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A Statistical Study of the Size of Fat Globules in Cows' Milk

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A Statistical Study of the Size of Fat Globules in Cows' Milk

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The observation that the fat in freshly drawn milk occurs in the form of minute particles or globules of varying size is credited to Van Leewenhoeck (1674) one of the first to work with the compound microscope. However, it is only within the last 75 years that any detailed study has been devoted to the determination of the variation in the size of these globules and to the more important factors which influence their mean size.

These studies revealed great differences in the size of the fat globules in the milk of various mammals. Pizzi (1895) states that the fat globules of rabbit milk are extremely large while the milk of the mare and the ass contain relatively small globules. As would be expected, the milk of the various breeds of dairy cattle has been studied rather extensively and considerable variation has been noted. While Fleischmann (1920) states that the fat globules in cow's milk may be as large as 22 micra in diameter, practically all observers find the variation in size between the limits of 0.1-10 microns.

BREED VARIATION

In the studies of the size of the fat globules of the various breeds of dairy cattle, the investigators have almost without exception reported only the mean or average size, whereas it is of equal if not greater importance to determine the variation in size as well. Thus Sturtevant (1872), Lamson (1888), Woll (1890-1892-1894), Collier (1891-1892), Schellenberger (1893), Gutzeit (1895), Cooper, Nuttall and Freak (1912) report the mean size of the fat globules in the milk of a number of breeds of cattle but fail to give the distribution in the various size classes. Weigmann (1927), in a recent study, is the first to indicate the frequency distribution of the globules in eight breeds of cattle.

The objects of the present study were: (1) To determine the most suitable method of measuring the diameter of large numbers of fat globules, and (2) To determine the type of frequency distribution and the most suitable statistical measures of the mean and the dispersion.

*The data presented in this paper were taken from a thesis submitted by the junior author in partial fulfillment of the requirements for the degree of Master of Arts in the Graduate School of the University of Missouri, 1928.

PREPARATION OF THE MILK FOR EXAMINATION

The samples of milk obtained from the dairy herd of the Missouri Experiment Station were thoroughly mixed and prepared for examination soon after milking to prevent clumping of the globules. One-half cubic centimeter of milk from each sample was placed in a watch glass and to it was added a cubic centimeter of a glycerin-cochineal stain mixture.* After thorough mixing, several drops of the prepared milk were mounted on slides for examination. The following method was employed. Two or three rings of vaseline, about one and one-half centimeters in diameter, were made on each slide to hold the cover glass and seal the preparation. In placing on the cover glasses, a little edge of the vaseline ring was left uncovered to allow the air to escape while the cover glass was being pressed down until the area surrounded by the vaseline ring was filled by a thin film of milk. The cover glass was then gently pushed over to close the opening and thus seal the preparation. The slides remained in good condition for several days. The slides were then left in a flat position for an hour or more to allow the fat globules to rise.

A permanent record of the field was made by means of a micro-photograph as suggested by Cooper, Nuttall and Freak (1912). A Leitz microscope with a two millimeter objective and 10X ocular was used giving a magnification of 920 times. Four by five inch photographs were taken with a micro-photographic camera adjusted to give a magnification of 1000 times. The photographs were taken on enlarging paper instead of negatives. Several photographs were taken of each sample A little practice is necessary to estimate the intensity of the light and the length of exposure necessary to obtain the best photographs.

ENUMERATION AND MEASUREMENT OF FAT GLOBULES

The size of the globules was determined by comparing the photograph of the globules with holes (of known diameter), in a transparency (celluloid). The holes were made in half-millimeter sizes from one to twelve millimeters. With the magnification used (1000 times) each millimeter represented one micron. Each globule was punched, with a pin, to prevent recounting, when it was measured. The frequency of the various sizes was recorded as they were measured.

*The cochineal stain is prepared by boiling 6 grains of powdered cochineal in 90 c.c. of distilled water for one-half hour, filtering while hot and adding enough distilled water to bring the volume to 90 c.c. One c.c. of this stain was then added to 10 c.c. of glycerin.

STATISTICAL STUDY OF THE DATA

The mean size of fat globules has been studied by several investigators, as indicated, but none have reported studies on the frequency distribution and the statistical constant (standard deviation and coefficient of variation). The microphotographic method used in this investigation made it possible to study a large number of samples. It was, therefore, possible to study the distribution and statistical constants of a large population of fat globules.

TABLE 1.—FREQUENCY DISTRIBUTION OF FAT GLOBULES

Class μ	Ayrshire Milk			Jersey Milk			Holstein Mlik		
	Frequency Distribution			Frequency Distribution			Frequency Distribution		
	Diameter in micra	Percentage of Distribution	Cubic micra*	Diameter in micra	Percentage of Distribution	Cubic micra*	Diameter in micra	Percentage of Distribution	Cubic micra*
	μ			μ			μ		
0.5	116	3.32	14.5	6	0.22	.7	309	2.08	39.0
1.0	464	13.30	464.0	74	2.71	74.0	1316	8.86	1316.0
1.5	591	16.93	1994.6	105	3.85	354.3	1842	12.40	6217.0
2.0	672	19.26	5376.0	277	10.16	2116.0	2794	18.81	22352.0
2.5	669	19.17	10453.1	452	16.57	7062.5	3282	22.10	51281.0
3.0	381	10.92	10287.0	441	16.17	11907.0	1821	12.26	49167.0
3.5	224	6.42	9604.0	380	13.93	16292.5	1269	8.54	54408.0
4.0	140	4.01	8960.0	252	9.24	16128.0	792	5.33	50688.0
4.5	84	2.41	7654.0	215	7.88	19591.9	494	3.32	45016.0
5.0	68	1.95	8500.0	230	8.43	28750.0	431	2.90	53875.0
5.5	40	1.15	6655.0	108	3.96	17968.5	221	1.49	36769.0
6.0	20	0.57	4320.0	73	2.68	15768.0	118	0.79	25488.0
6.5	12	0.34	3295.5	56	2.05	15379.0	92	0.62	25265.0
7.0	6	0.17	2058.0	33	1.21	11319.0	42	0.28	14406.0
7.5	3	0.08	1265.6	7	0.26	2953.0	21	0.14	8859.0
8.0				6	0.22	3072.0	5	0.03	2560.0
8.5				7	0.26	4298.9	2	0.01	1228.0
9.0				5	0.18	3645.0	2	0.01	1458.0
9.5									
	3490.0	100.00	80901.3	2727	100.00	176680.3	14853	100.00	450392.0

*The frequency in cubic micra or the volume of frequency was obtained by multiplying the cube of the diameter by the frequency. See text.

In Table 1 is presented a summary of the diameter frequency distribution for samples of milk of the Ayrshire, Jersey, and Holstein breeds. For purposes of comparison, the percentage frequency distribution of the fat globules is also calculated. These results are also plotted in Figure 1.

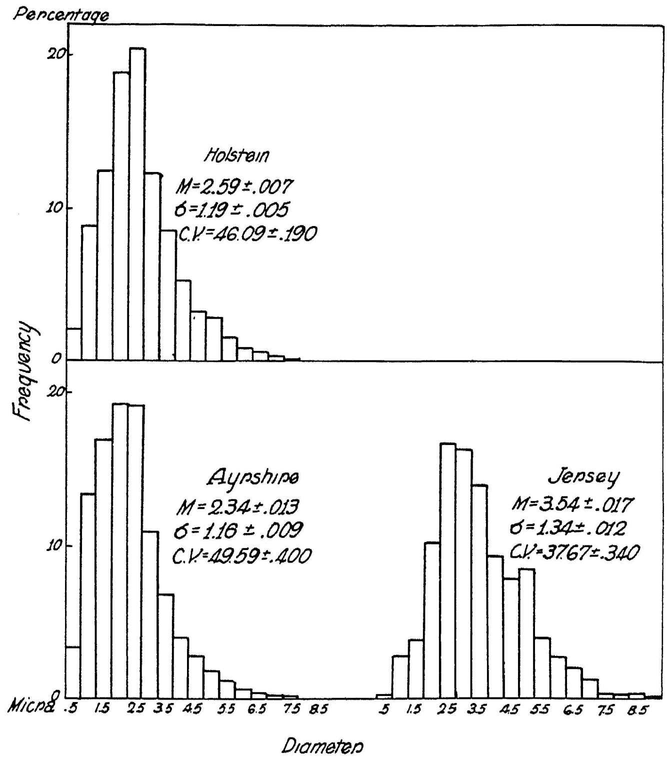

Figure 1.—Histogram showing the percentage frequency distribution of the diameter of fat globules in the milk of the Ayrshire, Jersey, and Holstein breeds.

As the arithmetic mean, standard deviation, and coefficient of variation is based on the concept that the frequency distribution is normal, the data were plotted on graph paper to note the shape of the frequency curve. (See figures 2, 3 and 4.) These figures show that there was a marked degree of positive skewness in the curves.

For further confirmation, the data were plotted on arithmetic and logarithmic probability paper, and it was found that the frequency distribution did not follow either a normal or logarithmic curve. The normal (calculated) frequency curves for these frequency distributions did not coincide in the least with the curves plotted from the observed data.

The Chi-square test of goodness of fit of the frequency curve showed that the curve plotted from the observed frequency did not follow a normal frequency curve.

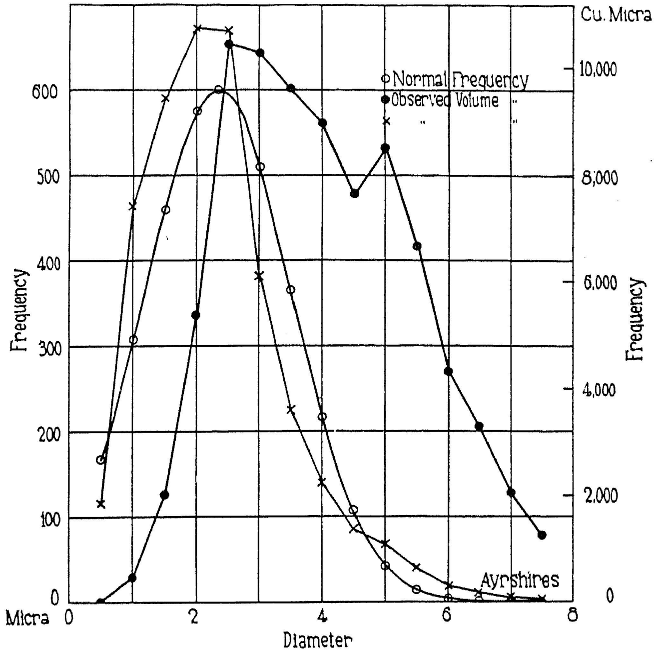

Figure 2.—Shows the observed frequency, the frequency expressed in cubic micra (volume frequency) and the normal arithmetic frequency distribution calculated from the data for the Ayrshire breed.

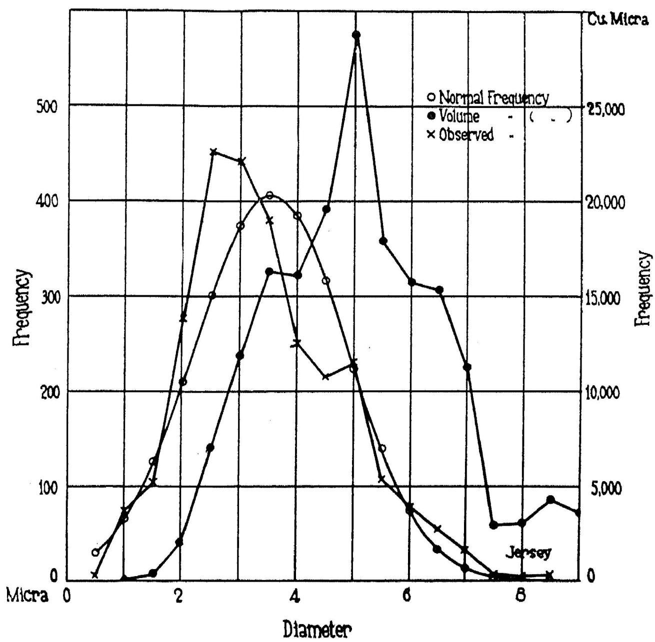

Figure 3.—Shows the observed frequency, the frequency expressed in cubic micra (volume frequency) and the normal arithmetic frequency distribution calculated from the data for the Jersey breed.

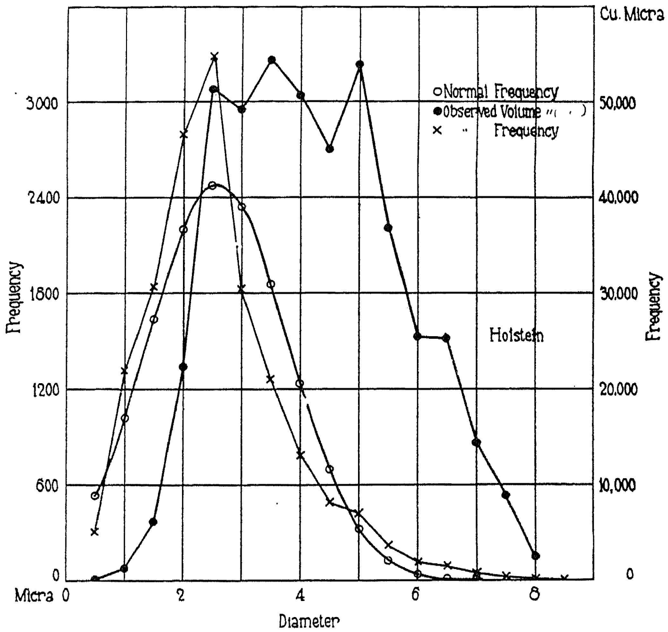

Figure 4.—Shows the observed frequency, the frequency expressed in cubic micra (volume frequency), and the normal arithmetic frequency distribution calculated from the data for the Holstein breed.

The volume frequency* was then studied to determine whether or not the data followed a normal curve of distribution. When the volume frequency was plotted on arithmetic probability paper it gave a curved line indicating that the data did not follow a normal frequency curve. However, when plotted on logarithmic probability paper, a line was obtained which was practically straight, (fig. 5) indicating that the data follows a logarithmic frequency curve fairly well.

It has already been noted that the calculations of the statistical constants are based on the concept that the frequency distribution is normal; the fact that the diameter frequency is neither normal nor logarithmic, indicates that neither the arithmetic nor geometric mean is entirely suitable as a statistical measure. Consequently the standard deviation is subject to some error. (The diameter frequency distribution appears to approach Pearson's type III curve, but the fit of this curve to the data was not determined.)

*The volume frequency was obtained by multiplying the cube of the diameter by the frequency. This does not give the true volume, but it is comparable to the true volume, which is obtained by multiplying the figure used for the volume frequency by the constant .5236. The volume of a sphere is obtained by multiplying the cube of the diameter by .5236. The volume is given in cubic micra.

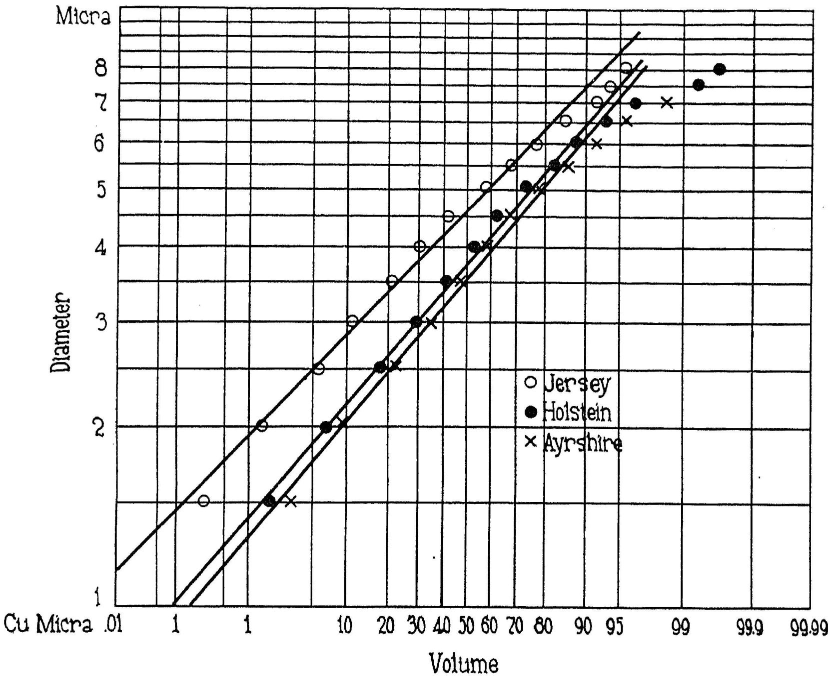

Figure 5.—The volume frequencies for the Ayrshire, Jersey, and Holstein breeds plotted on logarithmetic probability paper. If the distribution is normal the cumulative percentage distribution when plotted should follow a straight line.

This observation indicated that the statistical constants for the arithmetic volume frequency were not the most suitable measures. The geometric mean and the standard deviation ratio were believed to be more suitable measures. The geometric means is defined as the nth root of the product of a series of n terms. It was calculated by using the logarithms of the classes (diameter) and multiplying these by their frequency. The summation of the products thus obtained was divided by the total volume frequency. This result gave the logarithm of the geometric mean. The antilog of this was the geometric mean.

The standard deviation ratio was calculated by determining first the product of the logarithms of the classes times their volume frequency, and using this as the frequency distribution; then, the calculation was the same as for the standard deviation. The result is expressed as the logarithm of the standard deviation ratio; the antilog of this is the standard deviation ratio.

The geometric mean and standard deviation ratio were determined only for the volume frequency for the Holstein, Jersey and Ayrshire breeds, but not for the individuals in these breeds.

Table 2 summarizes the statistical constants calculated for the frequency, and for the arithmetic and geometric means of the volume frequency, for each breed.

TABLE 2.—STATISTICAL CONSTANTS FOR BREEDS

Frequency in Cubic Micra	Jersey	Holstein	Ayrshire
Geometric Mean	4.85 u (dia.)	4.00 u (dia.)	3.75 u (dia.)
Standard Deviation Ratio	1.00	1.04	1.03
Arithmetic Mean	5.06 ± .0025	4.29 ± .0015	4.02 ± .0035
Standard Deviation	1.57 ± .0018	1.51 ± .0011	1.46 ± .0024
Coefficient Variation	30.87 ± .0350	35.28 ± .0240	36.23 ± .0610
Diameter of Av. Volume	4.01	3.12	3.27
Frequency of globules (diameter in micra)			
Arithmetic Mean	3.54 ± .0170	2.59 ± .0066	2.34 ± .0130
Standard Deviation	1.34 ± .0120	1.19 ± .0047	1.16 ± .0094
Coefficient Variation	37.67 ± .3400	46.09 ± .1900	49.59 ± .4000

SUMMARY

1—A statistical study of the size of fat globules in cow's milk is reported. The micro-photographic method was employed in this study because of its rapidity in examining large numbers of samples and the permanency of the record of the observations.

2—Glycerin and cochineal stain were used to prevent Brownian movement and refraction of light by the globules; thus, the outline was made more distinct.

3—The globules were enumerated by the comparison of the photograph of the fat globule with graduated holes in a transparency, made in half millimeter sizes varying from one to twelve millimeters in diameter, each millimeter representing one micron.

4—The statistical constants for the arithmetic diameter frequency distribution were determined for each breed. The mean diameter and standard deviation for the Jersey breed was found to be 3.54 and 1.33 micra, for the Holstein 2.59, and 1.19 micra, and for the Ayrshire 2.34 and 1.16 micra.

5—The statistical constants for the arithmetic mean using the volume distribution were determined for each breed. The mean and standard deviation for the Jerseys were 5.07 and 1.56 micra, for the Holsteins 4.29 and 1.51 micra, and for the Ayrshires 4.02 and 1.46 micra.

6—The geometric mean and standard deviation ratio were determined for each breed. They were found to be for the Jerseys 4.85 and 1.00 micra; for the Holsteins 4.00 and 1.04 micra; and for the Ayrshires 3.75 and 1.03 micra.

7—The diameter of the globule of average volume was found to be 4.01 micra for the Jerseys; 3.12 micra for the Holsteins; and 3.27 micra for the Ayrshires.

Since it was found that the logarithmic distribution for the volume frequency follows more nearly a normal logarithmic curve, the geometric mean and standard deviation ratio are believed to be more appropriate statistical constants than the arithmetic mean and standard deviation.

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