

JUNE, 1932

BULLETIN 341

University of Vermont and State Agricultural College

Vermont Agricultural Experiment Station

BURLINGTON, VERMONT

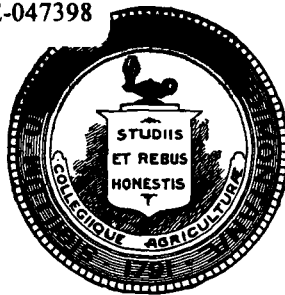
A STUDY OF THE FAT-GLOBULE SIZE
IN MILK

By M. H. CAMPBELL

Dhananjayrao Gadgil Library



GIPE-PUNE-047398



A critical comparison of different methods of measuring fat globules in cow's milk indicated that when diluted to 100 times its volume with equal parts of water and glycerin and mounted in a hemacytometer chamber relatively easy and very accurate measurements were made using a euscope in connection with a microscope.

Several environmental factors were found to affect the size of the fat globules. The largest globules were found present during the first two weeks of lactation, their size quickly lessened during the ensuing two months and thereafter declined more slowly to the end of lactation.

The mean fat-globule volume at the outset of lactation, during the forty-second week thereof and the averages for the entire lactation in terms of cubic microns were: Guernsey 28.4, 13.4, 18.8; Holstein 22.1, 6, 10.4, the former being more variable than the latter. The Angus showed a higher mean fat-globule size than the Guernsey.

Multiple factors seem involved in the inheritance of the size of fat globules in cow's milk.

BULLETIN 341: A STUDY OF THE FAT-GLOBULE SIZE IN MILK¹

By M. H. CAMPBELL

INTRODUCTION

The size of the fat globules in milk is of importance to the dairy industry because of its relation to the manufacture of butter and the creaming quality of milk. The presence of large fat globules in the milk results in a more rapid churning of the butter, with less loss of butterfat, than may be expected when the fat globules are very small. The large fat globules also produce more favorable creaming qualities than do the small ones, due to the the greater rapidity with which they rise. This is important in milk used for a city milk supply where a distinct cream line is desired.

The size of the fat globules in milk from purebred and grade cattle has been studied, but no studies appear to have been made where crossbred animals are involved. The Bowlker hybrid herd at the University of Illinois furnished excellent material for such a study, which was started in 1925. The history of this herd, with matings of the animals, has been published by Castle (1919) and by the writer (1924). The principal plan of breeding followed was the making of reciprocal crosses of Holsteins and Guernseys, a mating together of the first generation, and the back crossing of the second generation females to purebred Holstein and Guernsey males.

The plans for this study were formulated at the University of Illinois, where the method used in measuring the fat globules was developed, and the data pertaining to the Bowlker hybrid herd were collected from 1925 to 1927. The data for the purebred Guernseys and Holsteins were collected at this Station during the period 1927 to 1931. The data relating to the purebred Angus and the Angus-Holstein crossbreds were secured at the University of Wisconsin during 1931 to 1932.

REVIEW OF LITERATURE

The presence of fat globules in milk was first reported by A. Van Leeuwenhoek (1674). After discussing the globules of blood

¹A thesis submitted to the Graduate School of the University of Wisconsin in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

Paper No. 140 from the Department of Genetics, Agricultural Experiment Station, University of Wisconsin.

he states, "I have likewise observ'd some of the sweet Milk of Cows, and find that also to be made up of small transparent globules, carried in the same manner as in the Blood thorough a clear liquor." The microscopic observation was made by filling a very fine capillary tube with milk. When the tube was laid in a level position the fat globules raised to the upper surface where they were easily observed by use of a microscope.

Secretion of the fat globules.—The manner in which the fat globules of milk are liberated by the mammary gland has led to much study. Three hypotheses have been advanced to account for this process. They have been well summarized by Marshall (1922, pp. 591-593). According to the first of these, the cells themselves break loose and become disintegrated, after which their contents are set free in the alveoli of the glands. There has been opposition to this view on the basis that it would require extensive cell multiplication in the mammary gland, which has not been found to be the case.

That the mammary gland cells simply excrete substances, including the fat globules, into the alveolar lumen without becoming detached or destroyed themselves has been the most commonly accepted hypothesis. This point of view gains considerable support as such a process is analogous to that existing in most secretory glands.

The third hypothesis is, in a sense, a combination of the first two. Part of the cells liberate the fat globules into the lumina. Others lengthen so their ends project freely into the lumina of the alveoli. These projecting ends become detached with disintegration taking place before or after detaching. Then the fat globules in the disintegrated portion of the cell are set free in the milk. The part of the cell remaining in place will grow and soon another portion will become detached.

Size of the fat globules.—Bouchardat and Quevenne (1857) observed that the size of the fat globules varied appreciably in the milk of different species. They believed that the size of the fat globule in the milk from one cow was not the same at all times and under all conditions and that there was a difference between breeds. They applied the method used in the determination of yeast and blood corpuscle size to determine the numbers and size of the fat globules in milk. Their work indicated that the fat globules in cow's milk varied from 0.01 to 0.002 mm. in diameter (10μ to 2μ).

Sturtevant (1873) reported the milk globules, under a magnifying power of 800, to appear as circles of unequal diameters. By illuminating the field of the microscope from the edge the circles appeared

to be globules with darkened borders and light centers characteristic of fat. These, he states, are "globules of fat, inclosed in a thin membrane or pellicle of extreme tenuity." The measurements of these globules were reported as varying in size from mere granules to a diameter of $\frac{1}{1500}$ in. (16.93μ). Those reported as granules were less than $\frac{1}{27000}$ in. (0.94μ). The average size varied from $\frac{1}{4400}$ in. (5.77μ) at the beginning of the lactation period to $\frac{1}{6339}$ in. (4.01μ) at the end. This was the first report made of the apparent decrease in size of the globule coincident with the advance in lactation.

Blythe (1882) reported the number of fat globules per cmm. of milk to vary from 1,102,500 to 3,700,000.

Babcock (1885) did not agree with Sturtevant and others that the fat globules were inclosed in a thin membrane or pellicle. He compared the fat globules in milk with artificial emulsions and concluded that they are not inclosed in a membrane but are surrounded by a thin film of serum. This film produces the sharp refractive ring which appears to surround the fat globule when examined under the microscope. In this study Babcock reported a method for determining the number and relative size of fat globules in milk. This method consisted of filling three or four capillary glass tubes, of approximately 100 divisions of the micrometer scale in diameter, with milk diluted to 50 times its volume (one division of the micrometer scale was equivalent to 0.24 mm. or 2.4μ). These tubes were mounted on a slide and the number of fat globules counted in several fields of 50 micrometer divisions in length. From these counts the number of fat globules that would be found in a standard tube of 100 divisions in diameter and 50

divisions in length was calculated by the formula $\frac{10,000 n}{d^2}$. The result-

ing number divided by 1.085 gave the number of globules in 0.0001 cmm. of milk. From this number was further calculated the relative

size of the fat globules by the formula, $\frac{\text{percent fat content} \times 10,000}{\text{number globules in 0.0001 cmm.}}$

Determinations made by Babcock showed a wide variation in the milk of different cows with respect to the number and relative size of the globules. He found also that there was a variation between morning and evening milks when the periods between milkings were of unequal length. This variation was almost eliminated when the periods between milkings were of equal length.

The method used by Lamson (1888) for measuring the fat globules of milk was to dilute the milk with three to 400 parts of water, place a drop on a cover glass, and invert over a cell on an ordinary microscope slide. The percentage distribution of the globules as determined by this method are shown in table 1. No mention is made of the period of lactation at which the samples were taken.

Collier (1891-92), using the method developed by Babcock, studied

TABLE 1.—PERCENTAGE DISTRIBUTION OF THE FAT GLOBULES IN MILK OF DIFFERENT BREEDS AS DETERMINED BY LAMSON

	<u>1</u>	<u>1</u>	<u>1</u>	<u>1</u>	<u>1</u>	<u>1</u>	<u>1</u>
Inches	20000	10000	6600	5000	4000	3300	2850
Microns	1.27	2.54	3.85	5.08	6.35	7.70	8.91
Jersey	9.04	20.30	30.15	24.32	13.15	0.85	0.05
Durham	22.71	34.33	24.21	12.22	5.45	1.08	...
Ayrshire	23.32	35.45	24.98	12.43	3.65	0.27	...
Holstein	31.24	38.04	20.35	8.32	1.84	0.21	...

the fat globules in milk throughout the lactation period of individual animals representing different breeds. He recorded the number of globules from 0-1, 1-2, 2-3, etc., divisions of the micrometer scale in diameter. Each division, provided the magnification was the same as that used by Babcock, was equal to 2.4 μ . The method of sampling is not given, but apparently samples were taken from morning milk only, for he makes this statement, "The second table gives the averages of the actual milk yields of each animal on those mornings when samples were taken for microscopic examinations for each month of lactation and these amounts are just half of the daily milk yield." The conclusions which Collier has drawn from his results are as follows:

1. The number and relative size of the fat globules in the last pint of milk drawn was more than four times as great as in the first pint drawn.

2. There was a fairly steady increase in the number and relative size of the fat globules from the first to the last pint of milk when successive pints of the milk from one milking were examined. This increase accompanied the increase in percent fat content.

3. As the period of lactation advances the number of the smaller globules increases, with a corresponding decrease in the larger globules.

4. Holstein-Friesian milk contained a large number of small globules and a relatively small number of the larger globules as contrasted with a more even distribution of the various sized globules in Guernsey milk.

5. The number of small globules showed a relative increase during successive periods of lactation. A greater change in size was observed from the first to the second than from the second to the third lactation. This relative increase in small globules results in a smaller mean volume during successive lactations, which is similar in character to the change taking place during the advance in lactation. Results obtained for Holsteins and Guernseys are given in table 2.

Woll (1889, '90, '92, '94) compared the Babcock method with measurements made by use of Panum's hematometer. He found the readings made by the hematometer were, on an average, 6.5 percent lower than those by the Babcock method. Concerning this difference Woll states, "This is perhaps explained by the fact that the magnifying power used was different in the two cases, viz.: 950 for Babcock's method and 400 for the hematometer; even with the higher magnifying power, the smallest globules appear as mere granules or points discernible only by the closest search and it is therefor probable that these infinitesimal globules (less than 1μ) may have escaped notice with the lower magnifying power. The fact that the greatest discrepancies occur with milks having the smallest globules would seem to substantiate this view. With a too low number of globules found, their estimated relative size will of course be proportionately increased."

TABLE 2.—DISTRIBUTION OF 1,000 FAT GLOBULES IN HOLSTEIN AND GUERNSEY MILK DURING SUCCESSIVE LACTATION PERIODS AS DETERMINED BY COLLIER

Micrometer divisions	Holstein			Guernsey	
	First period	Second period	Third period	First period	Second period
0-1	278	372	412	174	309
1-2	467	420	410	370	277
2-3	202	178	151	308	248
3-4	44	31	23	111	128
4-5	8	2	4	32	33
5-6	1	5	4

The diameter of the fat globules was computed from the relative size by Woll according to the formula:

$v = 0.00000000011097s$ and $d = 1.2407 \sqrt[3]{v}$ where s equals the relative size of the average fat globules, d the diameter* of the same, 1.032 the specific gravity of milk, 0.93 the specific gravity of fat, and v the volume of the average fat globule.

The number of fat globules per 0.0001 cmm. of milk and the average relative size of the globules at the beginning and the end of the lactation periods of 18 cows were determined by Woll. His results

show that the size of the fat globules decreases with the advance of lactation period. There is, at the same time, an increase in the number of globules in a certain volume of milk. The number of globules per 0.0001 cmm. of milk ranged from 103 to 213 at the beginning of the lactation period and between 231 and 575 at the end. The average relative size of the fat globules at the beginning of the lactation period ranged between 170 and 458, equivalent to diameters of 3.3μ and 4.6μ respectively. The range at the end of the lactation period was between 66 and 197, equivalent to diameters of 2.41μ and 3.47μ respectively. Some examinations made of colostrum milk showed globules of a somewhat smaller size and fewer in number than was found in milk at the beginning of normal milk secretion. The diameters of the average sized globules for the breeds, determined from the relative size obtained by averaging results from the beginning and end of the lactation periods, were Jerseys 3.89μ ; Holsteins 3.39μ ; natives 3.64μ . In table 3 are given the average diameters of the fat globules obtained from samples taken during three different periods at the Columbian Exposition (1893).

While comparing the feeding values of succulent and dry feeds Woll found that the succulent feeds appeared to decrease the size of the fat globules and to increase their number in a certain volume of milk. This conclusion was based on results of trials with two cows, and the difference due to the change was not very marked. Any disturbance, such as sickness or excitement, appeared to affect the size of the globules. The average size of the globules was somewhat larger in the morning than in the evening milk, although the number was essentially the same for a certain volume. Thus, the increase in size of the globules in the morning milk accompanied a higher percentage of fat than was found in the evening milk.

Further studies were made to determine the effect of age on the size of the fat globules by computing the relative size at the beginning and end of successive lactation periods. There was a slight tendency toward an increased number of globules in 0.0001 cmm. of milk, with a similar decrease in the average relative size, as the animals advanced in age. This was most pronounced in the case of the Shorthorns, with the relative size decreasing from 664 for animals from two to five years of age to 400 for animals above eight years. The results for the Jerseys gave the highest relative size of 415 for animals from four to seven years of age. Those from two to three years had an average size of 337, while those over eight years averaged 334.

Merrill (1890) by use of the Babcock method, found the relative size of fat globules to be 182 for one Holstein cow, 130 and 178 for each of two Ayrshires, and 332 and 390 for each of two Jerseys.

TABLE 3.—AVERAGE DIAMETERS OF FAT GLOBULES FROM JERSEYS, SHORTHORNS AND GUERNSEYS AS DETERMINED BY WOLL AT THE COLUMBIAN EXPOSITION

	First period	Second period	Third period
	μ	μ	μ
Jersey	3.95	4.15	4.03
Shorthorn	3.35	3.57	4.79
Guernsey	3.58	3.84	...

These figures can not be used for direct comparison as the stage of lactation at which the samples were taken is not given.

Jones (1890) made fat globule measurements by mounting on flat slides as in ordinary microscopic preparations and by using the Babcock method. The globules varied from $\frac{1}{1800}$ in. (14.11μ) to $\frac{1}{4000}$ in. (6.35μ) in diameter. These measurements were made with a magnification of 500 and that writer states that doubtless many were smaller than $\frac{1}{4000}$ in. (This latter figure appears to be in error since measurements were made for much smaller globules, as shown in table 4.) The percentage distribution of the globules of varying diameters for three Jersey, three Holstein and one registered and two grade Ayrshire cows are given in table 4. The Jerseys were in the fourth month of lactation, the registered Ayrshire in the third, the grade Ayrshires in the eighth to twentieth, while the period of lactation for the Holsteins was unknown. The grade Ayrshires are included in this table because they illustrate the large percentage of small fat globules found late in the lactation period. Grade Jerseys and Holsteins are omitted because their lactation period corresponds to that of the registered animals.

TABLE 4.—PERCENTAGE DISTRIBUTION OF FAT GLOBULES OF VARIOUS DIAMETERS IN THE MILK OF DIFFERENT BREEDS AS DETERMINED BY JONES

Breed	1.40 μ	1.40-2.79 μ	2.79-4.19 μ	4.19-6.99 μ	6.99-9.78 μ
Jersey	17.4	28.6	27.	25.4	1.5
Jersey	30.5	23.7	20.3	20.3	5.1
Jersey	11.7	32.2	30.5	20.3	5.1
Holstein	44.1	29.4	19.1	5.9	1.5
Holstein	22.1	36.7	22.1	17.7	1.5
Holstein	31.2	33.4	16.7	16.7	2.
Ayrshire	40.	13.	31.	13.	3.
Grade Ayrshire	57.4	28.4	11.7	2.5	..
Grade Ayrshire	55.7	25.	16.7	2.5	..

In making careful measurements of samples prepared by the Babcock method, and computing the percentage fat content on the basis of the measured volume, Jones found that the percentage of fat so determined showed wide variations when compared with the results of chemical analyses. There was no direct relation between the size of the globules and the richness of the milk.

Fleischman (1893) states that in the milk of cows the diameter of the smallest and largest fat globules is respectively 1.6μ and 10μ . These globules, he states, are present in varying proportions and the number of different sized globules is in inverse ratio to their size. He later (1908) gives the size of the globules as ranging from 0.1 to 22.2μ .

Schellenberger (1893) made measurements of fat globules by using a Thoma blood-counting chamber and diluting the milk 100 times. The magnification used was 865. The average diameter of the fat globules was found to vary from 2.20μ for the Angler cattle to 2.95μ for the Jersey. These figures do not, however, appear to represent an average of the entire lactation period. The individual globules varied from 0.8μ to 9μ , with one exception where a globule 22μ in diameter was found in the milk of a Jersey cow with a fat test of 9.42 percent. The size of the fat globules decreased as the lactation period progressed. During the first month of lactation 43.6 percent of the globules averaged 1.96μ as compared to 90 percent averaging 1.96μ during the seventh month. This percentage of small globules appears to be high but may be due to a high class interval, the next larger class being 3.67μ . The number of fat globules averaging 1.96μ was found to be smaller, while those averaging 3.67 and 6μ were more numerous in high- than in low-testing milk. This result is based on a comparison of morning and evening milks.

Leufven (1894) found that oil cake, palmtree cake, peanut cake, cottonseed cake, sunflower cake, and rapeseed cake, when fed with a ration of hay, straw, roots, bran and ground grain, had no appreciable effect on the size of the fat globules in the milk. The decrease in size of the globules during successive feeding periods was not greater than might be expected from advance in lactation. The percentage of globules less than 1.1μ in diameter varied from 27.8 to 65, with the majority of results falling within the range of 40 to 55 percent. The portion of the globules from 1.1μ to 2.2μ in diameter varied from 32.6 percent to 47.5 percent, while most samples showed close to 40 percent within these limits. But few samples had more than 20 percent of the

fat globules with a diameter greater than 2.2μ , while one sample had but 1.9 percent of its globules equal to this diameter.

The size of the fat globules in milk from purebred and grade Ayrshires is also reported by Leufven. The grades resulted from crossing Ayrshires with four different breeds. From the results Leufven concludes that the size of the fat globules in the milk was determined by the breed with which the crossing was made. His data do not appear sufficient, however, to warrant this conclusion.

M'Donnell and Fields (1895) report the average diameter of fat globules to be about 0.000146 in. (3.71μ). The percentages of globules greater than 4μ are reported for six-day periods on a Jersey cow during the first four months of lactation. This percentage increases with progress of lactation, being as low as 36.5 percent at the end of one month and reaching 66.1 percent during the fourth month. This increase of globules above 4μ in diameter is not in agreement with most results. The relative size of the fat globules was found by M'Donnell and Fields to lessen with decrease in milk production. They state, "It is evident that the size of the fat globules varies in direct ratio with the milk yield." This might be expected, since milk yield and relative size of fat globules have been found, as a rule, to decrease with advance in lactation.

Pankowski (1895) refers to the size of the fat globules in milk, without presenting any data, as depending on the breed of cattle or the percentage fat content of the milk. He reports the largest globules in the milk of the Guernsey, with those of the Ostfreise smaller than the Angler. This latter observation does not agree with that previously made by Schellenberger, who reports the globules of Angler milk smaller than those of the Ostfreise. Green feeds, according to Pankowski, produced an increase in size with a decrease in the number of globules, while a reverse condition was produced by dry feeds.

Gutzeit (1895), in making a study of the factors affecting the size of the fat globules in milk, used the method of measurement employed by Babcock, except that the divisions of the micrometer scale were equivalent to 0.0025 mm. Instead of expressing the results in relative size, Gutzeit calculated the volume by taking into consideration the specific gravity of milk and butterfat. The factors used were 1.0315 and 0.94 respectively, or $1.0315/0.94 = 1.1$. Thus the average volume of the fat globules was expressed by the formula $f/n \times 0.000001 \text{ mm.}^3 \times 1.1$, where f equals the percentage fat content, and n equals the number of globules in 0.0001 cmm. of milk. This result divided by

0.000000001 cmm. gave the average volume in cubic microns. From this the average diameter was calculated by the formula, $D = 1.2407 \sqrt[3]{v}$.

This study was made on the milk of four different breeds, each represented by three cows. Daily samples for examination were taken each week throughout the lactation period. During shorter periods samples were taken on successive days. The greatest single factor affecting the size of the fat globules within a breed was found to be the advance in lactation. There was a slight increase in the size of the globules during the first of the lactation period, one to three weeks, followed by a gradual decrease. Turning the animals to pasture in the spring, or stabling them in the fall, resulted in an increase in the average size of the globules in each case. The decline in size following this increase extended over a longer period in the spring than in the fall. Thus the succulent feed of pastures in the spring, as well as dry feed when animals were placed in the barn, caused an increase in average size of the globules. This would indicate that distinct change in conditions from those to which animals are accustomed tended to cause an increase in the size of fat globules. This was further borne out by finding an increase in the size of fat globules following stormy days after which the animals were covered. The size of the fat globules as determined by Gutzeit for the lactation period are summarized in table 5.

Barthel (1907) reports the diameter of fat globules of milk to vary mostly between 1.6μ and 10μ , but in extreme cases the variation may be from 0.1μ to 22μ . The number of globules was found to be greater near the end than at the beginning of the lactation for the same volume of milk.

Cooper, Nuttall, and Freak (1911) made a very good study of the fat globule size by a different method than had been used by the previous investigators. They used a Thoma-Zeiss hemocytometer with a depth of 15μ . A small portion of milk was placed on this slide by means of a platinum loop. The cover glass was then put in place and pressed gently until in contact with the sides of the chamber. Photomicrographs were taken of the slides prepared in the above manner. The exposure was made sufficiently short to prevent blurring due to the Brownian movement. The diameters of the globules were measured by placing a transparency, containing a series of circles representing diameters equivalent to 1, 2, 3, etc., μ over the photomicrographs. This method of measuring the globules gave a frequency distribution which could not be obtained by the Babcock method. The

authors make the following statement concerning their method: "It seems that our method, though far more laborious than Gutzeit's or

TABLE 5.—SUMMARY OF THE AVERAGE FAT-GLOBULE SIZE FOR THE LACTATION PERIOD AS DETERMINED BY GUTZEIT

Breed	Volume in μ^3			Diameter in μ			Number per 0.0001 cmm.	2.5 μ	5 μ
	Average	Maximum	Minimum	Average	Maximum	Minimum			
Angler	13.	24.4	6.9	2.92	3.60	2.36	294	32.6	3.2
Montavoner..	9.4	30.4	4.2	2.62	3.87	2.	452	28.1	2.3
Holländer...	9.	21.8	4.1	2.58	3.47	1.99	379	25.2	1.9
Breitenburger	7.8	14.8	4.2	2.46	3.29	1.99	481	24.1	1.7

Babcock's, is more accurate, in that one can not miss the small globules of 1 μ diameter, which are so difficult to determine on account of their movement, and the omission of which makes a great difference in the results." The percentage of fat in the milk, when computed on the basis of the measured volume, was found to be in fairly close agreement with the results obtained by analytical methods.

The distribution of the different sized globules was determined for the mixed milk of four breeds and for individual cows of the Short-horn and Guernsey breeds. Results for the five breeds are given in table 6, those for the Guernseys being obtained by totaling the number of globules in each class for the individual cows.

This mean volume, as illustrated by these authors in a later report (1913), was determined by computing the total volume of fat measured and dividing by the number of globules. From this the mean diameter

was computed by the formula, $D = \sqrt[3]{\frac{6v}{\pi}}$. The mean diameter computed

by this method was compared with that of counting the total number of globules in a certain volume and using the percentage fat content of the milk, a method of calculation similar to the Babcock method as modified by Gutzeit. The mean diameters obtained by the two methods were found to agree very closely.

This method of computing the mean diameter of the fat globules was also used in making a study of the effect of feed on fat-globule size. High-, medium- and low-protein rations were fed to three groups of cows consisting of two animals each. The size of the fat globules was determined every third day for a period of 26 days, after which the rations fed the low- and high-protein groups were reversed. This change of feed did not produce any noticeable effects on the size of the

fat globules. It is concluded that "food has little or no influence on the size of the globules, if the 'ratio' is to be taken as the criterion."

TABLE 6.—PERCENTAGE DISTRIBUTION OF THE FAT GLOBULES IN MILK OF FIVE BREEDS OF CATTLE AS DETERMINED BY COOPER, NUTTALL, AND FREAK

Diameter μ	Shorthorn	South Devon	Jersey	Red Poll	Guernsey
1	41.19	39.62	37.76	38.78	29.71
2	26.65	26.16	17.40	27.29	21.35
3	19.24	21.09	18.44	16.45	23.19
4	8.77	9.63	13.99	10.	14.69
5	2.62	2.35	5.68	3.68	6.07
6	0.90	1.05	3.85	2.65	3.97
7	0.45	0.10	2.27	0.97	0.87
8	0.09	...	0.52	...	0.15
9	0.09	0.06	...
10	0.09	0.06	...
11	0.06	...

Eckles and Shaw (1913) report the average relative size of fat globules, determined by the Babcock method, to be 328 for Jerseys, 282 for Shorthorns, 150 for Ayrshires, and 142 for Holsteins. The size was found to decline very sharply during the first six weeks, then to remain fairly constant for five or six months, after which the decline was much more rapid to the end of the lactation period. They were unable to correlate the variation in relative size of the fat globules with the variation of any constituents of the milk or to the physical constants of the fat.

Hills and Boland (1913) state that the fat globules vary in size from 1 μ to 5 μ . They made a study of milks of 2.8, 3.2, 4.2, 5.2, 6.2 and 7.2 percent fat contents. The fat globules were grouped in classes with the first class including those under 1.6 μ , second from 1.6 μ to 3.2 μ , and the third over 3.2 μ . The milk of 2.8 percent fat content was found to contain 66 percent of the globules in the first class, 28 percent in the second and six percent in the third. In the highest testing milk there was 47 percent in the first class, 40 percent in the second and 16 percent in the third. They found a correlation of 0.19 between the percentage fat content of the milk and the number of globules of different sizes.

Weigmann (1927) studied the degree of dispersion of the fat globules in milk. The system of measuring was that developed by Van Dam and Sierks (1922) and later modified by Rahn (1928). This method consisted of diluting milk to 100 times its quantity with a 1½ percent gelatin solution containing one percent phenol. The gelatin inhibited the Brownian movement. A drop of the milk-gelatin solution was

placed on an ordinary slide and covered with a cover glass. With such a preparation it was found that the measurement of 600 globules was sufficient to give an accurate picture of the fat distribution in a sample of milk.

A summary of the percentage distribution of the globules, their mean volume, and the percentage fat content of the milk from eight breeds of cattle are given in table 7. These results show a distinct average volume for each breed. Accompanying this was found a breed difference in the frequency distribution of size of the globules. The percentage of the globules above six microns diameter varied from 1.37 in the "Holsteiner" to 8.16 for the "Elbmarschvieh." The former had the largest portion, or 61.57 percent of the globules, less than three microns in diameter as compared to 29.68 percent for the "Oberschwäbisches." The latter, on the other hand, had 63.61 percent of the globules ranging from three to six microns in diameter as compared to 41.92 percent for the next highest class.

The effect of change in lactation on the size of the fat globules was studied by Weigmann. He did not study this change during any one lactation, but used the latter part of one and the fore part of that which followed. Eight samples were taken during the year, but he was

TABLE 7.—SUMMARY OF THE PERCENTAGE FREQUENCY DISTRIBUTION OF THE FAT GLOBULES AND THEIR AVERAGE SIZE FOR EIGHT BREEDS OF CATTLE AS REPORTED BY WEIGMANN

Diameter	Elb- marsch- vieh	Breiten- burger Vieh	Dith- marscher Vieh	Geest- vieh	Shorthorn- vieh	Angler Vieh	Oberschw. Fleckvieh	Schwarzb. Holsteiner
μ								
0- 1	8.61	10.46	6.91	8.04	13.29	8.87	2.31	9.02
1- 2	27.39	23.73	20.13	27.54	22.11	26.54	8.08	26.92
2- 3	21.48	19.02	28.02	24.75	23.25	24.10	19.29	25.63
3- 4	16.88	21.12	26.25	23.50	22.54	24.65	30.71	24.04
4- 5	10.56	11.27	10.96	8.46	9.86	8.32	10.96	8.96
5- 6	6.92	8.25	4.71	4.96	5.48	5.27	12.94	4.06
6- 7	2.89	2.48	2.13	1.48	2.08	1.46	4.75	1.04
7- 8	2.39	2.21	0.60	0.81	1.06	0.60	1.46	0.29
8- 9	1.65	0.79	0.27	0.25	0.33	0.17	0.38	0.04
9-10	0.73	0.17	0.02	0.13	...	0.02	0.12	...
10-11	0.33	0.06
11-12	0.13	0.02
12-13	0.04
Mean volume μ^3	38.77	31.74	23.22	21.98	23.25	20.59	41.23	18.06

unable to obtain these samples at regular intervals. The first sample of the lactation period varied from 11 to 77 days after freshening on eight cows used for this study. The volume of the fat globules was always greater at the beginning than at the end of lactation. The dif-

ference in volume varied from 15.02 to 38.32 cubic microns. Colostrum milk was found to contain globules with the largest average size on the first day after calving, with a gradual decrease until about the sixth day.

The influence of feeding was found to be the most important factor, other than the breed of cattle or lactation period, affecting the size of the fat globules. The change from pasture to stall feeding, with the exception of one breed, caused an increase in the size of the globules, which decreased when the animals returned to pasture. The exception was that of the "Oberschwäbisches," which showed a decrease in fat globule volume with stall feeding and an increase with pasture feeding. The stall feeding in the latter case consisted mostly of roughages while the other breeds received concentrated feeds. Weigmann concludes that the increased size of the fat globules during stable feeding was due to the use of concentrated feeds and, most likely, of oil cakes.

Turner and Haskell (1929) measured the fat globules in milk by means of microphotographs. The milk was prepared by diluting with glycerin and cochineal stain. Data were apparently collected from mixed herd milk of different breeds without considering lactation period. A summary of these results is given in table 8. The class interval in this instance is 0.5μ as compared to 1μ or more in other investigations.

TABLE 8.—PERCENTAGE FREQUENCY DISTRIBUTIONS OF THE FAT GLOBULES AS REPORTED BY TURNER AND HASKELL

Diameter	Ayrshire	Jersey	Holstein
μ			
0.5	3.32	0.22	2.08
1.	13.30	2.71	8.86
1.5	16.93	3.85	12.40
2.	19.26	10.16	18.81
2.5	19.17	16.57	22.10
3.	10.92	16.17	12.26
3.5	6.42	13.93	8.54
4.	4.01	9.24	5.33
4.5	2.41	7.88	3.32
5.	1.95	8.43	2.90
5.5	1.15	3.96	1.49
6.	0.57	2.68	0.79
6.5	0.34	2.05	0.62
7.	0.17	1.21	0.28
7.5	0.08	0.26	0.14
8.	...	0.22	0.03
8.5	...	0.26	0.01
9.	...	0.18	0.01
9.5
Mean diameter	2.34	3.54	2.59
Diameter of the mean volume	3.27	4.01	3.12

The greatest frequency falls at 2 to 2.5 μ for the Ayrshire, 2.5 μ for the Holstein, and 2.5 to 3 μ for the Jersey. A relatively small percentage of the globules was found with a diameter less than 1 μ . The mean diameter calculated from the frequency of globules for the Jerseys was found to be 3.54 μ , for the Holsteins 2.59 μ , for the Ayrshires 2.34 μ . The average diameter calculated from the average volume was found to be 4.01 μ for the Jersey, 3.12 μ for the Holstein, and 3.27 μ for the Ayrshire.

DISCUSSION OF PREVIOUS WORK

The results of the previous investigations are in general agreement concerning the effects of three factors, namely: breed of cattle, stage of lactation and fractional milking on the size of the fat globules in milk. Breeds like the Jersey and Guernsey produce, on the average, fat globules that are distinctly larger than those produced by the Ayrshire and Holstein. This difference is not due to a much higher maximum in the size of the globules from the first two breeds, but to a higher percentage of the large globules in proportion to the small ones.

The advance in lactation has been shown to be one of the most important factors affecting the size of the fat globules in normal milk. Most investigators have not made a study of the globules throughout the lactation period, but their results from portions of lactation periods are in agreement, in this relation, with the longer studies. The most detailed data bearing on this point were obtained by Gutzeit. He concluded that there was a slight increase in the size of the fat globules during the first one to three weeks with a gradual decrease thereafter. This early increase in size was not reported by other workers.

There is some disagreement concerning the way in which other factors affect the size of the fat globules. Collier reported a distinct decrease in the size of the globules from the first to the second lactation, with a slight decrease during successive lactations. It should be remembered that these results were apparently obtained by the examination of morning milk only. Babcock has shown that the fat globules of morning and evening milk are not uniform in size and number unless the period between milkings is uniform. It is doubtful whether such uniformity would be maintained during three years. Irregular milking hours may have caused some of the change which Collier has concluded was due to successive lactations. Woll found a similar result when comparing two- to five-year-old Shorthorn animals with those over eight years. This was due mostly to two heifers in the

first group whose fat globules were exceptionally large. The results secured with the Jerseys did not bear out this fact as four- to seven-year-old animals produced the largest relative sized globules. This latter result could easily be due to individual differences, and Woll concluded that there could be but a slight decrease in size of the fat globules during successive lactation periods.

Considerable effort has been made to determine the effect of feed on the size of fat globules, with a lack of uniformity in the results. Woll concluded that succulent feeds tended to decrease the size of the globules. His conclusions were based on results from two cows and a small difference in the results. Leufven found no significant effect from feeding different kinds of oil cakes. Cooper, Nuttall, and Freak could not determine any effect by changing from high- to low-protein rations.

Gutzeit and Weigmann have made the most extensive studies on the effect of feed, being concerned mostly with pasture versus stable feeding. The former found a distinct increase in the size of the globules when turning cattle to pasture, or when returning them to the barn. After each increase there was a gradual decrease down to, or even below, the average size existing before the change was made. The increase in size was greater, and the period of gradual decrease longer, in the case of turning to pasture. This is due, according to Gutzeit, to the change of feed being more abrupt upon turning cattle to pasture than when bringing them into the stable. Weigmann found an increase in the size of the globules upon stabling cattle and stall feeding in the case of seven breeds, but a decrease on the part of another. These seven breeds tended to show some decrease while stall fed, without any increase when turned to pasture. The reverse was the case with the other breed, which showed an increase when going to pasture. Weigmann believed this latter case to be due to feeding roughage, while the seven breeds were fed varying amounts of oil cake when stabled. The amount of increase in the globule size upon stabling the animals was closely associated with the amount of oil cake being fed. This alleged effect of oil cake is not in agreement with the findings of Leufven.

It is difficult to account for the difference in these findings of Gutzeit and Weigmann. The methods used in determining the average size were unlike in the two cases, but according to Weigmann they gave very comparable results when applied to the same sample. One of the main sources of variance seems to have been located in differences in the time of sampling as well as in the number of samples which

were studied. Gutzeit examined a daily sample each week throughout a complete lactation period. Weigmann examined eight samples per animal during the year. These were taken at irregular intervals, and included parts of two lactation periods in most cases. The period between the taking of the last sample while the cattle were stabled and the first one after turning to pasture is of long duration, except in the case of the "Oberschwäbisches Fleckvieh." This is the breed which showed a different effect from the other seven. The length of time from turning to pasture to taking of the next sample is not indicated. It is possible that this period was of sufficient length to permit an increase in globule size with the following decrease before the sample was taken. This may account for the difference in results obtained by these investigators.

There is a noticeable difference in the frequency distribution of the fat globules as reported by a number of investigators. Lamson, Jones, and Cooper, Nuttall and Freak (tables 1, 4 and 6 respectively) report a much higher percentage of small globules than do Weigmann, and Turner and Haskell (tables 7 and 8). The class interval is not the same in all cases, so that allowance must be made for this when making comparisons. Lamson found from 9.04 percent of the globules in the case of the Jersey to 31.24 percent for the Holstein having an average diameter of 1.27 μ . He does not mention the period of lactation at which the determinations were made so they cannot be directly compared with average results. Jones found in Jersey milk, examined during the fourth month of lactation, that from 11.7 percent to 30.5 percent of the globules were less than 1.40 μ in diameter. An Ayrshire cow during the third month of lactation was found to have 40 percent of the globules less than 1.40 μ , while two grade Ayrshires late in lactation showed 55.7 and 57.4 percent in this class. Cooper, Nuttall, and Freak report an average of 29.71 percent for Guernseys to 41.19 percent for Shorthorns, which average 1 μ in diameter. In contrast to these results Weigmann found as low as 2.31 percent and not more than 13.29 percent of the globules, as an average for the lactation period, from zero to 1 μ in diameter. There were from 8.08 to 27.54 percent of the globules from 1 to 2 μ in diameter. Not more than 36 percent of the globules were found in these combined classes. Turner and Haskell used a class interval of 0.5 μ . The totals of the first three classes, which would include all globules less than 1.75 μ in diameter, are 6.78 percent for the Jersey, 23.34 percent for the Holstein and 33.55 percent for the Ayrshire. These results are hardly equivalent to the percentage Jones

found with a diameter less than 1.4μ , and Cooper, Nuttall, and Freak report with an average diameter of 1μ .

Gutzeit did not measure the globules in such a manner as to have a frequency distribution. He does report the percentage of the globules greater than 2.5μ in diameter, as well as those greater than 5μ . On an average the Angler milk contained 32.6 percent, the Holländer (Schwarzbunte, Holsteiner as reported by Weigmann) milk 25.2 percent and the Breitenburger milk only 24.1 percent of the globules with a diameter greater than 2.5μ . This left 67.4, 74.8, and 75.9 percent of the globules, in the milk of these respective breeds, with a diameter less than 2.5μ . These figures are higher than those obtained by Weigmann in his first three classes. Accompanying this difference Weigmann found a much higher average volume of the fat globules for each of these breeds than did Gutzeit, as is shown by a comparison of tables 5 and 7. He recognized this difference, but, as previously pointed out, he concluded that it was not due to the method of computation. He believes, therefore, that there has been a change in the average fat globule size as produced by these breeds during the past 30 years, which may be due in part to improved breeding and in part to better methods of feeding. It does not seem to the writer that either of these factors would materially affect the fat globule volume without producing some change in the percentage fat content and no such change took place.

Closely related to the above comparison are the results obtained by Weigmann for Shorthorn cattle and those obtained by Cooper, Nuttall, and Freak (tables 6 and 5 respectively). The former found 58.65 percent of the globules under 3μ diameter, while the latter found 87.08 percent of this size. Similarly, in Jersey milk, Cooper, Nuttall, and Freak found 73.6 percent of the globules with a diameter of 3μ or less, while Turner and Haskell report but 49.68 percent in this class. This difference is due mainly to the number of globules under 1μ in diameter. These variations observed in the results secured by recent workers is further evidence indicating that there has not been a change which can safely be ascribed to improved breeding or feeding. Rather do they imply that they may be ascribed to differences in the methods used in sampling or measurement. If faulty sampling was the cause it would seem to involve a procedure which would break some of the large globules into smaller ones, thus accounting for the high percentage of the latter obtained by one group of investigators. If the difficulty were one of mensuration it must have been due to lack of proper magnification or focusing, so that the very minute globules were not observed.

These conjectures will be discussed later in relation to the methods used in the present investigation.

PRESENT INVESTIGATION

The object of the present investigation was to study the manner in which the fat-globule size in cow's milk is inherited. Various environmental and physiological factors affecting the fat-globule size were to be observed with the cows maintained under as nearly uniform conditions as possible.

PLAN OF PROCEDURE

Method of measuring.—Fat globules are present in normal milk as very small spheres. When observed under the microscope they appear opaque with a dark boundary, which with proper focusing appears as a thin line. The diameter of these spheres can readily be measured. It should be remembered, however, that the volume of the fat globules does not vary as do their diameters but as the cubes of their diameters. Their volume is determined by the formula $\frac{4}{3}\pi r^3$ or $0.5236d^3$.

When whole milk is mounted as a thin film of 50 to 100 microns in depth on an ordinary microscope slide or in a capillary tube and is examined under the microscope the individual fat globules can not be distinguished. If, however, the milk is diluted from 50 to 100 times its volume with water the globules are so spread that each can be observed, provided they have not coalesced. In a milk of high fat content it is necessary to use the higher dilution in order that individual globules may be observed with certainty. As a result, all dilutions in this study have been made to 100 times the original volume.

When the milk is diluted with water and examined under the microscope the fat globules exhibit a Brownian movement to such an extent that it is difficult to measure them with accuracy. Glycerin was tried as a diluting medium and was found to be too dense to permit the fat globules to rise. However when equal parts of glycerin and water were used the globules soon rose to the surface and at the same time the Brownian effect was obviated. The question arose, however, whether the glycerin might affect the fat sufficiently to alter the normal globule size. Consequently, comparison was made of samples of Guernsey and Holstein milks diluted with water and with a water and glycerin mixture. The water and glycerin mixture used for the former was in the ratio of three to one while for the latter equal parts were

employed. Each dilution was mounted in a capillary tube and in a hemacytometer chamber and the globules measured by direct microscope, using a magnification of 1,746 \times . The globules were grouped in classes having a range of one micron and the class center as 1, 2, 3, etc. The results of these measurements are shown in table 9. The percentage frequency distribution, the volume of fat represented by the globules in each class, the total volume of fat in all classes and the mean fat-globule volume have been computed for each sample. The mean fat-globule volumes obtained by using the capillary tube and hemacytometer, when the samples were diluted with the water and

TABLE 9.—DIRECT MICROSCOPIC MEASUREMENTS OF FAT GLOBULES IN GUERNSEY AND HOLSTEIN MILKS DILUTED TO 100 TIMES THEIR VOLUMES WITH WATER AS COMPARED TO THE SAME DILUTION IN WATER AND GLYCERIN, AND THE USE OF CAPILLARY TUBES AS COMPARED TO THE HEMACYTOMER CHAMBER. MAGNIFICATION 1,746 \times

Guernsey Milk												
Diameter	Dilution with water						Dilution with water and glycerin					
	Capillary tube			Hemacytometer			Capillary tube			Hemacytometer		
	Frequency	Frequency	Volume	Frequency	Frequency	Volume	Frequency	Frequency	Volume	Frequency	Frequency	Volume
μ	%			%			%			%		
1	82	16.1	42.6	47	20.7	24.4	54	14.3	28.1	25	13.4	13.
2	119	23.4	498.6	61	26.9	255.6	92	24.4	385.5	46	24.6	192.7
3	80	15.7	1,131.2	42	18.5	593.9	81	21.5	1,145.3	44	23.5	622.2
4	96	18.9	3,217.	39	17.2	1,306.9	70	18.5	2,345.7	25	13.4	837.8
5	62	12.2	4,057.9	21	9.3	1,374.4	41	10.9	2,683.5	24	12.8	1,570.8
6	40	7.9	4,524.	14	6.2	1,583.4	26	6.9	2,940.6	20	10.7	2,262.
7	22	4.3	3,951.	1	0.4	179.6	8	2.1	1,436.7	1	0.5	179.6
8	8	1.5	2,144.6	1	0.4	268.1	4	1.1	1,072.3	2	1.1	536.2
9				0			0					
10				0			1	0.3	523.6			
11				1	0.4	696.9						
Total	509	100.	19,566.9	227	100.	6,283.2	377	100.	12,561.3	187	100.	6,214.3
Mean volume μ^3			38.4			27.7			33.3			33.2

Holstein Milk												
Diameter	Dilution with water						Dilution with water and glycerin					
	Capillary tube			Hemacytometer			Capillary tube			Hemacytometer		
	Frequency	Frequency	Volume	Frequency	Frequency	Volume	Frequency	Frequency	Volume	Frequency	Frequency	Volume
μ	%			%			%			%		
1	109	19.	56.7	23	21.9	12.	106	25.4	55.1	66	32.4	34.3
2	165	28.8	691.3	37	35.2	155.	125	29.9	523.8	62	30.4	259.8
3	129	22.5	1,824.1	18	17.1	254.5.	92	22.	1,300.9	27	13.2	381.8
4	91	15.9	3,049.4	17	16.2	569.7	50	12.	1,675.5	26	12.7	871.3
5	50	8.7	3,272.5	7	6.7	458.1	33	7.9	2,159.9	15	7.4	981.8
6	24	4.2	2,714.4	3	2.9	339.3	8	1.9	904.8	6	2.9	678.6
7	4	0.7	718.4				3	0.7	538.8	1	0.5	179.6
8	1	0.2	268.1				1	0.2	268.1	1	0.5	268.1
Total	573	100.	12,594.9	105	100.	1,788.6	418	100.	7,426.9	204	100.	3,655.3
Mean volume μ^3			22.			17.			17.8			17.9

glycerin mixture, checked more closely than might be expected, being 33.3 as compared to $33.2 \mu^3$ for the Guernsey, and 17.8 as compared to $17.9 \mu^3$ for the Holstein. The measurements made from the water dilutions showed more variation. The mean volumes for the Guernsey were $38.4 \mu^3$ with the capillary tube and $27.7 \mu^3$ with the hemacytometer. The corresponding volumes for the Holstein were $22 \mu^3$ and $17 \mu^3$. The agreement of the results obtained by using the water and glycerin mixture was apparently due to the fact that the globules remained relatively still and were much more easily measured. It was difficult in the water dilutions to determine whether small globules were being omitted or remeasured. The dilution made with one part glycerin to one part water reduced the Brownian movement more than did the dilution made with one part glycerin to three parts water. The former did not appear to interfere with the globules rising to the surface nor did the glycerin appear to affect the size of the fat globules. As a result, it was decided to use these proportions of glycerin and water during further work and this mixture will be referred to as a glycerin dilution.

The measurement of the fat globules by direct microscopic examination proved to be a very tedious task because it was necessary to keep

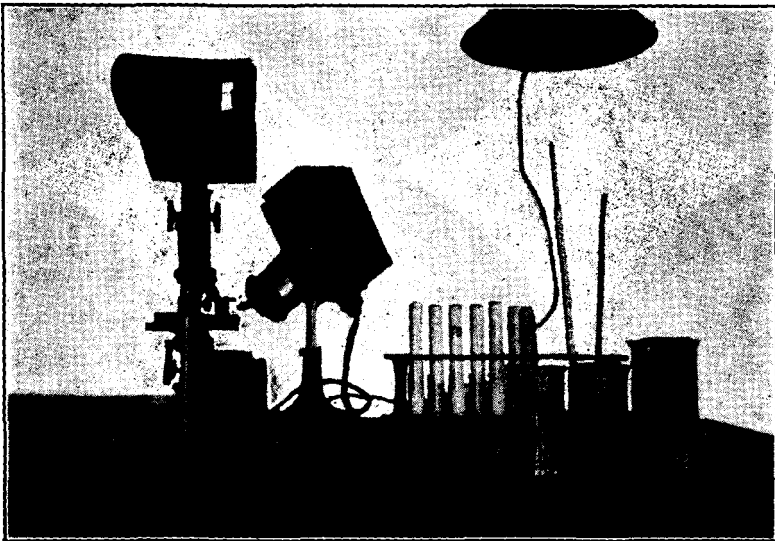


FIGURE 1.—Euscope and microscope used to make fat-globule measurements.

changing the focus continually to prevent missing the very small globules. The use of the euscope (Bausch and Lomb) in connection with the microscope gave promise of making this task less difficult (figure 1).

With this equipment the fat globules are projected on the white opaque screen where they appear greatly enlarged.

In order to make measurements with the euscope, a stage micrometer scale was projected on a white cardboard in front of the screen. A square field measuring 100 μ on a side was laid off on this cardboard. It was ruled one way, with each space equivalent to two microns. In making this scale the microscope was equipped with an oil immersion objective, a 10 \times ocular, and an aplanatic condenser. Thus the microscopic magnification was approximately 970 \times but the globules appeared more enlarged when projected on the screen.

The gelatin dilution as used by Rahn and Weigmann was compared to the glycerin dilution. Direct microscopic measurements of globules in the former were made by use of an ocular micrometer and a magnification of 1,746 \times , while globules in the latter were measured with the euscope (table 10). It was planned to measure globules in

TABLE 10.—MEASUREMENTS OF FAT GLOBULES IN MILK DILUTED TO 100 TIMES ITS VOLUME WITH A 1½ PERCENT GELATIN SOLUTION AND MEASURED BY DIRECT MICROSCOPE (MAGNIFICATION 1,746 \times) AS COMPARED TO A SIMILAR GLYCERIN DILUTION AND MEASUREMENTS MADE WITH THE EUSCOPE (MAGNIFICATION 970 \times)

Diameter μ	Gelatin dilution			Glycerin dilution		
	Frequency	Frequency	Volume	Frequency	Frequency	Volume
		%			%	
1	18	4.9	9.4	26	10.8	13.5
2	67	18.1	280.7	45	18.8	188.5
3	86	23.2	1,216.	57	23.7	806.
4	65	17.6	2,178.1	31	12.9	1,038.8
5	62	16.8	4,057.9	36	15.	2,356.2
6	32	8.6	3,619.2	23	9.6	2,601.3
7	20	5.4	3,591.8	15	6.3	2,693.9
8	15	4.	4,021.2	5	2.1	1,340.4
9	3	0.8	1,145.1	1	0.4	381.7
10	1	0.3	523.6	1	0.4	523.6
11	1	0.3	696.9			
Total	370		21,339.9	240		11,943.9
Mean volume μ^3			57.7			49.8

the gelatin dilution by use of the euscope, but the heat from the lamp melted the gelatin and permitted Brownian movement. Measurements of the gelatin dilution gave a mean volume of 57.7 μ^3 as compared to 49.8 μ^3 with the glycerin dilution. The main difference in the percentage frequency distribution is found in the 1 μ class, which is compensated for in the 4 and 5 μ classes. The very small globules were more readily observed by use of the euscope, which would indicate that they may have been missed in the direct microscopic measurements. If

the percentage of globules in the $1\ \mu$ class of the gelatin dilution should be increased from 4.9 to 10.6 percent the average globule volume would be reduced to approximately $54\ \mu^3$. This would leave a difference of about 7.5 percent which may lie within the range of experimental error.

The use of the regular hemacytometer or blood counting chamber was found to be a very satisfactory method of mounting the diluted milk samples. Instead of the ordinary cover glass it was necessary to use a special cover glass having a thickness of 0.18 mm. and an inside diameter of 12 mm. The cover glass was put in place and the chamber filled by capillary attraction to prevent crushing the globules. After filling the chamber it was necessary to let it stand in a level position for several minutes to permit the globules to rise.

In table 11 are given the results of six different methods of measuring the globules in a sample of milk. The six preparations, lettered from A-F, were made from dilutions with glycerin and water and measured as follows:

A. Three hemacytometer chambers were filled from three different dilutions of fresh milk. Each was allowed to stand from 15 to 20 minutes and then measured by direct microscopic examination with a magnification of $970\times$.

B. The diluted fresh milk was mounted in a vaseline ring on an ordinary slide as described by Turner and Haskell (1929). This was allowed to stand for 30 minutes and was measured by use of the euscope.

C. A piece of glass tubing drawn out to have an inside diameter of approximately 150 microns was filled by capillary attraction with the diluted fresh milk. This tube was mounted on a slide and allowed to stand 30 minutes, after which measurements were made with the euscope.

D. Three hemacytometer chambers were filled from three separate dilutions of fresh milk and let stand from 10 to 20 minutes. They were then measured by use of the euscope.

E. The three slides measured in "D" were allowed to stand for one hour from the time of filling and were again measured by use of the euscope.

F. The fresh milk was preserved with formaldehyde and left standing at room temperature for nine days. Three dilutions were then made from this sample and a hemacytometer chamber filled from each.

TABLE 11.—COMPARISON OF MEASUREMENTS MADE BY DIRECT MICROSCOPE AND WITH THE EUSCOPE; BY MOUNTING IN THE HEMACYTOMETER CHAMBER, CAPILLARY TUBES, AND A VASELINE RING ON A SLIDE; BY LETTING THE SLIDES STAND FROM TEN MINUTES TO ONE HOUR; AND THE SAME SAMPLE AFTER STANDING AT ROOM TEMPERATURE FOR NINE DAYS. ALL MAGNIFICATIONS 970 X

Diameter	A*			B*			C*			D*			E*			F*		
	Frequency	Frequency	Volume	Frequency	Frequency	Volume	Frequency	Frequency	Volume	Frequency	Frequency	Volume	Frequency	Frequency	Volume	Frequency	Frequency	Volume
		%			%			%			%			%			%	
1	189	26.7	98.3	75	33.8	39.	134	36.8	69.7	193	30.1	100.4	256	38.2	133.1	168	28.9	87.4
2	213	30.	892.5	62	27.9	259.8	92	25.3	385.5	210	32.8	879.9	187	27.8	783.5	200	34.4	838.
3	133	18.8	1,880.6	34	15.3	480.8	61	16.8	862.5	120	18.7	1,696.8	114	17.	1,612.	100	17.2	1,414.
4	73	10.3	2,446.2	29	13.1	971.8	38	10.4	1,273.4	61	9.5	2,044.1	54	8.	1,809.5	63	10.9	2,111.1
5	56	7.9	3,665.2	15	6.8	981.7	21	5.8	1,374.5	31	4.8	2,028.9	33	4.9	2,159.9	17	2.9	1,112.7
6	32	4.5	3,619.2	4	1.8	452.4	14	3.8	1,583.4	19	3.	2,148.9	20	3.	2,262.	22	3.8	2,488.2
7	6	0.9	1,077.5	3	1.3	538.8	4	1.1	718.4	6	0.9	1,077.5	6	0.9	1,077.5	6	1.	1,077.5
8	6	0.9	1,608.5							1	0.2	268.1	1	0.1	268.1	5	0.9	1,340.4
9													1	0.1	381.7			
Total	708		15,288.	222		3,724.3	364		6,267.4	641		10,244.6	672		10,487.3	581		10,469.3
Mean volume			21.6			16.8			17.2			16.			15.6			18.

* A-E, fresh milk; F, same milk preserved nine days; A, measured by direct microscope; B-F, with euscope; A, D, E and F, mounted in hemacytometer chamber; B, in vaseline ring; and, C in capillary tube; A-D and F, stood 10-30 minutes before measuring; E, stood one hour.

They were left standing from 15 to 20 minutes and then measured by use of the euscope.

Again the results showed the highest average volume when the measurements were made by direct microscope. This method gave an average volume of $21.6 \mu^3$ as compared to a variation from 15.6 to $18 \mu^3$ obtained by the five other methods. The lowest average volume was obtained by letting the slides stand for one hour. This same method showed the highest percentage of small globules, namely 38.2 percent. The dilution in the hemacytometer chamber manifested some signs of evaporation after standing for one hour which may have caused a slight difference in results. The globules were much easier to measure in the hemacytometer chamber or on the common slide than in the capillary tube due to the greater concentration of globules at the upper curvature of the tube.

A comparison of the measurements obtained from each of the three hemacytometer chambers filled from different dilutions in D, E and F, is set forth in table 12. The fat globules appearing in 10 fields, 100 microns square, were measured from each slide. The total number

TABLE 12—A COMPARISON OF THE FREQUENCY DISTRIBUTION OBTAINED WITH EACH OF THREE SLIDES WHEN FILLED WITH DIFFERENT DILUTIONS OF MILK DERIVED FROM THE SAME SAMPLE

Diameter μ	D*				E*				F*			
	I	II	III	Total	I	II	III	Total	I	II	III	Total
1	61	69	63	193	91	78	87	256	52	62	54	168
2	74	68	68	210	71	49	67	187	68	70	62	200
3	43	38	39	120	37	43	34	114	41	30	29	100
4	21	19	21	61	25	15	14	54	17	25	21	63
5	11	8	12	31	11	12	10	33	5	6	6	17
6	3	6	10	19	5	6	9	20	8	6	8	22
7	2	2	2	6	3	2	1	6	2	1	3	6
8	1			1	1			1	1	1	3	5
9						1		1				
Total	216	210	215	641	244	206	222	672	194	201	186	581

* D and E, fresh milk; F, preserved nine days; slides D and F stood 10-20 minutes before measuring, while E stood one hour.

of globules measured from three slides of one dilution was from 581 to 672. This is approximately the number which Van Dam and Sierks (1922) concluded was large enough to afford reliable results. The number of globules measured per slide varies from 210 to 216 in D, from 206 to 244 in E, and from 186 to 201 in F. These numbers were considered to be relatively close since they must necessarily represent a very small quantity of milk. During the course of the investiga-

tion there have been some cases where a much wider variation was observed between the three slides derived from one sample. This was most noticeable when many small globules were present. As a general rule, however, the variation between the three slides from one sample has not been large.

From the above results the following conclusions may be drawn:

1. Glycerin may be used with water in diluting a sample of milk without affecting the size and number of fat globules. When used in the proportion of one part water to one part glycerin, the latter does not prevent the fat globules from rising but practically eliminates the Brownian movement.

2. The hemacytometer may be used for mounting the diluted milk where it is not allowed to stand more than one hour before measurements are made. Measurement of the globules in 10 fields on three slides derived from one sample proved to be sufficient.

3. Milk when properly preserved and carefully handled may be allowed to stand for some days before the fat globules are measured.

4. The euscope may be used with the microscope to measure fat globules. By this method it is easier to detect the small fat globules than when making direct microscopic measurements. This larger number of globules observed when the euscope is used will give a slightly lower mean fat-globule volume than is obtained with direct microscopic measurements.

With the results of these preliminary trials as a basis the following method was adopted for measuring the fat globules throughout this investigation. A sample of milk was thoroughly mixed, but care was taken not to agitate it enough to break up the fat globules. One cc. of this mixed milk was then added to a test tube containing nine cc. of water, making a dilution of one in ten. This was thoroughly mixed and from it was taken one cc., which was added to another test tube containing nine cc. of glycerin and water in equal parts. This gave a dilution of one in 100, with approximately 45 parts of glycerin to 55 parts of water. The two steps were taken in making the dilution so that a smaller amount of glycerin would be required. Three such dilutions were made from each sample of milk.

Three hemacytometer chambers were prepared by washing with water, drying, washing with alcohol and again drying. The last step was found necessary after a few trials in which the hemacytometer and cover glass were washed only with water, since this procedure did not remove all the fat residues from preceding samples. Indeed, such

globules as remained attached to the inner walls were broken up into a smear during the process of drying and these were apt to appear in the next sample as a long streak of very minute globules. Washing with alcohol removed these and thus eliminated a possible chance of error from this source. Cover glasses were then placed on the hemacytometers, which were filled by placing one or two drops of the dilution next to the cover glass, so that the chambers filled by capillary attraction. The three hemacytometer chambers were filled from three different dilutions of one sample of milk, being filled at intervals of 10 to 15 minutes. The first was allowed to stand 15 to 20 minutes and then measurements were made in the order of filling.

All measurements were made with the euscope as previously mentioned. The fat globules in 10 square fields on the euscope were measured on each slide. Care was taken to move each slide about so there would be no duplication of fields. During the measurement of each field the microscope was focused up and down to be certain that all small globules were observed. The sizes of the globules were called off to an assistant, who did the recording. In this way measurements could be made on three slides from one sample in approximately 30 to 40 minutes.

Method of sampling.—The measurements of the fat globules in individual milkings were compared with measurements from a composite sample of the same milkings during a 48-hour period (table 13). The number of globules measured from individual milkings of cow No. 657 varied from 269 to 380 with an average of 317, as compared to 341 for the composite sample. The mean volume of the individual milkings varied from 35.8 to 58.2 μ^3 with an average of 43.8, as compared to a mean volume of 53 μ^3 for the composite sample. The percentage frequency distribution for the total of the four milkings and for the composite sample agreed fairly well except for the two large globule groups in the composite sample. The volume from these two groups was sufficient to raise the average volume by approximately 8.5 μ^3 . The results from cow No. 688 check more closely than do those from No. 657, even though there appears to be a greater variation between milkings. This is particularly noticeable in the case of the second afternoon milking, which contained a very large number of globules with a relatively low mean volume. The fact that the total volume of fat agrees very closely with those of the other milkings, as does also the percentage fat content, would indicate that the measurements were reasonably accurate, and that there may be considerable variation in the size of the

TABLE 13.—A COMPARISON OF THE FAT-GLOBULE SIZE DETERMINED FOR EACH OF FOUR MILKINGS AND FOR A COMPOSITE SAMPLE OF THE CORRESPONDING MILKINGS. DILUTIONS MADE WITH WATER AND GLYCERIN AND MEASUREMENTS MADE WITH A EUSCOPE

Cow No. 657

Diameter	P. M.			A. M.			P. M.			A. M.			Total four milkings			Composite sample		
	Frequency	Frequency	Volume	Frequency	Frequency	Volume	Frequency	Frequency	Volume	Frequency	Frequency	Volume	Frequency	Frequency	Volume	Frequency	Frequency	Volume
μ	%			%			%			%			%			%		
1	37	12.7	19.2	59	18.5	30.7	71	18.2	36.9	30	11.2	15.6	197	15.5	102.4	63	18.5	32.8
2	37	12.7	155.	46	14.5	192.7	72	18.4	301.7	52	19.3	217.9	207	16.3	867.3	45	13.2	188.5
3	51	17.5	721.1	76	23.9	1,074.6	76	19.5	1,074.6	77	28.6	1,088.8	280	22.1	3,959.2	71	20.8	1,003.9
4	48	16.4	1,608.5	54	17.	1,809.5	67	17.2	2,245.2	48	17.8	1,608.5	217	17.1	7,271.7	58	17.	1,943.6
5	53	18.1	3,468.9	39	12.3	2,552.5	47	12.	3,076.1	32	11.9	2,094.4	171	13.5	11,191.9	44	12.9	2,879.8
6	35	12.	3,958.5	25	7.9	2,827.5	28	7.2	3,166.8	17	6.3	1,922.7	105	8.3	11,875.5	33	9.7	3,732.3
7	18	6.2	3,232.6	16	5.	2,873.4	21	5.4	3,771.4	9	3.4	1,616.3	64	5.	11,493.8	19	5.5	3,412.2
8	10	3.4	2,680.8	0			5	1.3	1,340.4	4	1.5	1,072.3	19	1.5	5,093.5	3	0.9	804.2
9	3	1.	1,145.1	2	0.6	763.4	3	0.8	1,145.1				8	0.6	3,053.6	3	0.9	1,145.1
10				0									0			0		
11				1	0.3	696.9							1	0.1	696.9	0		
12																0		
13																1	0.3	1,150.3
14																0		
15																1	0.3	1,767.1
Total	292		16,989.7	318		12,821.2	390		16,158.2	269		9,636.5	1,269		55,605.8	341		18,059.8
Mean volume μ^3			58.2			40.3			41.4			35.8			43.8			53.
Babcock test			4.8			4.			5.			3.7						4.2
Babcock test \times	1.1	5.3				4.4			5.5			4.1						4.6
Percent fat by volume			5.7			4.3			5.4			3.2			4.6			6.

TABLE 13.—*Concluded*
Cow No. 688

Diameter	P. M.			A. M.			P. M.			A. M.			Total four milkings			Composite sample		
	Frequency	Frequency	Volume	Frequency	Frequency	Volume	Frequency	Frequency	Volume	Frequency	Frequency	Volume	Frequency	Frequency	Volume	Frequency	Frequency	Volume
μ	%			%			%			%			%			%		
1	95	20.4	49.4	66	16.8	34.3	266	29.7	138.3	80	16.4	41.6	507	22.6	263.6	103	17.6	53.6
2	89	19.2	372.9	77	19.6	322.6	245	27.3	1,026.5	118	24.3	494.4	529	23.6	2,216.5	139	23.8	582.4
3	105	22.6	1,484.7	92	23.4	1,300.9	187	20.9	2,644.2	127	26.1	1,795.8	511	22.8	7,225.5	151	25.8	2,135.1
4	75	16.1	2,513.3	64	16.3	2,144.6	116	13.	3,887.2	88	18.1	2,948.9	343	15.3	11,493.9	102	17.5	3,418.
5	56	12.	3,665.2	48	12.2	3,141.6	53	5.9	3,468.9	43	8.8	2,814.3	200	8.9	13,090.	48	8.2	3,141.6
6	30	6.5	3,393.	28	7.1	3,166.8	21	2.3	2,375.1	21	4.3	2,375.1	100	4.4	11,310.	24	4.1	2,714.4
7	9	1.9	1,616.3	11	2.8	1,975.5	8	0.9	1,436.7	5	1.	897.9	33	1.5	5,926.5	9	1.5	1,616.3
8	5	1.1	1,340.4	3	0.8	804.2				5	1.	1,340.4	13	0.6	3,485.	5	0.9	1,340.4
9	0			4	1.	1,526.8							4	0.2	1,526.8	1	0.2	381.7
10	0												0			1	0.2	523.6
11	0												0			0		
12	0												0			0		
13	1	0.2	1,150.3										1	0.1	1,150.3	0		
14																1	0.2	1,436.8
Total	465		15,585.5	393		14,417.3	896		14,976.9	487		12,708.4	2,241		57,688.1	584		17,343.9
Mean volume μ^3			33.5			36.7			16.7			26.1			25.7			29.7
Babcock test			5.1			4.4			4.7			4.4						4.5
Babcock test $\times 1.1$			5.6			4.8			5.2			4.8						5.
Percent by volume			5.2			4.8			5.			4.2			4.8			5.8

globules of different milkings. The mean volume for the four individual milkings is 25.7 as compared to 29.7 μ^3 for the composite sample.

The task of making measurements of individual samples was too laborious to be undertaken. These results indicated that a 48-hours composite sample was sufficiently accurate and, consequently, that method of sampling was adopted. In this case it was necessary to preserve the samples. This was done by using $\frac{1}{2}$ cc. of formaldehyde per sample. In order to prevent the cream from adhering to the side of the bottle, samples were rotated sufficiently after each milking to make certain that the entire contents were well mixed. This also tended to reduce the likelihood of souring. When samples were so handled they usually arrived at the laboratory in good condition. When in this shape they could be kept in the refrigerator for several days before measuring with no apparent indication of change. If stored in the refrigerator it was necessary to warm them gradually before mixing.

When samples are not thus carefully taken the fat globules have a tendency to coalesce into large clusters. This was particularly noticeable in a few samples which were slightly soured or had become frozen. After some experience it was found to be relatively easy to obtain satisfactory composite samples to keep them for some time without danger of coalescence.

The 48-hour composite samples were taken at four-week intervals throughout the lactation period. The first sample was not taken at a definite time after parturition, but was included with the first regular set of samples drawn after calving, provided the cow had been fresh for at least a week.

Collection of the data.—At the time this investigation was started the Bowlker hybrid herd at the University of Illinois consisted mainly of F_1 , F_2 and some back-cross animals. The original purebred parents were gone and the number of F_1 females was small. Since it was necessary in any event to measure fat globules from unrelated purebred Guernseys and Holsteins, it was thought advisable to defer these measurements and to make them from all available hybrid animals as they freshened. In 1927 the writer assumed a position at the University of Vermont. In order to complete the study on the hybrid animals the composite samples were taken by staff members of the Dairy Department at the University of Illinois and forwarded to the writer. Most of these samples arrived in good condition and measurements made from them did not appear to differ widely from the results previously secured. However the results thus obtained are so indicated. Early in

1928 the Bowlker hybrid herd was dispersed and no further samples could be secured. Thus the number of samples that had been expected would be made available for use in this particular study was greatly curtailed.

During the period 1928-31 data were obtained on purebred Guernseys and Holsteins at the University of Vermont. During 1931-32 data have been collected on Angus-Holstein crossbred animals and two purebred Angus at the University of Wisconsin.

MANAGEMENT OF THE ANIMALS

The animals in the Bowlker hybrid herd were maintained under fairly uniform condition. They did not have access to pasture but were stable-fed during the entire year and turned into dry lots during the summer. The feed consisted of a fairly uniform grain ration, corn silage and alfalfa hay. The most noticeable change in feed occurred in the fall when the silos were filled.

The management of the Guernseys and Holsteins was somewhat varied. Some of the animals were on official test and were barn-fed during the complete lactation period. Two of the cows were under controlled experimental conditions throughout the lactation with the principal change occurring in the spring when fresh-cut lawn clippings were substituted for silage, and the reverse in the fall. Other animals were turned to pasture during the summer. The following brief statement indicates the changes of feed or care in relation to the period of sampling for the Holsteins and Guernseys. The term "period" indicates the four-week period of the lactation during which the samples were taken.

HOLSTEINS

Cow No. 3. Stable-fed on mineral balance trials as described by Ellenberger, Newlander and Jones (1931). Silage replaced with grass clippings $2\frac{1}{2}$ weeks before period seven.

Cow No. 5. Turned to pasture one week before period eight.

Cow No. 10. On feeding trial during period four. Details have been published by the writer (1931). Turned to pasture one week before period six.

Cow No. 11. On feeding trials (see No. 10) during periods five, six and seven. Turned to pasture immediately after period seven.

Cow No. 18. On feeding trials (see No. 10) during periods three four and five. Turned to pasture immediately after period five.

Cow No. 19. Same as No. 3 except that grass clippings replaced silage 2½ weeks before period six.

Cow No. 20. Suffered from udder trouble during period six. Turned to pasture just after period seven.

Cow No. 42. Suffered from foot-rot during period four. Turned to pasture two weeks before period six.

Cow No. 46. Turned to pasture one week before period four.

GUERNSEYS

Cow No. 62. On mineral balance trial during period two, grass clippings being fed. Turned out to pasture the day sampling was started for period three.

Cow No. 63. On feeding trial (see No. 10) during periods four, five and six. Turned out to pasture two weeks previous to period seven.

Cow No. 64. Stable-fed, grass clippings being used in the place of silage during period one. Turned out to pasture the day sampling was started for period two.

Cow No. 66. Same as No. 63.

Cow No. 68. Same feeding trials as No. 63 during periods seven, eight, and nine, and turned out to pasture two weeks previous to period 10.

Cow No. 69. Stable-fed throughout the lactation period, the feeding of silage being discontinued one week before period 10.

Cow No. 71. Suffered from foot-rot during periods four and five. On same feeding trials as No. 63 during periods 8, 9 and 10. Turned out to pasture two weeks before period 11.

Cow No. 74. Turned out to pasture two weeks before period three.

Cow No. 81. Stable-fed throughout the lactation period, the feeding of silage being discontinued three weeks before period 10.

Cow No. 86. Turned out to pasture three weeks before period seven.

Animals turned out to pasture during the summer were fed some grain and during the late summer were fed some hay so the change of feed in the fall was made very gradually.

The Angus-Holstein crossbred animals were maintained without pasture, being stable-fed throughout the year much as were the Bowlker hybrid animals.

Table 14 lists all the animals used in this investigation with their ages, dates of freshening and of subsequent service resulting in conception.

TABLE 14.—A LIST OF ANIMALS INCLUDED IN THE INVESTIGATION WITH THEIR AGES, DATES OF FRESHENING AND DATES OF SUBSEQUENT SERVICE RESULTING IN CONCEPTION

Cow No.	Age yr.	Date of freshening Mo., day, yr.	Subsequent service Mo., day, yr.	Cow No.	Age yr.	Date of freshening Mo., day, yr.	Subsequent service Mo., day yr.
Holsteins				Guernseys			
3	7	12- 6-28	2-21-29	62	7	4-26-29	12-10-29
5	8	11-14-28	1-18-29	63	3	12- 6-29	3-13-30
10	4	1-15-29	3-25-29	64	9	5-16-29
11	3	11-25-28	12-28-28	66	6	12-15-29	3-20-30
18	8	1-20-29	3-16-29	68	4	9-26-29	4-29-30
19	6	12-27-28	3-27-29	69	7	9-15-29	2-26-30
20	4	1- 8-29	4-12-30	71	4	9- 8-29	1- 4-30
42	7	1-26-29	6-26-29	74	2	4-17-30	6- 2-30
46	7	3- 5-29	6-17-29	81	2	9-23-30	12- 1-30
				86	2	1- 2-31	2- 8-31
Bowlker hybrid animals							
F ₁							
657	11	3-26-26	5-29-26	688	9	1- 4-26	5- 9-26
657	12	2-22-27	690	5	3- 1-27
F ₂							
723	6	6- 3-26	739	5	12-17-26
724	7	12-19-26	3-18-27	740	3	6- 3-26	1-26-27
729	5	4-19-26	6- 1-26	741	3	7- 6-26	10- 5-25
729	6	3-12-27	743	2	12-10-26	3-16-27
732	6	1-14-27	742	4	1-13-27
735	4	7- 6-26	805	4	9-18-26	4- 4-27
F ₂ x Holstein back-cross				Miscellaneous			
801	5	9-25-26				
804	4	10- 6-26	50	2	5-11-27
806	3	4-23-27	646	6	1-12-27	5-24-27
807	2	5-29-26	1-17-27	803	5	9-11-26	2-16-27
Angus							
65	2	12-12-31	89	4	1-15-32
Angus-Holstein cross							
F ₁				F ₂			
55A	7	2-26-31	9- 5-31				
62D	4	11-11-31	71B	4	8-19-31	1-18-32
64D	3	7-18-30	72D	2	11-25-31	2- 2-32
69C	4	5- 3-31	12-22-31	76B	3	9-28-31	1- 1-32
69D	3	8-13-31	12- 5-31	78A	4	3-24-31	2-22-32
75A	4	3-16-31	12-11-31	83A	2	4-22-31	8-15-31
77A	4	3-24-31	8- 5-31	92A	2	10- 6-31

PRESENTATION OF RESULTS

The percentage frequency distribution, the total volume of fat and the mean fat-globule volume have been computed for the measurements made at four-week intervals throughout the period of lactation of the individual cows. Due to the great mass of data in hand it was deemed necessary to combine the frequency distributions of corresponding periods into separate groups for the Guernseys, Holsteins, the

Bowlker hybrid F_1 , F_2 and back-cross animals. From these combined frequency distributions have been calculated the percentage frequency distributions and the mean volumes for each group. The mean volume of each period from two to ten, inclusive, has been expressed in percentage of the mean volume of period one. This is done by dividing the mean volume for the first period into each of the succeeding mean volumes. These results for the five groups are given in tables 15 to 19, inclusive.

The mean fat-globule volumes, determined for the individual animals of the above mentioned groups, are given in tables 20 to 24, inclusive. Included in these tables for the Guernsey, Holstein and F_2 groups are the mean and the standard deviation of these individual means for the first 10 periods. Determinations have not been made past the tenth period, nor for the F_1 and back-cross animals, because of the small numbers.

Full lactation periods have not been studied for the Angus nor for the F_1 and F_2 animals of the Angus-Holstein cross. Samples have been taken from the Angus at two-week intervals since the calves were removed from them March 3, 1932, to determine any change that might take place in the fat-globule size as a result of altered conditions. The percentage frequency distributions and the mean fat-globule volumes for each of the Angus cows are given in table 25.

The frequency distributions obtained for more than one of the Angus-Holstein F_1 animals at any one period have been combined. From these have been computed the percentage frequency distribution and the mean volume for each of the first 10 periods during lactation (table 26). Similar results are given for the F_2 animals in table 27. The mean fat-globule volumes for the individuals in these groups are given in tables 28 and 29. As these results are computed from incomplete lactation periods a tendency toward greater variation is manifested than would have occurred had data been available during complete lactations.

Cow No. 742, an F_2 from the Bowlker hybrid herd, was slaughtered after measurements had been made for two periods of the lactation. She was used by Nevens (1928) in a study of the digestive process in dairy cattle, which required a six-day fasting period preceding slaughter. During this period water was offered but she consumed only 34 pounds. She was more seriously affected than any of her mates and was unable to arise to her feet during the last day or

TABLE 15.—GUERNSEYS: COMBINED PERCENTAGE FREQUENCY DISTRIBUTIONS, THE MEAN FAT-GLOBULE VOLUMES AND THE MEAN VOLUMES EXPRESSED IN PERCENT OF THE FIRST

Period	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
μ															
1	26.56	23.36	23.23	21.55	22.64	25.15	25.52	27.81	27.86	29.47	29.78	25.66	23.22	32.70	34.53
2	20.10	23.87	25.90	26.72	28.71	28.99	29.80	30.90	30.79	31.78	32.	31.38	30.22	34.20	30.63
3	20.53	21.28	24.43	24.57	24.78	24.53	25.44	23.38	23.47	23.32	22.43	24.71	25.46	20.73	19.13
4	14.37	15.77	14.32	14.70	13.16	12.49	11.24	10.68	10.93	9.44	10.30	11.51	12.36	7.94	10.42
5	10.19	9.37	7.89	8.39	6.71	6.30	5.53	5.03	4.67	4.08	4.21	4.43	6.25	3.51	3.85
6	5.74	4.37	3.18	3.23	2.94	1.97	1.81	1.46	1.75	1.39	0.91	1.73	1.94	0.77	0.91
7	1.75	1.56	0.85	0.68	0.76	0.40	0.51	0.58	0.45	0.35	0.29	0.41	0.55	0.10	0.48
8	0.38	0.22	0.17	0.13	0.24	0.17	0.10	0.15	0.07	0.10	0.02	0.14			0.05
9	0.28	0.15	0.02	0.03	0.04		0.04		0.01	0.04	0.04			0.05	
10	0.05	0.05					0.01			0.03		0.03			
11								0.01							
12															
13															
14															
15															
16	0.05										0.02				
Mean vol. μ^3 Percent of the first	28.4 100.	24.7 87.	20.3 71.5	20.4 72.	19.1 67.4	16.5 58.2	15.8 55.8	14.8 52.2	14.6 51.3	13.4 47.2	13.	14.9	16.4	10.7	12.2

TABLE 16.—HOLSTEINS: COMBINED PERCENTAGE FREQUENCY DISTRIBUTIONS, THE MEAN FAT-GLOBULE VOLUMES AND THE MEAN VOLUMES EXPRESSED IN PERCENT OF THE FIRST

Period	1	2	3	4	5	6	7	8	9	10	11	12	13
μ													
1	27.90	32.20	38.20	40.66	41.62	45.98	48.11	46.39	50.06	48.93	48.43	46.96	53.12
2	25.25	25.66	29.14	30.45	30.02	29.22	29.90	29.77	29.93	30.97	31.76	32.46	30.22
3	18.27	18.74	17.96	17.56	17.76	16.28	14.46	16.85	14.28	14.57	13.93	14.36	11.46
4	13.37	12.47	8.69	7.30	7.18	5.74	5.34	4.91	4.15	4.22	4.32	4.35	3.67
5	9.80	7.21	4.39	3.05	2.72	2.26	1.78	1.74	1.24	1.10	1.32	1.26	1.31
6	4.26	2.81	1.31	0.86	0.68	0.43	0.38	0.28	0.29	0.18	0.22	0.48	0.22
7	0.68	0.77	0.29	0.12	0.02	0.07	0.03	0.04	0.05	0.03	0.02	0.09	
8	0.38	0.11						0.02				0.04	
9	0.09												
10		0.03	0.02			0.02							
Mean vol. μ^3	22.1	17.8	11.9	9.6	9.	7.9	7.	7.1	6.2	6.	6.2	6.7	5.5
Percent of the first	100.	80.6	53.8	43.5	40.6	35.7	31.7	32.1	27.9	27.2			

TABLE 17.—BOWLKER HYBRID F₁ ANIMALS: COMBINED FREQUENCY DISTRIBUTIONS AND MEAN FAT-GLOBULE VOLUMES

Period	1	2	3	4	5	6	7*	8*	9*
μ									
1	28.46	26.67	32.52	32.58	40.12	44.26	38.67	38.19	38.57
2	20.14	21.98	29.35	26.72	28.97	29.70	30.21	31.94	32.01
3	10.42	16.58	18.92	19.59	17.16	14.29	16.79	16.22	18.39
4	11.81	13.78	10.08	12.12	8.24	6.77	8.05	8.33	6.70
5	13.19	9.64	5.36	5.65	3.56	3.12	4.06	3.46	2.81
6	8.33	5.59	2.53	2.39	1.48	1.41	1.52	1.42	1.02
7	5.56	3.78	1.12	0.52	0.36	0.33	0.44	0.33	0.35
8	1.39	1.08	0.06	0.26	0.11	0.03	0.15	0.07	0.07
9	0.35	0.54	0.06	0.09			0.07	0.04	0.04
10		0.09		0.04		0.03	0.04		0.04
11									
12						0.03			
13		0.09				0.03			
14				0.04					
15	0.35	0.09							
16									
17		0.09							
Mean volume μ^3	45.6	37.8	16.2	17.3	11.5	10.9	12.6	11.4	10.5

* Results from samples that had been shipped are included in these averages.

TABLE 18.—BOWLKER HYBRID F₂ ANIMALS: COMBINED PERCENTAGE FREQUENCY DISTRIBUTIONS, THE MEAN FAT-GLOBULE VOLUMES AND THE MEAN VOLUMES EXPRESSED IN PERCENT OF THE FIRST

Period	1	2	3	4	5	6	7	8	9	10
μ										
1	27.39	34.87	33.18	36.95	41.06	43.83	44.70	46.88	49.74	44.98
2	23.33	27.01	30.77	32.42	32.26	32.36	33.82	33.27	32.13	31.45
3	17.95	20.26	20.22	18.75	17.06	16.25	14.63	13.65	12.44	15.82
4	13.74	9.40	9.33	7.67	6.62	5.44	4.96	4.52	4.07	5.74
5	9.39	5.54	4.96	3.06	2.17	1.63	1.42	1.31	1.18	1.56
6	5.04	1.88	1.16	0.84	0.66	0.42	0.42	0.33	0.34	0.41
7	2.31	0.72	0.27	0.30	0.12	0.03	0.04	0.03	0.04	0.04
8	0.51	0.17	0.03	0.01	0.03	0.04		0.01	0.01	
9	0.19	0.11	0.08				0.01		0.02	
10	0.05	0.04			0.01				0.01	
11	0.05								0.01	
12									0.01	
13										
14					0.01					
15	0.05									
Mean volume μ^3	27.7	15.5	12.9	10.3	8.9	7.4	6.9	6.4	6.3	7.3
Percent of the first	100.	55.8	46.4	37.1	32.1	26.8	24.9	23.1	22.5	26.2

TABLE 19.—BOWLKER HYBRID F_2 AND HOLSTEIN BACK-CROSS ANIMALS: COMBINED PERCENTAGE FREQUENCY DISTRIBUTIONS, THE MEAN FAT-GLOBULE VOLUME AND THE MEAN VOLUMES EXPRESSED IN PERCENT OF THE FIRST

Period	1	2	3	4	5	6	7	8	9	10
μ										
1	36.31	44.34	46.39	45.55	47.26	47.77	49.57	53.34	55.45	59.37
2	29.40	27.12	27.69	31.75	31.46	31.37	29.24	27.93	28.95	26.91
3	17.02	15.69	14.83	14.01	14.10	14.45	13.20	12.03	10.59	9.31
4	8.51	7.36	7.06	5.74	5.10	4.52	5.12	4.60	3.52	2.88
5	4.58	3.38	2.77	2.04	1.66	1.40	2.12	1.57	1.03	1.24
6	2.51	1.57	0.91	0.54	0.34	0.41	0.61	0.47	0.30	0.20
7	1.16	0.46	0.25	0.25	0.08	0.08	0.11	0.06	0.08	0.03
8	0.26	0.04	0.10	0.08			0.03		0.05	0.03
9	0.06	0.04							0.03	
10	0.13			0.04						0.03
11	0.06									
Mean volume μ^2	16.7	11.1	9.4	8.3	6.9	6.7	7.4	6.4	5.6	5.
Percent of the first	100.	66.7	56.6	49.8	41.3	39.9	44.5	38.2	33.5	30.2

two during the fasting period. An opportunity was thus afforded to study the effect of fasting upon the fat-globule size.

During the six days preceding fasting two three-day composite samples were taken, but during the six-day fasting period individual milking samples were drawn. The percentage frequency distribution of measurements from these samples, together with the mean fat-globule volume, the Babcock test, and the percentage of fat as determined by the measured volume, are given in table 30, which also contains corresponding data for the first two periods of the lactation.

DISCUSSION

In considering the combined results secured with the several groups it should be borne in mind that many of the cows completed their lactations after the ninth or tenth period. The results attained during the periods after the tenth manifested greater variation than was observed in the earlier periods, because a lesser number of cows contributed data; hence the results are not strictly comparable to those which were secured in the earlier periods. Because of this fact, and because the last few lots of samples derived from the Bowlker hybrid herd were shipped from Illinois to Vermont, only the results for the first 10 periods for these animals have been combined. The mean volumes are given for complete lactation periods of the individuals. The number of animals used in computing the combined frequency distribution and the mean volume for 10 periods in any one group are variable as a result of certain

TABLE 20.—GUERNSEYS: MEAN FAT-GLOBULE VOLUMES, IN CUBIC MICRONS, FOR INDIVIDUAL COWS, THE MEANS AND THE STANDARD DEVIATIONS OF THESE MEANS

Period	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Cow No.																
62		25.5	26.9		14.1	12.9	13.	13.7	10.4	10.9	15.6	14.9	12.9	9.4	9.1	
63	22.6	21.3	19.8	15.7	16.7	19.7	16.6	16.2	15.8	14.1						
64	57.5	43.6		19.4	18.9	18.3	16.1	17.8	17.1	17.6	15.	21.	21.6			
66	32.6	22.	23.8	16.3	21.7	15.8	22.2	20.1	23.6							
68	26.0	26.4	15.8	21.6	22.7	15.7	15.5	18.7	14.8	14.8	17.1	15.9	16.5	12.9	17.	17.3
69	26.5	33.8	30.2	36.4	32.	32.	28.3	24.	26.9	30.5	26.6*	33.3				
71		17.5	10.6	33.2†	15.5†	8.	7.5	8.2	8.4	9.2	8.7	10.7				
74		29.5	23.9	20.8	19.	16.	14.7	12.	10.7	11.2						
81	20.	16.4	16.1	18.3	14.2	13.4	13.4	14.4	14.8	11.1	12.7					
86		27.2	26.8	22.9	21.8	27.1	22.6	15.3								
Mean	30.9	26.3	21.5	21.4	20.1	17.9	17.	16.	15.8	14.9						
σ	13.7	8.1	6.5	6.6	5.4	7.	5.9	4.5	6.2	6.9						

* No. 69 aborted July 10, 1930, this being two days before the sample was taken for period 11.

† Periods four and five for No. 71 have been omitted from computation due to an abnormal fat-globule size caused by a severe case of foot-rot.

TABLE 21.—HOLSTEINS: MEAN FAT-GLOBULE VOLUMES, IN CUBIC MICRONS, FOR INDIVIDUAL COWS, THE MEANS AND THE STANDARD DEVIATIONS OF THESE MEANS

Period	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Cow No.														
3	22.8	16.3	11.8	8.9	8.1	8.4	7.	7.9	6.3	5.7	5.5			
5		18.8	12.6		7.5	7.8	8.	7.	5.4					
10		13.7	7.1	6.8	6.3	4.9	5.8		4.8	5.5				
11	21.9	20.5	13.7	7.6	8.7	9.	4.7	8.3		8.3				
18	31.6	14.5	11.9	12.	10.5	9.1		6.7	7.7					
19	19.4	10.6	14.5	6.8	7.7	6.7	6.6	4.8	5.6	5.				
20		28.5	13.3	8.2	8.8	17.5*	9.1		5.8	6.4	6.9	6.2	4.7	5.6
42	21.6	19.7	13.4	17.3†	13.2	11.4		7.6	7.2	5.5	5.9	7.4	6.3	
46	18.6	22.8	12.4	13.8	10.9		8.6	7.8	6.7	6.	6.4			
Mean	22.7	18.4	12.3	10.2	9.1	8.2	7.1	7.2	6.2	6.1				
σ	4.5	5.2	2.1	3.8	2.1	2.	1.6	1.	0.9	0.9				

* The sixth period of No. 20 is not included in the computations due to an abnormal size of the fat globules caused by a teat injury.

† This slight increase may have been due to a case of foot-rot, but was not considered serious enough to omit.

TABLE 22.—BOWLKER HYBRID F₁ ANIMALS: MEAN FAT-GLOBULE VOLUMES, IN CUBIC MICRONS FOR INDIVIDUAL COWS

Period	1	2	3	4	5	6	7	8	9	10
Cow No.										
657		53.	17.8	12.8	10.7	11.	15.5	10.8	7.7	
657		26.8	12.8	12.4	5.8	6.5	8.9*	8.7*	8.5*	
688			41.5†	29.7	19.7	17.2	19.2	15.9	16.4	
690‡	45.6	37.1	20.1	14.5	16.1	11.1	9.7*	11.7*	13.8*	10.3*

* Samples shipped.

† This volume was computed before the uniform method of measurement had been adapted so was not used in calculating the mean volume of table 3.

‡ Mean volumes of 7.8 and 6.4 μ^3 were found for periods 11 and 12 respectively for No. 690.

abnormal conditions and the loss of a few samples. The number of animals involved in the combined frequency distributions of any period may be determined by comparison with the same period of the corresponding group in tables 20 to 24.

Computation of the volume.—The volume of fat in each class has been computed by the formula, $f \times 0.5236D^3$, where f represents the frequency and D the diameter in microns. The mean volume is then determined by the formula
$$\frac{\sum (f \times 0.5236D^3)}{n}$$
 where \sum represents

summation and n the total number of globules measured. The use of the first formula, $f \times 0.5236D^3$, would not be correct if the globules were evenly distributed in each class. If in any class, as, for example, 3 μ , they were distributed evenly in ten classes from 2.55 to 3.45 μ the mean volume of that class would be above the volume determined by the formula in which D equals 3 μ . This is due to cubing the diameters.

The distribution of the globules has not been uniform in each class. Other than the 1 μ class there is a tendency for more globules to be in the lower than in the upper part of the class. A correction might be made to take care of this condition. This correction might best be based on the third of Shepherd's moments, which would involve the mean diameter. If the volume was computed by using D^3 it would be necessary to subtract $\frac{1}{4}$ of the mean diameter from the mean volume. Since the factor 0.5236 is used in computing the volume the correction would be $\frac{1}{4} \times 0.5236$ or approximately $\frac{1}{8}$. This fraction of the mean diameter was found in most cases to be from 0.2 to 0.3 μ . Since the amount of the correction was small and relatively uniform throughout the lactation period it was thought unnecessary to apply it.

Volume frequency.—The frequency distribution of the volume of fat appears in many cases to be that of a normal distribution. If plotted

TABLE 23.—BOWLKER HYBRID F_2 ANIMALS: MEAN FAT-GLOBULE VOLUMES, IN CUBIC MICRONS, FOR INDIVIDUAL COWS, THE MEANS AND THE STANDARD DEVIATIONS OF THESE MEANS

Period	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Cow No.																				
723	55.	26.2	18.2	13.9	10.	7.3	9.9	7.9	9.2	7.3	7.6	7.7	7.5	6.3	4.1	4.3	5.7	4.8	9.	6.2
724		15.	14.	13.8	9.7	10.	9.4	7.3	5.2	5.2*	5.3*									
729†	23.	12.7	9.2	6.5	5.9	5.3	4.2	4.7	4.7	5.1										
732	16.2	7.9	8.5	8.3	8.6	5.1	6.1	5.8*	4.7*	6.9*										
735	32.8		9.4	10.1	8.2	7.2		6.3	5.9	7.6	5.2	5.7	5.7	5.8	7.3*	5.7*				
739		20.1	18.2	16.5	9.8	7.9	7.2	6.9	6.1	7.3*	5.1*									
740	21.9	27.4	19.1	10.7	8.7	9.3	7.4	7.1	6.7	8.	8.4	7.8	7.3	6.4	7.2	7.9				
743		11.2	15.5	10.1	9.4	6.8	6.1	4.8	5.1	5.8*	5.8*									
805		26.5	14.4	12.4	15.2	10.6	8.4	8.3	10.	8.4	7.7	5.9								
Mean	29.8	18.4	14.1	11.4	9.5	7.7	7.3	6.6	6.4	6.9										
σ	15.3	7.7	4.1	3.	2.5	2.	2.	1.2	1.9	1.										

* Samples shipped.

† Periods two to five, inclusive, for No. 729 are an average of two lactation periods.

TABLE 24.—BOWLKER HYBRID F_2 AND HOLSTEIN BACK-CROSS ANIMALS: MEAN FAT-GLOBULE VOLUMES, IN CUBIC MICRONS, FOR INDIVIDUAL COWS

Period	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Cow No.																	
801	10.4	7.9		8.5	6.4	6.2	7.1	6.3	4.6	4.4	5.8*	6.3					
804		13.8	11.8		7.8	7.1	8.	5.9	6.1	4.3	4.4	4.7*	5.1*	5.8*	5.1*	5.1*	4.4*
806	13.4	8.	5.2	6.5	5.6*	6.6*	5.9*										
807	43.	19.2	13.2	10.2	8.4	6.7	9.1	7.1	6.2	6.7	6.2	5.8	5.9	7.3	5.	5.7	

* Sample shipped.

TABLE 25.—ANGUS: PERCENTAGE FREQUENCY DISTRIBUTIONS, THE MEAN FAT-GLOBULE VOLUMES AND THE PERCENT FAT CONTENT FOR EACH OF TWO COWS

μ	Cow No. 65				Cow No. 89			
	March 5-7	March 19-21	April 2-4	April 16-18	March 5-7	March 19-21	April 2-4	April 16-18
1	22.48	25.80	19.21	15.85	25.39	22.21	16.99	10.57
2	35.86	38.17	30.87	41.66	34.78	38.55	28.40	29.65
3	20.41	21.30	19.73	25.93	21.46	17.95	19.17	23.53
4	14.07	10.70	13.04	10.92	10.80	12.31	12.86	18.35
5	4.83	2.55	8.40	4.92	4.21	5.29	8.50	8.24
6	2.07	1.18	5.32	0.60	2.52	2.76	5.58	5.18
7	0.28	0.20	1.72	0.12	0.28	0.46	3.88	1.88
8			1.20		0.42	0.23	2.18	1.65
9			0.34		0.14		1.46	0.47
10		0.10	0.17			0.12	0.97	0.24
11						0.12		0.24
Mean volume μ^3	15.2	12.2	28.6	13.3	16.	17.8	43.6	34.5
Babcock test	4.	4.2	5.6	4.	4.1	5.4	5.5	5.4
Babcock test $\times 1.1$	4.4	4.6	6.2	4.4	4.5	5.9	6.1	5.9
Percent fat by volume	3.7	4.1	5.6	3.7	3.8	5.2	6.	4.9

TABLE 26.—ANGUS-HOLSTEIN F₁S: COMBINED PERCENTAGE FREQUENCY DISTRIBUTIONS AND THE MEAN FAT-GLOBULE VOLUMES

Period	1	2	3	4	5	6	7	8	9	10
μ										
1	22.38	24.79	33.16	34.19	28.73	30.81	31.66	34.67	38.18	37.47
2	22.38	31.25	35.08	33.19	35.23	36.38	36.13	32.49	30.89	32.93
3	22.38	17.92	17.28	21.81	20.53	20.50	19.35	18.63	19.81	19.33
4	12.35	12.71	9.08	6.65	10.27	8.69	9.62	10.16	7.36	6.78
5	12.35	7.71	3.66	2.61	3.40	2.63	2.35	2.84	2.80	2.89
6	6.07	4.37	0.87	1.18	1.70	0.88	0.78	0.97	0.83	0.40
7	1.86	0.62	0.70	0.31	0.07	0.13		0.06	0.09	0.16
8		0.42	0.17	0.06	0.07		0.11	0.12	0.04	
9		0.21						0.06		0.04
10										
11	0.23									
Mean volume μ^3	28.3	21.3	12.2	10.5	12.4	10.4	10.4	11.2	9.8	9.4

on the basis of the class marks of the diameters it would approach the form of a normal curve. This treatment of the volume frequency is not justified because it is based on the cubes of the diameters. This produces a distorted class interval, which should be considered if a curve was to be plotted. Because of this abnormal condition curves have not been plotted for these frequencies.

Percentage fat content.—The percentage fat content has been computed on the basis of volume for the individual animals for comparison

TABLE 27.—ANGUS-HOLSTEIN F₂S: COMBINED PERCENTAGE FREQUENCY DISTRIBUTIONS, THE MEAN FAT-GLOBULE VOLUMES AND THE MEAN VOLUMES EXPRESSED IN PERCENT OF THE FIRST

Period	1	2	3	4	5	6	7	8	9	10
μ										
1	21.75	27.72	29.91	29.02	30.62	32.78	30.61	30.86	38.65	33.88
2	20.68	27.03	30.71	31.48	31.56	35.46	33.94	37.76	31.43	33.93
3	22.65	25.16	20.45	22.06	20.16	18.02	21.24	19.73	19.78	19.70
4	13.67	11.40	9.86	10.93	11.03	9.13	9.68	8.55	5.83	8.29
5	11.29	5.14	6.06	4.63	4.32	3.29	3.39	2.34	3.52	2.70
6	5.68	2.92	2.47	1.35	1.78	1.28	1.07	0.76	0.49	1.14
7	2.80	0.56	0.47	0.45	0.49	0.04	0.06		0.18	0.30
8	0.91	0.07	0.07	0.08	0.04				0.12	0.06
9	0.41									
10	0.16									
Mean volume μ^3	32.5	16.5	15.4	13.8	13.9	10.9	11.4	9.8	9.8	10.9
Percent of the first	100.	50.8	47.4	42.5	42.8	33.5	35.1	30.2	30.2	33.5

TABLE 28.—ANGUS-HOLSTEIN F₁S: MEAN FAT-GLOBULE VOLUMES, IN CUBIC MICRONS, FOR INDIVIDUAL COWS*

Period	1	2	3	4	5	6	7	8	9	10	11	12	13
Cow No.													
62D	28.3	21.3	12.2	13.9	14.7	12.7							
69C								10.4	11.3	11.6	12.2	12.1	
69D				8.8	10.6	7.2	10.4	12.1					
75A									7.9	6.3	6.	6.9	7.5
77A									10.5	10.3	7.8	8.7	9.7

* The last four cows were milking when the measurements were begun and have not freshened since.

TABLE 29.—ANGUS-HOLSTEIN F₂S: MEAN FAT-GLOBULE VOLUMES, IN CUBIC MICRONS, FOR INDIVIDUAL COWS*

Period	1	2	3	4	5	6	7	8	9	10
Cow No.										
71B				12.0	10.2	7.8	9.6	8.6		
72D	34.8	16.3	15.7	15.2	19.7					
76B	26.5	14.3	13.6	11.5	12.1	11.5	14.2			
78A†									8.6	8.5
83A‡								11.4	11.3	13.8
92A	37.6	19.8	16.8	17.4	14.1	14.3	13.7			
Mean	33.	16.5	15.4	14.	14.	11.2	12.5	10.	10.	11.2

* Three cows of this group were milking when the measurements were begun and they have not freshened since.

† Mean volumes of 8.2, 8.7 and 8.7 μ^3 were obtained for periods 11, 12 and 13 respectively for No. 78A.

‡ Mean volumes of 11.7 and 10.5 μ^3 were obtained for periods 11 and 12 respectively for No. 83A.

with the Babcock test (tables 13 and 25). This computation was based on the volume of diluted milk in which the fat globules were measured. The hemacytometer chamber was $100\ \mu$ deep and the field in the euscope $100\ \mu$ square. This made the volume of diluted milk equal $1,000,000\ \mu^3$ for each field. Thirty such fields were measured for each sample making $30,000,000\ \mu^3$ of diluted milk. This is equivalent to $300,000\ \mu^3$ of undiluted milk. The percentage fat content by volume

is then determined by the formula
$$\frac{\text{total volume of fat} \times 100}{300,000}$$
. This

result should be larger than the percentage fat content determined by the Babcock test because the latter is based upon weight. The percentage fat content obtained by the Babcock test may be converted to a volume basis if it is multiplied by the specific gravity of milk divided by the specific gravity of fat. The figures for these two factors will

vary somewhat but may be taken as $\frac{1.032}{0.93} = 1.1$. Therefore, the

percentage fat content determined by the Babcock test has been multiplied by 1.1 for comparison with the percentage of fat determined by the measured volume. These results checked more closely than had been expected in the preliminary work. The greatest difference as is shown in table 13 was in the composite sample for No. 657, where the corrected Babcock test was 4.6 percent as compared to 6 percent when determined by volume. The other results checked more closely than had been expected. It happened that these samples represented a relatively large fat-globule size. In other samples with smaller globules the two figures did not check so closely. The percentage fat content by volume has in general proved to be lower than that determined by the Babcock test, in some cases being as much as 50 percent lower. The two determinations, however, tend to vary directly so that the results of different samples are quite comparable.

Environmental factors affecting fat-globule size.—It is difficult to separate the environmental and physiological factors which affect the fat-globule size. An attempt will be made, however, to point out certain variations in the results which it is believed, are due to certain environmental factors which may react indirectly by producing an abnormal condition, such, for example, as infection in the animal body.

A serious injury resulting in an infection has been shown to produce a noticeable change in the fat-globule size, as illustrated by the Holstein cow No. 20 (table 21). In the sixth period there was a dis-

tinct increase in the mean volume. The sample for this period was taken after a severe teat injury resulting in a badly infected udder and a material decrease in milk production. The infection was practically checked by the time that the seventh period sample was taken and the mean volume returned nearly to normal.

Closely related to the condition of No. 20 is that of the Guernsey cow No. 71. This animal was taken with a severe case of foot-rot between the third and fourth periods. During the next two periods she suffered severe pain which resulted in a greatly increased fat-globule volume, but her condition had become apparently normal when the time arrived for the sampling representing the sixth period. From that time onward to the end of the lactation this cow showed a much lower mean fat-globule volume than did the other Guernseys. It might have been thought that this result was caused by the abnormal condition obtaining during periods four and five had not the mean volume already dropped to $10.6 \mu^3$ during the third period. It is believed, therefore, that the results secured with this cow during the periods other than four and five represent a normal condition, indicating that some Guernsey cows produce milk containing relatively small fat globules.

The Holstein cow No. 42 had a case of foot-rot similar to that of No. 71. The serious condition did not extend over as long a period nor did it affect her as severely. An increase in mean volume was noted during the fourth period, but it was not considered abnormal enough to be omitted from the calculations.

Measurements were made for two lactation periods on No. 657 (table 22). The mean volumes of the first period are somewhat higher than those for the second. Both lactation periods started in the spring so the time of year should have no effect on the corresponding periods. During the first lactation this cow was apparently in normal health. She gave birth to large twin calves at the second lactation, and as a result of calving trouble her milk production was low as compared to the first period. She showed no signs of estrum during the second period, which would indicate an abnormal condition. This may be the reason for the lower mean fat-globule volume noted during the second lactation. In but one other case were measurements made on two lactation periods of the same cow. This was for cow No. 729. In only one instance was the difference between the mean volumes of corresponding periods of the two lactations greater than $1 \mu^3$. This one variation

was $1.9 \mu^3$ and may have been due to the fact that the sample representing the second lactation was shipped to the writer.

Previous investigators have obtained conflicting results in regard to the effect of feed upon the size of the fat globule. There were no abrupt changes made in the feed of the Bowlker hybrid and the Angus-Holstein crossbred animals, yet noticeable variations occurred which are unexplainable. Cow No. 657 shows an increase in the mean volume of $4.5 \mu^3$ during the seventh as compared to the sixth period of the first lactation (table 22). This was accompanied by a decrease in milk production and an increase in percentage fat content. The sample was taken on September 4 to 6, which was too early for the feeding of new silage. No reason is known for this increase. A similar variation in the mean volume occurred during the fifth period of No. 72 D (table 29), but was not accompanied by a noticeable change in quantity of milk or percentage fat content. Again no reason can be given for this variation. As a rule, however, the variations from one period to the next during the lactations of the crossbred animals were relatively small, except such as were due to the normal decline to be discussed later.

Certain variations in the mean fat-globule volume of Guernseys and Holsteins may be attributed to changes of feed, while in other cases no apparent effect is manifest. Holstein cows 10, 11, 18 and Guernsey cows 63, 66, 68 and 71 were fed in a trial in which rations containing fish-meal were compared with check rations (Campbell 1931). No. 10 was gradually being changed from the standard dairy ration to the fish-meal ration when the third sample was taken. She was eating well at the time but, later, completely refused her grain ration. The fish-meal could not be said to have affected the size of the globules as the decrease in that period is not abnormal. Cow No. 11 showed no apparent effects from change of feed during periods four, five and six. The sample for the seventh period was taken during the last two days of the third feeding trial, when the feed was the same as for the fifth period. She was not turned out to pasture until the forenoon after the last milking for the seventh period had been sampled. Therefore, the decrease in the size of the globules was not caused by an abrupt change of feed, nor by pasture feeding. Had the sample been taken two days later the change undoubtedly would have been considered as due to the effect of pasture. No. 63 showed a high mean volume during the sixth period, when her feed was exactly the same as it had been during the fourth period. No. 66 showed a low mean volume during each the fourth and sixth period, at which time she was being fed the fish-meal ration.

She was one of the cows that disliked this ration and refused part of her feed. The change of the fat-globule size was apparently due in this case to the refusal of feed more than to the feed itself. No. 68 had a higher mean volume during period eight, when she was getting a check ration, than when getting a fish-meal ration during periods seven and nine. This increase in period eight could hardly be considered as due to the feed for it was very similar to that of period six.

Cows 5, 10, 11, 18, 46, 63, 66, 68, 74, and 86 were turned out to pasture, but one to two weeks had elapsed before the next samples were taken. Details regarding this length of time have been given under the management of the animals. No. 10 showed a slight decrease in mean volume during period six. There was a small increase in volume during period four for No. 46. Normally a decrease in this period as compared to the third would have been expected. The remainder of the group appeared to have a normal fat-globule size in samples taken from one to two weeks after turning out to pasture. It is interesting to note that No. 86 had an increase in the fat-globule size during the sixth period. This sample was taken approximately ten days before pasture feeding began and no known reason can be advanced for the increase.

Cows 3, 5, 69 and 81 were stabled-fed but varied as much in the mean fat-globule volume as did the animals turned to pasture. The change from silage to grass clippings two and one-half weeks before the seventh period of No. 3 and the sixth period of No. 19 may have caused a slight drop in the fat-globule size of the former, but not the latter. The silage was withdrawn from Guernsey cows Nos. 69 and 81 between the ninth and tenth periods with opposite effects on the fat globules of the two animals. The former showed an increase in size, while the latter showed a decrease.

Gutzeit and Weigmann came to unlike conclusions with regard to changes in size of the fat globules as a result of turning cows to pasture. The results of this investigation do not bear out the conclusions of either of these investigators. There was very little, if any, effect on the size of the fat globules as a result of turning the Guernsey and Holstein cows to pasture, unless the effect it produced had disappeared within one to two weeks. This may be accounted for in part by the fact that these cows were fed a grain ration while on grass, and that during the first few days they were fed hay before being turned out to pasture. The change may have been more abrupt in the case of the animals studied by Gutzeit and Weigmann. The results published

by Gutzeit show as large variations in the globule size due to change in weather as was produced by turning the cows to pasture. This fact, together with the fact that two cows under carefully controlled experimental conditions in this investigation showed little if any effect from substituting grass for silage, leads the writer to believe that the sudden change of conditions is as great a factor as is the change of feed in producing a variation in the globule size when cows are turned out to pasture.

One other factor, that of fasting, causes a material change in the fat-globule size, as was shown by cow No. 742 (table 30). The increase in the mean volume began with the second milking of the fasting period and increased rapidly during the next few milkings. The highest mean volume observed was $97 \mu^3$ for the third milking before slaughter. There is considerable variation in the mean fat-globule volume of the individual milkings. This is probably due to the number of globules measured being too few in comparison to their size, although the fat percentage by volume, in some cases, is close to that of the Babcock test. The largest globule recorded in the measured fields was 31μ in diameter, but careful searching of the entire area disclosed one globule with a diameter of 36μ . One such globule is equivalent to 46,656 globules of 1μ diameter. It is possible that the extremely large globules produced by this cow were due as much to lack of water as to lack of feed.

Frequency distribution.—The general frequency distribution of the fat globules for all animals studied shows a larger portion of small globules than was found by Weigmann (1926) and Turner and Haskell (1929). In this investigation all globules up to 1.5μ were included in the 1μ class as it was at first thought that very few globules were less than 0.5μ , but this does not account for all the discrepancy. The percentage of globules in the first two classes, or those with a diameter up to 2.5μ , is greater than the total percentage of the globules up to 3μ diameter as determined by these former investigators. The results of this investigation more nearly approach those obtained by Jones (1890), Gutzeit (1895) and Cooper, Nuttall and Freak (1913). This larger portion of small globules is reflected in the mean volume of the fat globules as will be discussed later in this article.

The Holsteins and Guernseys differ with respect to the frequency distribution of the fat globules. The former (table 16) after the third period have nearly as large a percentage of the globules in the 1μ and 2μ classes as do the latter (table 15) in the first three classes. As high as 50 percent of the globules of the Holstein milk are found in the 1μ

TABLE 30.—THE PERCENTAGE FREQUENCY DISTRIBUTIONS, MEAN FAT-GLOBULE VOLUMES, THE BABCOCK TEST AND THE PERCENT FAT CONTENT AS DETERMINED BY VOLUME FOR COW No. 742

	Period 1	Period 2	Days preceding fasting		1st day		2nd day		3rd day		Fasting period		4th day		5th day		6th day	
			3-6	0-3	P.M.	A.M.	P.M.	A.M.	P.M.	A.M.	P.M.	A.M.	P.M.	A.M.	P.M.	A.M.	P.M.	A.M.
μ																		
1	22.35	25.15	26.30	30.08	27.31	24.83	22.45	17.99	24.82	17.11	28.86	16.75	16.59	20.30	16.72	21.82		
2	17.42	23.24	28.76	26.50	28.87	26.65	22.08	20.84	17.79	16.86	15.90	12.26	18.15	17.85	17.75	23.08		
3	18.18	21.35	17.98	15.14	18.30	15.95	16.14	13.	12.53	13.54	10.67	8.71	10.04	11.69	13.32	13.93		
4	13.64	14.59	15.73	13.36	15.21	15.49	13.54	12.81	15.54	19.	13.39	13.23	10.42	10.15	15.40	12.89		
5	14.77	8.92	5.62	9.35	5.41	10.71	12.06	13.38	13.53	15.44	13.39	14.52	13.90	8.	13.32	10.40		
6	8.33	4.32	4.04	3.79	2.32	5.69	7.61	10.90	9.02	8.55	9.21	18.07	11.20	10.15	11.23	9.15		
7	4.92	1.62	1.35	1.56	1.55	0.68	4.08	6.69	4.26	6.41	5.86	10.65	9.27	10.77	6.53	2.91		
8	0.39	0.54	0.22		0.77		2.04	3.06	2.01	2.85	1.88	4.52	4.25	5.54	2.87	3.53		
9				0.22	0.26			0.38	0.25		0.63	0.32	3.47	2.77	1.04	0.62		
10								0.38				0.65	0.77	1.23	0.78	0.83		
11		0.27						0.57	0.25				0.77	0.31		0.42		
12											0.21		0.39	0.62	0.78			
13													0.39		0.26			
14										0.24			0.39	0.31				
15																		
16												0.32						
17																		
18																		
19														0.31		0.21		
—																		
—																		
30																		0.21
Mean volume μ^3	36.9	26.	20.4	21.9	21.	23.4	37.2	55.9	42.7	51.4	45.8	79.	91.7	97.	66.9	85.7		
Babcock test	4.	4.8	4.3	4.2	4.2	4.7	9.2	11.2	9.6	9.7	8.7	10.3	8.3	9.7	10.1	10.2		
Babcock test $\times 1.1$	4.4	5.3	4.7	4.6	4.6	5.2	10.1	12.3	10.6	10.7	9.6	11.3	9.1	10.7	11.1	11.2		
Percent fat by volume	3.3	3.2	3.	3.3	2.7	3.4	6.7	9.7	5.4	7.2	7.3	8.2	7.9	10.5	8.5	13.7		

class, while the maximum in this class for the Guernseys is approximately 30 percent. No attempt has been made to plot curves for these distributions as it is evident that such curves would be greatly skewed.

The Guernseys show a slight decrease in the percentage of globules in the $1\ \mu$ class during the first four periods, with a gradual increase from that time to the end of the lactation. There is a gradual increase in the percentage in the $2\ \mu$ class during the fore part of the lactation period, with a more stabilized condition in the latter part. The $3\ \mu$ class shows a slight rise during the first two periods, with a fairly uniform percentage maintained during the remainder of the lactation. The $4\ \mu$ class shows uniformity during the first four periods with a gradual decrease thereafter. In the remainder of the classes there is a tendency toward a gradual decrease in percentage with advance in lactation. The Holsteins show an increase in the percentage of globules in the $1\ \mu$ class with advance in lactation. The $2\ \mu$ and $3\ \mu$ classes of this breed show somewhat the same trend as the $3\ \mu$ and $4\ \mu$ classes of the Guernseys, except that the percentage is higher. In each class above $3\ \mu$ the Holsteins manifest a tendency toward a gradual decrease in percentage with advance in lactation, as did the Guernseys after the $4\ \mu$ class. These percentages for the first four classes for these two breeds are represented graphically in figure 2.

The decrease in the percentage frequency of the small globules during the fore part of the lactation period of Guernseys does not mean a decrease in the total number of these globules. The small globules must increase much more rapidly in proportion than the large ones decrease, in order to maintain the volume. This increment is more rapid in the $2\ \mu$ and $3\ \mu$ than in the $1\ \mu$ class during the fore part of the lactation, with the globules in the latter class increasing more rapidly during the last part of lactation. In contrast to this the Holsteins evidence a noticeable increase in the smallest globules shortly after the onset of lactation. This would indicate a more rapid decrease in the mean volume of the Holstein fat globules than of the Guernseys. The accuracy of this observation will be borne out in the later discussion of the volume of the globules.

The frequency distributions for the individual Holsteins followed quite uniformly the trend shown by the combined results for the breed. The individual Guernseys were more variable in this respect, as was most noticeable during early lactation. No. 64 had a very low percentage of globules in the $1\ \mu$ and $2\ \mu$ classes with a high percentage in the next two classes, during the first period. In the second period

there was a distinct increase in the 1μ as compared to a decrease in the 3μ class. After this period the trend was much like that of the group. No. 69 was much unlike the remainder of the Guernseys. She produced a higher percentage of globules in the 1μ class during the fore than during the latter part of the lactation. The 2μ class did not show an increase until the eighth period. The decrease in the 4μ and 5μ classes did not occur until near the end of lactation. The frequency

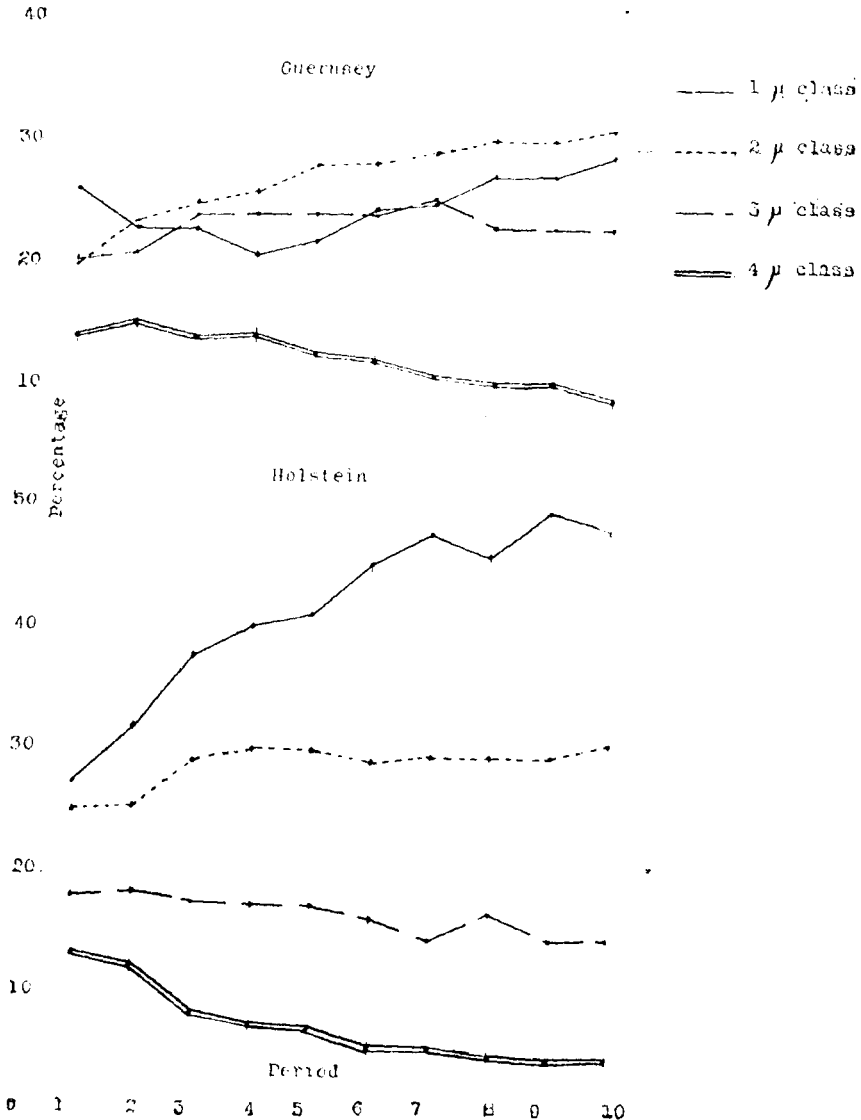


FIG. 2.—Graphical representation of the percentages of the globules in each of the 4μ classes during 10 periods.

distributions for No. 71 appear much like those for the Holsteins. Different blood lines, as well as high and low testing animals, are represented in each breed. Therefore the greater variation of the frequency distributions for the Guernseys as compared to those for the Holsteins can not be attributed to breeding.

There is more fluctuation from one period to the next for the Bowlker hybrid F_1 group (table 17) than for either the Guernsey or Holstein. This may be due to the small numbers and, too, there is but one cow represented in the first period. The first lactation of No. 657 shows a trend more like the Guernseys than Holsteins, although the percentage of globules is somewhat higher in the 1μ and lower in the 3μ class than is the case with the Guernseys. The second lactation for this cow shows more fluctuation than the first, with more than 50 percent of the globules in the 1μ class during periods five and six. The frequency distribution for No. 688 is much like that of the combined Guernseys, while that for No. 690 is more comparable to the Holsteins.

The F_2 group (table 18) is similar to the Holsteins in regard to the frequency distribution of the globules. There is a distinct increase in the frequency of the globules in the 1μ and 2μ classes with advance in lactation. The 3μ class shows a slight rise during the second and third periods, after which there is a decrease (fig. 3). This increase in the percentage frequency of the small globules for the F_2 animals was reflected in a greater number of these globules measured per sample than for the Holsteins. The reason for this was a slightly higher fat content in the milk of the F_2 s, which required a large number of the small globules to make up this volume. Measurements in this group amounted to as high as 1,800 globules per sample as compared to 1,400 for the Holsteins.

The individual F_2 animals appear to show more variability in the first three periods than do the Holsteins or Guernseys. Some of the animals start the lactation with a low percentage of globules in the 1μ and 2μ classes, but the decrease in size is in evidence during the third or fourth period. Others show a relatively high percentage of small globules during the first and second periods.

The Bowlker hybrid back-cross animals show much the trend of the Holsteins in respect to the frequency distribution. The percentage of small globules in this group is even higher in the early part of the lactation than is shown by the Holsteins. This may be due to the fact that a smaller number of animals contributed data. At least one back-

cross animal, No. 807, shows only 19 percent in each of the first two classes, with 17 and 15 percent respectively in the 3 μ and 4 μ classes. The globules soon decreased in size, for by the second period the percentage of globules in the 1 μ class had nearly doubled.

The measurements for the last two periods of the Angus (table 25), which probably represent a normal condition, indicate the high percentage to occur in the 2 μ class with the proportions in the 1 μ and 3 μ classes nearly equal.

Bowlker hybrid F_2 group

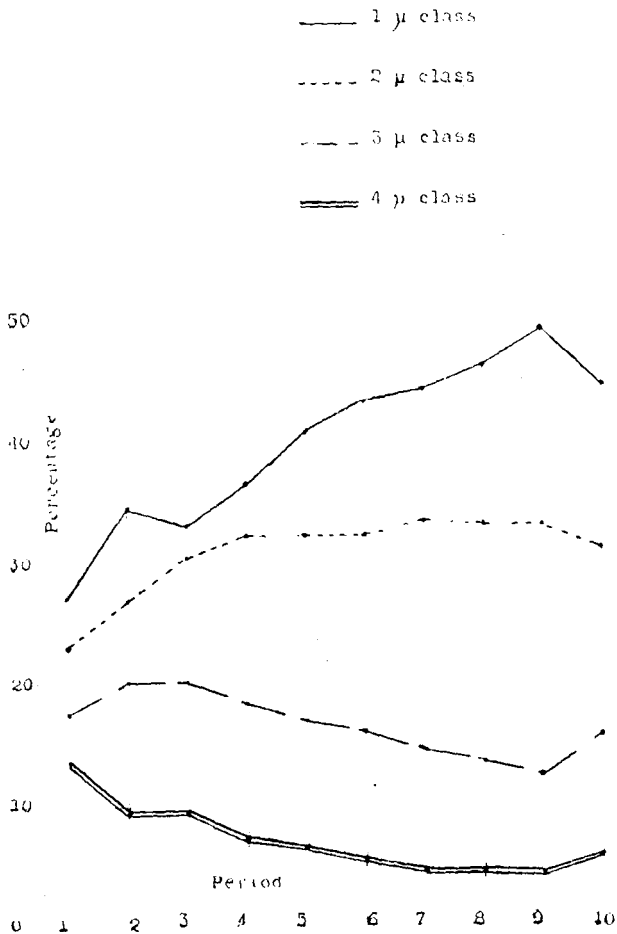


FIG. 3.—Graphical representation of the percentages of the globules in each of the 4 μ classes during 10 periods.

Only one animal is represented in the first three periods of the Angus-Holstein F_1 group (table 26). In the first period she produced an equal number of globules in each of the first three classes. In the next two periods the percentage of globules is greatest in the 2μ class with that of class one being above class three. The combined individuals indicate a tendency for the percentage distribution to exhibit the same trend as that of the Holsteins, except that a smaller percentage is represented in the 1μ class with a higher percentage in classes three and four.

The F_2 group of the Angus-Holstein cross (table 27) is based on results from three or more animals in each period. This group shows almost an equal percentage of globules in the first two classes, with a gradual increase in each during the lactation. The percentage in the third and fourth classes runs above that for the Holsteins.

Mean volume of the fat globules.—The mean volumes of the fat globules as determined in this investigation are on the whole lower than those found by Weigmann (1926). This was indicated by the differences in the frequency distributions, and appears to be due to the much smaller portion of very minute globules observed by Weigmann. If the Holsteins of this country may be deemed to be comparable to the Holländer or Schwarzbunte Holsteiner as studied by Gutzeit and Weigmann, then the results of the present investigation compare very well with those obtained by Gutzeit (1895). He found the mean volume to be $9 \mu^3$ for the Holländer cattle, while the results obtained by Weigmann was approximately twice this amount, or $18.06 \mu^3$. The mean of the mean volumes for the first 10 periods of the Holsteins (table 16) is $10.4 \mu^3$, while that for 13 periods is $9.5 \mu^3$.

The mean volumes obtained for the Guernseys appear to be substantiated in results obtained by Woll (1892) and Cooper, Nuttall and Freak (1911). Their results are reported in mean diameters, but these are based on the volume. The mean diameters of 3.58μ and 3.84μ reported by Woll for the Guernseys at the Columbian Exposition are equivalent to volumes of $24 \mu^3$ and $29.7 \mu^3$ respectively. It may be assumed that cows entered in a test such as that at the Columbian Exposition were relatively fresh, and that these figures apply to the fore part of the lactation. On the basis of this assumption the results are in close agreement with the mean volumes determined for the first two periods of the Guernseys in this study.

Cooper, Nuttall and Freak (1911) report mean diameters for the fat globules of Guernsey milk determined on the basis of volume, as

3.28, 3.07, 2.84 and 2.80 μ . These measurements were made at 25, 48, 70 and 174 days, respectively, after freshening. The respective volume equivalents are 18.5, 15.2, 12 and 11.5 μ^3 . These results are below the mean volumes of the Guernseys at corresponding periods of this investigation, but they are comparable to results obtained with some of the individual cows.

It will be recalled that Weigmann concluded that the methods used by Gutzeit were correct, and that there had been an increase in the mean size of the fat globules during the past 30 years. He believed this increase had been brought about partly by breeding. The writer expressed his belief earlier that breeding was not responsible for this change. In view of the agreement between the results of the present investigation and those of Gutzeit, Woll, etc., it appears that there has been no marked increase in the size of the fat globules produced by the cattle of today as compared to those of 30 years ago. It would seem that the increase obtained by Weigmann was brought about by a failure on his part to observe all of the very small globules.

The mean volumes for periods two to ten inclusive for the different groups have been expressed in percentage of the mean volume of period one. The Guernseys show the least decrease of any of the groups with the mean volume of the tenth period being 47.2 percent of that for the first period. The decrease is most rapid in the first two periods. The Holsteins, also, show the largest amount of decrease in the first two periods, but it is greater than was the case with the Guernseys. The mean volume of the third period is 53.8 percent of the first, which is nearly as much as the total decrease for the Guernseys. The mean volume for the tenth period of the Holsteins is 27.2 percent of the first.

A comparison of the mean volumes of the different periods of the Bowlker hybrid F_1 group is not justifiable since the first period is based on the results of one animal. The F_2 group shows a greater decrease from period one to two than do either of the purebred groups. The mean volume of the second period is but 55.8 percent of the first, while for period nine it is down to 22.5 percent with a slight rise in period 10. The back-cross animals do not show as great a percentage decrease as the F_2 group but this is due to a much lower mean volume in the first period.

The Angus-Holstein F_1 group is composed of too few animals to enable one to determine a fair percentage decrease. The first three periods of the F_2 group represent three animals while a fourth is included in periods four and five. In the second period this group showed

more decrease in size of the globules than did the Bowlker hybrid F_2 group. The mean volume in this period was only 50.8 percent of that in the first. The decrease in the next three periods is small. Although the results secured during the last periods were on the milks of different cows than were used in the first period, they indicate that the total decrease in mean volume for this group is hardly as great as that for the Bowlker hybrid F_2 group.

The mean volumes of the fat globules for each period throughout the lactation have been given for the individual animals of each group. The mean and the standard deviation of these means have been determined for each of the Guernsey, Holstein and Bowlker hybrid F_2 groups. This mean of the means does not weight the different sized globules and, therefore, is a false mean, but it is used in a statistical treatment of the data. In most cases it is higher than the true mean. The probable errors of these constants are not used because of the small numbers upon which they are based.

The standard deviation is much higher for the Guernseys than for the Holsteins showing the former to be more variable. It is almost as large during the last part of the lactation for the Guernseys as during the fore part for the Holsteins. The F_2 s exhibit a higher standard deviation in the first period than do the Guernseys, but this deviation decreases rapidly during the first three periods. During the last seven periods it is somewhat comparable to that of the Holsteins.

The formula developed by Fisher (1930, p. 105), for testing the significance of the mean of a small sample, has been applied to the standard deviations of the Guernseys, Holsteins and Bowlker hybrid F_2 . This formula is as follows:

$$\bar{x} = \frac{1}{n'} s(x).$$

$$s^2 = \frac{1}{n'(n'-1)} s(x - \bar{x})^2.$$

$$t = \frac{\bar{x} \sqrt{n'}}{s}.$$

$$n = n' - 1$$

s = the differences of the standard deviations.

\bar{x} = the mean of the differences.

Values for t are given by Fisher, p. 139. When n equals 9, as in each of these cases, and P is equal to 0.01, only one value in a hundred will exceed 3.250 by chance, so that a difference as great as, or greater than, this figure is clearly significant.

TABLE 31.—A COMPARISON OF THE STANDARD DEVIATIONS OF THE MEANS FOR THE GUERNSEYS AND HOLSTEINS, ACCORDING TO FISHER'S FORMULA FOR TESTING THE SIGNIFICANCE OF THE MEANS OF SMALL SAMPLES

Standard deviation Guernsey	Standard deviation Holstein	Difference x	$x - \bar{x}$	$(x - \bar{x})^2$
13.7	4.5	9.2	4.5	20.25
8.1	5.2	2.9	-1.8	3.24
6.5	2.1	4.4	-0.3	.09
6.6	3.8	2.8	-1.9	3.61
5.4	2.1	3.3	-1.4	1.96
7.	2.	5.	0.3	.09
5.9	1.6	4.3	-0.4	.16
4.5	1.	3.5	-1.2	1.44
6.2	0.9	5.3	0.6	.36
6.9	0.9	6.	1.3	1.69
$t = 7.73$			$\bar{x} = 4.7$	$n = 9$
				32.89

Comparisons by this method have been made for the Guernseys and Holsteins (table 31), Guernseys and Bowlker hybrid F_2 s (table 32), and Holsteins and Bowlker hybrid F_2 s (table 33). The comparison for the Guernseys and Holsteins gives a value of t equal to 7.72.

TABLE 32.—A COMPARISON OF THE STANDARD DEVIATIONS OF THE MEANS FOR THE GUERNSEYS AND THE BOWLKER HYBRID F_2 S, ACCORDING TO FISHER'S FORMULA FOR TESTING THE SIGNIFICANCE OF THE MEANS OF SMALL SAMPLES

Standard deviation Guernsey	Standard deviation F_2	Difference	$x - \bar{x}$	$(x - \bar{x})^2$
13.7	15.3	-1.6	-4.6	21.16
8.1	7.7	0.4	-2.6	6.76
6.5	4.1	2.4	-0.6	.36
6.6	3.	3.6	0.6	.36
5.4	2.5	2.9	-0.1	.01
7.	2.	5.	2.	4.
5.9	2.	3.9	0.9	.81
4.5	1.2	3.3	0.3	1.69
6.2	1.9	4.3	1.3	1.69
6.9	1.	5.9	2.9	8.41
$t = 4.33$			$\bar{x} = 3.$	$n = 9$
				43.65

This value being greater than 3.250 shows that the difference between these breeds is clearly significant. When comparing the Guernseys and the Bowlker hybrid F_2 s the value of t is 4.41. This result shows a significant difference between the Guernseys and the F_2 s. The value

of t in the latter case is not as large as in the former, which would indicate that the difference between the two purebred breeds is greater than between the Guernseys and F_2 s. The comparison for the Holsteins and F_2 s gives a value of t equal to 1.54. This shows a value for P of

TABLE 33.—A COMPARISON OF THE STANDARD DEVIATIONS OF THE MEANS FOR THE HOLSTEINS AND THE BOWLKER HYBRID F_2 S, ACCORDING TO FISHER'S FORMULA FOR TESTING THE SIGNIFICANCE OF THE MEANS OF SMALL SAMPLES

Standard deviation F_2	Holstein	Difference	$x - \bar{x}$	$(x - \bar{x})^2$
15.3	4.5	10.8	9.1	82.81
7.7	5.2	2.5	.8	.64
4.1	2.1	2.	.3	.09
3.	3.8	-.8	-2.5	6.25
2.5	2.1	.4	-1.1	1.21
2.	2.	0	-1.7	2.89
2.	1.6	.4	-1.4	1.96
1.2	1.	.2	-1.5	2.25
1.9	.9	1.	-.7	.49
1.	.9	.1	-1.6	2.56
		$\bar{x} = 1.7$		101.15
Ten periods $t = 1.58$		$n = 9$	Last eight periods $t = 0.73$	
			$n = 7$	

approximately 0.15, which indicates that about 15 percent of the time a difference as great as or greater than that between these two groups would be due to chance, and that there is no significant difference between them. The first two periods of the Holsteins and F_2 show

TABLE 34.—A COMPARISON OF THE MEANS FOR THE HOLSTEINS AND ANGUS-HOLSTEIN F_2 S, ACCORDING TO FISHER'S FORMULA FOR COMPARING THE MEANS OF SMALL SAMPLES

Means	Holstein $x - \bar{x}$	$(x - \bar{x})^2$	Means	Angus-Holstein F_2 $x - \bar{x}$	$(x - \bar{x})^2$
22.7	11.9	141.61	33.	18.2	331.24
18.4	7.6	57.76	16.5	1.7	2.89
12.3	1.5	2.25	15.4	0.6	.36
10.2	-.6	.36	14.	-.8	.64
9.1	-1.7	2.89	14.	-.8	.64
8.2	-2.6	6.76	11.2	-3.6	12.96
7.1	-3.7	13.69	12.5	-2.3	5.29
7.2	-3.6	12.96	10.	-4.8	23.04
6.2	-4.6	21.16	10.	-4.8	23.04
6.1	-4.7	22.09	11.2	3.6	12.96
$\bar{x} = 10.8$		281.53	$\bar{x} = 14.8$		413.06
$t = 1.44$		$n = 18$			

the highest standard deviation. If they are omitted from the calculation the value of t is found to be 0.83 with a corresponding value for P of 0.40. This indicates that approximately 40 percent of the time the difference between these last eight periods would be due to chance.

Therefore the greater difference existing between these breeds in respect to globule size is observed during the early stages of lactation.

The Angus-Holstein F_2 group contained too few numbers upon which to determine a standard deviation. The means for each period have been determined and compared with those of the Holsteins by Fisher's method (p. 107-109). This comparison (table 34) gives a value of t equal to 1.33 with n equal to 18. This indicates that the difference between these groups is not significant, as in approximately 20 percent of the cases the difference is due to chance. It is realized that this comparison is based on the means of very small numbers in case of the Angus-Holstein F_{2s} , but it is significant that this result should be in close agreement with that of the F_{2s} from the Guernsey-Holstein cross.

It is regretted that the results secured with the Angus are too few to make comparison with the Holsteins and the Angus-Holstein F_2 , similar to those made above. If, however, the results obtained from the third and fourth samples of the Angus are indicative of their normal condition, it may be assumed that the differences between the Angus and Holsteins would be greater than that between the Guernseys and Holsteins. On the same basis it would be expected that the difference between the Angus and the Angus-Holstein F_2 would also be significant.

The Guernsey-Holstein F_{1s} , although only three in number, appear more variable than do the F_{2s} . No. 688 exhibited a fat-globule size which appears to approach more nearly that of the Guernsey than of the Holstein. Cows Nos. 657 and 690 showed a high mean volume at the outset, but after the third or fourth period of the lactation the fat-globule size was but slightly above that of the Holsteins. Each of the three cows was produced from the same type of mating, namely, a Guernsey male mated to a Holstein female. The first two cows, Nos. 657 and 688, were from the same dam but by a different sire.

In the Bowlker hybrid F_2 group there were three F_1 sires involved. Nos. 724 to 740, inclusive, were by the same sire. No. 729 was from the F_1 cow No. 657, the daughter tending to produce a much smaller globule than did her dam. Nos. 740 and 743 were from the same F_1 dam, but were by different sires. The former produced slightly larger fat globules than did the latter.

The first two Bowlker hybrid back-cross animals, No. 801 and No. 804, were by the same Holstein sire, while the other two, Nos. 806 and 807, were by his son. These last two were from the F_2 cows, No. 723 and No. 729 respectively. No. 807 produced the largest globules of

the back-cross group and larger than did her dam. The variations between these closely related animals does not appear, however, to be sufficiently great to have significance.

The difference in conformation of the Angus and Holstein breeds offers an opportunity to compare the type of the crossbreds with the size of the fat globules in their milks. The F_1 animals included in this study appear, in conformation, more like the Holsteins than the Angus. They show many dairy characteristics and have been quite persistent in milk production. These characteristics are particularly noticeable in Nos. 62D and 75A. The former appears to produce a slightly higher mean globule volume than do the Holsteins, but the samples from the last part of the lactation for No. 75A show a fat globule size characteristic of the Holsteins. Nos. 69C and 77A exhibit the least dairy appearance of any in the group. The former is not very persistent in milk production and shows a fat globule size above that for the Holsteins. The latter is more persistent in production with a fat-globule size little if any above that of the Holsteins for the last part of lactation.

The group of F_2 animals studied appears to be more of the Angus than the Holstein conformation. Two of these, Nos. 71B and 92A, show the most dairy conformation and have been the most persistent in production. During periods four to eight the former has shown a mean fat-globule size and a frequency distribution closely related to that of the Holsteins. The latter has been producing larger globules than the Holsteins. Of the other four animals No. 72D has the highest mean fat-globule volume, which is much above that of the Holsteins. The last part of the lactation for No. 78A would indicate a fat-globule size but little above that of the Holsteins. There appears to be a little more variability in the mean fat-globule volume of the Angus-Holstein F_2 than was found with the Bowlker hybrid F_2 . This may be due partly to the Angus having a larger fat-globule size than the Guernseys.

This study was undertaken with the expectation that it might throw some light on the manner of inheritance of the fat-globule size. It has been found, however, that there are many factors which cause considerable variation in the size of the globules of which inheritance is but one and, perhaps, not the most important. Such being the case a large number of crossbred animals would need to be studied before one would be warranted in drawing definite conclusions as to the effect of inheritance per se on fat-globule size. Unfortunately it was not possible to secure these large numbers because of the unexpected dispersal of the Bowlker hybrid herd.

Of the three F_1 s studied, two appear to have a fat-globule size approaching that of the Holstein, except during the early part of lactation. The results for the third were much like those for the Guernseys. The F_2 s show more variation during the early part of lactation than do either the Guernseys or Holsteins, but after the first few weeks they are comparable to the Holsteins. The four back-cross animals by Holstein sires gave results much like the Holsteins. It is probable that a large number of individuals in the F_2 group would have brought out more variation, with some animals almost comparable to the Guernseys.

Although the available results are limited they indicate that fat-globule size is determined by multiple factors. These may act more like the factors determining milk production than percentage fat content. In milk production Castle (1919) found the crossbred animals to be more like the Holstein than the Guernsey parents. Gaines (1923) shows the percentage fat content of the F_1 and F_2 animals to be almost intermediate between the parents. The fat-globule size for the F_2 group, although somewhat intermediate, tends to be more like that for the Holstein than the Guernsey.

SUMMARY AND CONCLUSION

A comparison was made of different methods of measuring the fat globules in cow's milk. A dilution of the milk to 100 times its volume with equal parts of water and glycerin was made without the glycerin affecting the size of the fat globules. When this dilution was mounted in a hemacytometer chamber the fat globules could be readily measured by use of the euscope in connection with the microscope. This equipment made it easier to observe the very small globules and to measure them with greater accuracy than was the case when direct microscopic measurements were attempted.

Environmental factors were found to affect the size of the fat globules in milk. Injury, sickness and sudden changes of condition usually produce a marked increase in the fat globule size. A change of feed produces an increase in some cases, in some it may produce a decrease, while in others it exerts no apparent effect on the size of the fat globules. Turning the cows to pasture, where the change was made gradually and feeding of grain was continued, had no apparent effect on the size of the fat globules at the end of one or two weeks after the change was made. Neither did the substitution of grass for silage, where animals were stable fed, manifest any marked effect.

The fat globules are usually largest during the first two weeks of lactation with the most rapid rate of decline occurring during the next two months. Thereafter the rate of decline in size is slow but usually continues to the end of the normal lactation period.

The mean fat-globule volume of the Guernsey milk was found to be $28.4 \mu^3$ at the beginning of the lactation period with a decline to $13.4 \mu^3$ at approximately the forty-second week. The average for the 42-week period is $18.8 \mu^3$. Approximately 65 to 80 percent of the fat globules of this breed are less than 3.5μ in diameter, with a fairly even division between the 1 and 3 μ classes. The number in each of these classes is slightly lower than in the 2 μ class.

The Holsteins were found to have a mean fat-globule volume ranging from $22.1 \mu^3$ at the beginning to $6 \mu^3$ during the forty-second week of the lactation. The average for this period is $10.4 \mu^3$. The portion of the fat globules under 3.5μ in diameter ranges from 70 to 94 percent with from 1.5 to 3.5 times as many in the 1 μ as in the 3 μ class.

The mean fat-globule volume of the individual Guernsey cows is more variable than that of the Holsteins.

The Angus appear to show a higher mean fat-globule size than do the Guernseys.

The F_2 s resulting from the Guernsey-Holstein cross and from the Angus-Holstein cross show a more rapid rate of decline in the fat-globule size during the first six weeks of lactation than do the Guernseys or Holsteins. There is much more variation in the means for the individual animals during the first two periods than during the remainder of the lactation.

There is a significant difference between the means of the Guernseys and Holsteins, and between the Guernseys and the F_2 s of this cross. A comparison of the means for the Holsteins with that of the Guernsey-Holstein F_2 s, or the Angus-Holstein F_2 s does not show a significant difference.

A number of factors appear to be involved in the inheritance of the size of the fat globules in cow's milk.

ACKNOWLEDGMENTS

The writer wishes to acknowledge credit due to the University of Illinois for data collected from the Bowler hybrid herd and to the University of Wisconsin for the data relating to the purebred Angus and the Angus-Holstein crossbred animals. The University of Illinois

loaned the microscopic equipment used in collecting data at the Vermont and Wisconsin Stations. The writer is grateful to Professors H. A. Ruehe, W. L. Gaines, W. W. Yapp of the University of Illinois for help in planning and carrying out of the study; to Professors L. J. Cole and R. A. Brink of the University of Wisconsin for aid in summarizing the data; and to the assistants who faithfully have carried out the tedious task of recording the measurements.

LITERATURE CITED

- Babcock, S. M. 1885. Study of the fat globules of milk. N. Y. (State) Sta., Rpt. 4, pp. 266-275.
- Barthel, Chr. 1907. Die Methoden zur Untersuchung von Milch und Molkereiprodukten. Translation by Goodwin, W., p. 4.
- Blyth, A. W. 1882. Foods: Their composition and analysis, p. 201.
- Bouchardat, M., and Quevenne, T. A. 1857. Du Lait, pp. 4 and 181.
- Campbell, M. H. 1924. Inheritance of black- and red-coat colors in cattle. Genet. 9, pp. 419-434.
- . 1931. The supplemental value of fish meal as a feed for dairy cattle. Vt. Sta., Bul. 333.
- Castle, W. E. 1919. Inheritance of quantity and quality of milk production in dairy cattle. Proc. Nat. Acad. Sci., 5, pp. 428-434.
- Collier, P. 1891-92. Microscopic examination of milk. N. Y. (State) Sta., Rpt. 10, pp. 129-162; Rpt. 11, pp. 153-162.
- Cooper, W. F., Nuttall, W. N., and Freak, G. A. 1911. The fat globules of milk in relation to churning. Jour. Agric. Sci., 4, pp. 150-176, 3 pl.
- . 1913. Observations on the fat globules of milk. Jour. Agric. Sci., 5, pp. 331-356.
- . 1913. The enumeration and measurement of fat globules in milk. Jour. Agric. Sci., 5, pp. 357-376.
- Eckles, C. H., and Shaw, R. H. 1913. The influence of the stage of lactation on the composition and properties of milk. U. S. Dept. Agr., Bu. An. Ind., Bul. 155, 88 pp.
- . 1913. The influence of breed and individuality on the composition and properties of milk. U. S. Dept. Agri., Bu. An. Ind., Bul. 156, 27 pp.
- Ellenberger, H. B., Newlander, J. A., and Jones, C. H. 1931. Calcium and phosphorous requirements of dairy cows. I. Weekly balances through lactation and gestation periods. Vt. Sta., Bul. 331, 27 pp.
- Fisher, R. A. 1930. Statistical methods for research workers. 3rd edition, London: Oliver and Boyd.
- Fleischmann, Wm. 1893. Lehrbuch der Milchwirtschaft. (Translation by Aikman, C. M., and Wright, R. P., 1896. The book of the dairy, pp. 19-24); 1908, 4th edition, p. 43.
- Gaines, W. L. 1923. The inheritance of fat content of milk in dairy cattle. Amer. Soc. An. Proc. —, pp. 29-32.
- Gutzeit, E. 1895. Die Schwankungen der Mittleren Grösse der Fettkügelchen in den Kuhmilch nach Laktation, Fütterung und Rasse, sowie über den physikalischen und chemischen Unterschied der grössten und Kleinsten Fettkügelchen. Landwirtschaft. Jahrb., 24, pp. 539-668, Tafel VL-XV.
- Hills, F. B., and Boland, E. N. 1913. Segregation of fat factors in milk production. Proc. Iowa Acad. Sci., 20, pp. 195-198.
- Jones, L. R. 1890. Study of milk globules. Vt. Sta., Rpt. 4, 65-69.
- Lamson, H. H. 1888. Report of the microscopist. N. H. Sta., Rpt. 1, pp. 85-86.
- Leufven, Gust, J. son. 1894. Undersökningar inom Ultima meieri och ladugård 5 Fettkuleundersökningar. Redogörelse För Verksamheten vid Ultuna Landbruksinstitut, pp. 39-46. Abstract E. S. R., Vol. viii, p. 161.
- M'Donnell, M. E., and Fields, J. W. 1895. On the variation in the number and size of the fat globules in milk. Pa. Sta., An. Rpt. (not numbered), pp. 75-85.
- Marshall, F. H. A. 1922. The physiology of reproduction. 2nd edition. Longmans, Green and Co., London and New York.

- Merrill, L. H. 1890. The fat globules of milk. Maine Sta., Rpt. 6, p. 58.
- Nevens, W. B. 1928. Effects of fasting and the method of preparation of feed upon the digestive process in dairy cattle. Jour. Agr. Rsch., 36, 9, pp. 777-794.
- Pankowski, Dr. 1895. Einflüsse auf die Zahl und Grösse der Fettkügelchen in Kuhmilch. Milch-Zeit., 24, p. 386.
- Rahn, O., and Sharp, P. F. 1928. Physik der Milchwirtschaft, pp. 35-69.
- Schellenberger, O. 1893. Ueber die Grösse und die Zahl der Fettkügelchen in der Milch von Kühen verschiedener Rassen. Milch-Zeit., 22, 49, pp. 817-819.
- Sturtevant, E. L. 1872-76. Milk: Physiological and miscellaneous. N. Y. State Agri. Soc., Trans. 32, pp. 91-124, pl. XII-XIV.
- Turner, C. W., and Haskell, A. C. 1929. A statistical study of the size of fat globules in cow's milk. Mo. Sta., Rsch. Bul. 130, 11 pp.
- Van Dam, W., and Sirks, H. A. 1922. Verlagen Landbouwkund. Onderz. d. Rijkslandbouwproefstations. Bd. 26, S. 106. (Reference by Rahn and Sharp—Physik der milchwirtschaft.)
- Van Leeuwenhoeck, A. 1674. Philosophical Transactions. 9, 102, p. 23; 106, p. 122.
- Weigmann, H. H. 1926. Ueber den Despersionsgrad des Fettes in der Milch. Milchwirtschaf. Forsch., 4, pp. 259-311.
- Woll, F. W. 1889-90. The number and size of fat globules in cow's milk. Wis. Sta., Rpt. 6, pp. 99-102 and 119-122; Rpt. 7, pp. 238-247.
- . 1892. On conditions influencing the number and size of fat globules in cow's milk. Agr. Sci., 6, pp. 441-453; 510-528; 537-553.
- . 1894. The fat globules in cow's milk. Wis. Sta., Rpt. 11, pp. 223-239.