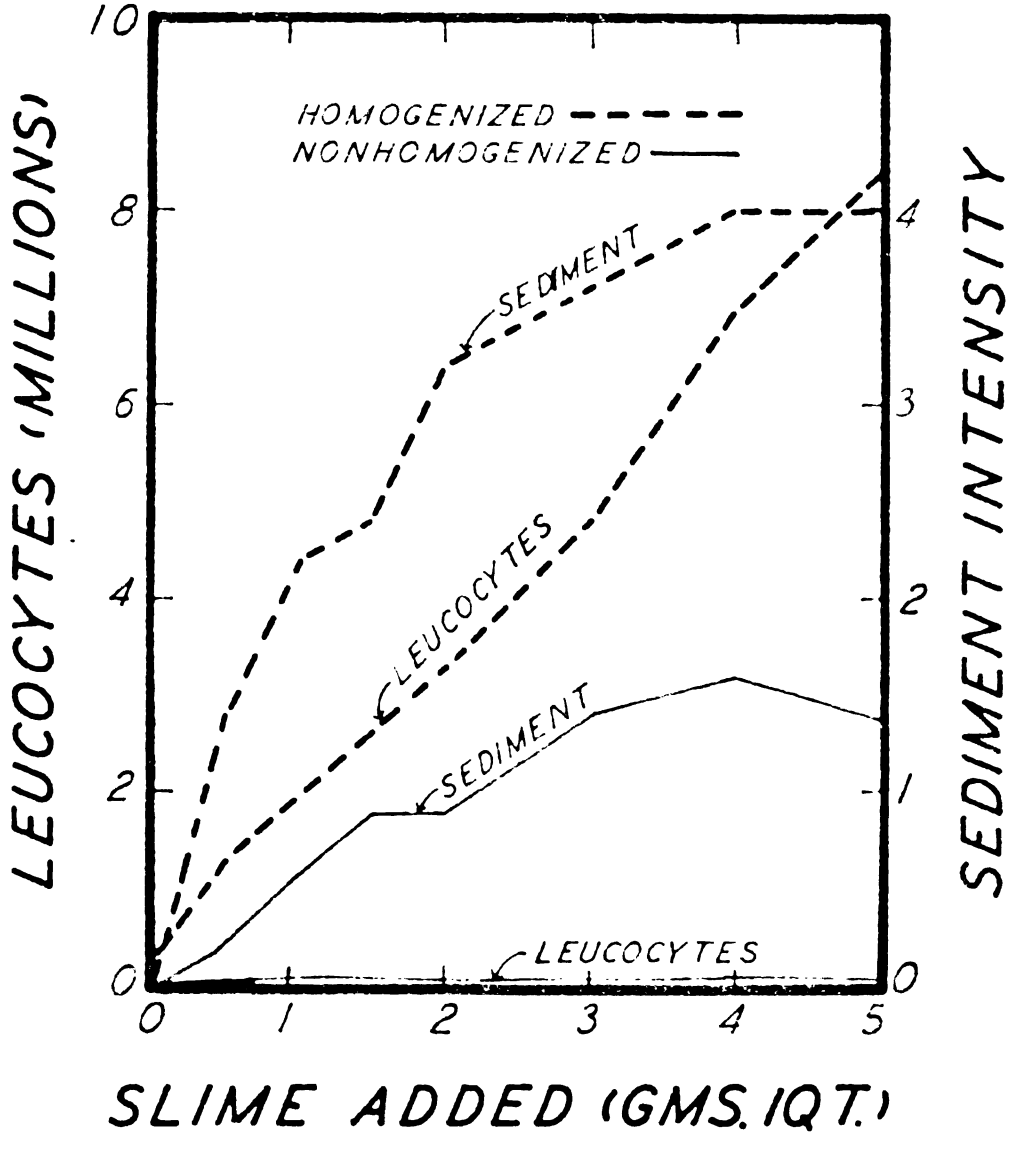


Figure 3. The relationship between sediment and the number of leucocytes in nonhomogenized and in homogenized milk.

2. Effect of adding various amounts of washed leucocytes to fresh pasteurized milk on the creaming of the milk.

Since previous trials showed the migration of leucocytes with the fat, it seemed of interest to find whether the fat would follow the leucocytes if sufficiently large amounts of washed leucocytes were added to the milk. Such a procedure would make possible a closer study of the attraction between the fat and the leucocytes.

Fresh washed leucocytes were added to pint bottles of fresh pasteurized milk in which creaming had not taken place. Increasing amounts of washed leucocytes were added as shown in table 3. For photographic purposes ten ml. of saturated Sudan III was added to each pint bottle. Each bottle was shaken twenty-five times and placed in storage at 40° F. for sufficient length of time to allow for the natural undisturbed migration of both the fat and the leucocytes. After two weeks time the bottles were examined for cream volume, sediment formation, distribution of leucocytes, and the percentages of fat and total solids in the upper middle and lower thirds of each pint bottle. The results are shown in tables 3, 4, 5 and 6 and in figures 4, 5, 6, 7, 8 and 9.

The addition of washed leucocytes had various effects on the creaming ability of milk depending upon the numbers added. The depth of the cream layer increased with added increments of washed leucocytes until finally both upper and lower layers were formed. While no sediment was observed upon the addition of up to 12.5 gms. of washed leucocytes per pint of milk, the cream volume was increased noticeably, as shown in table 3 and figure 9. At this point the upper third of the bottle contained over one hundred million leucocytes per ml., as shown in table 4, which was 95.8 per cent of the total

number present in the milk. These were associated with 92.8 per cent of the total fat present.

Upon the addition of 2.5 gms. more of leucocytes the "breaking point" of the creamline was reached, the leucocyte count dropped in the upper layer to approximately thirty-five million per ml. and continued to decrease in numbers upon the further addition of increasing weights of leucocytes, as shown in column 2, table 4. The divided cream volume was associated with marked decrease of fat and total solids in the upper layer.

The data in figure 8 show clearly the action of the leucocytes in general upon the cream volume. Data showing the migration of the fat with the leucocytes towards the bottom of the bottle are presented in table 5 and figure 5. The correlation between the number of leucocytes and the percentage of fat is shown in figure 6. It is of interest to note that the fat curve follows the leucocyte curve, although it tends to lag behind from the "breaking point" of the leucocyte curve on toward the right. However, upon the addition of between 30 and 40 gms. of added leucocytes, the upper and lower fat curves cross and thus, from the 40 gm. addition on, there was more fat in the lower one-third of the milk than there was in the upper one-third. From this graph (Fig. 6) it can be seen also that the fat had a strong tendency to remain in the upper part of the bottle, but that the stronger downward sweeping action of the leucocytes prevented the rising of the fat. The data show that there must exist a very strong attraction between the fat and the leucocytes, otherwise the fat would obey the laws of gravity and rise to the top.

The data in table 6 and figure 7 show the distribution of total solids in the milk to which various amounts of washed leucocytes had been added. The upper and lower total-solid curves follow closely the leucocyte and fat curves respectively and cross at the addition of between 20 and 25 gms. of

washed leucocytes per pint. A steep slope was observed between 12.5 and 15 gms. samples in all three curves, due to the shifting of both the fat and the leucocytes.

The cream volumes, both on the top and bottom of the bottle showed a greyish-red color upon the addition of increasing weights of leucocytes. In general, the color of the "cream" in the lower layer was of a darker greyish-red hue than that of the upper layer.

Table 3. The influence of added leucocytes on the creaming ability of milk

Leucocytes added gms./pint	Volume, in ml. per pint of the		
	Cream layer	Skimmilk layer	Sediment layer
0.0	63	412	0
2.5	58	417	0
5.0	66	409	0
7.5	70	405	0
10.0	74	401	0
12.5	77	398	0
15.0	45	390	40
20.0	40	390	45
25.0	35	385	55
30.0	30	390	60
45.0	23	365	87
60.0	15	360	100

Table 4. The influence of the number of leucocytes in milk on their distribution

Leucocytes added gms./pint	Leucocytes per ml. before storage	Leucocytes per ml. after storage in the Upper one-third of pint bottle No.	%	Middle one-third of pint bottle No.	%	Lower one-third of pint bottle No.	%
0.0	400,000	1,440,000	71.3	200,000	9.9	380,000	18.8
2.5	9,800,000	16,000,000	94.2	60,000	0.4	920,000	5.4
5.0	20,000,000	44,800,000	93.5	80,000	0.1	3,060,000	6.4
7.5	28,400,000	64,000,000	92.8	160,000	0.2	4,820,000	7.0
10.0	39,600,000	83,200,000	93.5	120,000	0.1	5,660,000	6.4
12.5	48,000,000	111,200,000	95.8	380,000	0.3	4,520,000	3.9
15.0	56,500,000	34,400,000	25.0	2,180,000	1.6	101,000,000	73.4
20.0	74,500,000	32,200,000	16.8	560,000	0.3	159,200,000	82.9
25.0	101,000,000	31,400,000	15.1	340,000	0.2	175,600,000	84.7
30.0	109,500,000	21,700,000	8.7	280,000	0.1	228,800,000	91.2
45.0	175,000,000	11,200,000	2.2	300,000	0.1	492,000,000	97.7
60.0	220,000,000	6,600,000	1.2	60,000	0.0	544,000,000	98.8

Table 5. The influence of the number of leucocytes in milk on the distribution of the fat after storage (Babcock test)

Leucocytes added gms./pint	Distribution of fat in the Upper one-third of pint bottle			Middle one-third of pint bottle			Lower one-third of pint bottle		
	Fat %	Gms.of fat	% of total	Fat %	Gms.of fat	% of total	Fat %	Gms.of fat	% of total
0.0	10.1	16.463	93.5	0.3	0.489	2.8	0.4	0.652	3.7
2.5	10.1	16.463	94.4	0.3	0.489	2.8	0.3	0.489	2.8
5.0	10.1	16.463	92.6	0.4	0.652	3.7	0.4	0.652	3.7
7.5	10.0	16.300	95.2	0.2	0.326	1.9	0.3	0.489	2.9
10.0	9.7	15.811	91.5	0.5	0.815	4.7	0.4	0.652	3.8
12.5	10.3	16.789	92.8	0.4	0.652	3.6	0.4	0.652	3.6
15.0	7.1	11.573	68.9	0.5	0.815	4.9	2.7	4.401	26.2
20.0	6.7	10.921	63.8	0.4	0.652	3.8	3.4	5.542	32.4
25.0	5.8	9.454	54.2	0.2	0.326	1.9	4.7	7.661	43.9
30.0	5.9	9.617	56.2	0.2	0.326	1.9	4.4	7.172	41.9
45.0	3.5	5.705	34.3	0.2	0.326	2.0	6.5	10.595	63.7
60.0	4.4	7.172	39.6	0.2	0.326	1.8	6.5	10.595	58.6

Table 6. The influence of the number of leucocytes in milk on the distribution of the total solids after storage (Mojonnier method)

Leucocytes added gms./pint	Distribution of total solids in the Upper one-third of pint bottle T.S. %	Gms.of T.S.	% of total	Middle one-third of pint bottle T.S. %	Gms.of T.S.	% of total	Lower one-third of pint bottle T.S. %	Gms.of T.S.	% of total
0.0	18.32	29.8616	49.4	9.26	15.0938	25.0	9.51	15.5013	25.6
2.5	17.86	29.1118	48.6	9.32	15.1916	25.4	9.56	15.5828	26.0
5.0	18.85	30.7255	49.2	9.65	15.7295	25.2	9.78	15.9414	25.6
7.5	17.69	28.8347	48.6	9.49	15.4687	26.1	9.20	14.9960	25.3
10.0	17.97	29.2911	48.4	9.60	15.6480	25.9	9.52	15.5176	25.7
12.5	17.91	29.1933	48.6	9.29	15.1427	25.2	9.67	15.7621	26.2
15.0	14.52	23.6676	41.1	9.36	15.2568	26.5	11.41	18.5983	32.4
20.0	14.30	23.3090	38.6	9.51	15.5013	25.7	13.22	21.5486	35.7
25.0	13.47	21.9561	36.7	9.18	14.9634	25.0	14.01	22.8363	38.3
30.0	13.19	21.4997	35.4	9.22	15.0286	24.8	14.81	24.1403	39.8
45.0	12.03	19.6089	30.8	9.11	14.8493	23.4	17.86	29.1118	45.8
60.0	11.11	18.1093	28.4	9.05	14.7515	23.1	18.96	30.9048	48.5

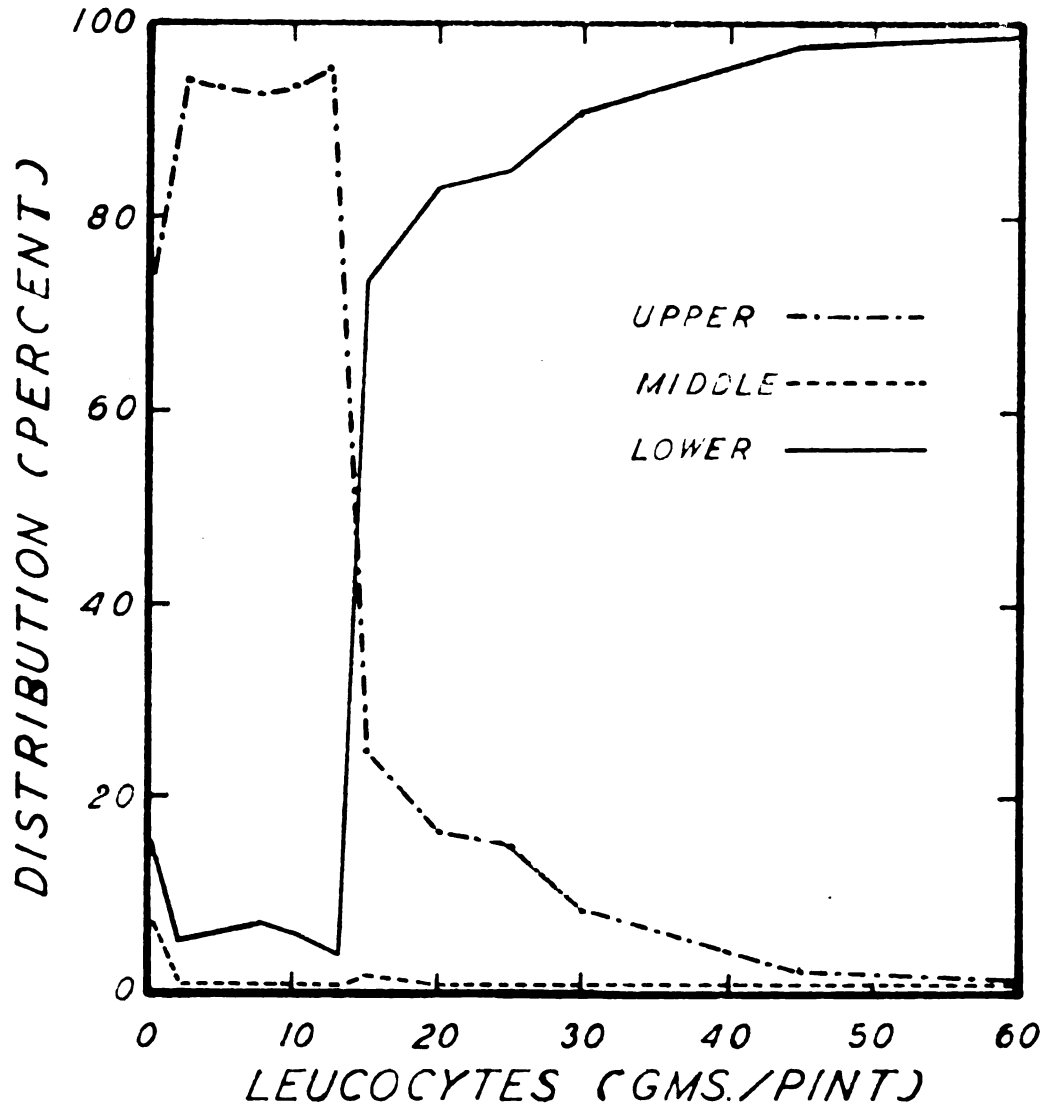
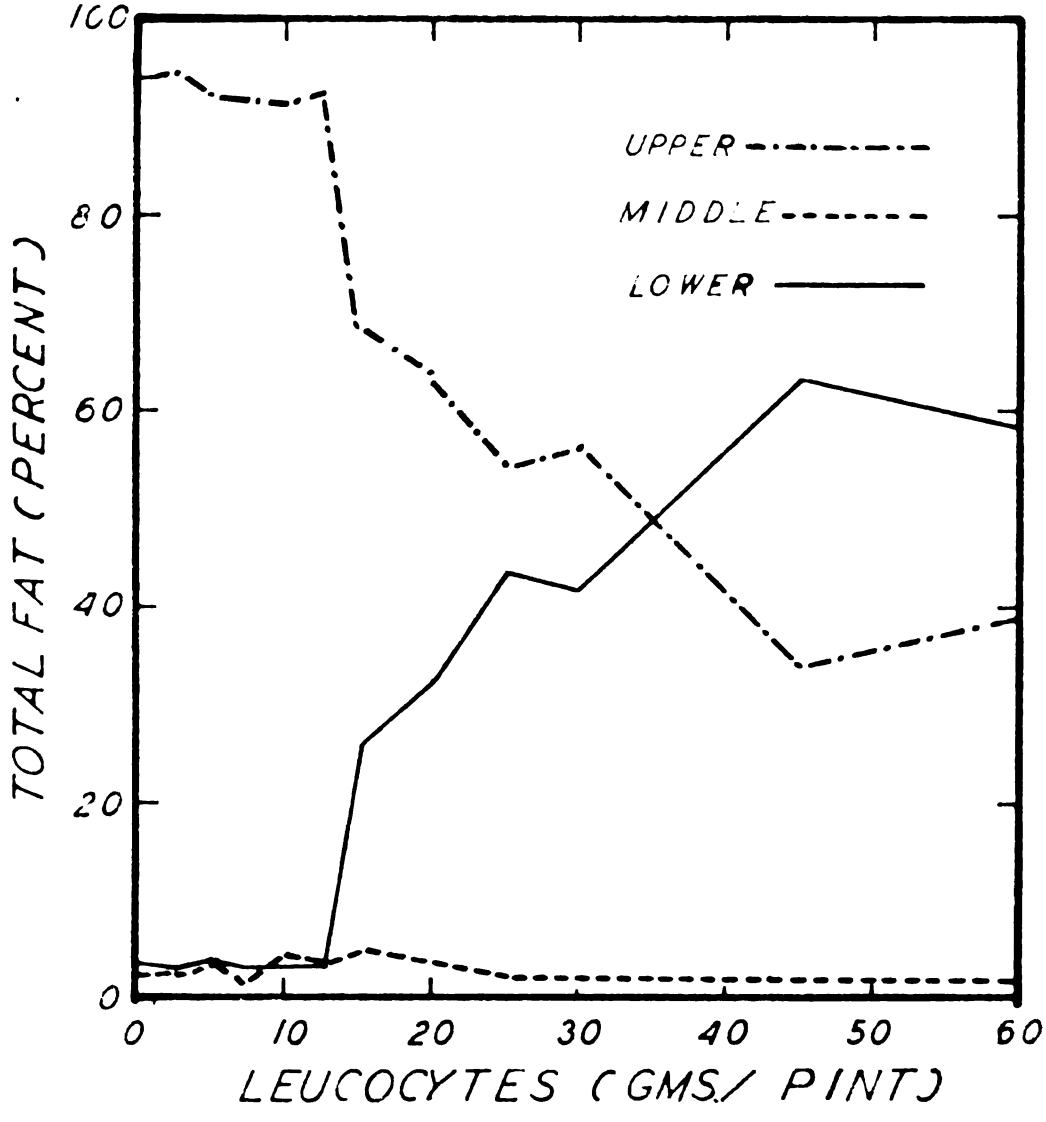
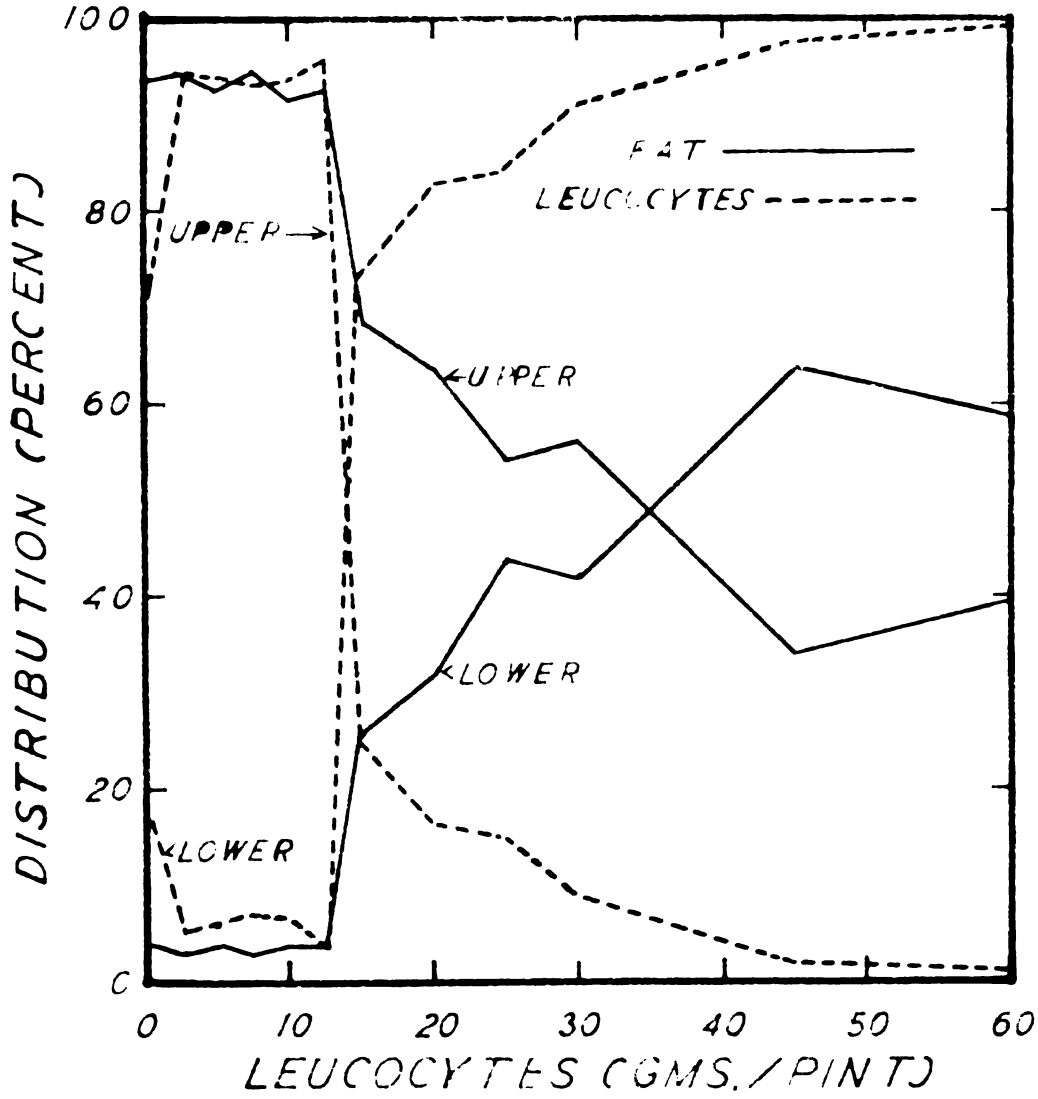


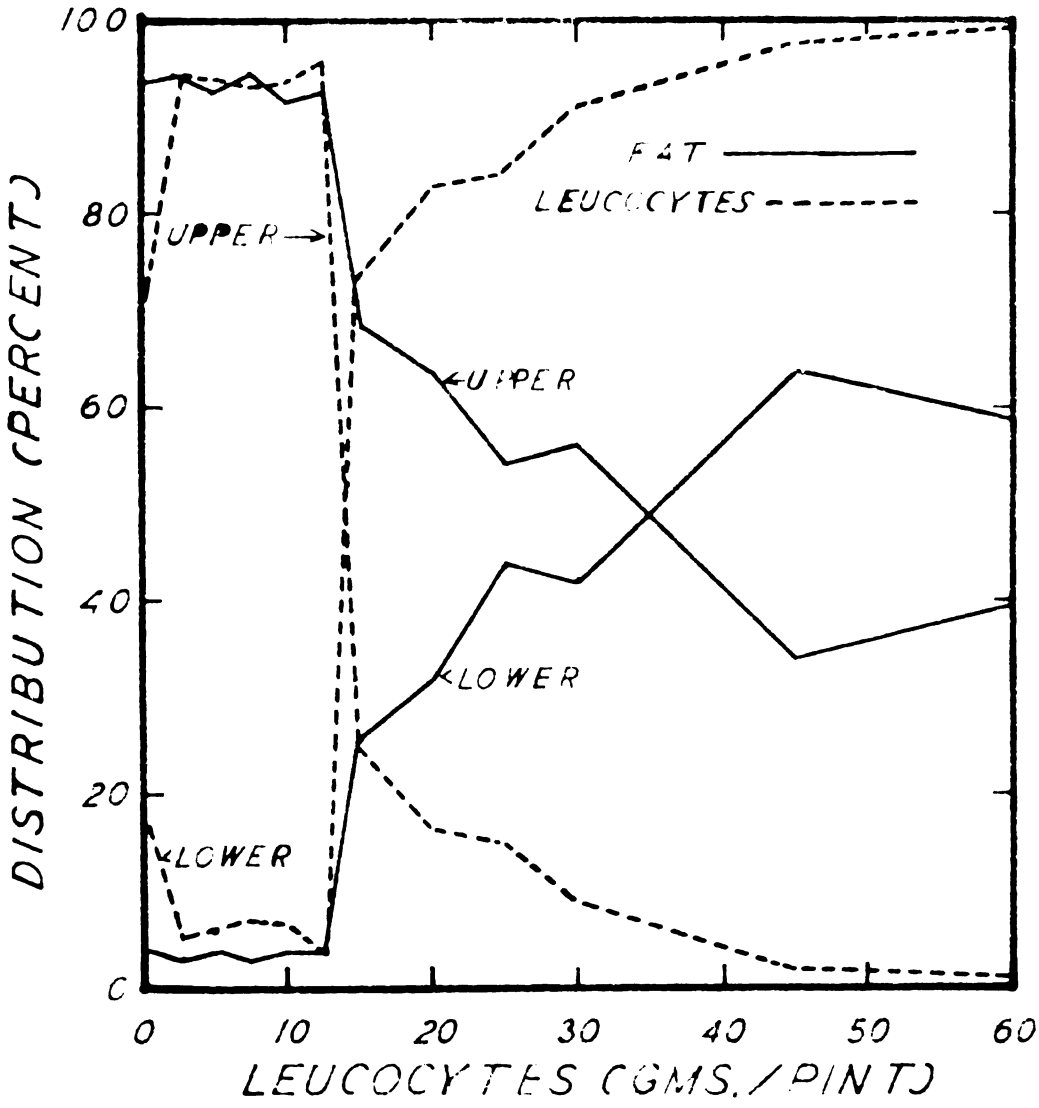
Figure 1. The distribution of leucocytes in the upper, middle and lower fifths of plant fertilizer, to which various amounts of leucocytes were added. Distribution of leucocytes in the fifths was determined by differential counting.



[illegible] of fat in the upper, middle and lower [illegible] females to which various amounts of leucocytes were added.



[illegible] upper and lower tiers of pint bottles or kits to which various amounts of leucocytes were added.



[illegible] the [illegible] cytes [illegible] the
upper [illegible] lower thirds of pint bottles of milk to which
[illegible] amounts of leucocytes were added.

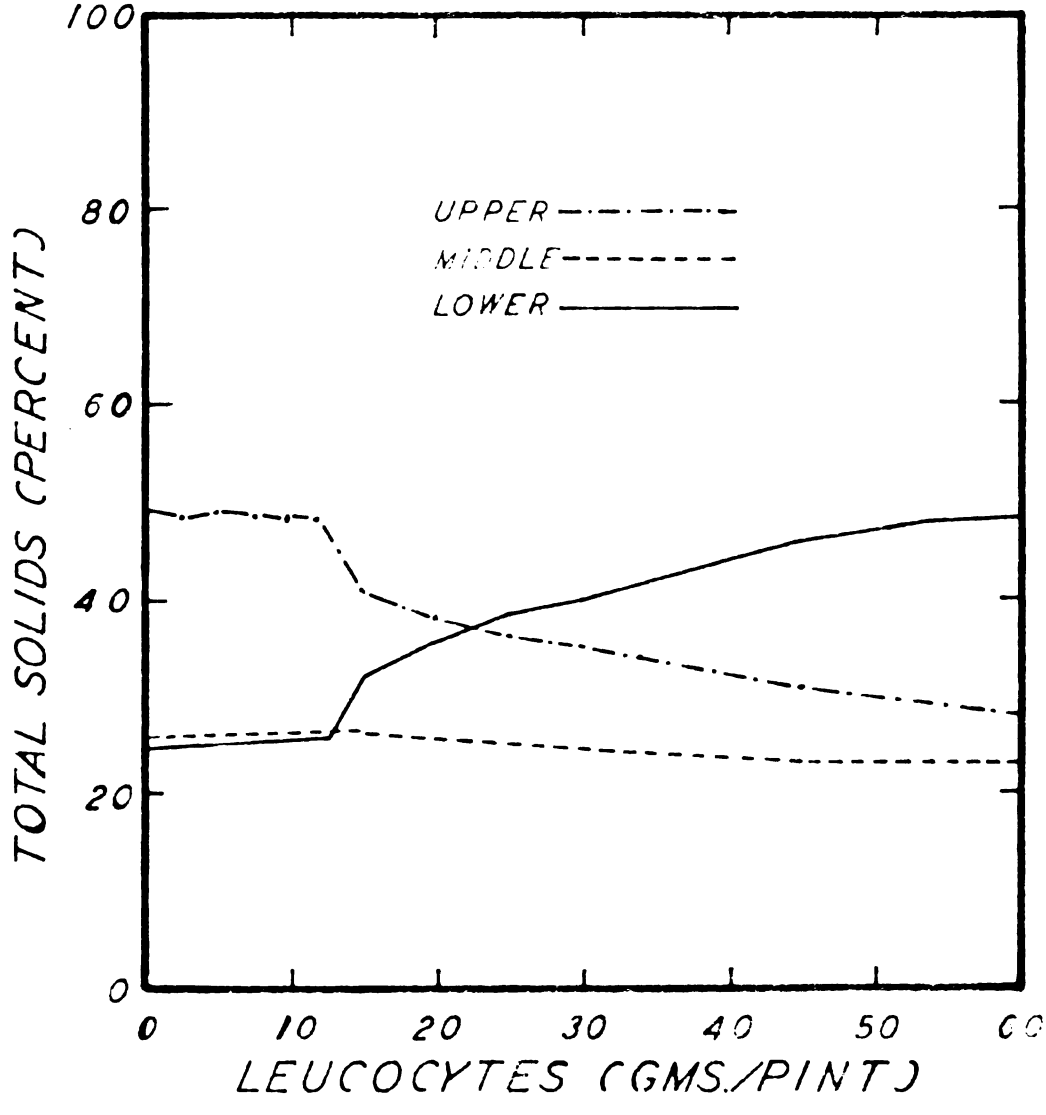


Fig. 1. The distribution of total solids in the upper, middle and lower one-thirds of pint bottles to which various amounts of leucocytes were added.

Figure 8

The creaming of milk to which various amounts of leucocytes had been added. From left to right respectively additions of 0, 7.5, 15, 30, and 60 gms. of washed leucocytes were made.

Figure 9

The influence of certain amounts of added leucocytes on the creaming ability of milk. From left to right respectively 0, 12.5, 15.0, and 60 gms. of washed leucocytes were added.

3. Effect of reversing the electrical charge on the fat globules upon the migration of washed leucocytes.

Since fat globules carry a negative charge normally, (Mommsen, 1932) with its isoelectric point at pH 4.3 (North and Sommer, 1935) below which point they obtain a positive charge, and the leucocytes carry a positive charge (Bechhold 1929), it was felt that by lowering the pH below the isoelectric point of the fat globules, the behavior of the similarly charged leucocytes toward the fat globules could be observed. If the rising of the leucocytes with the fat were due only to the sweeping action of the fat, then the number of leucocytes migrating to the top of the bottle should be the same in both cases, whether the fat and the leucocytes carried the same or opposite charges. If, however, the rising was due to attraction of opposite charges, then the leucocytes should rise with the negatively charged fat globules, but settle down, when the fat carried a positive charge.

Pasteurized milk, held for 24 hours at 40° F. was tempered to 80° F. and separated. The cream was diluted with physiological salt solution at 80° F. and separated again. This was repeated two times, or until the skim portion was fairly clear. The skimmilk was heated to 86° F., rennet added to coagulate the casein, and the curd cooked, raising the temperature to 120° F. in order to expel most of the whey. The whey was strained and heated to 180° F., cooled to 80° F., and clarified in order to remove all suspended matter.

The whey and washed cream were remixed, washed leucocytes added at the rate of 0.5 gm. per quart and the mixture acidified using N/1HCl, taking samples at decreasing pH values, down to pH 1.5. Pint bottles were used and milk ranging in pH from 6.5 to 1.5 was stored for 48 hours at 40° F., after

which time examinations were made as to cream volume, sediment formation, distribution of leucocytes and so forth. The results are presented in tables 7 and 8 and in figures 10 to 14 inclusive.

The effect of acidifying the artificial preparation of washed cream in sweet whey showed an interesting picture in the behavior of both the fat globules and the leucocytes. At pH 4.3, the approximate isoelectric point of the fat globules, fat rising was most complete, leaving a clear whey below, with no sediment at the bottom (Figure 12).

With increasing and decreasing pH values the cream volume was less (Table 7, Figure 12) until at both extremes the volumes were of equal size, the whey portion showed the same milky color and some white sediment was found at the bottom of the bottles.

The distribution of leucocytes in the upper middle and lower one-third portion of each pint bottle is shown in table 8 and figure 10. Above the isoelectric point the leucocyte count followed the distribution of the fat, while at lower pH values, the leucocyte count in the cream layer decreased, which seemed to indicate that the fat, although rising toward the top had failed to carry the leucocytes with it. Data in figure 11 illustrates the behavior of the leucocytes with respect to the amount of fat rising. As more acid was added the positive electric charge on the fat globules and the leucocytes, which carry a positive charge (Bechhold, 1929) became more pronounced, preventing the leucocytes to be carried up by the sweeping action of the rising fat.

The data in figure 11 show that above the isoelectric point of fat the leucocyte curve excels in slope the cream-volume curve, indicating that the leucocytes are carried up proportionally more than the fat. At pH values below the isoelectric point, however, the leucocytes are carried up at decreas-

ing proportionally less than the fat, particularly at pH 3.0 or below where the difference is most pronounced.

While it can be seen from table 7 and figure 12 that at pH 6.0 and pH 3.0, 2.5, 2.0, 1.5 the cream volumes are all the same, namely 45 ml., yet the leucocyte count in the upper one-third protions ranged from 540,000 to 20,000 per ml. Assuming that fat rising with clumping had taken place at both pH 6.0 and 3.0 then it would follow that equal numbers of leucocytes could be expected in the two cream layers. The only reason for the difference in the behavior of the leucocytes, or the fat must then be due to the fact that both carry the same electric charge and thus repel each other. This would tend to explain the decreasing number of leucocytes carried into the upper portion with decreasing pH values.

Table 7. The influence of pH on fat rising in a washed-cream heat-treated whey mixture

pH of sample	Cream volume (ml.)	Appearance of lower portion	
		Clearness of whey	Intensity of sediment*
6.5	53	milky	1.0
6.0	45	"	1.0
5.0	182	fairly clear	0.0
4.5	212	clear	0.0
4.0	170	fairly clear	0.0
3.5	53	milky	0.5
3.0	45	"	1.0
2.5	45	"	1.0
2.0	45	"	1.0
1.5	45	"	1.0

*The sediment had a white color.

Table 8. The influence of pH on the migration of leucocytes in a washed-cream, heat-treated whey mixture

pH of sample	Leucocytes per ml. in the Upper one-third of pint bottle	% of total	Middle one-third of pint bottle	% of total	Lower one-third of pint bottle	% of total
6.5	300,000	15.6	80,000	4.2	1,540,000	80.2
6.0	540,000	25.5	100,000	4.7	1,480,000	69.8
5.0	1,420,000	83.5	200,000	11.7	80,000	4.8
4.3	1,860,000	88.5	200,000	9.5	40,000	2.0
4.0	1,100,000	80.9	120,000	8.8	140,000	10.3
3.5	260,000	43.3	40,000	6.7	300,000	50.0
3.0	20,000	1.3	40,000	2.6	1,480,000	96.1
2.5	20,000	1.3	20,000	1.3	1,500,000	97.4
2.0	80,000	4.9	40,000	2.4	1,520,000	92.7
1.5	40,000	2.4	10,000	0.6	1,600,000	97.0

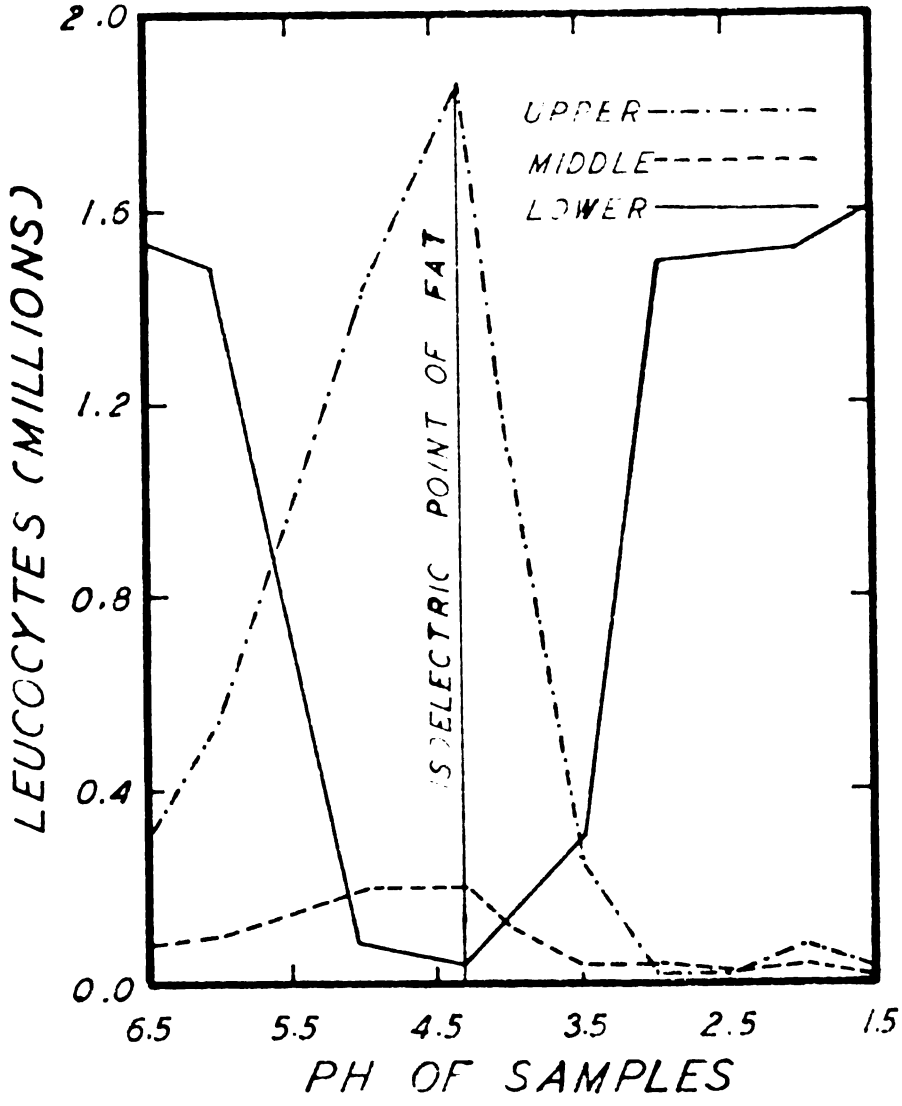


Figure 1. The distribution of leucocytes in upper, middle and lower centrifuged fat bottles of emulsion samples, plotted from pH determinations.

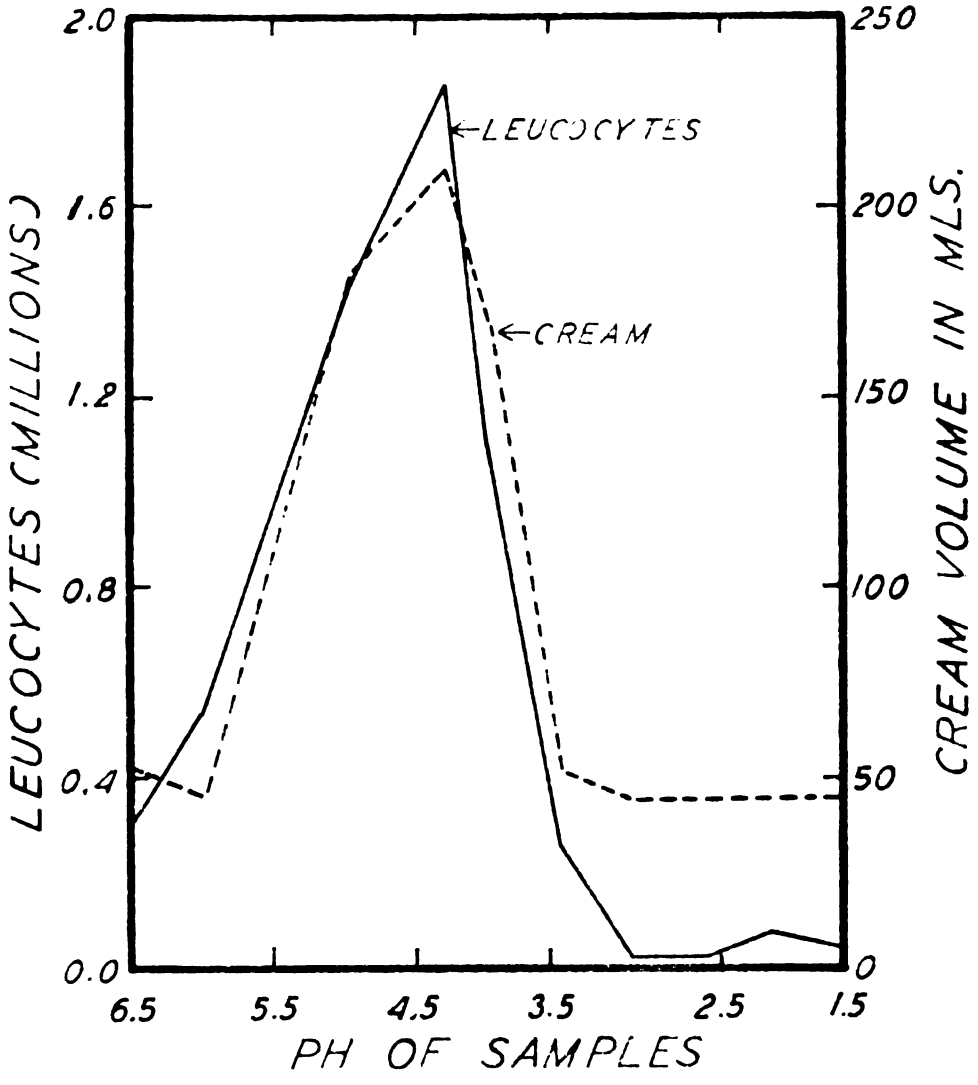


Fig. 1. The relation of leucocyte count in upper one-third of pint bottles to the cream volume in the respective bottles.

Figure 12

Pint bottles of washed-cream, heat-treated whey mixtures, having pH values from left to right of 6.5, 6.0, 5.0, 4.3, 3.5, 2.5 and 1.5 respectively. Note the cream volume in each bottle and also the clearness of the whey in the sample 4, having a pH of 4.3, the isoelectric point of fat.

Figure 13

Pint bottles of washed-cream, heat-treated whey mixtures having pH values from left to right of 6.0, 5.0, 4.3, 4.0, and 3.0.

Note the decrease of cream volume in the samples having pH values above and below that at the isoelectric point and also the clearness of whey in the lower portions.

Figure 14

Pint bottles of washed-cream, heat-treated whey mixtures ranging in pH from 6.5 to 2.5.

From left to right:

(1) pH 6.5 - normal range. Fat carries a negative charge.

(2) pH 4.3 - isoelectric point of fat. No charge.

(3) pH 2.5 - high-acid range. Fat carries a positive charge.

Note the cream volumes and the similarity of opacity of the lower portions in bottles 1 and 3.

4. Effect of pasteurization and homogenization temperatures upon the number and distribution of leucocytes in milk.

The work of Russell and Hoffman (1908), and of Campbell (1909) had shown that when portions of the same milk were heated to increasingly higher temperatures an increase in the leucocyte count took place. Since this work was done with nonhomogenized milk only, it was felt to be of interest to repeat their experiments using both nonhomogenized and homogenized milk.

Three trials were run, using raw clarified milk to which fresh separator slime was added at the rate of 1.5 to 3.0 gms. per quart of milk. The milk was divided into five two-gallon portions. Temperatures of 140°, 150°, 160°, 170° and 180° F. were employed, with a holding time of 30 minutes. Each portion, pasteurized at a different temperature, was homogenized separately at 2500 pounds pressure. Samples were taken before and after homogenization. All samples were held for 48 hours at 40° F. before examination of any kind was made.

Increasing the pasteurization temperature resulted in increased leucocyte counts both in the nonhomogenized and homogenized portions of milk (Table 9, column 1 and figure 15). The results are similar to those obtained by Campbell (1909) with nonhomogenized milk. The homogenized milk counts are lower than those of the nonhomogenized milk at all temperatures. This is due, as will be shown later, to the destruction of some of the leucocytes during the process of homogenization. Otherwise the slopes are identical.

While it has been known for a long time that only mature white blood cells enter the blood stream, which do not multiply (Sabin, 1923) and the

incubation trial by Slanetz and Naghski (1939) showed the same results, it must be assumed that raw milk actually contains a higher leucocyte population than that generally obtained by counting procedures. Campbell (1909) used both the volumetric Doane-Buckley method and the Stokes-Stewart smear-stain method, and found that higher counts were obtained in each case, when milk was heated to higher ranges of increasing temperatures. Thus it would seem that some leucocytes will not stain unless heat treatment is practiced. Sabin (1923) and Sabin et al (1925) who practiced vital staining on living cells obtained from the blood, found that a certain group, called "non-motile form" would not stain with neutral red, which was commonly used. They gave the following explanation. "As seen in the living preparations, the cell first stops moving and rounds up, and the granules no longer stain with neutral red. It is obvious that these granules have either lost all power of reacting to vital dyes, or that the cell membrane has become impermeable to the dye." Schilling, as quoted by the same authors (1925) referred to this type of cell as "physiological death of leucocyte." The probability exists that some leucocytes found in milk may possess some such properties which prevent their staining, unless heat treatment is practiced.

While the leucocyte counts increased with increasing pasteurization temperatures resulting in a fairly uniformly inclined slope, the percentages of total leucocytes in the upper, middle and lower thirds of the bottle in the nonhomogenized milk showed broken curves (Fig. 16). Failure of the fat globules to clump and form a cream layer at 160° F. resulted in an abnormal distribution of leucocytes in the nonhomogenized sample.

Data in figure 16 show the various curves, while those in table 10 and figure 17 show the relation of cream volume to the per cent of total

leucocytes in the upper one-third portion of the five samples of nonhomogenized milk. The data in this table show that the depth of the cream volume is a decisive factor in the distribution of leucocytes, and is in agreement with the observations made previously that the leucocytes tend to rise with the fat globules, while in the absence of fat rising they will settle to the bottom.

Increasing the pasteurization temperature resulted in an increased intensity of sediment, both in the nonhomogenized and homogenized milk samples. However, the intensity of the sediment in the homogenized milk was greater in each case than that of the correspondingly heat-treated nonhomogenized milk sample. Data in table 11 and figure 18 show the relation of the intensity of sediment of the nonhomogenized and homogenized milk to the pasteurization temperature employed. The maximum increase in intensity of sediment was between the pasteurization temperatures of 150° and 160° F., resulting in a large increase of sediment. Since all milk portions were held at the respective pasteurizing temperatures for 30 minutes, it can be expected that some coagulation of albumin, such as observed by Rowland (1933) took place at higher temperatures. This albumin, although white in color would tend to settle and carry with it suspended matter, which otherwise would not have been deposited. This would explain the reason for the intensity of sediment in the milk pasteurized at 170° and 180° F. respectively, since both of these lots showed cream rising, but still had a sediment equal in intensity to that of the sample pasteurized at 160° F. where no fat rising had taken place.

It is of interest to note that the same relation exists between the intensity of sediment and the per cent leucocytes in the lower one-third portion of the bottle, in both the nonhomogenized and in the homogenized

milk as was reported in section 1. While the sediment in the homogenized milk is due primarily to the settling of leucocytes, such is not the case in the nonhomogenized samples, especially at the pasteurizing temperatures of 170° and 180° F.

Table 9. The influence of the pasteurization temperature on the distribution of leucocytes in nonhomogenized and in homogenized milk (Average of three trials).

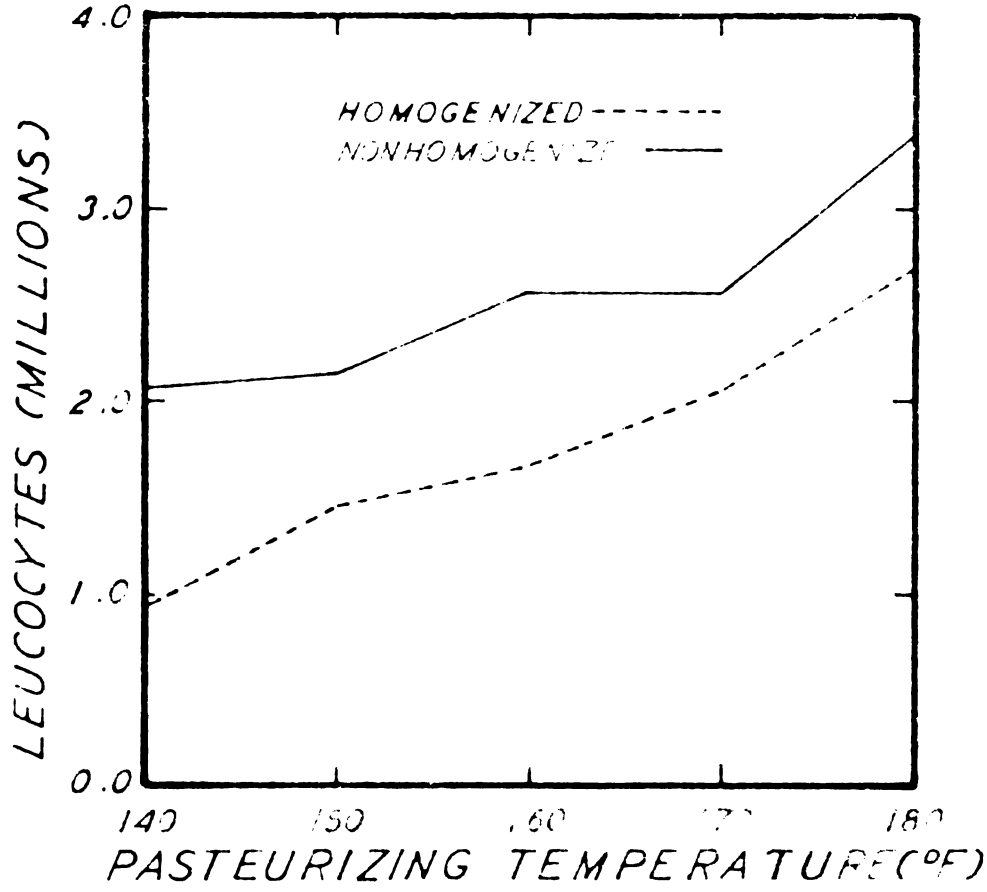
Past. temp. °F.	Leucocytes per ml. before storage	Leucocytes per ml. after storage in the Upper one-third of bottle		Middle one-third of bottle		Lower one-third of bottle	
				Nonhomogenized			
			% of total		% of total		% of total
140	2,087,000	3,962,000	94.9	139,000	3.3	72,680	1.8
150	2,158,000	6,240,000	94.0	23,400	0.4	374,200	5.6
160	2,582,000	4,658,000	71.0	942,400	14.4	959,000	14.6
170	2,572,000	7,006,000	89.9	248,500	3.2	535,800	6.9
180	3,370,000	6,741,000	88.0	199,000	2.6	718,800	9.4
				Homogenized			
140	945,800	585,400	20.9	532,700	19.0	1,684,000	60.1
150	1,455,000	225,200	6.2	421,800	11.6	2,978,000	82.2
160	1,676,000	395,600	8.9	453,500	10.1	3,619,000	81.0
170	2,042,000	695,800	11.0	643,100	10.2	4,977,000	78.8
180	2,696,000	748,300	9.1	806,400	9.8	6,690,000	81.1

Table 10. The relationship between the cream volume and the leucocyte count of the upper one-third when the milk was pasteurized at different temperatures (Average of three trials).

Past. temp. °F.	Per cent of total leucocytes in upper one-third of bottle	Cream volume per bottle (ml.)
140	94.9	165
150	94.0	115.5
160	71.0	0.0
170	89.9	127.3
180	88.0	122.3

Table 11. The influence of the temperature of pasteurization upon the intensity of sediment in nonhomogenized and in homogenized milk (Average of three trials).

Past. temp. °F.	Intensity of sediment on bottom of quart bottle	
	Before homogenization	After homogenization
140	1.0	3.3
150	1.3	3.3
160	2.3	3.6
170	2.6	3.6
180	2.6	3.6


4.0
3.0
2.0
1.0
0.0
HOMOGENIZED
NONHOMOGENIZED
LEUCOCYTES (MILLIONS)
140
150
160
180
PASTEURIZING TEMPERATURE(°F)

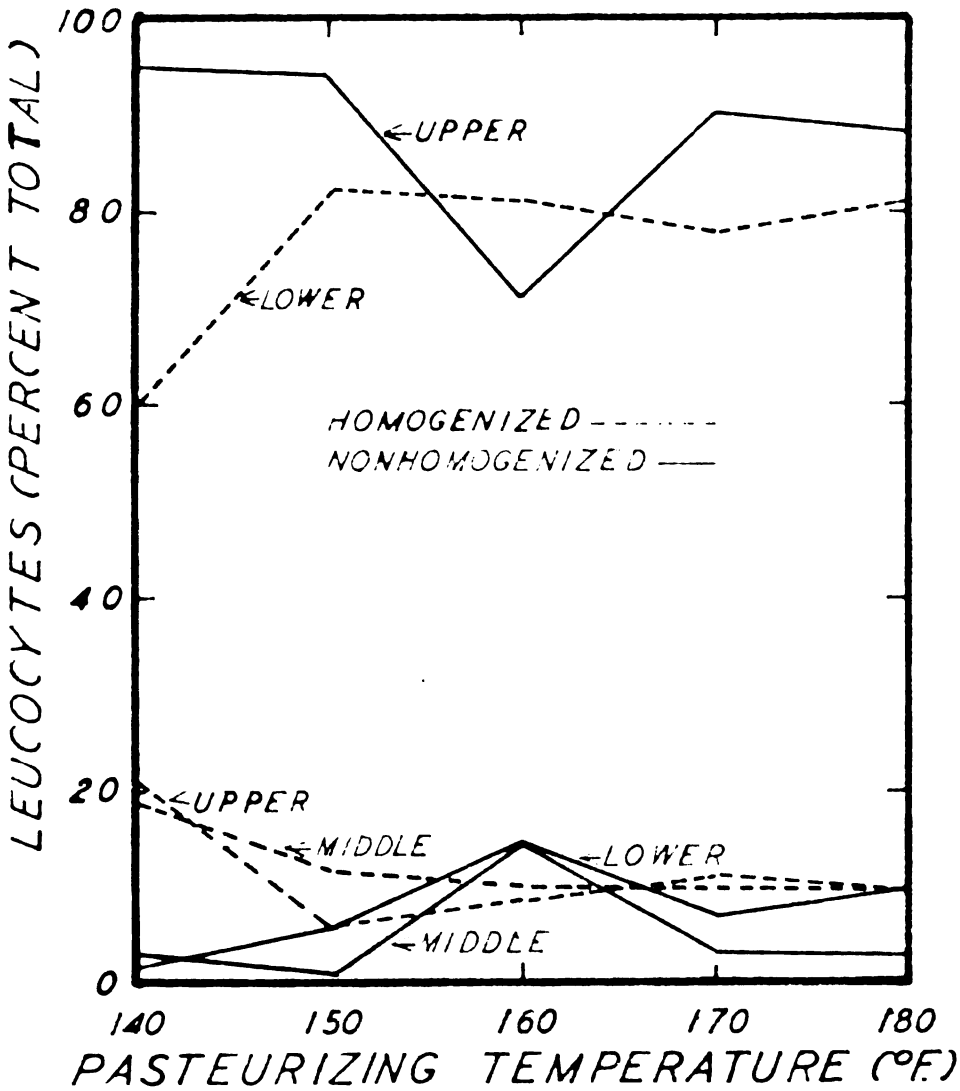


Fig. 1 The percentage distribution of leucocytes in the upper, middle and lower fourths portion of a quart bottle of milk before and after homogenization when pasteurized at different temperatures for 5 minutes.

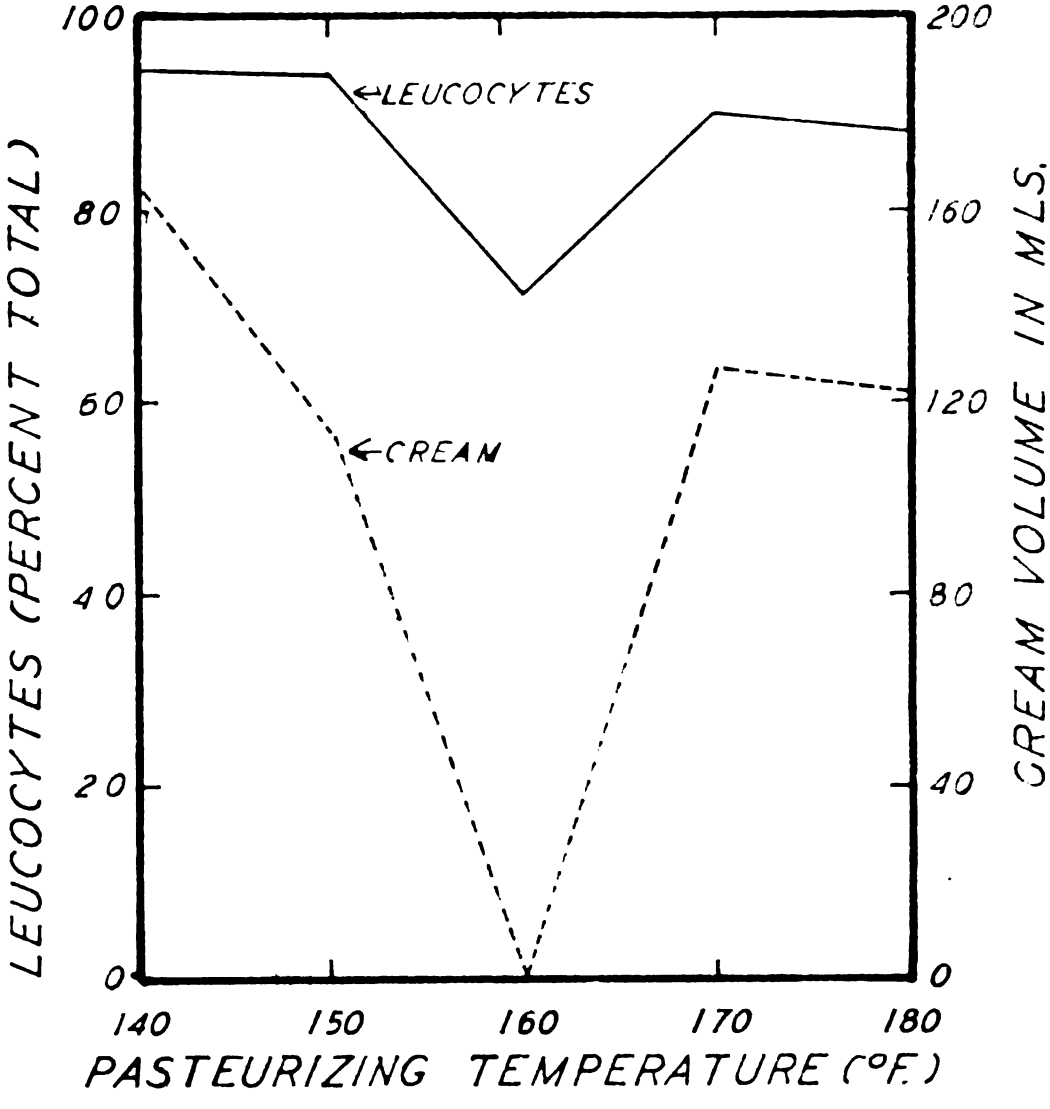
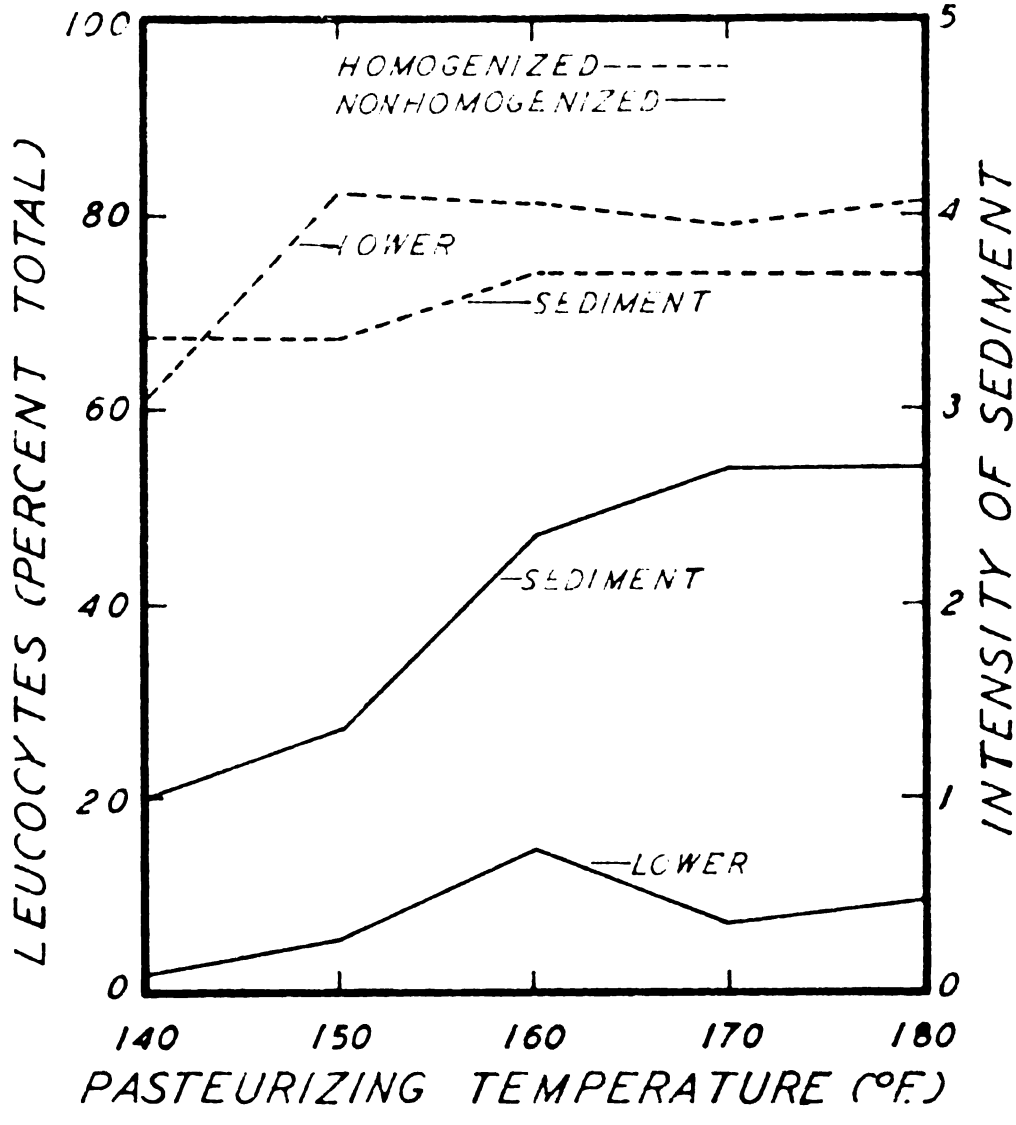


Figure 1. The relationship between cream volume and leucocyte count of upper one-third of pint bottle of milk pasteurized at different temperatures.



[illegible] of sediment and leucocytes in the lower centrifuged portion of quart bottles of nonhomogenized and homogenized milk pasteurized at [illegible] temperatures for 30 minutes.

5. Effect of homogenizing before or after pasteurization on the number and distribution of leucocytes in milk.

Since homogenization before or after pasteurization are both practiced commercially, and a difference in opinion exists in the preference of one or the other method, it was felt that a comparative study of the two methods would be of value. By varying the sequence of the procedure, special attention was to be paid to the number of leucocytes after processing, and to the intensity of sediment produced in each case.

To ten gallons of fresh, raw, clarified milk was added fresh separator slime at the rate of 1.5 gms. per quart. One-half portion of the milk was heated to 100° F., homogenized and pasteurized, while the other one-half was pasteurized and homogenized. Each one-half portion was divided into five equal lots. Pasteurizing temperatures of 140°, 150°, 160°, 170° and 180° F. were employed, holding the milk for 30 minutes. Homogenization was performed at 2500 pounds pressure. Samples taken at various stages of processing were held for 48 hours at 40° F. before examinations of any kind were made.

The data, shown in table 12 and figure 19, indicate that homogenization before pasteurization reduced the leucocyte count to a greater extent than if homogenization after pasteurization were practiced. This, however, was not the case when pasteurization temperatures of 140° to 150° F. were used. No definite conclusions could be reached as to why this was so, but the fact remained that all three trials showed the same trend. The leucocyte count for the pasteurized-homogenized milk yielded a slope similar to that observed when the milk was heat-treated at a high temperature as was shown in part 4 figure 15. Thus the difference in the slope of the leucocyte-count curve of the homogenized-pasteurized milk was due to the reversed

sequence of treatment. Some explanation might be found in the following: While all the homogenized-pasteurized milk was homogenized at the same temperature, namely 100° F., the pasteurized-homogenized milk was homogenized at a different temperature, namely at the pasteurizing temperature of each respective lot of milk. The distribution of leucocytes after storage was very much the same with both treatments. Some difference was encountered at temperature treatments above 160° F. as shown in figure 20. This difference in behavior was attributed to a variety of factors, which require further study.

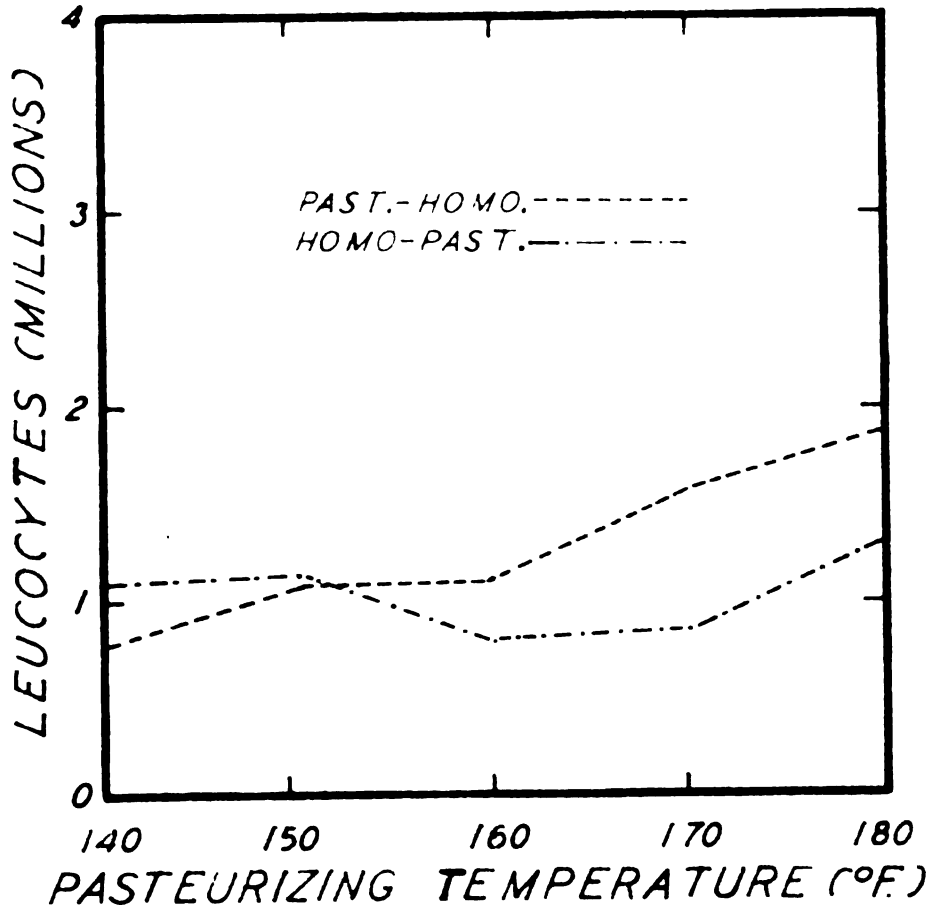
While all samples showed a large amount of sediment, the intensity varied with the individual lots (Table 13). A slight increase in the intensity of sediment was observed between 140° and 150° F. heat treatment of pasteurized-homogenized milk, while the others of this series were given the same score. All samples of the homogenized-pasteurized milk showed a uniform intensity of sediment, which was greater than the highest value given to the pasteurized-homogenized lots. Thus, according to the results obtained from the above trials, pasteurization at 140-150° F. before homogenization would be preferable to homogenization prior to pasteurization.

Table 12. The leucocyte counts in pasteurized-homogenized and homogenized-pasteurized milk samples, before and after storage, pasteurized at different temperatures for thirty minutes (Average of three trials).

Past. temp. °F.	Leucocytes per ml. before storage	Leucocytes per ml. after storage in the Upper one-third of bottle	Per cent	Middle one-third of bottle	Per cent	Lower one-third of bottle	Per cent
			Pasteurized-Homogenized				
140	775,100	429,400	17.2	410,000	16.5	1,651,000	66.3
150	1,080,000	299,500	10.2	396,600	13.9	2,188,000	75.9
160	1,113,000	477,400	11.3	447,300	11.0	3,137,000	77.2
170	1,545,000	563,000	12.3	767,500	17.5	3,067,000	69.7
180	1,865,000	729,300	13.6	389,000	7.3	4,242,000	79.1
			Homogenized-Pasteurized				
140	1,098,000	353,400	13.3	395,400	15.5	1,806,000	70.7
150	1,140,000	317,500	11.0	338,800	11.3	2,219,000	77.2
160	820,000	245,000	9.5	455,700	17.3	1,863,000	72.7
170	856,700	179,300	8.4	168,700	7.9	1,792,000	83.7
180	1,315,000	599,700	18.2	640,600	19.4	2,060,000	62.4

Table 13. The intensity of sediment in pasteurized-homogenized and homogenized-pasteurized milk, pasteurized at different temperatures (Average of three trials).

Past. Temp. °F.	Intensity of sediment in bottom of quart bottle of milk	
	Pasteurized-homogenized	Homogenized-pasteurized
140	2.3	3.0
150	2.6	3.0
160	2.6	3.0
170	2.6	3.0
180	2.6	3.0


LEUCOCYTES (MILLIONS)
4
3
2
1
0
PAST.-HOMO.
HOMO-PAST.
140
150
160
170
180
PASTEURIZING TEMPERATURE (°F.)

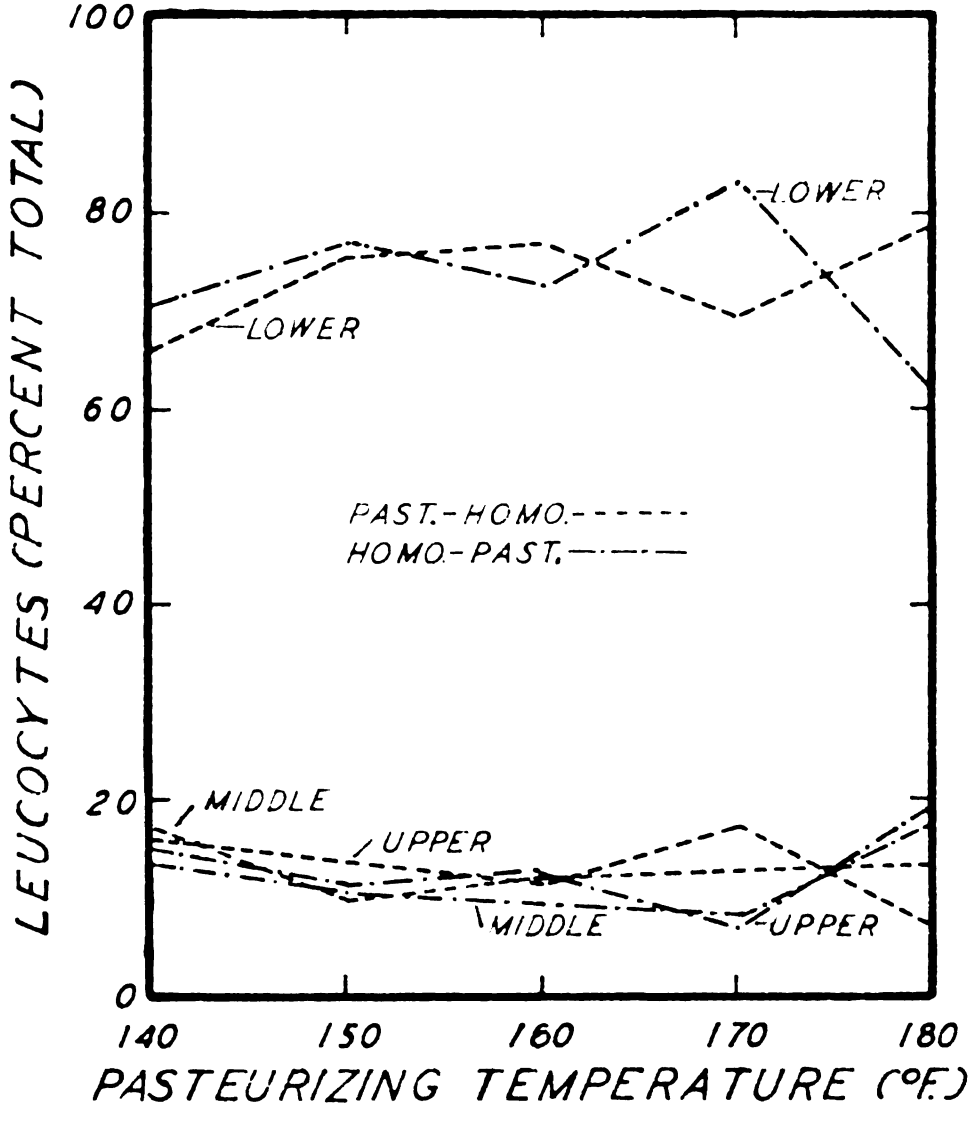


Fig. 1. The percentage distribution of leucocytes in the upper, middle and lower one-fifth portions of a pint bottle of raw milk and raw homogenized-pasteurized milk, pasteurized at different temperatures for 30 minutes.

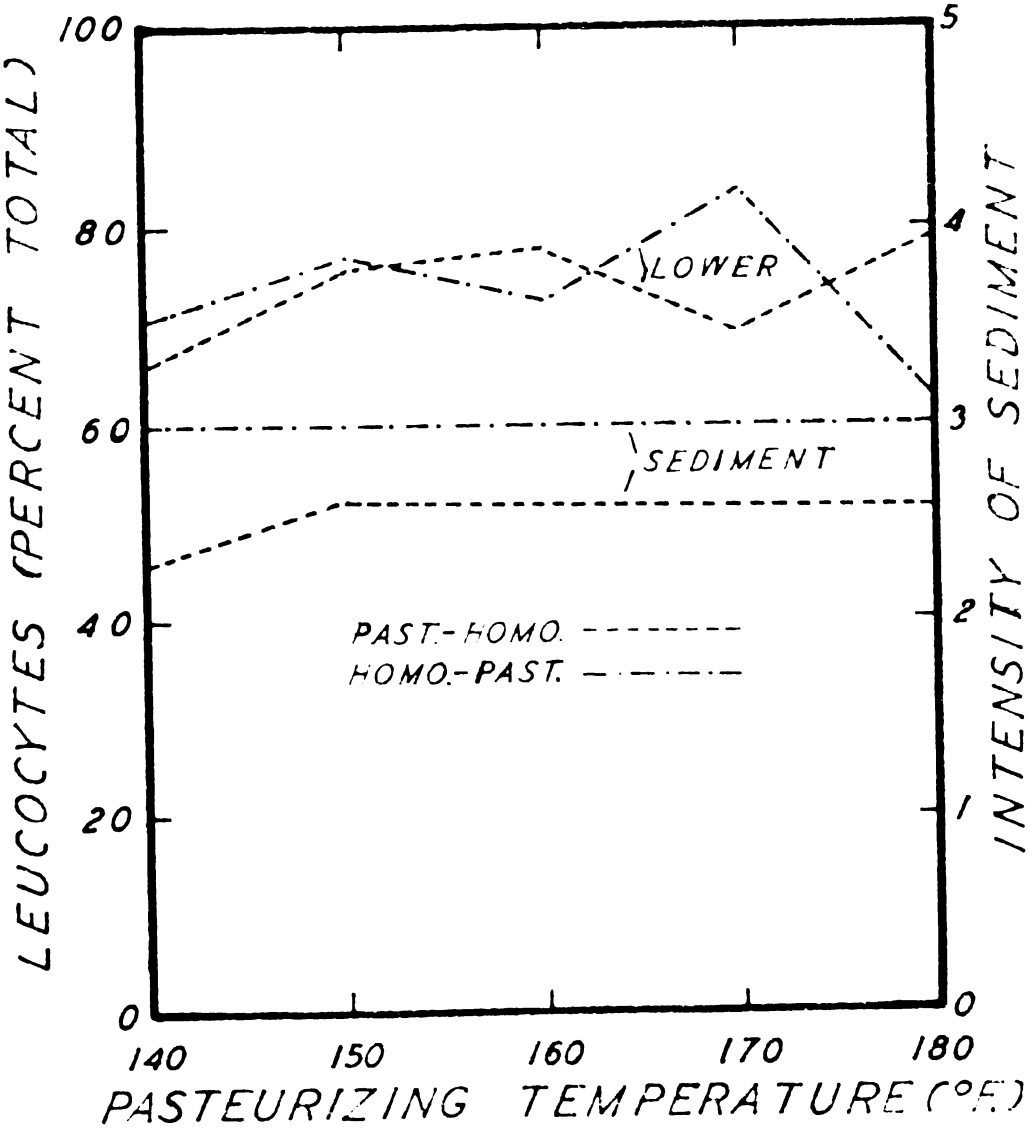


Figure 11. The influence of the time of pasteurizing with respect to homogenization on the leucocyte count in the lower one-third of bottled milk and on sedimentation

6. Effect of delivery-route agitation on the formation of sediment in homogenized milk.

Since agitation of homogenized milk, such as in unavoidable during delivery had been found to aid the settling of leucocytes, as reported by Hahn and Tracy (1940), a close study was made over a period of time, using clarified, filtered, and nonclarified-nonfiltered homogenized milk to ascertain the specific effects of route agitation on sedimentation.

Twenty-four trials were conducted over a period of five months, using regular clarified homogenized milk. Six trials were made with nonclarified, filtered regular homogenized milk, and nine trials with nonfiltered, nonclarified vitamin D milk, over a shorter period of time.

Quart bottles were picked at random during the bottling process, two at a time, one of which was used as control and held at 40° F. for 48 hours, while the other one was taken on the delivery route, but returned and placed with the control bottle for the remainder of the 48 hours. Examinations as to formation of sediment and leucocyte count were made at the end of the holding time. Experiments were started late in July, and carried on with intervals until the end of December.

The influence of delivery route agitation was observed. All three sets of trials conducted showed higher leucocyte counts in the lower portion of the bottle in the return milk as compared with the control. This difference in counts was attributed to the agitating effect that the milk had to undergo during delivery. Although the danger existed that heat shocking, such as reported by Tracy (1935), would also produce similar results, temperature observations showed that the milk leaving at 40° F. would return at a temperature not higher than 50° F. Thus the effect of a change in

temperature, although possibly a factor, was considered to be negligible. The data are shown in tables 14, 15 and 16, and in figures 22, 23, and 24. While the increase of the number of leucocytes per milliliter in the lower portion of the bottle upon delivery-route agitation was only slight, the increase in the intensity of sediment was more pronounced. This was especially the case with the nonclarified series, as shown in table 17 and figure 25. The beneficial effect of clarification is clearly shown in these trials.

The higher intensity of sediment in the regular filtered, nonclarified milk, as compared with the intensity of sediment observed in the vitamin D, nonfiltered, nonclarified milk may be partially due to the higher leucocyte count in the former milk, as shown in table 18. The data show that if milk is not clarified, high intensities of sediment may be obtained even with leucocyte counts below 100,000 per milliliter. Hahn and Tracy (1940) found that if the milk were normal in every respect then it was possible to eliminate sedimentation when the milk contained approximately 100,000 $\pm$ 25,000 cells per milliliter. The above results are in agreement with their observations.

No special study was made on influence of heat shocking on the settling of leucocytes and the intensity of sediment produced. However, it was generally observed that in examining the milk in the laboratory, with a temperature of 70° to 85° F. in the room, the milk from the cooler having a temperature of 40° F. would increase in temperature during the examination period. Observations made in 15-minute intervals showed that the intensity of sediment in the control samples of the nonclarified quart bottles of milk would increase within the first 15 minutes and show the same intensity of sediment as was found in the return samples on the first

observations. Since little agitation had taken place, the change in intensity of sediment was attributed to the effect of increasing temperature of the milk. The sediment of all the nonclarified milk studies was usually of a yellowish-grey to greyish color.

Table 14. The influence of delivery-route agitation on the migration of leucocytes in regular clarified homogenized milk (Average of 24 trials).

	Leucocytes per ml. in the upper, middle and lower one-third portions, after storage, in regular clarified, homogenized milk.					
	Control			Return		
Trial No.	Upper one-third	Middle one-third	Lower one-third	Upper one-third	Middle one-third	Lower one-third
1	10,000	20,000	120,000	120,000	100,000	100,000
2	120,000	80,000	80,000	40,000	40,000	40,000
3	20,000	10,000	80,000	60,000	10,000	40,000
4	80,000	120,000	160,000	20,000	20,000	80,000
5	40,000	10,000	60,000	20,000	20,000	60,000
6	60,000	60,000	40,000	20,000	10,000	160,000
7	40,000	20,000	60,000	20,000	60,000	280,000
8	40,000	40,000	100,000	10,000	60,000	80,000
9	40,000	80,000	100,000	60,000	100,000	100,000
10	40,000	10,000	40,000	60,000	20,000	20,000
11	80,000	20,000	80,000	60,000	20,000	120,000
12	60,000	60,000	120,000	20,000	10,000	260,000
13	100,000	10,000	220,000	100,000	80,000	160,000
14	40,000	60,000	220,000	40,000	60,000	160,000
15	20,000	60,000	60,000	20,000	20,000	20,000
16	40,000	60,000	60,000	60,000	20,000	20,000
17	20,000	20,000	20,000	20,000	60,000	60,000
18	40,000	40,000	60,000	20,000	40,000	60,000
19	40,000	160,000	200,000	80,000	40,000	360,000
20	40,000	10,000	180,000	40,000	120,000	80,000
21	40,000	60,000	60,000	80,000	10,000	320,000
22	10,000	10,000	80,000	10,000	10,000	20,000
23	100,000	80,000	240,000	20,000	40,000	140,000
24	40,000	100,000	140,000	40,000	10,000	100,000
Log. Average	40,460	38,200	90,120	34,450	29,600	84,700
Per cent of total	24.0	22.6	53.4	23.2	19.9	56.9

Table 15. The influence of delivery-route agitation on the migration of leucocytes in filtered, nonclarified regular homogenized milk (Average of six trials).

	Leucocytes per ml. in the upper, middle and lower one-third portions after storage in filtered, nonclarified homogenized milk					
	Control			Return		
Trial No.	Upper one-third	Middle one-third	Lower one-third	Upper one-third	Middle one-third	Lower one-third
1	40,000	220,000	460,000	80,000	60,000	340,000
2	60,000	80,000	340,000	80,000	160,000	480,000
3	140,000	80,000	200,000	10,000	40,000	260,000
4	180,000	160,000	260,000	40,000	140,000	400,000
5	220,000	220,000	640,000	260,000	200,000	640,000
6	60,000	120,000	200,000	100,000	100,000	260,000
Log. Average	96,320	134,600	318,300	63,640	101,400	376,000
Per cent of total	17.5	24.5	58.0	11.8	18.8	69.4

Table 16. The influence of delivery-route agitation on the migration of leucocytes in nonfiltered, nonclarified homogenized vitamin D milk (Average of nine trials).

	Leucocytes per ml. in the upper, middle and lower one-third portions, after storage, in nonfiltered, nonclarified, homogenized vitamin D milk					
	Control			Return		
Trial No.	Upper one-third	Middle one-third	Lower one-third	Upper one-third	Middle one-third	Lower one-third
1	140,000	180,000	640,000	220,000	160,000	520,000
2	80,000	20,000	220,000	60,000	40,000	200,000
3	20,000	20,000	60,000	40,000	10,000	120,000
4	40,000	100,000	300,000	20,000	140,000	180,000
5	120,000	80,000	180,000	180,000	60,000	180,000
6	80,000	60,000	160,000	40,000	80,000	80,000
7	10,000	40,000	10,000	10,000	10,000	40,000
8	120,000	80,000	300,000	120,000	120,000	300,000
9	40,000	10,000	280,000	40,000	100,000	220,000
Log. Average	53,330	46,930	158,000	53,600	55,700	164,000
Per cent of total	21.0	18.0	61.0	19.6	20.4	60.0

Table 17. The influence of delivery-route agitation on the intensity of sediment in quart bottles of homogenized milk

Type and treatment of milk prior to homogenization	The intensity of sediment in the	
	Control	Return
Regular, clarified	0.0	.2
Regular, filtered, not clarified	0.5	3.0
Vitamin D, not filtered, not clarified	0.55	2.33

Table 18. The relation between the number of leucocytes per milliliter in quart bottles and the intensity of sediment observed

Type and treatment of milk prior to homogenization	Control		Return	
	Leucocytes per ml.	Intensity of sediment	Leucocytes per ml.	Intensity of sediment
Regular, clarified	56,260	0.0	49,580	0.2
Regular, filtered, not clarified	183,070	0.5	180,220	3.0
Vitamin D, not filtered, not clarified	86,420	0.55	91,100	2.33

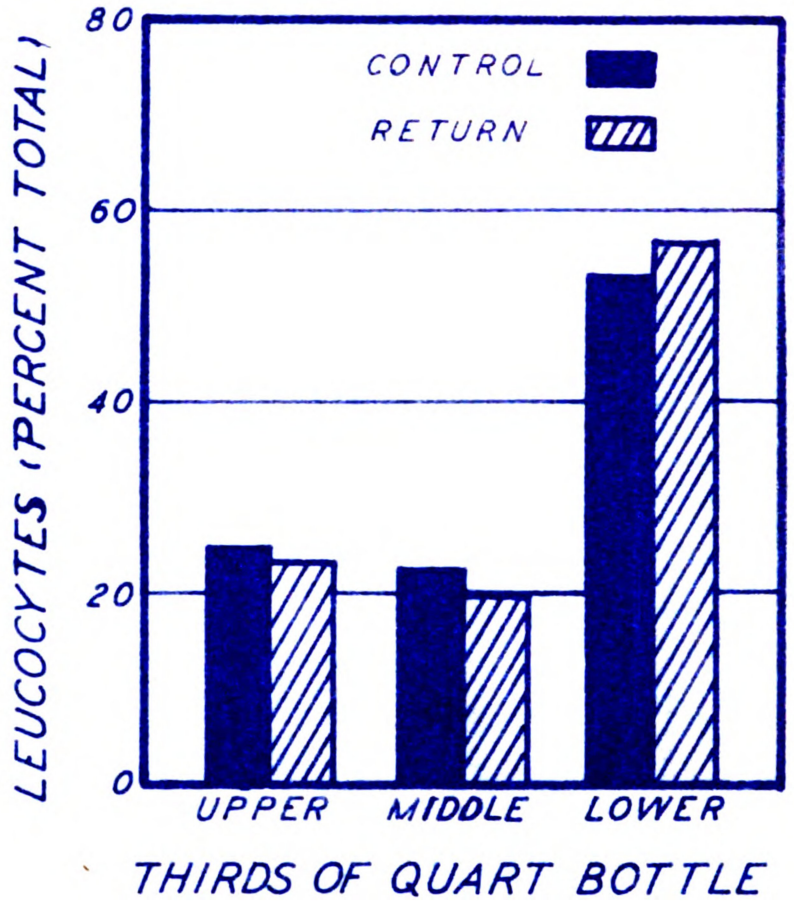


Figure 22. The per cent distribution of leucocytes in the upper, middle and lower one-third portions of quart bottles of regular clarified, homogenized milk.

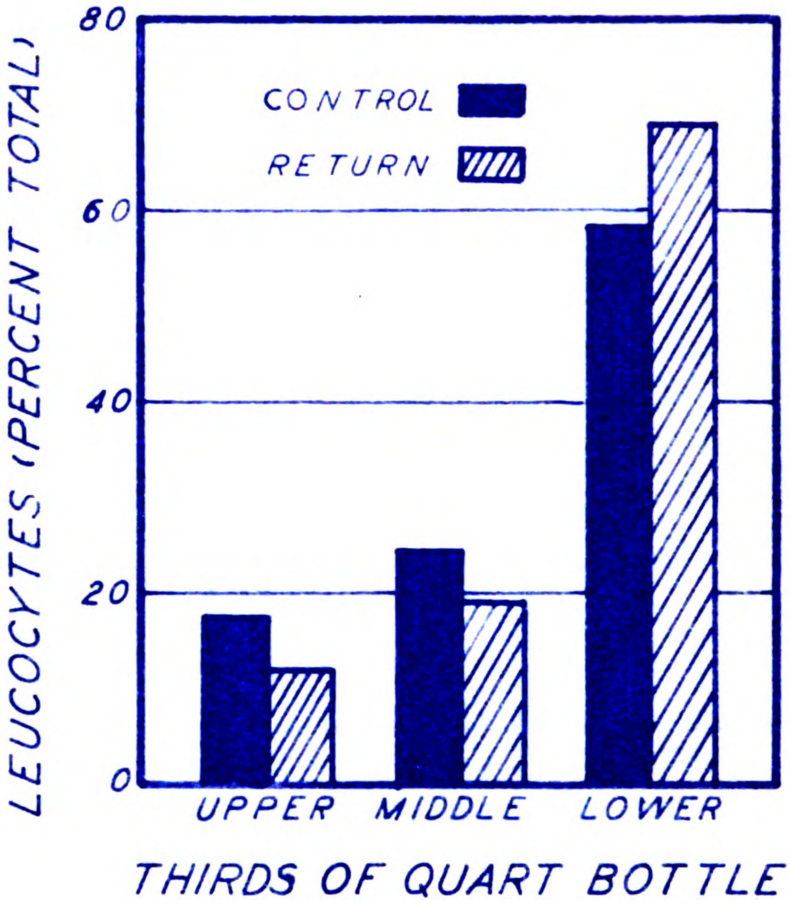


Figure 16. The per cent distribution of leucocytes in the upper, middle and lower one-third portions of quart bottles of filtered, dechlorinated, regular homogenized milk.

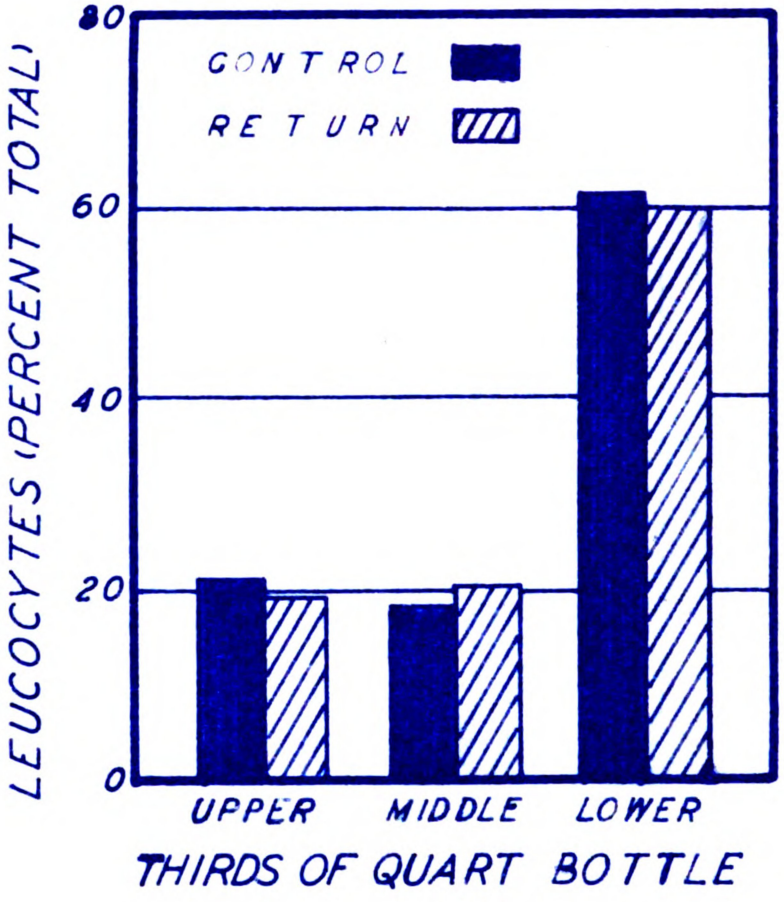


Figure 24. The per cent distribution of leucocytes in the upper, middle and lower one-third portions of quart bottles of nonfiltered, nonclarified homogenized vitamin D milk.

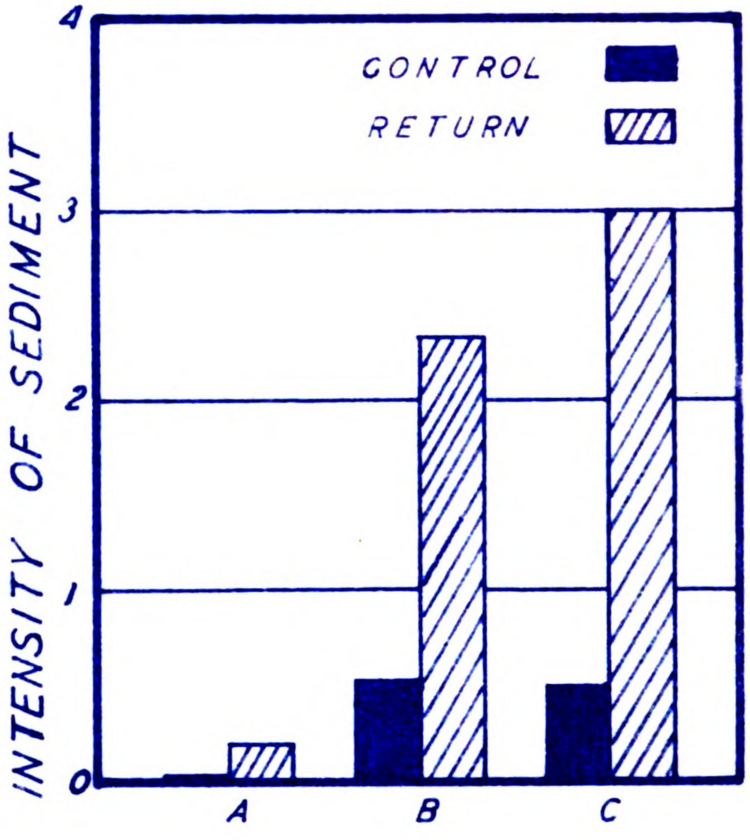


Figure 25. The relationship between the intensities of sediment as found in quart samples of control and return homogenized milk. Milk treatment previous to homogenization:

A. Regular, clarified

B. Vitamin D, not filtered, not clarified

C. Regular, filtered, not clarified

7. Effect of normal homogenization pressure on the leucocyte count of milk.

The observation was made, while conducting the various trials, that the homogenized milk usually contained a lower leucocyte count per milliliter, than the homogenized sample of the same milk. Thus it was felt of interest to find out what the average percentage reduction in the number of leucocytes was when normal homogenization pressures were employed.

All milk used in this experiment was pasteurized at 142° - 144° F. for 30 minutes and homogenized at pressures ranging from 2000 to 2500 pounds per square inch. Leucocyte counts were made from samples of milk collected before and after homogenization. A total of thirty trials were conducted. The data secured are presented in table 19.

The data show that in all but four cases the number of leucocytes found in the nonhomogenized milk exceeded the count in the homogenized milk. The inconsistency of these four trials, however, might be explained by the fact the counts were within the range of experimental error, and were made also on low count milk to which no leucocytes had been added.

The reduction, based on the logarithmic average of the above trials, was found to be 41.28 per cent.

The decrease in the number of leucocytes in the homogenized milk must be attributed to the destructive action of the homogenization process. No other explanation can be given, since in making the counts, it was much easier to see the leucocytes in the microscopic field when homogenized milk was used, than with nonhomogenized milk. Thus, as far as observing the leucocytes in making the counts was concerned, no lower numbers should have been encountered.

Table 19. The influence of normal homogenization pressure on the destruction of leucocytes

Trial No.	Leucocytes per ml. in milk when	
	Nonhomogenized	Homogenized
1	3,900,000	2,320,000
2	1,620,000	480,000
3	1,440,000	760,000
4	1,140,000	1,040,000
5	1,800,000	640,000
6	1,000,000	700,000
7	1,060,000	460,000
8	880,000	700,000
9	940,000	760,000
10	2,240,000	960,000
11	640,000	440,000
12	260,000	340,000
13	100,000	120,000
14	80,000	100,000
15	180,000	100,000
16	440,000	160,000
17	160,000	100,000
18	200,000	140,000
19	120,000	180,000
20	320,000	320,000
21	140,000	120,000
22	2,260,000	580,000
23	3,000,000	1,140,000
24	4,060,000	2,160,000
25	1,460,000	700,000
26	1,900,000	560,000
27	1,580,000	460,000
28	960,000	480,000
29	1,060,000	500,000
30	800,000	480,000
Log. average	725,600	426,000
Per cent reduction	41.28	

8. Effect of repeated high pressure homogenization on the leucocyte count of milk.

Since the results of previous trials had shown that normal homogenization pressures reduced the leucocyte count per milliliter in the homogenized milk, an experiment was conducted to show the influence of repeated high pressure homogenization on the leucocyte count of milk. It was felt that such procedure would demonstrate the destructive action of homogenization on the leucocytes in the milk, at high homogenization pressures.

Three trials were conducted, using raw, clarified milk to which was added fresh separator slime at the rate of 1.5 gms. per quart of milk. The milk was pasteurized at 142° - 144° F. for 30 minutes and homogenized, first at 2500 pounds pressure and then followed by five repetitions at 5000 pounds pressure. Quart samples were collected, which were cooled and held for 48 hours at 40° F. at the end of which period examinations were made.

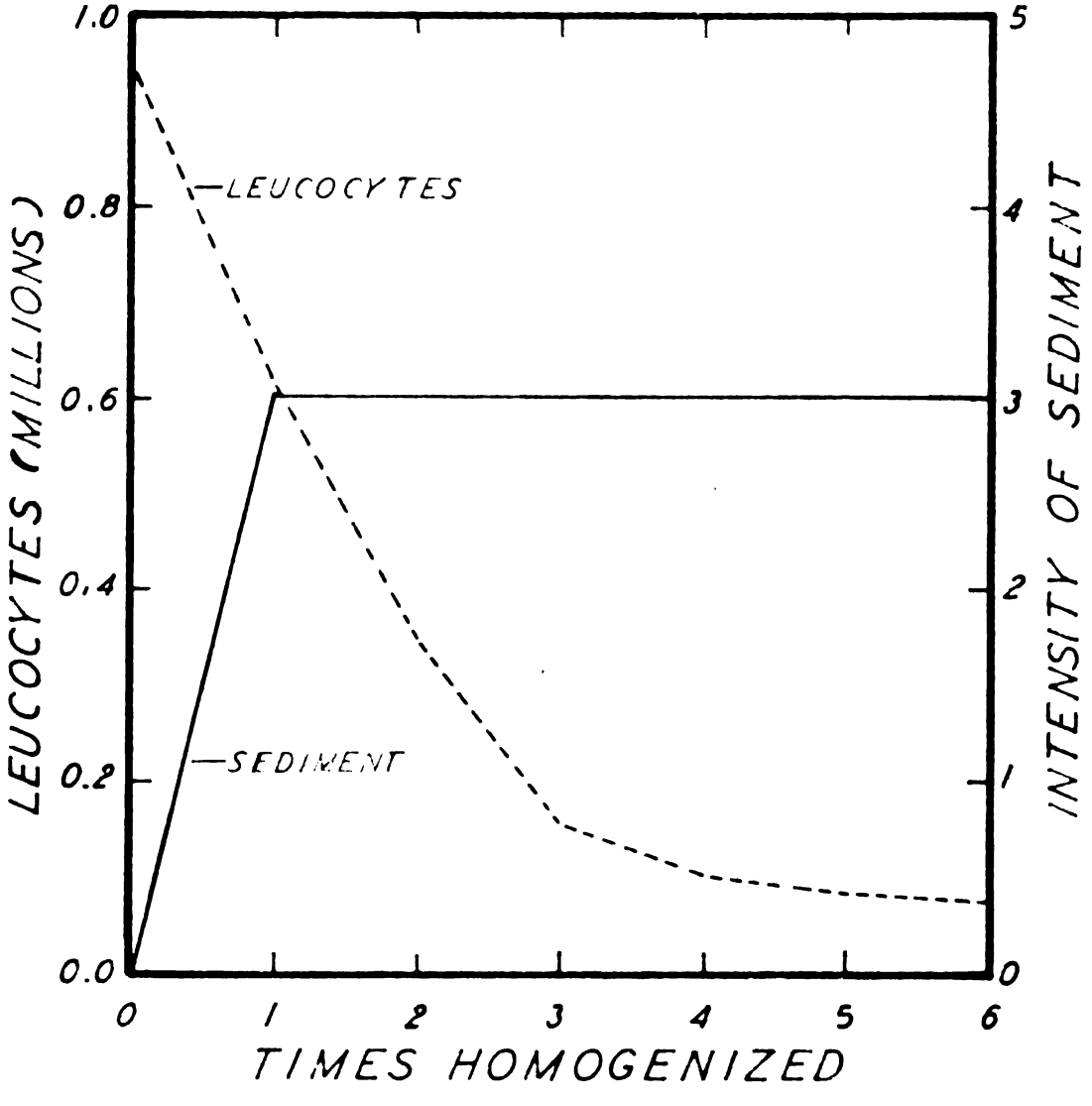
The leucocyte count per milliliter decreased rapidly with the first two trials of high pressure homogenization which reduced the count over 80 per cent. After that the rate of reduction was markedly less. The data are shown in table 20 and figure 26. The highest total reduction obtained after the last homogenization was 92.4 per cent.

Although the leucocyte count decreased with repeated high pressure homogenization, no reduction in the intensity of sediment was observed. All bottles of homogenized milk showed sediment of a greyish color and were given an intensity rating of 3.0. Some difference was noticed in the general appearance of the sediment. The sediment deposits of the milk homogenized from one to five times resembled sand in appearance, while the sediment of the last bottles was smooth and even throughout. The conclusion was reached that

the broken leucocytes tended to settle down and form sediment, similerly to the nonbroken leucocytes found in milk.

Table 20. The influence of repeated high pressure homogenization on the leucocyte count and the intensity of sediment in quart bottles of homogenized milk (Average of three trials)

Number of times homogenized	Homogenization pressure (lbs.per sq.in.)	Leucocytes (No.per ml.)	Leucocyte reduction (% of total)	Intensity of sediment
0	0	957,000	0	0.0
1	2500	625,400	34.6	3.0
2	5000	357,200	62.7	3.0
3	5000	159,100	83.4	3.0
4	5000	106,300	88.9	3.0
5	5000	84,300	91.2	3.0
6	5000	72,700	92.4	3.0



[illegible] part of [illegible] pressure homogenization on the leucocyte count and on the intensity of sediment.

9a. Effect of continuous, high-pressure homogenization on the leucocyte count of milk.

Since previous experiment had shown the destructive action of high-pressure homogenization upon leucocytes, a trial was conducted with the purpose of attempting to accomplish the complete destruction of leucocytes.

To five gallons of pasteurized milk was added fresh separator slime at the rate of 1.5 gms. per quart. The milk was heated to 140° F. and homogenized, first at 2500 pounds pressure, and again at 5000 pounds pressure for ten minutes continuously. Quart samples of milk were collected before and after the first homogenization as well as at two-minute intervals during the continuous process. All samples were cooled and held at 40° F. for 48 hours at the end of which time examinations for sediment and leucocytes were made.

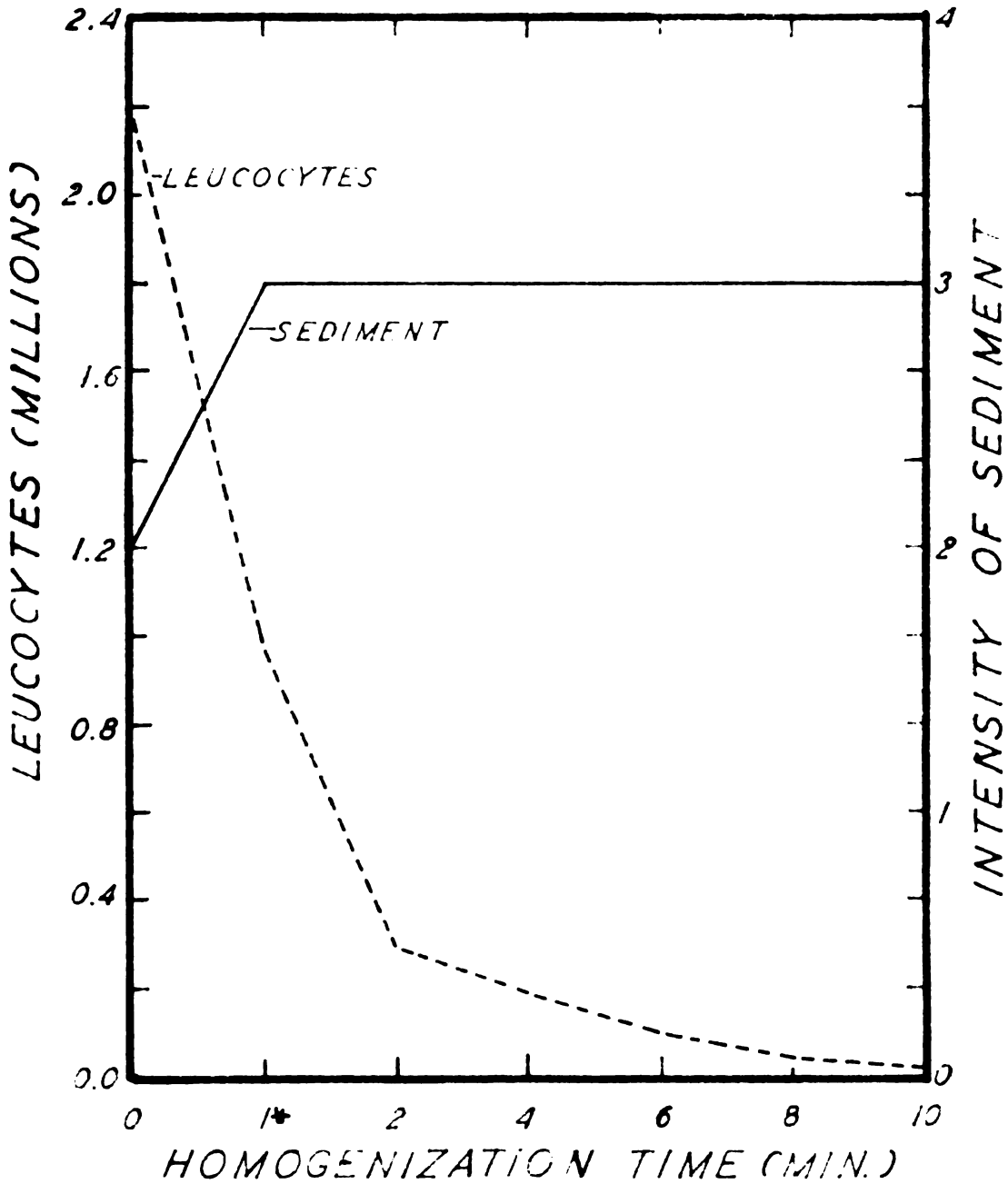
The data, shown in table 21 and figure 27, give the results obtained in this experiment. Virtually complete destruction of leucocytes was accomplished. The leucocyte count was reduced from 2,240,000 per milliliter to 20,000 per milliliter as a result of homogenizing at 5000 pounds pressure for 10 minutes. Microscopic examination of the homogenized milk showed the last samples to contain leucocytes of small size only. The leucocyte fragments, although not readily stained could be seen as small particles in the microscopic field. The intensity of sediment was found to be the same in all samples of homogenized milk. This would indicate that the broken leucocytes settled and formed sediment, similarly to the nonbroken leucocytes.

Since the temperature of the milk increased during the process of homogenization from 140° F. to 190° F. it might be expected that the sediment of the latter samples contained some destabilized portions together

with the fragmented leucocytes. Also, the high temperature must have liberated more leucocytes thus resulting in a higher percentage of total reduction than the 99.1 per cent as calculated.

Table 21. The influence of continuous high pressure homogenization on the leucocyte count and on the intensity of sediment

Time homogenized (min.)	Homogenization pressure (lbs. per sq. in.)	Leucocytes per ml.	Leucocyte reduction (% of total)	Intensity of sediment
0	0	2,240,000	0	2.0
0	2500	960,000	57.1	3.0
2	5000	320,000	85.8	3.0
4	5000	180,000	92.0	3.0
6	5000	100,000	95.5	3.0
8	5000	40,000	98.2	3.0
10	5000	20,000	99.1	3.0



[illegible] The influence of continuous high-pressure homogenization on the leucocyte count and the intensity of sediment in [illegible] milk.

9b. Effect on sedimentation of adding increasing portions of continuous, high-pressure-homogenized milk to normal homogenized milk.

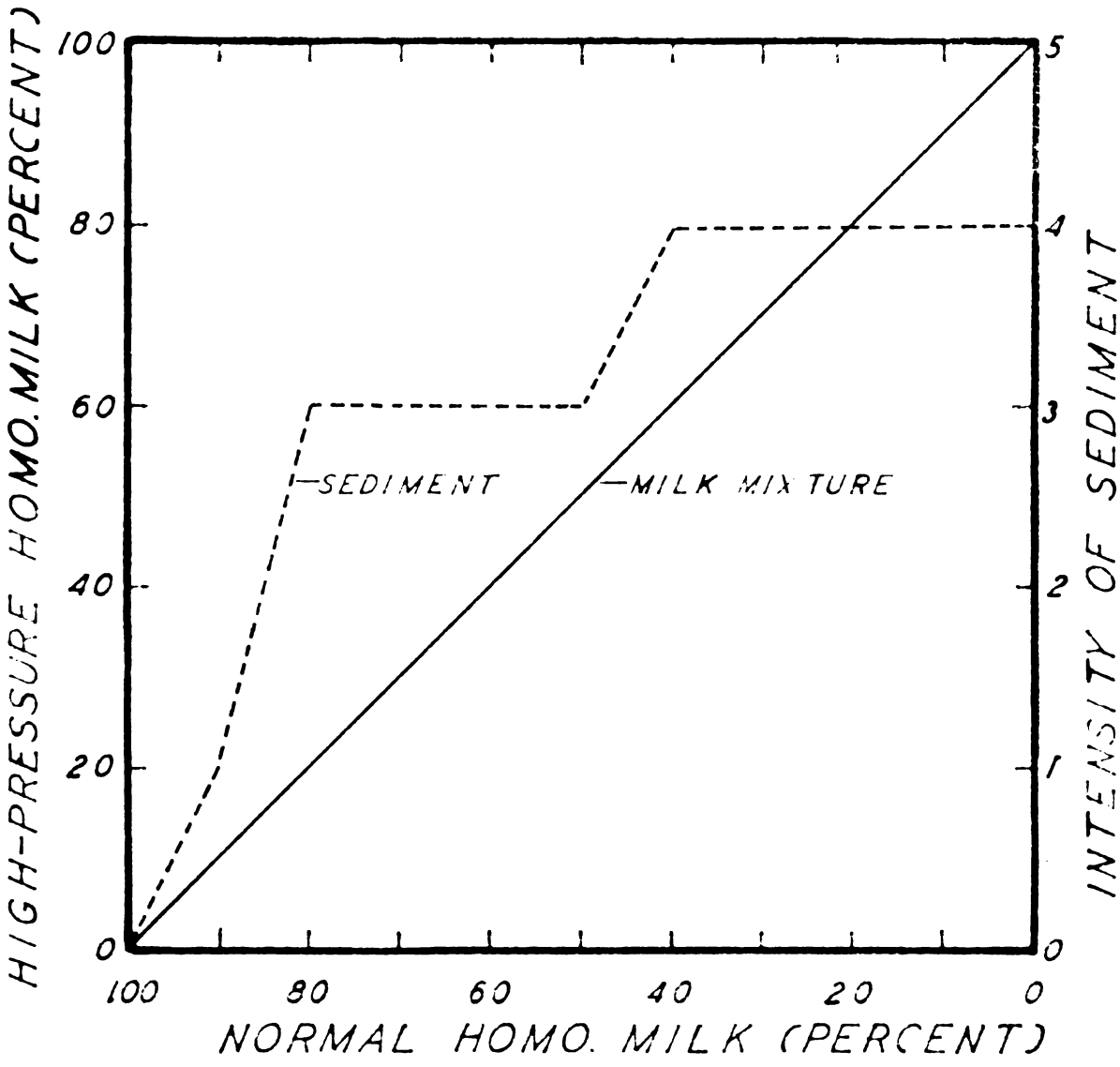
The object in this experiment was to find out whether or not mixtures of normal homogenized milk and milk subjected to a continuous high pressure treatment, which was high in leucocyte fragments, would form sediment of greater intensity.

To a series of eleven quart bottles containing decreasing amounts of normal homogenized milk, were added increasing amounts of the continuous, high-pressure-homogenized milk prepared earlier in this experiment. All samples were mixed well, and allowed to remain undisturbed for 48 hours at 40° F. Sediment studies were made at the end of the storage period.

The intensity of sediment increased with the addition of increasing portions of continuous, high-pressure-homogenized milk. This is shown in the data of table 22 and figure 28. The sediment was not due primarily to leucocytes, since none of the samples contained a leucocyte count above 80,000 per milliliter. The sediment curve in figure 28 follows the straight line of the milk mixture. Thus no doubt can exist as to the settling of the broken leucocytes in normal homogenized milk.

Table 22. The influence of adding increasing portions of continuous high-pressure treated milk to normal homogenized milk on the intensity of sediment produced

Sample No.	Per cent of normal homogenized milk per bottle	Per cent of continuous high-pressure treated milk per bottle	Intensity of sediment
1	100	0	0
2	90	10	1
3	80	20	3
4	70	30	3
5	60	40	3
6	50	50	3
7	40	60	4
8	30	70	4
9	20	80	4
10	10	90	4
11	0	100	4


HIGH-PRESSURE HOMO. MILK (PERCENT)
100
80
60
40
20
0
SEDIMENT
MILK MIXTURE
5
4
3
2
1
0
INTENSITY OF SEDIMENT
100
80
60
40
20
0
NORMAL HOMO. MILK (PERCENT)

10. Effect of temperature of clarification on the intensity of sediment in homogenized milk.

While the results of previous workers (Jacobsen and Olson, 1931) had shown that the temperature at which milk was clarified plays a role in the efficiency of the removal of leucocytes from milk, a further study seemed to be of interest in view of sedimentation in homogenized milk.

Six gallons of fresh raw milk were divided into three equal lots. The three lots of milk were clarified at temperatures of 60°, 100° and 145° F. respectively. Washed leucocytes were then added to the clarified milk at the rate of 2 gms. per quart. The mixtures were pasteurized at 142° to 144° F. for 30 minutes, followed by homogenization at 2500 pounds pressure. Quart samples were collected, cooled and stored at 40° F. for 48 hours. Examinations for sediment formation and leucocytes were made at the end of the storage period.

The use of lower clarification temperatures resulted in slightly lower leucocyte counts in the clarified milk, as shown in the data of table 23. This difference in count, based on the results of three trials only, seems not to be of any significance. All the clarified milk had very low leucocyte counts, and no sediment formation would have taken place, according to the results obtained in previous trials.

The addition of similar weights of washed leucocytes to the milk which had been clarified at different temperatures with subsequent pasteurization and homogenization showed a similar trend in the leucocyte count to that observed in the clarified milk. Lower leucocyte counts were obtained in milk samples which had been clarified at lower temperatures.

The correlation of the leucocyte count per milliliter of milk after homogenization to the intensity of sediment produced is shown in the data of table 23 and in figure 29.

Whether or not the slight increase in leucocyte count at the higher temperature and the increase in the intensity of sediment were due only to the clarification temperature employed, is not certain. Since, however, the same trend, namely a higher count with higher clarification temperature, was observed in the clarified milk, both before and after the addition of washed leucocytes, and the intensity of sediment increased also with higher clarification temperatures it would follow that low clarification temperatures would be more desirable.

The data in table 24 show the distribution of leucocytes in the upper, middle and lower one-third portions of quart bottles of milk after storage. The distribution, calculated as percentage of total number of leucocytes per bottle, in the upper, middle and lower portions were about the same in all three lots of milk.

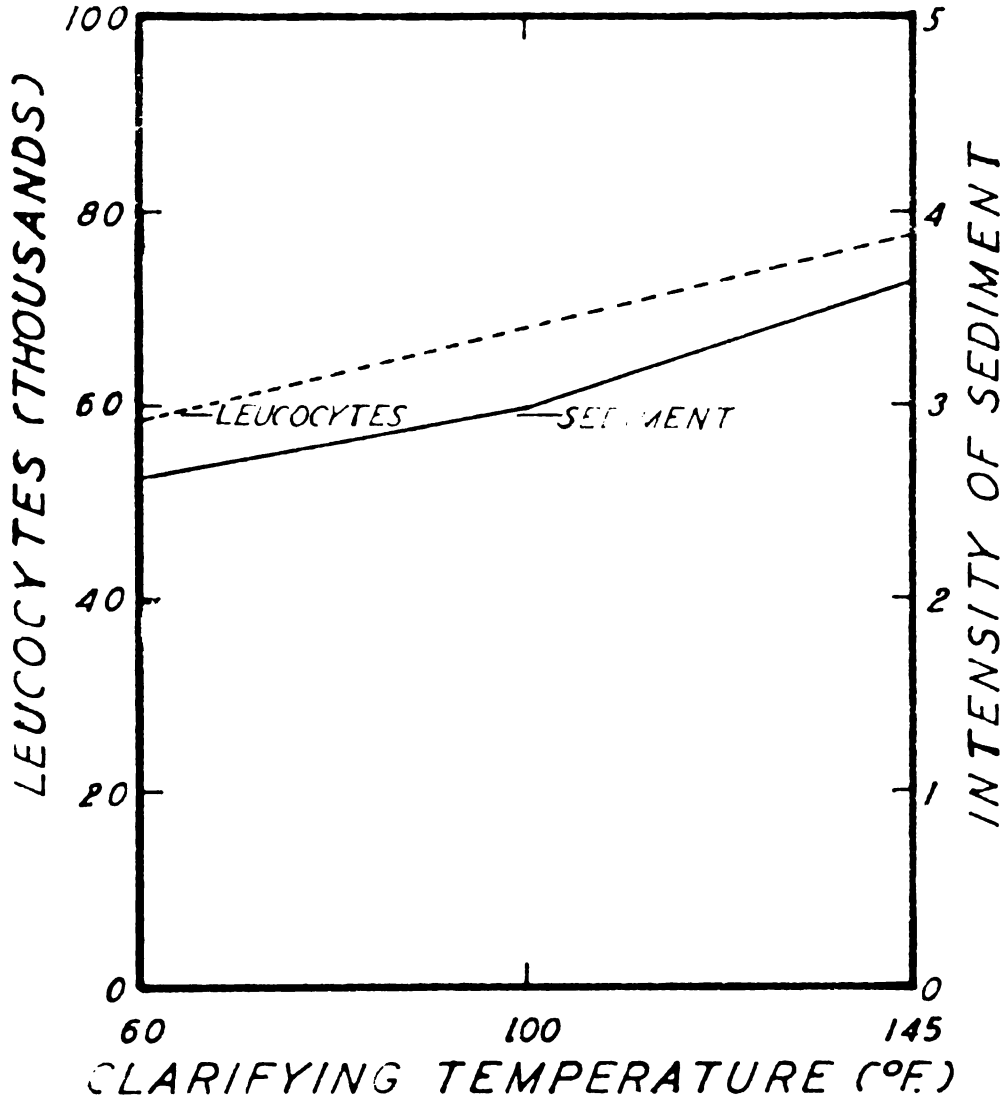
Table 23. The influence of the temperature of clarification on the leucocyte count and the intensity of sediment observed (Average of three trials)

Temperature of clarification (°F.)	Leucocytes per milliliter of the milk when			Intensity of sediment
	Raw	Clarified	Homogenized*	
60	31,740	10,000	589,600	2.66
100	31,740	12,600	683,400	3.0
145	31,740	15,870	781,200	3.66

*2 gms. washed leucocytes per quart of milk were added prior to homogenization.

Table 24. The influence of the temperature of clarification on the distribution of leucocytes in quart bottles of homogenized milk to which washed leucocytes were added after clarification (Average of three trials)

Temperature of milk at clarification (°F.)	Leucocytes per milliliter in the					
	Upper one-third of quart bottle		Middle one-third of quart bottle		Lower one-third of quart bottle	
		% of total		% of total		% of total
60	252,000	17.2	405,100	27.7	807,500	55.1
100	300,000	16.5	464,500	25.5	1,055,000	58.0
145	433,200	19.9	546,000	25.0	1,199,000	55.1



[illegible] Showing relation between intensity of sediment and leucocyte count

11. Effect of clarification at different temperatures and at different stages of processing on the weight of dry matter removed.

Since clarification removes the unstable suspended particles it was thought to be of interest to study the weight of dry matter removed from equal weights of milk, when clarification was performed at different temperatures of the milk and at different stages of processing. The weights of dry matter removed per given quantity of milk would then serve as an indication as to the efficiency of the clarifying process at different temperatures and at different stages of processing.

To twelve gallons of fresh raw milk were added washed leucocytes at the rate of 0.5 gms. per quart. The milk was divided into six equal portions or lots. Lots 1, 2, 3 and 4 were clarified at temperatures of 40°, 60°, 100° and 145° F. respectively. Lot 5 was clarified at 145° F. after pasteurization, and lot 6 at the same temperature after pasteurization and homogenization.

All six lots of milk were pasteurized and homogenized in the regular manner, quart samples collected, cooled and stored for further studies. The clarifier slime of each lot was collected separately by scraping and rinsing the bowl with small amounts of distilled water, until perfectly clean. The slime and the washings were placed in petri plates and dried in a drying oven at 100° C., cooled and weighed.

The reduction in leucocyte count due to clarification was very great in all cases, ranging from 85.2 to 97.2 per cent of total number of leucocytes as shown in the data in table 25. The difference in leucocyte count between the individual lots of milk, however, were not considered significant enough in order to pay too much importance to these values. The same might be said about the slight differences in numbers of leucocytes per

milliliter of milk in the clarified, homogenized samples of the different lots of milk. The differences in leucocyte count are all in the range of experimental error.

The data in table 26 and in figure 30 show the grams of dry matter removed by clarification of each two-gallon lot of milk. The weight of the total dry matter removed increased with increasing heat treatment, but decreased with homogenization. This would tend to indicate that more efficient clarification can be obtained by clarifying the milk at pasteurization temperatures, prior to homogenization.

According to total-solids analysis made by the Mojonnier method the washed leucocytes contained 21 per cent total solids. Since each lot of milk contained four grams of washed leucocytes, which would mean 0.84 gms. of dry matter, the rest of the removed matter must have been present in the milk prior to the addition of the washed leucocytes. The high values obtained in the two lots of milk clarified at 145° F. before and after pasteurization would show the effect of heat treatment, both on the leucocytes and also on the milk proteins.

The fact that the pasteurized homogenized lot showed a lower value must be attributed to the homogenizing action upon the suspended particles. The possibility exists, that some particles must have been reduced to a small enough size so that they would not be removed by the centrifugal force of clarification.

Fat tests made by the Mojonnier method on each lot of dry matter showed a decrease in percentage fat with higher clarifying temperatures. A slight increase, however, took place with homogenization. The data are shown in table 26 and figure 31. Some difficulty was encountered in making the tests, since the dry matter was used and it seemed difficult to dissolve

the dry slime. Since all samples were treated in the same manner it was felt that the data would show the trend of fat removal with clarification at different temperatures of milk, at different steges of homogenization.

The slight increase in the percentage of fat in the dry matter obtained from the homogenized lot over that of the nonhomogenized lot, clarified at the same temperature might be attributed to the reduced size of the fat globules in the homogenized milk. The smaller size of the fat globules, together with their increased specific gravity would aid in removing some of the fat during the process of clarification.

According to the fat analysis made on washed leucocytes by the Mojonnier method it was found that 23.58 per cent of the total solids were made up of fatty substances. This value is much higher than the highest value found in the dry separator slime, which would indicate that a large percentage of the dry separator slime was made up of fat-free substances. As the milk was clarified at higher temperatures the percentage of fat in the dry separator slime decreased, showing that the percentage of solids-not-fat increased.

Table 25. The influence of clarifying temperature and subsequent homogenization on the leucocyte count of milk (Average of three trials)

Stage of processing and temperature at which clarified	Leucocytes per milliliter in the milk			
	Before clarifying	After clarifying	% reduction	After clarifying & homogenization
1. Raw at 40° F.	446,000	31,740	92.9	25,400
2. Raw at 60° F.	446,000	18,170	95.9	25,200
3. Raw at 100° F.	446,000	12,600	97.2	18,600
4. Raw at 145° F.	446,000	25,200	94.3	20,000
5. Pasteurized at 145°F.	446,000	66,000	85.2	31,300
6. Homogenized at 145°F.	446,000*	28,810	93.5	12,600

*Leucocyte count before homogenization.

Table 26. The influence of the temperature of the milk and the stage of processing on the weight of dry matter removed by clarification (Average of three trials)

Stage of processing and temperature at which clarified	Dry matter removed from 2 gallons of milk (gms.)	Per cent fat in dry matter
1. Raw at 40° F.	1.5277	13.7
2. Raw at 60° F.	1.8720	11.6
3. Raw at 100° F.	2.2325	9.0
4. Raw at 145° F.	3.1616	5.1
5. Pasteurized at 145° F.	3.1671	4.2
6. Homogenized at 145° F.	2.2459	4.6

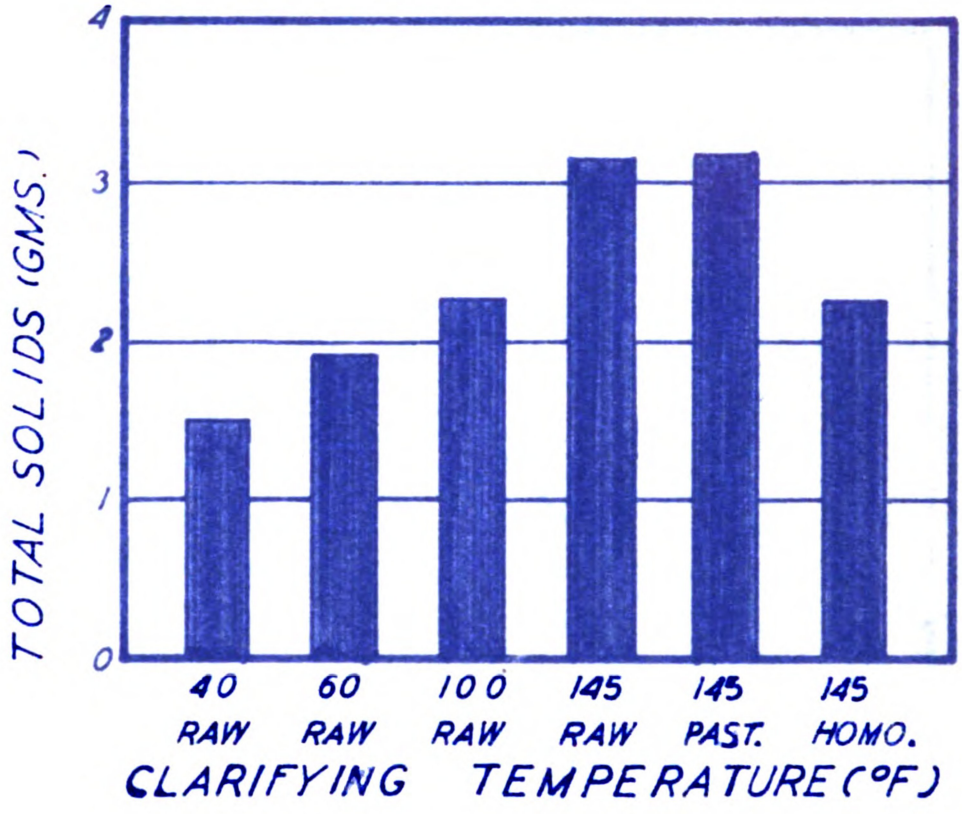
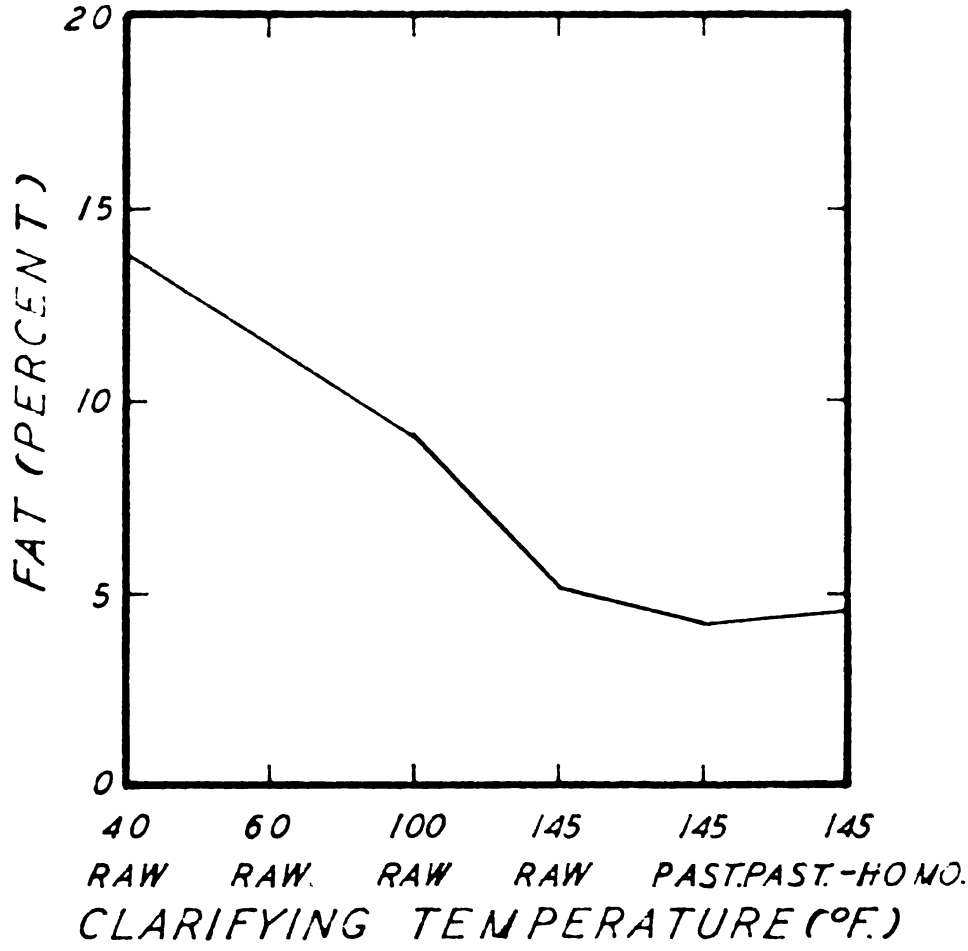


Figure 30. Grams of dry separator slime removed by clarifying 2 gallon portions of the same milk at different temperatures and different stages of processing.


20
15
10
5
0
FAT (PERCENT)
40
RAW
60
RAW.
100
RAW
145
RAW
145
PAST.
145
PAST.-HO MO.
CLARIFYING TEMPERATURE (°F.)

12. Microscopic observations of sediment in homogenized milk.

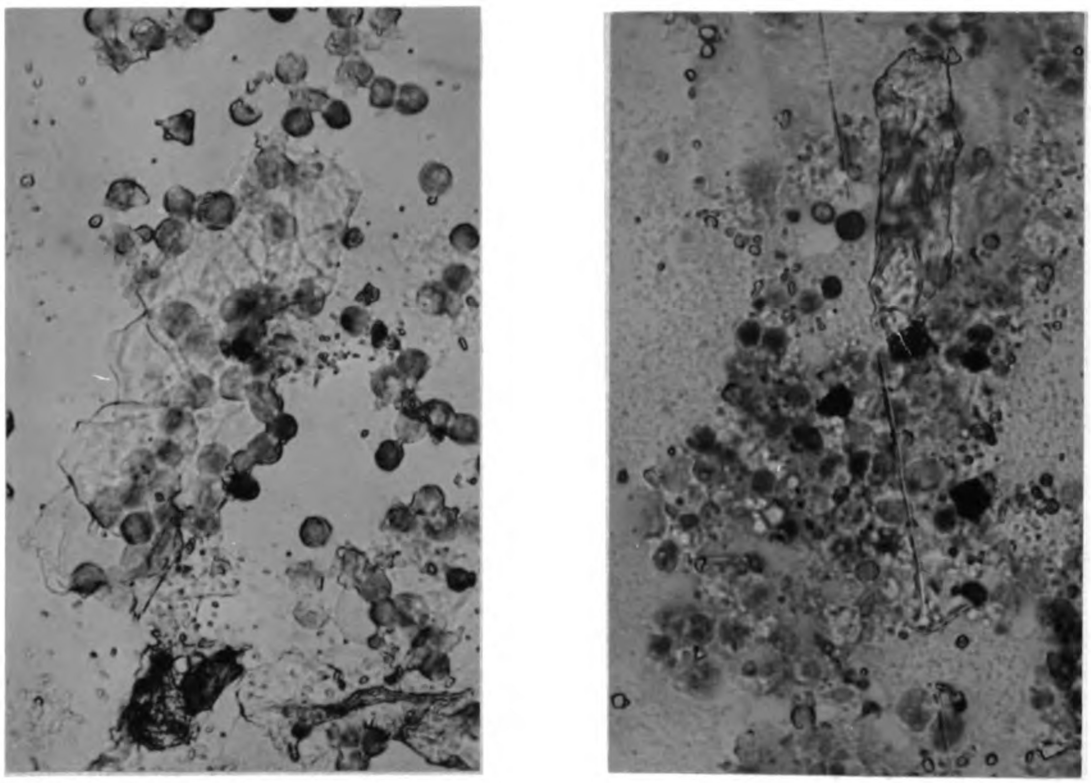
Many microscopic fields showing the sediment in homogenized milk had been studied while performing the previously described experimental work. Some of the more common and illustrative views were photographed and are presented in figures 32 to 36 inclusive.

The influence of the temperature of clarification upon the removal of cellular elements in raw milk is shown clearly in figures 32, 33 and 34. Higher clarification temperature allowed for the more complete removal of the large cellular elements, which usually tended to settle in the homogenized milk, and thus contributed to sediment formation. A temperature of 145° F., therefore, seemed to be more suitable for clarification, as far as the removal of large cellular elements was concerned. This could be attributed to the fact that the milk would be less viscous at a high temperature, and thus allow for greater ease with which the suspended cellular elements could be thrown out by the centrifugal force.

The clarifier slime obtained by clarifying the decanted milk, without disturbing the sediment in the bottom of the bottle was very similar in all three cases. Few leucocytes and epithelial cells were found in the microscopic fields of milk clarified at 60°, 100° and 145° F. respectively.

The sediment of nonclarified homogenized milk was commonly observed to be of a nature as shown in figure 35. Leucocytes and large epithelial cells, with few small fragments were predominant. The clarifier slime obtained by clarifying the same milk, which had been decanted without disturbing the sediment showed few large epithelial cells but many leucocytes and some smaller fragments.

While it had been shown previously that continuous high pressure homogenization reduced the leucocyte count by 99 per cent, the stained sediment showed very many small fragments, with few leucocytes. The small fragments were believed to be parts of the broken leucocytes. Figure 36 shows the typical view of the sediment.

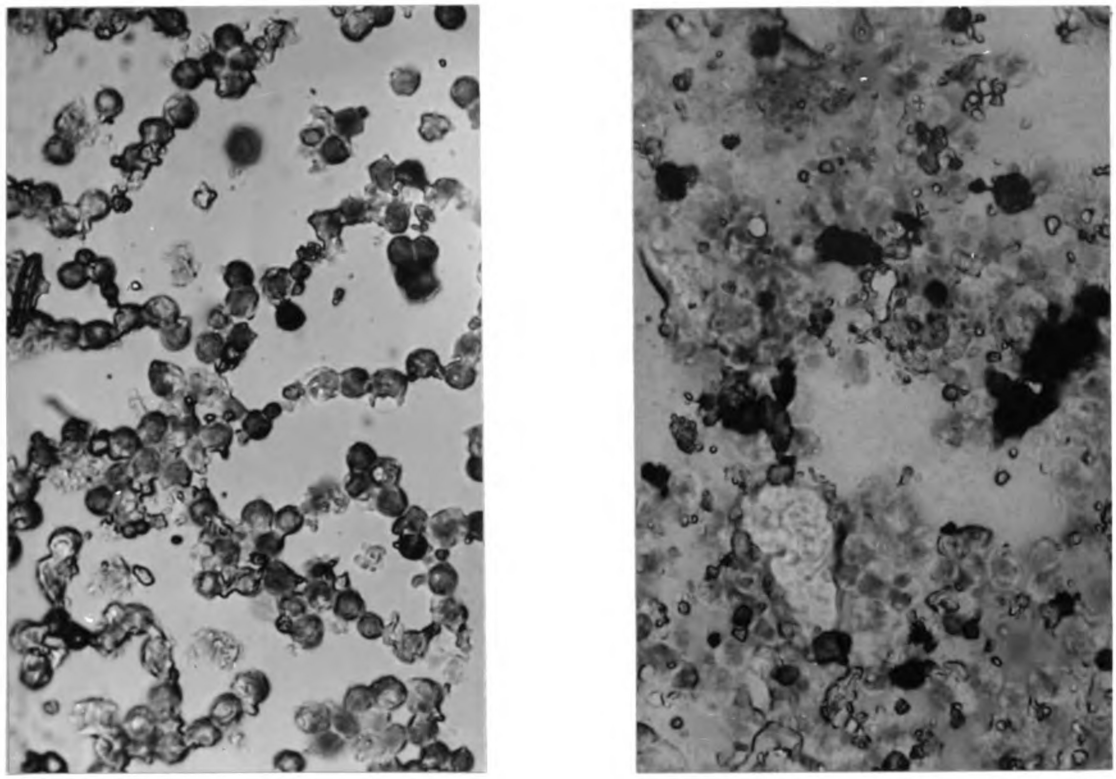


A B

Figure 32.

A. Sediment of homogenized milk which had been clarified at 60° F. and to which washed leucocytes were added at the rate of 2 gms. per quart of milk, followed by pasteurization and homogenization.

B. Clarifier slime obtained by clarifying the above milk, which had been decanted without disturbing the sediment. (Magnification 550x).

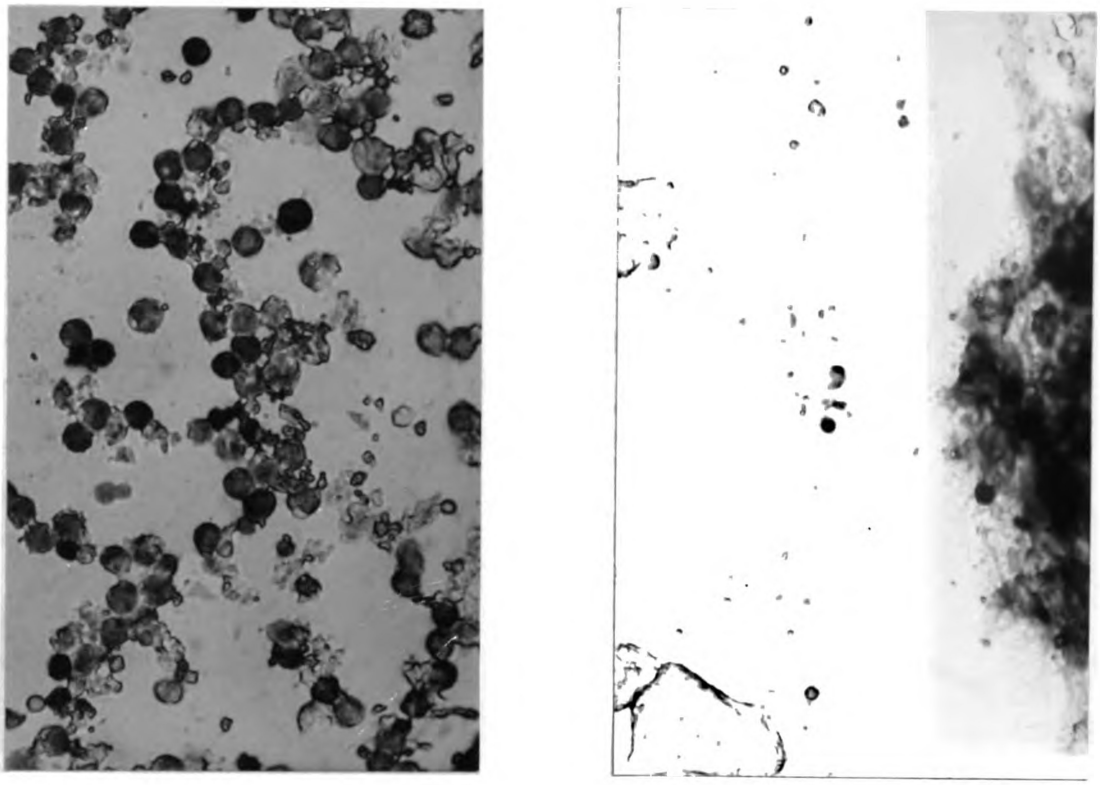


A B

Figure 33.

A. Sediment of homogenized milk which had been clarified at 100° F. and to which washed leucocytes were added at the rate of 2 gms. per quart of milk, followed by pasteurization and homogenization.

B. Clarifier slime obtained by clarifying the above milk, which had been decanted without disturbing the sediment. (Magnification 550x).

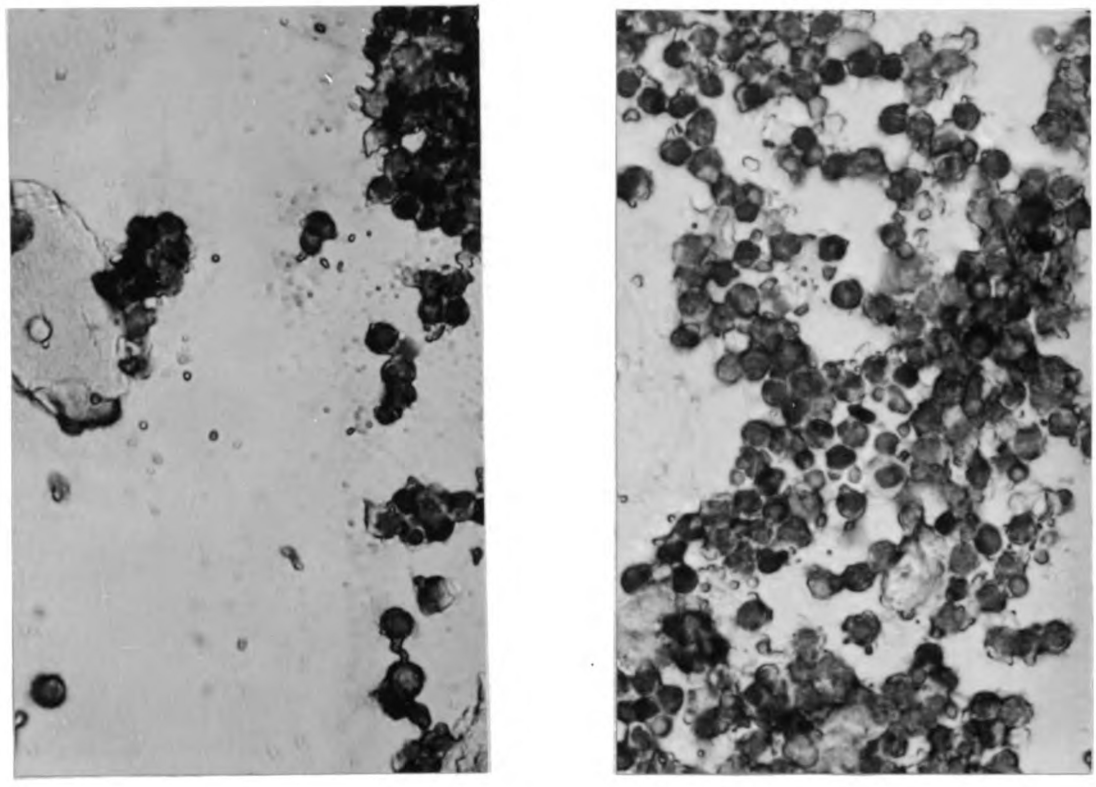


A B

Figure 34.

A. Sediment of homogenized milk which had been clarified at 145° F. and to which washed leucocytes were added at the rate of 2 gms. per quart of milk, followed by pasteurization and homogenization.

B. Clarifier slime obtained by clarifying the above milk, which had been decanted without disturbing the sediment. (Magnification 550x).



A B

Figure 35.

A. Sediment of nonclarified homogenized milk. A predominance of leucocytes and large epithelial cells was observed.

B. Clarifier slime obtained by clarifying the above milk, which had been decanted without disturbing the sediment. (Magnification 550x).

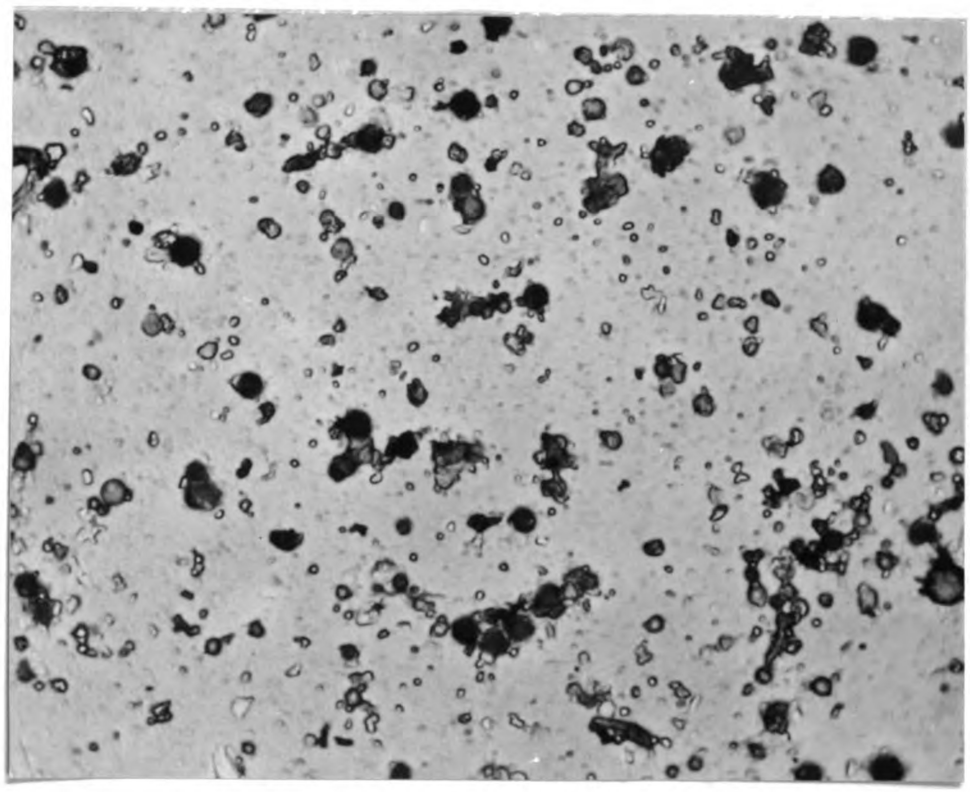


Figure 36. Sediment found in milk which had been homogenized continuously for ten minutes at 5000 pounds pressure. Fragments of broken leucocytes seemed to make up the largest part of the sediment. (Magnification 550x).

DISCUSSION

While performing the experimental work in the studies reported, the main emphasis was placed on the behavior of leucocytes in nonhomogenized and in homogenized milk under various conditions of temperature, pH, and homogenizer pressures. A careful study of the various results obtained allows for a better understanding of the behavior of leucocytes in milk and their influence upon sediment formation in homogenized milk.

There exists no doubt that leucocytes play an important part in sediment formation in homogenized milk. Thus, selection of milk with low leucocyte counts would eliminate this source of sediment, and may, therefore, be regarded as the first step in the prevention of sediment formation in homogenized milk. However, other sources of sediment exist and it is possible to have sediment formed in homogenized milk with a very low leucocyte count. It follows then that the selection of milk wholly on the basis of leucocyte count is a rather unreliable method to assure absence of sediment in the homogenized milk. In addition, other more dependable methods should be employed to eliminate sediment.

Ordinary filtration is not a satisfactory method to eliminate leucocytes and other suspended particles in milk in large enough quantities so as to render the average homogenized milk sediment free. Therefore, filtration should not be considered as a protective measure against sediment formation in the homogenized milk.

The most reliable method to be employed to prevent sediment formation to take place in homogenized milk is power clarification. This should be done at a temperature and at a stage of processing which would allow for the most complete removal of the suspended particles found in milk. Clari-

fication at the pasteurizing temperature before or after the holding period and previous to homogenization is recommended. At this temperature the fat is in the liquid state, and the milk is less viscous which allows for the easy removal of the suspended particles. The high temperature also helps to free the leucocytes by lowering the attraction between them and the fat, and thus allows for greater deposition in the clarifier slime. Since pasteurization tends to denature some proteins, especially albumin, and makes them unstable, clarification after pasteurization would remove also the possibility of this source of sediment. Clarification should be performed before homogenization since many leucocytes and other suspended particles are broken up during the process of homogenization, and thus may be too finely divided to respond to clarification.

Despite the fact that higher than usual temperatures of holder pasteurization liberate more leucocytes to be thrown out in subsequent clarification, it is believed regular pasteurization temperatures of 142° to 144° F. for 30 minutes are more desirable than higher temperatures for the same length of time for the reason that less protein will be destabilized and removed by clarification. Pasteurization should be done before clarification and homogenization in order to render the milk sediment-free.

While heat treatment, such as employed in pasteurization, tends to destabilize the proteins to some extent, it is felt that homogenization should follow pasteurization, rather than precede it. The chances are that if milk is pasteurized after homogenization more proteins will be denatured during pasteurization, since homogenization tends to destabilize the protein to some extent. If on the other hand, the milk is pasteurized before homogenization, the milk proteins enter the pasteurization process in a

more stable condition than if it were homogenized prior to pasteurization and the effect of homogenization upon the milk proteins, followed by immediate cooling of the milk, is less pronounced.

Since delivery-route agitation and heat shocking from the time the milk is bottled until it is consumed are commonly unavoidable, these conditions should be taken into account when processing the milk. To be assured of a satisfied customer a satisfactory product should be offered. This can be done only if all precautions are taken to remove the largest amount of suspended particles of the milk. There should be no traces of any kind of sediment whatsoever.

In summarizing, the procedure recommended according to the present knowledge, all milk should be pasteurized at regular pasteurizing temperatures, clarified at the pasteurizing temperature after pasteurization and homogenized at a high enough pressure to assure homogeneity of the milk, followed by immediate cooling to 40° F. or lower. Such milk should be able to reach the consumer sediment free and thus uphold the consumer's confidence in the product.

SUMMARY

The behavior of leucocytes in nonhomogenized and in homogenized milk has been studied from various angles.

The addition of 0.5 gm. of separator slime per quart of clarified, pasteurized, homogenized milk was sufficient to produce a noticeable sediment upon storage of the milk.

Adding increasing increments of washed leucocytes to freshly pasteurized, nonhomogenized milk resulted in increasing the depth of the cream layer to its "breaking point" beyond which a "cream layer" composed of fat and leucocytes formed in the bottom of the bottle.

When the electric charge of the fat globules was reversed from negative to positive, the leucocytes failed to be carried up by the "sweeping action" of the fat.

Higher leucocyte counts and a somewhat higher intensity of sediment were noted when higher pasteurization temperatures were employed.

Homogenization prior to pasteurization resulted in a slightly lower leucocyte count and in a higher intensity of sediment as compared with similar milk homogenized after pasteurization.

Delivery-route agitation had a pronounced influence on the settling of leucocytes and other suspended matter in homogenized milk, and thus aided in the formation of sediment. Heat shocking had a similar effect to that of delivery-route agitation.

Normal homogenization pressures destroyed large percentages of leucocytes in milk. Results of thirty trials showed a reduction of 41.28 per cent. Rehomogenizing the milk five times at 5000 pounds pressure reduced the leucocyte count 92.4 per cent, with little effect on the intensity of sediment.

Milk homogenized at 5000 pounds pressure for 10 minutes showed a reduction of leucocyte count of 99.1 per cent. The intensity of the sediment was the same in all homogenized samples. The additions of increasing portions of this milk to normal clarified homogenized milk resulted in a corresponding increase in the intensity of the sediment in the milk mixture.

Milk clarified at 60°, 100° and 145° F. respectively, showed slight differences in the leucocyte count of the clarified milk. This difference, however, was felt to be in the range of experimental error and thus was considered to be of little significance.

The weight of dry total solids removed from equal portions of similar milk when various clarification temperatures were used at different stages of processing, showed that milk clarified at 145° F. before or after pasteurization, resulted in the largest weights of total solids removed. The percentage of fat in the dry total solids decreased with increasing clarification temperatures.

Microscopic examinations of clarifier slime showed that more large cellular constituents were removed at clarification temperatures of 100° and 145° F. than at 60° F. In general, the sediment of nonclarified homogenized milk showed more large epithelial cells and other large fragments, than did the clarifier slime of the same decanted clarified milk.

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