JOURNAL OF DAIRY SCIENCE

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ANNUAL MEETING
JUNE 22-24, '54
STATE COLLEGE, PA.
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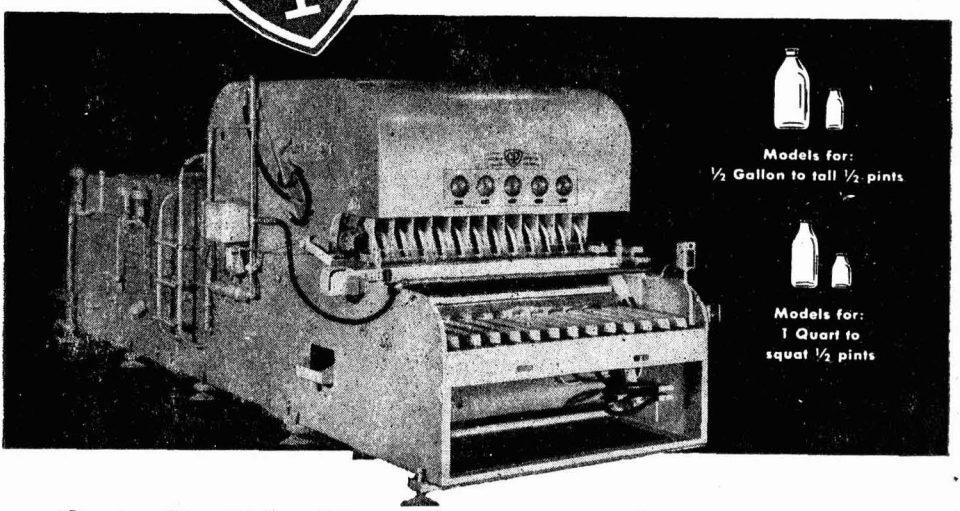

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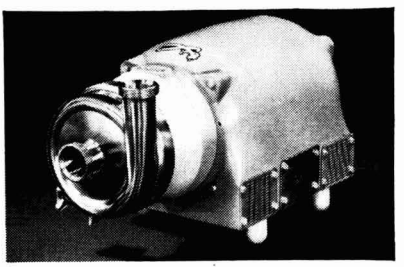

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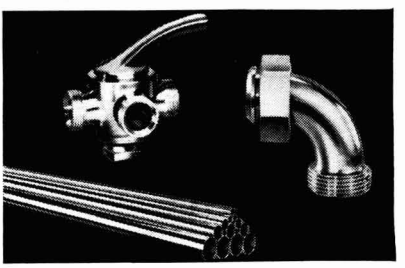

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VOLUME XXXVII February, 1954 NUMBER 2

RELATIONSHIP BETWEEN FAT GLOBULE SURFACE AREA AND CAROTENOID AND VITAMIN A CONTENT OF MILK IN SUCCESSIVE PORTIONS OF A MILKING[1]

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AND

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There have been several reports that the carotenoid and perhaps the vitamin A content of the fat remaining in skimmilk after the separation process may be higher than that of the original whole milk fat *(2, 9, 11, 15)*. Kon *et al. (11)* and Francois *(9)* have suggested that, since the fat of skimmilk represents only the smallest of the fat globules of whole milk, the reported differences in carotenoid and vitamin A content might be correlated with differences in globule size. Smaller globules have greater surface areas, relative to their volumes, than do larger globules, and Kon *et al.* suggested a relationship, for carotenoids, to the "surface-volume" ratio. They found no such differences for vitamin A. However, Francois found these differences for both carotenoids and vitamin A and proposed a surface layer, on the fat globule, highly concentrated in these substances. In general, differences in carotenoid concentration between skim and whole milk fat have been reported to be greater than those for vitamin A.

Average globule diameter has been reported to increase through successive portions of a milking *(5, 10, 12, 13, 15)*, and this, taken in the light of the above discussion, might explain the reported decline in carotenoids per gram of fat in such portions *(8)*. Eaton *et al. (8)* did not find that vitamin A per gram of fat showed any trend in successive portions of a milking. The present study was undertaken to make a strict quantitative study of the relationship between fat globule size and carotenoid and vitamin A content.

Received for publication April 30, 1953.

[1] The data were taken from a thesis submitted to the Graduate School of the University of Connecticut by the senior author in partial fulfillment of the requirements for the M.S. degree. The project was supported in part by funds provided by the Chas. M. Cox Co., Boston, Mass., and the Big-Y-Foundation, Norwich, Conn.

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กระทรวงอุตสาหกรรม

EXPERIMENTAL

Animals. Four first-lactation Holstein cows were assigned one at a time, for a 2-week period each, to the experiment. They were fed a 13.4% crude protein grain mixture (1 lb. to 4 lb. of actual milk produced) and U. S. No. 1 alfalfa hay ad libitum and were pastured on bluegrass-ladino clover (summer, 1951). Their

TABLE 1
Description of cows in experiment

	Cow			
	1	2	3	4
Mean daily production *(lb.)*	33.9	40.7	43.8	41.7
Stage of lactation *(days)*	94	190	72	56
Days in calf	7	104	0	0

mean daily productions through their 2-week periods, stages of lactation, and days in calf on assignment were as presented in Table 1.

Samples. Samples were taken at every third evening milking, using a special valve developed for the experiment *(19)*. By this method, the milk from a single quarter could be divided into four successive portions without disturbing the routine machine milking of the three other quarters. Each of the four quarters of every cow was sampled once during the experiment, making a total of 16 experimental milkings.

A sample for gravity skimming was taken from each portion. This was contrasted, in subsequent analyses, with whole milk from that portion in order to determine the effect of a change in globule size while holding stage-of-milking constant. All samples were analyzed for fat percentage by ether extraction *(1, 11)* and for carotenoid and vitamin A content *(3)*. Photomicrographs of the samples were taken, after suitable dilution, for globule size determination *(4, 6, 18)*. The diameters of 200 to 600 globules were measured in each sample at a magnification of exactly 1,000.

The portions were each identified by a decimal fraction representing the stage-of-milking. This was obtained by dividing the total weight accumulated up to halfway through each of the successive portions by the total weight of the milking (nearest gram). These fractions, varying from 0 to 1, are symbolized in the following discussion by the variable M.

The diameter, d, of each globule was measured, in microns, to the nearest half-micron. The sum of the squared diameters and the sum of the cubic powers of diameters then were calculated in each sample. Assuming that the globules were spherical, the total globule surface area was $\pi\Sigma d^2$. The total globule volume would then be $\pi\Sigma d^3/6$. Thus, by dividing the sum of the squares by one-sixth the sum of the cubes, the surface-to-volume ratio, $6\Sigma d^2/\Sigma d^3$, was obtained in reciprocal micron units, and is symbolized below by the variable G. This quantity is neither mathematically nor physically equal, in general, to six times the reciprocal of the mean diameter, $6/\bar{d}$. It is clear, moreover, that this latter quantity is meaningless for the testing of the hypothesis that the total carotenoid and

vitamin A content is related to the total surface area of fat globules in the sample of milk.

Statistics. Quantitative relationships were explored by the fitting of least-squares regressions. Differences between cows and milkings were eliminated by fitting average within-milking regressions *(16)*.

RESULTS

The fat globule surface-to-volume ratio and the carotenoids and vitamin A, both expressed as micrograms per gram of fat, were higher in the skimmilk than in the whole milk from which it was separated (Table 2). This offered qualitative support to the contention that carotenoids and vitamin A seem to be concentrated at the fat globule surface.

TABLE 2

Differences between skimmilk and the whole milk from which it was separated in carotenoids and vitamin A per gram of fat and in the fat globule surface-to-volume ratio

Criterion	Skimmilk[a]	Whole milk[a]	Difference	Significance
Carotenoids per g. of fat	18.06γ	9.26γ	8.80γ	$P < 0.01$
Vitamin A per g. of fat	9.71γ	7.37γ	2.34γ	$P < 0.01$
Fat globule surface to vol. ratio	2.2935μ^{-1}	1.6494μ^{-1}	0.6441μ^{-1}	$P < 0.001$

[a] These values are the averages of 60 (carotenoids and vitamin A) and 64 (globule surface-to-volume) milk samples. The mean of the four samples of a milking was considered a single independent observation. A carotenoids-vitamin A aliquot from one of the milkings was lost in processing, and the three other portion-samples of this milking were not considered in this stage of the analysis.

In the results below, the quantitative investigation was confined to the whole milk data. If the skimmilk data also had been used, a wider range of globule surface-to-volume ratio and carotenoid and vitamin A content would have been sampled. Such data no doubt would have demonstrated the point more impressively, but this would have confounded the description of trends by departing from the normal bovine secretion.

Trends with globule surface-to-volume ratio. Both carotenoids and vitamin A per gram of fat were found to increase directly with globule surface-to-volume ratio (whole milk values only). The average linear regressions were significant for both substances ($P < 0.05$), and neither showed a tendency to curvature (Figure 1).

Trends with stage-of-milking. Vitamin A per gram of fat, as previously reported *(8)*, showed no trend with stage-of-milking. Carotenoids per gram of fat, on the other hand, tended to decline to a minimum at a point approximately midway through the milking, and thereafter to increase, in contrast to the previous report *(8)*, which indicated a steady decline for carotenoids per gram of fat through the entire milking. This highly significant trend ($P < 0.001$) was described by the regression:

1. $R = 10.02 - 4.692\,M + 5.288\,M^2$

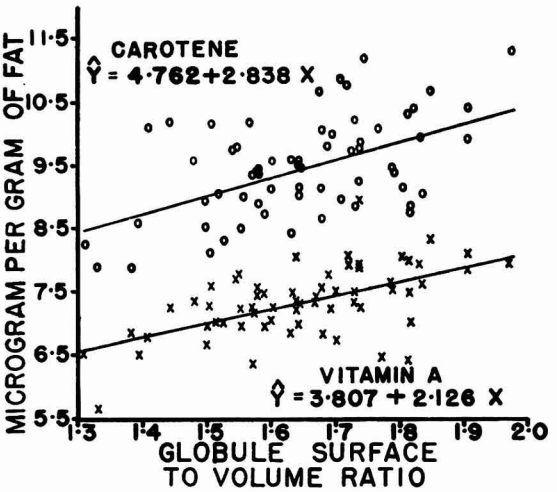

FIG. 1. Relationship between carotenoids, vitamin A, and globule surface-to-volume ratio in samples of whole milk. The plotted observations have been adjusted so as to eliminate differences among the 16 milkings, the necessary adjustments originating in a covariance analysis.

where M = stage-of-milking, varying from 0 to 1, and R = micrograms carotenoids per gram of fat. The minimum value of R with respect to M in this equation can be found by differentiation, showing the minimum value to occur at $M = 0.44$ (approximately midway). The previous report *(8)* investigated colostral milkings, whereas the present study was conducted on cows relatively advanced in lactation.

No significant trend with stage-of-milking was found for globule size, expressed either as surface-to-volume ratio or mean diameter. Consequently, there appears to have been some other and unknown stage-of-milking variable in addition to globule size affecting carotenoids per gram of fat.

Although not of interest to the main problem, the relationships of other whole milk variables to stage-of-milking also were investigated. Thus, carotenoids and vitamin A per gram of milk each increased with stage-of-milking ($P < 0.001$), not directly but in relationships that curved upwards with milking progress ($P < 0.001$ and < 0.05). The average regressions were:

2. $C = 0.259 - 0.110\,M + 0.398\,M^2$

and

3. $B = 0.199 - 0.00794\,M + 0.196\,M^2$

where C = micrograms carotenoids per gram of milk and B = micrograms vitamin A per gram of milk.

Fat percentage increased with stage-of-milking ($P < 0.001$), a fact which has long been recognized *(13)*. A tendency to curve upwards with milking progress was not significant. The average linear regression was:

4. $F = 2.211 + 2.771\,M$

where F = grams fat per 100 g. milk, indicating that fat percentage more than doubled between first- and last-drawn milk.

MATHEMATICAL CONSIDERATIONS

Perhaps the most interesting relationships indicated are those under *Trends with globule surface-to-volume ratio* and Figure 1. Apparently, there was a direct proportionality between either carotenoids or vitamin A per gram of fat, on the one hand, and globule surface-to-volume ratio, on the other. The relationship is not materially altered by canceling out "gram of fat" and "globule volume," since these would bear some constant proportionality to each other. This leaves direct linearity between total carotenoids and vitamin A and the total globule surface area. The linearity suggests surface layers of constant depths (i.e., independent of globule diameter). An attempt to expand this and other considerations follows.

Assume a sample of fat globules with total surface area $S\mu^2$ and total volume $V\mu^3$. The density of fat is $Q\gamma/\mu^3$, and the carotenoids per gram of fat is R (in units of γ/g.). Then:

5. Globule surface-to-volume ratio $= S\mu^2/V\mu^3 = G$
6. Total weight of fat in sample $= QV\gamma = QV \times 10^{-6}$ g.
7. Total weight of carotenoids in sample $= RQV \times 10^{-6}$ g. (in γ units)

If R is a linear function of G, viz.: $R = a + bG$, then:

8. Total weight of carotenoids $= (a + bG)\ (QV \times 10^{-6}$ g.)
$= (aQV \times 10^{-6}$ g.) $+ (bGQV \times 10^{-6}$ g.)
$= (aQV \times 10^{-6}$ g.) $+ (b[S\mu^2/V\mu^3]\ QV \times 10^{-6}$ g.)
$= (aQV \times 10^{-6}$ g.) $+ (bQS/\mu) \times 10^{-6}$ g. (in γ units)

Thus, the total weight of carotenoids can be factored into two components: one, varying with V, the total globule volume, will be called the "interior component"; the other, varying with S, the total globule surface area, will be called the "surface component."

Surface component. As indicated above, the weight of the "surface" carotenoids appears to be:

9. $(bQS/\mu) \times 10^{-6}$ g. (in γ units)

The quantity b is expressed in units of $\mu\gamma$/g. For simplicity, factor out the units and substitute for b the quantity b', the numerical value of b without units. The above quantity, 9, then becomes:

9a. $b'QS \times 10^{-6}\ \gamma$

If the density of the surface carotenoids is $W_C\gamma/\mu^3$, then:

10. Volume of "surface" carotenoids $= b'S(Q/W_C) \times 10^{-6}\mu^3$

If the "surface" carotenoids are in a layer-form on the globule surfaces, then, since layer thickness = layer volume per layer area, and the layer area is $S\mu^2$, the total globule surface area:

11. Apparent layer thickness $= b'\ (Q/W_C) \times 10^{-6}\mu$
$= b'(Q/W_C) \times 10^{-10}$ cm.

Sommer *(17)* has presented data supporting a monomolecular phospholipid membrane of thickness $4.4 \times 10^{-3}\mu$. Therefore:

12. Total volume of phospholipid membrane $= 4.4\,S \times 10^{-3}\mu^3$
13. Total weight of phospholipid membrane $= 4.4\,PS \times 10^{-3}\gamma$
    where $P\gamma/\mu^3$ is the density of the membrane.

If it is assumed that the surface carotenoids are entirely associated with the phospholipid membrane, then dividing the weight of the surface carotenoids (Equation 9a) by the weight of the phospholipid membrane (Equation 13) should yield the concentration of carotenoids in the surfaces of the globules. Thus:

14. Concentration in phospholipid membrane
    $= (b'QS \times 10^{-6}\gamma)/(4.4\,PS \times 10^{-3}\gamma)$
    $= b'(Q/P) \times 10^{-2}/44 \sim (b'/44)\ \%$

Note that Q and P, the densities of fat and phospholipid, respectively, are assumed to be almost exactly equal, following Sommer *(17)*.

If no assumption is made about the association of the "surface" carotenoids, then it is not possible to express the concentration in terms of units of volume or weight, since there is no knowledge of the depth of the zone in which the surface component lies. In equations involving adsorption, surface concentrations are often expressed per unit of area. Thus:

15. Concentration of carotenoids in surface $= b'Q \times 10^{-6}\ \gamma/\mu^2$
    $= b'Q \times 10^{2}\ \gamma/\text{cm.}^2$
    Assuming a fat density of 0.9 g./cm.^{3}, then $Q = 9 \times 10^{-7}$ (in units of γ/μ^3) and

15a. Concentration of carotenoids in surface $= 9b' \times 10^{-5}\gamma/\text{cm.}^2$

Interior component. The weight of the "interior" carotenoids (Equation 8) appears to be:

16. $aQV \times 10^{-6}$ g. (in γ units)

Making a substitution similar to that in 9a, we have:

16a. $a'QV \times 10^{-6}\ \gamma$

This is the carotenoids apparently not concentrated at the surface. The concentration in the fat may be found by dividing by the weight of fat, $QV\ \gamma$ (Equation 6):

17. Interior concentration $= a' \times 10^{-6} = a' \times 10^{-4}\ \%$

Application. All the foregoing remarks on carotenoids may be applied to vitamin A. For quantitative application, estimations of the values a' and b' are necessary. The slope terms fitted to the data (Figure 1) seem reasonable for this purpose. Because the intercepts fall outside of the range of experience of the data, they are not directly determined experimentally. This is inherent in the method, because the obtaining of a value G = 0 in actual experience requires globules of infinitely large diameters. Therefore, any conclusions based on the intercept values from these data must be considered as first approximations only.

DISCUSSION

If the relationship between carotenoids or vitamin A per gram of fat and globule surface-to-volume ratio is truly linear, and if the slope terms of Figure 1 are good estimations of the actual slope terms, it then follows that Equation 11 results in good estimations of the apparent thicknesses of the carotenoid and vitamin A surface layers. Inserting the experimentally determined values for b' into Equation 11 yields:

18. Apparent thickness of carotenoid "layer" $= 2.838 \times 10^{-10} (Q/W_C)$ cm.
19. Apparent thickness of vitamin A "layer" $= 2.126 \times 10^{-10} (Q/W_A)$ cm.

where Q is the density of milk fat, W_C the density of the carotenoid "layer," and W_A the density of the vitamin A "layer."

Q is a determinable quantity; the W's are not. However, if the respective layers were composed entirely of carotenoids or vitamin A, their densities, W_C and W_A, would be of the same order of magnitude as the density of fat, Q. This would leave zones of thickness less than 1/100 the order of molecular diameters *(7)*, an impossible situation. We are left with the alternative that the layers are not close-packed, but are highly dilute solutions or molecules widely spaced. From this point of view, W_C and W_A are much less than Q and the above values have meaning (provided the W's could be evaluated). However, some qualitative knowledge of the nature of the surface layers seems to have been gained.

TABLE 3

Apparent concentrations of carotenoids and vitamin A in various portions of the fat globule

Globule portion	Carotenoids	Vitamin A
Phospholipid membrane[a] *(% by wt.)*	(2.838/44) = 0.0645	(2.126/44) = 0.0483
Globule surface[a] *(γ/cm.²)*	2.6×10^{-4}	1.9×10^{-4}
Globule interior *(% by wt.)*	0.000476	0.000381

[a] Only one of these is applicable, depending on the assumptions accepted. See text.

Table 3 follows from the insertion of values from the data into Equations 14, 15a, and 17. The stated concentrations in the phospholipid membrane, based on the assumption that the surface components of carotenoids and vitamin A are entirely associated with the membrane, would seem to contradict the argument that the surface components are not close-packed. The assumption is equivalent to setting the apparent thickness of either of the components (Equations 18 and 19) equal to 4.4×10^{-3} μ, the thickness of the phospholipid membrane, according to Sommer *(17)*. Inserting a fat density of 0.9 g./cm.³ and solving for W_C and W_A, we find the apparent densities of surface carotenoids and surface vitamin A to be 5.8×10^{-4} g./cm.³ and 4.4×10^{-4} g./cm.³, respectively. Thus, even under this assumption, the layers are not close-packed.

Without this or some similar assumption regarding the depth of the surface zone, the data provide no means of arriving at estimates of the concentrations at the surface comparable to the "interior" concentrations of Table 3. Consequently, the concentrations given under *Globule surface* in Table 3, being expressed in units of area, must not be directly compared with those given under *Globule interior*, which are given as percentages by weight.

SUMMARY

Single-quarter milkings of first-lactation Holsteins were divided into successive portions, and skimmilk and whole milk samples were taken from each portion. These samples were analyzed for fat percentage, vitamin A and carotenoid content, and globule size distribution. Skimmilk was significantly higher in carotenoids and vitamin A per gram of fat and also in the globule surface-to-volume ratio than was the whole milk from which it was separated. This indicated a possible concentration of carotenoids and vitamin A at the globule surface.

Considering the whole milk data only, both carotenoids and vitamin A per gram of fat had significant positive linear trends with globule surface-to-volume ratio. By considering the magnitude of the trends, it was possible to reject the possibility of continuous surface layers of carotenoids and vitamin A and to advance, instead, a possible dilute solution or loose chemical complex on the globule surface. Values were advanced for the concentrations of carotenoids and vitamin A in the various portions of the fat globule.

Carotenoids per gram of fat showed a highly significant tendency to decline to a point near the milking mid-point and thereafter increase. Vitamin A per gram of fat showed no trend with stage-of-milking. Carotenoids and vitamin A per gram of milk increased, with stage-of-milking, in upward-curving trends.

Globule size, expressed either as surface-to-volume ratio or mean diameter, showed no trend with stage-of-milking. Fat percentage increased significantly with stage-of-milking, more than doubling its value from the beginning to the end of the milking. There was no apparent tendency to curvature.

ACKNOWLEDGMENTS

The authors are indebted to G. Beall for primary aid and advice in the course of this work. K. L. Dolge performed the carotenoid and vitamin A analyses and Mrs. E. S. White aided in the fat globule measurements and in the statistical analysis. E. G. Boettiger, L. D. Matterson, M. E. Morgan, and M. J. Walker gave technical advice at various stages. To all these the authors are deeply grateful.

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A METHOD FOR THE DETECTION OF FOREIGN FATS IN DAIRY PRODUCTS

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The Reichert-Meissl, Polenske, and Hehner number has served to distinguish butterfat from other edible fats and to detect admixtures of the latter with butterfat *(1)*. In addition, methods based on differences in the type of sterol *(4)*, the refractive index *(6)*, and the amount of tocopherol *(3)* have been suggested as a means of detecting foreign fats in butterfat. However, none of these methods seem to give reliable results if the foreign fat is lard, beef tallow, or a mixture composed of 60-80% coconut oil and 40-20% hydrogenated cottonseed oil and if these foreign fats are present in concentrations of less than 30% *(5)*.

In the present study, a method for the detection of admixtures of 10% or more of vegetable or animal fats with butterfat is described. This method is based on the fact that fats are composed of a mixture of triglycerides which have different solubilities in absolute ethanol and esterify at different rates under controlled conditions *(5)*.

EXPERIMENTAL PROCEDURE

Butter was melted and extracted with ether and washed once with water; the ether extract was dehydrated with sodium sulfate and freed of solvent on a water bath. Ten g. of melted fat was weighed into a 250-ml. Erlenmeyer flask, 100 ml. of hot absolute ethanol added with gentle rotation of the flask, and the clear solution allowed to cool to room temperature. When cool, the flask was immersed for 2 hours in a water bath kept at 20° C. The mixture was filtered and the precipitate washed once with 10 ml. of absolute ethyl alcohol. The filtrate and the washing were combined and the volume was noted. The precipitate was dried on a steam bath and transferred to a vacuum desiccator, and the refractive index [2] of the melted fat was determined at 40° C.

A 10-ml. aliquot of the filtrate or alcohol soluble fraction was transferred to a small tared petri dish, freed of solvent on a steam bath, transferred to a vacuum desiccator, and weighed. This value times the total volume of the alcohol soluble fraction gave the weight of the alcohol soluble fraction. The refractive index of the residue was determined at 40° C. Seventy-five ml. of the remaining filtrate was poured into a 250-ml. Erlenmeyer flask, and 5 ml. of 0.001% sodium methoxide solution was added with gentle rotation of the flask. After 1 hour, 3 drops of 1%

Received for publication June 12, 1953.

[1] Armour research fellow. Funds for partial support of these studies were made available by the American Dairy Association and Armour & Co., Chicago.

[2] All refractive indices were determined with a Zeiss refractometer.

phenolphthalein were added and the sodium methoxide was neutralized with dilute phosphoric acid. Ten ml. of water was added with gentle rotation of the flask, and the unesterified triglycerides were allowed to settle out. A 50-ml. aliquot of the clear solution was transferred to a 250-ml. separatory funnel, 50 ml. ethyl ether and 50 ml. of water were added, the separatory funnel was shaken gently, and the aqueous phase was discarded. The ether phase was washed with water, dried with anhydrous sodium sulfate, transferred to a 100-ml. beaker, and freed from solvent, and the refractive index of the residue was determined at 40° C.

The sodium methoxide catalyst was prepared by dissolving 4.3 g. of clean metallic sodium in 80 ml. of absolute methyl alcohol, cooling, and diluting to exactly 100 ml. with methyl alcohol. Ten ml. of this solution was diluted to 1 l. with absolute ethyl ether as needed. One ml. of the latter contained 1 mg. of sodium methoxide. The dilute phosphoric acid was prepared by diluting 0.71 g. of 85% phosphoric acid to 1 l. One ml. of this solution will neutralize 1.0 mg. of sodium methoxide.

The rearranged fat was prepared by the addition of 1 ml. of 0.001% sodium methoxide in absolute ether to every gram of moisture-free fat and allowing the mixture to stand at room temperature for 1 hour. The catalyst was inactivated by adding dilute phosphoric acid; the fat was washed thoroughly with water, dried over anhydrous sodium sulfate, and freed from the solvent. The rearranged lard was prepared by mixing 7.5% of tributyrin with lard before it was subjected to rearrangement.

RESULTS

Samples of butterfat which had been collected at various times of the year and in different parts of the United States[3] were found to contain 70 ± 4% of alcohol soluble triglycerides at 20° C. (Table 1). Coconut oil was completely soluble, and hydrogenated cottonseed oil and rearranged lard contained approximately 26% and 40% of alcohol soluble fractions, respectively. Furthermore, the

TABLE 1

The solubility of various fats in ethanol at 20° C. and the refractive indices of the alcohol insoluble and soluble fractions

Fat	Solubility (%)	Refractive index		
		Whole fat	Soluble	Insoluble
Butterfat	70±4	1.4540—1.4542	1.4538—1.4541	1.4539—1.4544
Coconut oil	100	1.4492	1.4492	.....
Hydrogenated cottonseed oil	26	1.4620	1.4624	1.4610
Hydrogenated cottonseed oil[a]	38	1.4605	1.4620	1.4600
Rearranged lard	40	1.4592	1.4590	1.4590

[a] 20% mono and diglycerides added.

[3] Obtained through the courtesy of H. F. Long, Sugar Creek Creamery Co., Danville, Ill., and G. A. Crapple, Wilson & Co., Chicago.

refractive indices of the alcohol insoluble and the alcohol soluble fractions of butterfat did not vary appreciably from the refractive index of the whole fat. The refractive index of the alcohol soluble and the alcohol insoluble fractions of pure butterfat varied from 1.4538 to 1.4541 and from 1.4539 to 1.4544, respectively. After ethanolysis of the alcohol soluble fraction, the refractive index was found to vary from 1.4476 to 1.4482.

Admixtures of butterfat and 10% of the various fats commercially available as substitute fats for butterfat had different solubilities in ethanol, different refractive indices, and different rates of ethanolysis from pure butterfat (Table 2). The admixtures of butterfat and 10% of coconut oil yielded more alcohol

TABLE 2

The effect of adding 10% various fats to butterfat on the percentage of soluble material and the refractive indices of the alcohol soluble, insoluble and soluble ester fractions

Fat	Solubility (%)	Refractive index		
		Insoluble	Soluble	Soluble esters
None	71.3	1.4541	1.4540	1.4480
Coconut oil	75.2	1.4539	1.4532	1.4472
Hydrogenated coconut oil	70.9	1.4538	1.4533	1.4472
Hydrogenated cottonseed oil	63.3	1.4550	1.4543	1.4481
Hydrogenated fat[a]	67.1	1.4549	1.4541	1.4480
Hydrogenated fat[b]	61.8	1.4554	1.4547	1.4482
Palm oil	65.8	1.4546	1.4541	1.4478
Hydrogenated cottonseed oil[c]	74.0	1.4549	1.4542	1.4482
20% cottonseed + 80% coconut oil	70.9	1.4541	1.4534	1.4471

[a] Crisco; [b] Velvet; [c] 20% mono and diglycerides added.

soluble triglycerides and an alcohol soluble and ethyl ester fraction of lower refractive index than butterfat. On the other hand, most of the other substitute fats tested to date yielded less alcohol soluble triglycerides and an alcohol insoluble fraction of higher refractive index than butterfat. Mixtures of 70-80% of coconut oil with hydrogenated cottonseed oil, beef tallow, or lard could be made which yielded approximately the same proportion of alcohol insoluble triglycerides as butterfat. However, such mixtures contained enough coconut oil to affect the refractive index and the rate of ethanolysis of the alcohol soluble fraction.

The average refractive index of the alcohol insoluble fraction of 20 different samples of authentic butterfat was found to be 1.4542 $\pm$ 0.00002 (S.E.M.) and was significantly different from 1.4550 $\pm$ 0.00003 (S.E.M.), the average value of the samples containing 10% of substitute fat other than coconut oil. The addition of 10% coconut oil significantly lowered the refractive index of the alcohol soluble fraction of pure butterfat from 1.4540 $\pm$ 0.00002 to 1.4532 $\pm$ 0.00005.

A synthetic butterfat with a mixed fatty acid composition similar to butterfat (Table 3) was prepared by mixing 50% rearranged lard containing 7.5% tributyrin, 25% hydrogenated coconut oil, and 25% beef tallow. This mixture, as well as pure butterfat. was rearranged and 10% of the resulting product was added to pure butterfat. The rearranged butterfat and the rear-

TABLE 3

Comparison of the mixed fatty acid composition of butterfat with a mixture of rearranged lard containing 7.5% tributyrin, coconut oil, and beef tallow

Fatty acid	Butterfat (%)	Synthetic mixture (%)
Butyric	3.7	3.8
Caproic	1.7	0.0
Caprylic	1.0	2.2
Capric	1.9	1.7
Lauric	2.8	11.5
Myristic	8.1	5.3
Palmitic	25.9	23.1
Stearic	11.2	10.3
Oleic	32.8	40.9
Linoleic	3.7	4.8

TABLE 4

The percentage of soluble material from various fats and the refractive indices of the whole fat, the insoluble fraction, soluble fraction and the soluble esters.

Fat	Solubility (%)	Refractive Index			
		Whole fat	Insoluble	Soluble	Soluble ester
Butterfat	71.7	1.4540	1.4540	1.4538	1.4482
Rearranged butterfat	78.8	1.4530	1.4535	1.4531	1.4473
Synthetic butterfat	59.2	1.4562	1.4564	1.4552	1.4482
Rearranged synthetic butterfat	55.5	1.4553	1.4560	1.4547	1.4500
Butterfat + 10% rearranged butterfat	72.6	1.4539	1.4541	1.4533	1.4464
Butterfat + 10% rearranged synthetic butterfat	65.8	1.4542	1.4545	1.4538	1.4470

ranged synthetic butterfat both decreased the refractive index of the alcohol soluble and the soluble ester fraction (Table 4). The synthetic butterfat also increased the percentage of alcohol insoluble triglycerides and increased the refractive index of this fraction.

It would be advisable to run a known sample of pure butterfat as standard with the unknown. A rancid butter of high free fatty acid value will give inaccurate data and should not be used as a standard.

DISCUSSION

The small but consistent differences in the solubility and refractive indices of the alcohol insoluble and alcohol soluble fractions, which were always noted when another fat was mixed with butterfat, were due to the physical characteristics contributed by component triglycerides. Although a thorough analysis of the component triglyceride mixture of butterfat has never been attempted, Sommer *(7)* has pointed out that over 5,000 are theoretically possible. Each triglyceride has a specific melting point and refractive index which is dependent on the component fatty acids and their relative position to each other in the molecule (Table 5). A shift from an alpha to a beta position of the same fatty acid causes a shift in both melting point and the refractive index. The largest

TABLE 5

The effect of chain length on the melting point and refractive index of synthetic triglycerides (2)

Glyceride	Melting point (°C.)	Refractive index
16-18-16	68	1.4471
18-16-16	62	1.4467
12-18-18	54	1.4448
10-18-18	49	1.4444

C_{10} Caproic, C_{12} Lauric, C_{16} Palmitic, C_{18} Stearic acid.

shift is caused by the replacement of a saturated by an unsaturated fatty acid (Table 6). As each fat is composed of a specific mixture of triglycerides, it would not be possible to duplicate the exact mixture of triglycerides in butterfat

TABLE 6

The effect of unsaturated fatty acids on the melting point and refractive index of synthetic triglycerides (2)

Glyceride	Melting point (°C.)	Refractive index
S-O-S	41	1.4485
S-O-O	23	1.4524
S-S-O	38	1.4494
P-O-P	35	1.4476
P-O-O	19	1.4511

O—Oleic, P—Palmitic, S—Stearic acid.

by mixing various proportions of fats so as to obtain the correct mixed fatty acid composition of butterfat. The synthetic butterfat prepared in our laboratory by mixing beef fat, rearranged lard containing 7.5% tributyrin, and coconut oil had almost the same fatty acid composition as pure butterfat. However, it could be detected in butterfat at a 10% level as easily as any of the substitute fats presently available. Improvements in the synthetic butterfat might be made by the use of hydrogenated lard and hydrogenated rearranged coconut oil which contains butyric acid. Such a mixture of fats may have the same refractive index as butterfat. However, it still would have to be rearranged sufficiently to produce the same triglyceride composition as butterfat, and that may not be possible. Our data indicated that even rearranged butterfat contained a different proportion of triglycerides than the original butterfat.

It seems evident that substitute fats could be prepared from coconut oil and cheap animal fats which would have approximately the same Reichert-Meissl, Polenske, and Hehner number as butterfat. Furthermore, these fats would have the same tocopherol and butyric acid content and contain the same sterols as butterfat. It is possible that differences in the refractive indices of mixtures of these fats with butterfat at the 10% level may be too small for positive identification. Moreover, an accurate refractometer is required. Nevertheless, the method described in this paper may serve as a rapid screening test of suspected samples. Furthermore, other means of separating and identifying triglycerides are possible and are now under study in our laboratory.

SUMMARY

The suspected sample is first separated into alcohol soluble and insoluble triglycerides in order to increase the concentration of the adulterant in one of these fractions and cause enough shift in the refractive index for the adulterant to be detected.

The average refractive index of the alcohol insoluble fraction of 20 different samples of authentic butterfat was found to be 1.4542 ± 0.00002 (S.E.M.) and was significantly different from 1.4550 ± 0.00003 (S.E.M.), the average value of the samples containing 10% of substitute fat other than coconut oil. The addition of 10% coconut oil significantly lowered the refractive index of the alcohol soluble fraction of pure butterfat from 1.4540 ± 0.00002 to 1.4532 ± 0.00005.

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STUDIES OF HERD MANAGEMENT RECORDS
II. RELATION OF GESTATION LENGTH TO BIRTH WEIGHT OF HOLSTEIN CALVES OF BOTH SEXES AT VARIOUS CALVINGS

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Normal gestations for dairy cattle vary from 265 to more than 300 days. The lower limit is an arbitrary figure used because it is believed to be the shortest period that represents a normal gestation *(19)*. Among dairy breeders there is considerable interest in the length of the gestation period because of its use in planning for calving dates and because of the believed relationship between its length and the sex and weight of the subsequent calf. Traditionally, male calves are believed to be carried longer than females and to weigh more at birth. Longer gestation periods are believed to result in heavier calves. To throw light on this question, reproduction records from 1897 to 1950 of the Holstein herd of the University of Nebraska were studied.

Numerous investigators, *(1, 2, 8, 10, 11, 13, 15, 21)* have reported upon gestation length. Brackel *et al. (3)* have presented a review of the literature and state that for Holsteins the average gestation varied from 276 to 281 days. They reported that male calves were carried an average of 279.2 days, as compared with 277.8 days for females. Advancing age of the dam did not affect the length of gestation, according to Copeland *(5)*, Knapp *et al. (12)*, Jafar *et al. (11)*, and Warren *(20)*. Taking the opposite view were Herman and Spaulding *(10)*, Braude and Walker *(4)*, and Knott *(14)*. Knott reported an increase, which averaged 1.5 days per gestation, for each advancing gestation up to 6 years.

Within the Holstein breed, sex differences in birth weight of calves seemed important. Various authors *(6, 7, 8, 9, 15, 17, 18, 21)* reported a range in birth weights from 92.9 to 101.0 lb. for males, and from 85.5 to 94.0 lb. for females. According to Eckles *(6)* there is an increase in birth weight of calves from the first through the third gestation, which is in agreement with the findings of Fitch *et al. (8)* and Morgan and Davis *(16)*.

Braude and Walker *(4)* found an increase of 0.91 lb. in the birth weight of the calf for each day it was carried beyond the normal term. Brackel *et al. (3)* reported a definite relationship between the mean gestation length and the birth weight of Holstein calves, the correlation coefficient being +0.24. Jafar *et al. (11)* studying 76 calves found a correlation of $+0.61 \pm 0.09$ on raw data, and 0.52 ± 0.10 when adjusted for sex. They also reported the per cent of variance due to sex as 28.6 and that due to calving sequence 0.1.

Received for publication June 17, 1953.

[1] This article is based upon a thesis submitted by the junior author to the Graduate College, University of Nebraska, as a partial requirement for the Master of Science Degree. Published with the approval of the Director of the Nebraska Agricultural Experiment Station as Paper 617 in the journal series.

TABLE 1

Relation of length of gestation, sex, and calving sequence on the birth weight of Holstein calves

Calving sequence	MALES			FEMALES			BOTH SEXES			DIFFERENCES[a]	
	No.	Av. gestation	Av. birth wt.	No.	Av. gestation	Av. birth wt.	No.	Av. gestation	Av. birth wt.	Gestation	Birth wt.
		(days)	*(lb.)*		*(days)*	*(lb.)*		*(days)*	*(lb.)*	*(days)*	*(lb.)*
1	128	278.9	92.0	114	278.2	85.7	242	278.6	89.1	0.7	6.3
2	89	279.2	99.3	89	278.2	90.7	178	278.7	95.0	1.0	8.6
3	59	278.4	100.7	62	278.8	93.3	121	278.6	96.9	−0.4	7.4
4	42	279.4	99.5	38	278.9	95.7	80	279.2	97.7	0.5	3.8
5	29	278.8	94.6	29	278.1	95.4	58	278.5	94.9	0.7	−0.8
6	20	279.0	99.9	19	277.9	92.4	39	278.5	96.2	1.1	7.5
7	11	277.0	92.1	12	278.4	84.7	23	277.7	88.2	−1.4	7.4
8	6	279.5	103.7	8	278.6	85.2	14	279.0	93.1	0.9	8.5
All calvings	384	278.9	96.7	371	278.4	90.3	755	278.6	93.5	0.5	6.4

[a] Values for differences computed by subtracting value for female calves from that for males.

PRESENTATION OF DATA AND DISCUSSION

This study included 755 gestations resulting in single births of 384 males and 371 female calves. Table 1 presents, for successive calvings, first through the eighth, the average gestation lengths and birth weights for males, for females, and for all calves. The difference in gestation lengths and birth weights for the various calving sequences are shown, together with a summary of the data for all gestations. The range in length for individual gestations was 265 to 297 days for male calves and 266 to 290 days for female calves. The range in birth weights for individual calves was 47 to 148 lb. for males and 56 to 130 lb. for females. It is evident from the data in Table 1 that advancing age, as indicated by calving sequences, had no effect upon the length of gestation. For all gestations, the mean of 278.9 days for male births was practically identical with 278.4 days, the mean for female births.

The average birth weights for both males and females at the first gestation were lower than for succeeding calving sequences up to the seventh. There was a rise in average birth weight for males to a high of 100.7 lb. at the third gestation and then an irregular trend downward to the eighth gestation, where there was another high. Small numbers of calves with one unusually heavy calf caused this situation. For females there was a steady rise to the fourth gestation and then a decline.

To test the significance of the differences in the birth weights an analysis of variance was made (Table 2). This analysis indicates that the difference in birth weight between male and female calves (6.4 lb.) was highly significant

TABLE 2
Analysis of variance

Source	Degrees of freedom	Sum of squares	Mean square	Expected mean square	Estimated variance components	%
Total	754	110,401				
Sex	1	7,704	7704[a]	$\sigma^2 E+377.39\sigma^2 S$	$\sigma^2 S=20.1$	12.5
Calving Sequence	7	9,022	1288.9[a]	$\sigma^2 E+85.74\sigma^2 C$	$\sigma^2 C=13.7$	8.6
Gestation Length	25	12,128	485.1[a]	$\sigma^2 E+28.29\sigma^2 G$	$\sigma^2 G=13.1$	8.2
Remainder	721	81,547	113.1	$\sigma^2 E$	$\sigma^2 E=113.1$	70.7

[a] $= P < 0.01$

and that the calving sequence of the dam had a significant influence upon the birth weight of the calf. Probably this latter influence was mainly due to the lighter calves born after the first gestation. Finally, it was shown that gestation length had a significant effect upon the birth weight of calves. The estimated variance components indicated the relative importance of sex, calving sequence, and gestation length in determining birth weight. The largest part of the variance was not accounted for by this analysis. An analysis of interaction indicated no interaction between the three factors.

TABLE 3

Correlation (r) between gestation length and birth weight and regression (b) of birth weight on gestation length

	r	b	99% Fiducial limits of r
		(lb. days)	
Total	0.26	0.66	0.28 – 0.22
Within sex	0.25	0.62	0.27 – 0.21
Within calving	0.26	0.64	0.28 – 0.22
Within sex and calving	0.26	0.61	0.28 – 0.22

Table 3 presents other statistics of the relationship between gestation length and birth weights. The 99% fiducial limits show that these statistics are highly significant.

SUMMARY

A study of 755 normal gestations, 384 male births and 371 female, of Holstein calves in the University of Nebraska herd during the period 1897-1950 was made to determine the relationship between length of gestation and birth weights of calves, by sexes and successive calvings. Gestation length for each sex and for the successive calvings showed no significant trend. The average gestation length for males was 278.9 days and for females 278.4 days. The average birth weight for males was 96.7 lb., and for females 90.3 lb. Analysis of variance showed that differences in birth weight due to sex, calving sequence, and gestation length were significant ($P < 0.01$). Correlation between gestation length and birth weight was 0.26 with 99% fiducial limits of 0.28-0.22. The regression of birth weight on gestation was 0.66 lb. per day.

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A QUANTITATIVE APPRAISAL OF THE FREE AMINO ACIDS IN FOREIGN TYPE CHEESE

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Cheese ripening is inalterably associated with the production of numerous compounds from protein. The nature and significance of a number of the higher molecular weight intermediate end products have been extensively developed, but the area embracing the water soluble protein constituents has yet to be fully explored. Significant advances have been made by Tuckey *et al. (13)* using X-ray diffraction patterns and by Harper and Swanson *(3)* and Reihard and Garey *(9)* utilizing microbiological methods. Certain limitations inherent in each of these methods have prevented the attainment of a more complete view of water soluble components in cheese. The advent of chromatographic techniques brings this objective closer to realization.

Cheddar cheese has been subjected to chromatographic analyses more extensively than other cheese. As reported by Kosikowsky *(5)*, many free amino acids and a number of amines were found to be present in commercial Cheddar cheese. Block *(2)*, also using paper chromatography, observed similar free amino acid patterns in Cheddar cheese. In another investigation *(8)* the quantitative aspects of free amino acids and amines in Cheddar cheese over the entire ripening period were noted.

Foreign type cheese have recently been analyzed for their water soluble nitrogenous compounds by chromatographic methods *(2, 4, 6, 11, 12)*, but these studies were largely restricted to the qualitative phases. The present study deals more with the quantitative aspects of free amino acids and amines in foreign type cheese.

METHODS

Thirty commercial foreign type cheese were analyzed during the course of this study. The history of most of these cheese, regarding date and place of manufacture, was generally unknown but in each instance the cheese, obtained from counters of food stores, were confirmed as typical of the type upon organoleptic testing.

Two dimensional paper chromatographic methods as applied earlier to Cheddar cheese by Kosikowsky *(5, 7)* were used. Quantitative estimation of the soluble nitrogenous compounds observed on chromatograms was by the method of Block *(1)*. As the methods used here were identical to those applied earlier to Cheddar cheese *(5, 7, 8)* and subject to the same experimental errors, the sensitivity and variability of results were considered to be of the same magnitude. In actual practice a number of amino acids were so heavily concentrated on the

Received for publication July 8, 1953.

chromatograms as to exceed the maximum limits (6.06 mg./g. cheese) used in the standard curves. These concentrations were listed in the present tables by the notation, >6.06 mg./g. cheese.

RESULTS

Chromatographic patterns of the free amino acids and related compounds of foreign type cheese, in general, were not unlike those found with Cheddar cheese

TABLE 1

Free amino acids in Emmenthaler and domestic Swiss cheese (milligrams per gram)

Amino acids and related compounds[a]	Emmenthaler		Domestic Swiss			
	1	2	1	2	3	4
Glutamic acid	>6.06	>6.06	>6.06	>6.06	>6.06	>6.06
Aspartic acid	0.00	1.50	0.11	0.08	0.11	0.11
Leucine-methionine	4.22	0.67	3.53	0.76	0.68	0.80
Basic	>6.06	0.95	6.06	0.46	0.91	0.46
Valine	4.56	1.52	5.02	1.66	0.11	1.71
Alanine	2.28	0.40	1.37	0.15	0.46	0.11
Glutamine	>6.06	0.42	0.68	0.11	0.46	0.11
Phenylalanine	1.25	1.06	1.94	0.31	1.03	0.34
Tyrosine	1.48	0.37	1.03	0.31	0.34	0.34
Glycine	1.25	0.14	0.46	0.10	0.11	0.11
Threonine	0.80	0.15	0.46	0.27	0.11	0.34
Proline	5.47	1.21	5.02	2.59	1.25	2.51
Tyramine	0.00	0.00	0.00	0.00	0.00	1.25
α-amino-butyric acid	0.00	0.10	0.11	0.00	0.00	0.00
Methionine sulphoxide γ-amino-butyric acid	3.31	0.71	3.76	0.91	0.68	0.91
Asparagine	0.80	1.52	6.06	0.40	1.48	0.46
Serine	0.68	0.18	0.23	0.23	0.23	0.23
Flavor development	Med.	Med.	Med.	Mild sl. sour	Mild	Mild

[a] Notation 0.00 indicates inability to obtain detectable amount of particular amino acid by this analytical method and not complete absence of amino acid.

during earlier studies *(8)*. Although some qualitative differences among these foreign cheese were apparent, it was not possible to effectively identify cheese types by their free amino acid patterns.

Quantitatively, amino acids varied markedly between different cheese and cheese types, as shown in Tables 1, 2, 3, 4, and 5, ranging from traces to amounts greater than 6.06 mg./g. cheese. In this respect the degree of ripeness of cheese within each classification was an important factor as the sharper flavored cheese usually showed higher levels of individual amino acids.

Glutamic acid, leucines, valine, and basic amino acids predominated in most foreign type cheese. Proline, present in many of the cheese, was consistently high in the Emmenthaler-Swiss group and low or nonexistent in the Camembert group. This observation regarding proline in Swiss cheese is in accord with that of Virtanen and Kreula *(14)* who by conventional chemical methods found levels of from 3.5 to 6.0 mg./g. for aged cheese of this type. Asparagine was

TABLE 2

Free amino acids in Roquefort and Blue cheese (milligrams per gram)

Amino acids and related compounds[a]	Roquefort			Gorgonzola	Domestic Blue			
	1	2	3	1	1	2	3	4
Glutamic acid	>6.06	6.06	>6.06	>6.06	>6.06	>6.06	>6.06	>6.06
Aspartic acid	0.23	1.48	0.23	1.14	0.46	1.14	0.91	0.14
Leucine-methionine	5.13	.....	2.85	>6.06	4.90	5.59	3.76	0.97
Basic	>6.06	4.60	1.86	0.46	0.23	>6.06	>6.06	1.23
Valine	>6.06	5.36	4.33	>6.06	5.01	5.59	4.67	0.88
Alanine	>6.06	0.57	3.76	4.60	2.62	1.71	5.36	0.70
Glutamine	0.91	>6.06	2.30	0.23	6.06	>6.06	>6.06	0.61
Phenylalanine	3.19	0.00	>6.06	0.00	0.23	0.23	0.57	0.60
Tyrosine	3.65	3.54	1.86	4.33	3.53	3.31	0.57	0.55
Glycine	0.34	0.11	0.11	0.34	0.57	0.11	0.23	0.00
Threonine	2.74	0.00	1.00	0.34	0.46	0.23	0.00	0.00
Proline	0.46	0.80	3.62	0.34	0.57	0.46	3.88	0.13
Tyramine	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
α-amino-butyric acid	0.00	0.46	0.00	0.00	0.00	0.00	0.00	0.00
Methionine sulphoxide γ-amino-butyric acid	1.93	>6.06	>6.06	4.79	>6.06	3.08	4.22	1.71
Asparagine	0.00	0.00	0.00	0.00	0.00	2.96	3.53	0.40
Serine	1.14	0.00	2.52	0.57	0.23	0.34	0.11	0.00
Flavor development	strong	strong	strong	strong	strong	strong bitter	medium	mild

[a] Notation 0.00 indicates inability to obtain detectable amount of particular amino acid by this analytical method and not complete absence of amino acid.

..... No observation made.

TABLE 3

Free amino acids in Limburger cheese and related types (milligrams per gram)

Amino acids and related compounds[a]	Liederkranz			Domestic Limburger			Brick
	1	2	3	1	2	3	1
Glutamic acid	1.71	>6.06	0.34	0.34	4.67	0.34	0.23
Aspartic acid	0.23	0.68	0.10	0.23	0.91	0.11	0.11
Leucine-methionine	2.85	>6.06	2.85	1.14	4.21	1.60	1.27
Basic	0.80	2.28	0.68	>6.06	3.19	0.34	0.46
Valine	3.99	6.06	2.17	0.57	4.56	0.34	0.46
Alanine	0.80	0.00	4.56	0.11	1.37	0.23	0.23
Glutamine	0.00	>6.06	0.23	0.80	0.11	0.11	0.77
Phenylalanine	1.25	4.10	0.57	0.00	0.46	0.00	0.00
Tyrosine	0.00	1.03	0.34	0.00	0.00	0.00	0.11
Glycine	0.80	0.46	0.23	0.11	0.11	0.00	0.00
Threonine	0.00	0.00	0.11	0.00	0.00	0.00	0.00
Proline	3.31	0.46	1.92	0.46	0.00	0.00	0.00
Tyramine	0.00	1.03	1.48	0.00	0.00	0.00	0.00
α-amino-butyric acid	0.00	3.65	0.00	0.00	0.11	0.11	0.11
Methionine sulphoxide γ-amino-butyric acid	1.25	1.37	5.93	>6.06	0.34	>6.06	>6.06
Asparagine	0.00	0.00	0.00	0.00	0.00	0.00	0.77
Serine	0.23	0.11	0.70	0.11	0.11	0.00	0.11
Flavor development	strong	strong	med.	strong	med.	mild	mild

[a] Notation 0.00 indicates inability to obtain detectable amount of particular amino acid by this analytical method and not complete absence of amino acid.

TABLE 4

Free amino acids in domestic Camembert cheese (milligrams per gram)

Amino acids and related compounds[a]	Domestic Camembert			
	1	2	3	4
Glutamic acid	>6.06	>6.06	0.11	0.23
Aspartic acid	1.09	0.23	0.00	0.11
Leucine-methionine	>6.06	1.02	0.80	1.60
Basic	2.08	0.57	2.05	0.46
Valine	6.06	0.80	0.11	2.62
Alanine	>6.06	0.34	0.11	0.11
Glutamine	0.23	0.57	0.00	0.00
Phenylalanine	2.19	1.48	0.11	0.46
Tyrosine	3.51	1.25	0.11	0.00
Glycine	1.53	0.23	0.00	0.00
Threonine	0.23	0.34	0.00	0.00
Proline	0.00	0.11	0.00	0.00
Tyramine	0.00	0.00	0.00	0.00
α-amino-butyric acid	0.00	0.00	0.00	0.00
Methionine sulphoxide γ-amino-butyric acid	0.34	0.46	1.48	4.56
Asparagine	0.00	0.00	0.00	0.00
Serine	0.11	0.34	0.46	0.11
Flavor development	strong v. ripe	medium opt. ripe	mild under ripe	mild under ripe

[a] Notation 0.00 indicates inability to obtain detectable amount of particular amino acid by this analytical method and not complete absence of amino acid.

observed in all Emmenthaler-Swiss cheese but inconsistently so in other cheese types. No evidence of tryptophane, within the sensitivity limits of the present method, was noted but amines such as glutamine and tyramine were present in a number of cheese.

A direct comparison of total quantities of free amino acids to flavor intensity is not possible under conditions of this study. It is possible, however, to make some general observations with reference to cheese types and individual amino acid concentration. Data shown in Tables 1, 2, 3, and 4 indicate surprisingly enough that the Roquefort-Blue and the Emmenthaler-Swiss types contain greater concentrations of many of the free amino acids than either the Limburger or Camembert types when the cheese are compared at relatively similar flavor intensities. In view of the fact that the latter cheese historically are associated with soft, highly broken-down bodies and pronounced aroma, both normally considered as criteria of extensive protein hydrolysis, these results were not expected.

In the 30 ripened cheese the concentration of individual free amino acids was to a certain degree related to their concentration in the casein of milk. For example, glutamic acid, leucine, and valine are present in largest concentration in casein. As free amino acids in the ripened foreign type cheese, these same compounds were also present in highest concentration. But such a relationship apparently does not hold true for all free amino acids, as indicated by the proline and tyrosine concentrations. In casein, according to

TABLE 5

Free amino acids in some miscellaneous type cheese (milligrams per gram)

Amino acids and related compounds[a]	Asiago	Provolone	Domestic Gouda	Gruyere Process	Primost Type
Glutamic acid	>6.06	0.34	0.36	>6.06	0.00
Aspartic acid	0.56	0.11	0.15	0.59	0.00
Leucine-methionine	>6.06	1.14	0.91	5.22	0.23
Basic	>6.06	2.17	0.00	1.49	0.00
Valine	>6.06	0.80	0.13	2.33	0.00
Alanine	5.59	0.23	0.11	0.52	0.00
Glutamine	6.06	0.11	0.11	0.11	0.00
Phenylalanine	>6.06	0.11	0.00	0.90	0.00
Tyrosine	0.00	0.11	0.15	0.11	0.00
Glycine	0.23	0.11	0.11	0.13	0.00
Threonine	0.34	0.34	0.00	0.24	0.00
Proline	>6.06	0.23	0.00	2.11	0.00
Tyramine	0.00	0.00	0.00	0.00	0.00
α-amino-butyric acid	0.11	0.00	0.00	0.11	0.00
Methionine sulphoxide γ-amino-butyric acid	>6.06	4.22	0.20	1.92	0.00
Asparagine	>6.06	0.00	2.05	2.26	0.00
Serine	3.08	0.11	0.11	0.11	0.00
Flavor development	sharp	medium	mild	medium	v. mild

[a] Notation 0.00 indicates inability to obtain detectable amount of particular amino acid by this analytical method and not complete absence of amino acid.

Rogers' Associates *(10)*, proline and tyrosine rank 3rd and 7th, respectively, among the amino acids, whereas in cheese analyzed in this study their relative positions in terms of concentration of free amino acids varied for each from 4th to 17th place. These examples, as well as others not presented here, serve only to emphasize that partial or complete transformation of many of the freshly split smaller molecular casein compounds is an inevitable part of cheese ripening.

A study of this type is designed primarily to provide more basic information on the composition of ripened cheese. It is hoped that information of this nature may be found useful at some later date to investigators working on problems dealing with the origin of flavor in cheese. Future investigations using new and more refined analytical techniques will undoubtedly show the presence of an even greater variety of substances as well as more accurate measurements of their concentrations. At the same time it is encouraging to note that substantial agreement on the nature of compounds present in foreign type cheese exists between results presented here and those recently reported by Storgards and Lindquist *(12)*.

SUMMARY

A quantitative estimation of the free amino acids and related compounds of 30 commercial foreign type cheese revealed that free amino acids were present in concentrations from a trace to more than 6 mg. per gram of cheese with glutamic acid, leucines, valine, and basic amino acids predominating.

No foreign cheese type could be solely identified by its chromatographic amino acid pattern, although some differences were observed. Roquefort-Blue

types and Emmenthaler-Swiss types were distinguished to some extent from Limburger and Camembert cheese types by the greater concentrations of many of the individual amino acids existing in the former cheese.

ACKNOWLEDGMENT

The authors are indebted to Francis P. Heffernan, now a member of the Armed Forces, for his aid in analyzing a number of these foreign type cheese.

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THE DIGESTIBILITY OF HONEYSUCKLE *(LONICERA JAPONICA)* FOR YEARLING DAIRY BULLS[1]

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For many years it has been observed by farmers in the southern states that cows and certain other livestock often graze honeysuckle *(Lonicera japonica)*, especially when other roughage is in short supply. Wild animals and birds have been observed to utilize honeysuckle as part of their food supply, especially during the winter *(1, 2, 3, 5, 7)*, but no published reports have been found concerning the value of this plant for ruminant domestic animals.

Since there is a definite need for an emergency pasture crop for drought and winter weather that is a perennial, is cheap to establish and maintain, and is resistant to adverse weather, insects, and diseases, it was decided to investigate the digestibility of honeysuckle as a roughage crop.

EXPERIMENTAL

One set of identical twin bulls of the Jersey breed was used in this experiment. Three 10-day digestion trials were conducted using digestion stalls patterned after plans obtained from the University of Tennessee Cooperative Project, Oak Ridge *(4)*. Each digestion trial consisted of: *(a)* a preliminary period of about 5–10 days between fecal collection periods, during which a constant intake was established without any weigh-back of the honeysuckle, and *(b)* a collection period of 10 days. During each period of the collection intervals the animals consumed all the honeysuckle fed.

A 3% aliquot sample of honeysuckle was obtained twice each day, and at the same time a 3% aliquot sample of the previous 12-hour feces sample was secured.

The honeysuckle, which was fed as the sole roughage, was harvested by hand each day and fed fresh to the animals. Only the tender terminal parts of the plants were harvested, and care was taken not to lose any fresh leaves; briars, pine needles, dry leaves, and foreign material were removed. The bulls were given all the water they would consume twice daily in a pail. They were weighed at the beginning of each trial and every 5 days during the trial.

All chemical analyses of the honeysuckle and the feces were made in duplicate in accordance with official methods adopted by the Association of Official Agricultural Chemists.

RESULTS AND DISCUSSION

It was observed from hand botanical sorting that the proportion of leaves and vine fed was approximately 85 and 15%, respectively, on the fresh basis. A

Received for publication July 23, 1953.

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TABLE 1

Chemical composition of honeysuckle and feces (fresh basis)

	Moisture	Ash	Fat	Protein	Fiber	NFE
	%	%	%	%	%	%
First trial 10/14–10/23						
Honeysuckle	59.7	4.4	2.7	4.5	7.4	21.3
Feces, Bull 1	77.6	2.9	2.2	1.5	8.1	7.7
Feces, Bull 2	77.3	3.5	2.3	1.7	6.0	9.2
Second trial 11/5–11/14						
Honeysuckle	57.0	2.8	1.7	3.9	10.7	23.9
Feces, Bull 1	76.2	2.1	2.0	2.1	8.1	9.5
Feces, Bull 2	76.0	2.1	2.0	2.0	6.7	11.2
Third trial 11/20–11/29						
Honeysuckle	61.6	2.6	1.5	3.9	10.0	20.4
Feces, Bull 1	75.4	1.9	1.8	2.1	6.7	12.1
Feces, Bull 2	75.4	2.0	1.7	2.1	10.8	8.0

constant intake of fresh honeysuckle of 20 lb. per day was achieved for the two bulls during the first 10-day collection period. The intakes were 22 lb. daily for the second collection period and 26 lb. daily for the third period. The experiment was conducted from October 1 to November 30, 1952. Table 1 shows the

TABLE 2

Digestion coefficients and total digestible nutrients of honeysuckle

	Fat	Crude protein	Crude fiber	NFE	TDN (fresh basis)
	%	%	%	%	%
First trial					
Bull 1	32.2	77.1	27.6	75.7	23.6
Bull 2	28.2	74.7	46.3	69.0	23.2
Average	30.2	75.9	37.0	72.4	23.4
Second trial					
Bull 1	(–3.5)	50.4	32.1	64.3	20.8
Bull 2	18.9	64.7	57.1	69.1	25.6
Average	7.7	57.6	44.6	66.7	23.2
Third trial					
Bull 1	26.1	66.7	57.9	62.7	22.0
Bull 2	30.9	66.9	34.6	76.3	22.7
Average	28.5	66.8	46.3	69.5	22.3
Av. for 3 trials	22.1	66.8	42.6	69.5	23.0

chemical composition of honeysuckle and feces during the experiment. Table 2 shows the digestion coefficients and total digestible nutrients for honeysuckle.

No apparent explanation can be given for variability in the digestion coefficients for crude fiber (Trials 1, 2, 3) and crude protein (Trial 2) between Bull 1 and Bull 2.

Using Morrison's 1948 figures *(6)*, it will be noted that the TDN content of fresh honeysuckle as fed in this experiment compares favorably with that of the

following green forages on an equivalent dry matter basis: Bermuda grass pasture; Brome grasses, wild; Dallis grass pasture; Johnson grass pasture; lespedeza annual, pasture, before bloom; lespedeza, annual, in bloom; and timothy, before bloom. On the basis of this limited trial, it seems that honeysuckle may offer some possibilities as an emergency green roughage for dairy cattle. It is possible, of course, that grazing animals would consume more leaves and less stems than offered in this trial, which might cause scouring. Certainly, further research is needed before practical suggestions can be made.

The bulls remained in good general condition throughout the experiment and gained an average of 0.8 lb. per day on an average daily consumption of 22.7 lb. of the honeysuckle. Bull 1 weighed 298 lb. at the start of the experiment and 320 lb. at the end, and Bull 2 weighed 304 lb. at the start and 330 lb. at the finish of the experiment.

SUMMARY

Three 10-day digestion trials were conducted with one set of identical twin dairy bulls to determine the digestibility of fresh green honeysuckle *(Lonicera japonica)*. The average digestion coefficients were as follows: fat, 22.1; crude protein, 66.8; crude fiber, 42.6; NFE, 69.5. The per cent TDN on a fresh basis was 23.0.

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SLIME FORMATION ON COTTAGE CHEESE[1]

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The formation of slime on cottage cheese held at relatively low temperatures is one of the important bacteriological defects of this product. The rate of formation, color, and general appearance of the slime suggest that the defect may be produced by a variety of microorganisms. Also, descriptions of the slime defect by various manufacturers indicate that the character of the slime, as well as the odor of the product, is variable.

Elliker *(2)* attributed the slimy-curd defect to contamination of milk from poorly cleaned equipment subsequent to pasteurization. The ropy-milk bacteria, *Alcaligenes viscosus* and *Aerobacter aerogenes,* were suggested as causative organisms. Parker *et al. (3)* demonstrated that three species of bacteria, *Alcaligenes metalcaligenes, Pseudomonas viscosa,* and *Pseudomonas fragi,* were responsible for the gelatinous or slimy curd defect of cottage cheese. Contaminated equipment and water used to wash the curd were believed to be the important sources of the organisms. Limited control of the defect was attained by regulating the pH of the final product.

This study was conducted to determine the relationship between storage temperature and production of the slime defect and also to determine the resistance of the causative organisms to heat and chlorine. Since Parker *et al. (3)* indicated that a pH below 5.0 was necessary for consistent control of the defect and since cheese known to be below pH 5.0 developed slime, it appeared desirable to obtain further information on this point.

METHODS

Isolation of organisms. Samples of cottage cheese exhibiting the slimy defect were received from several dairy plants in Indiana and Illinois. These samples were plated on tryptone glucose beef-extract milk agar, and, after incubation, colonies were picked from the plates, purified, and tested for their action on sweet-curd cottage cheese. In addition to cottage cheese samples, some isolations were made from various items of equipment used in the manufacture of cottage cheese in one of the plants.

Determination of pH. The pH determinations were made with a Leeds and Northrup Type K potentiometer fitted with a quinhydrone electrode and saturated calomel cell. Samples were prepared according to the method of Sanders *(4)*.

Received for publication August 7, 1953.

[1] Published with the approval of the Director of the Purdue Agricultural Experiment Station as Journal Series paper No. 736.

[2] Present address: Pet Milk Co., Greenville, Illinois.

Heat resistance. Capillary tubes 8 to 10 cm. in length, 1.5 to 2.0 mm. in diameter, and having a wall thickness of 0.15 to 0.20 mm. were aseptically filled with 24-hour cultures of the test organisms and sealed. After a definite exposure, the content of a capillary tube was emptied into sterile litmus milk. The inoculated litmus milk tubes were incubated at 21° C. and observed for growth.

Chlorine resistance. Cultures tested for chlorine resistance were grown on agar slants for 24 hours at 21° C. The growth was removed from the slant with sterile distilled water and shaken to break up clumps of bacteria; the number of bacteria in the suspension was determined by the standard plate count. The bacterial suspension (5 ml.) was mixed with a hypochlorite solution (5 ml.) of definite strength (determined by thiosulfate titration), and aliquots were removed at intervals into sterile milk. After incubation, the milk was examined for growth.

RESULTS

Rate of slime formation on cottage cheese held at various temperatures. The country-style cottage cheese used for this experiment was manufactured by an accepted commercial procedure and had an initial pH of 4.77. One lot of curd was used for the experiment. Portions of this lot were washed with bacterial suspensions prepared from 24-hour cultures of the test organisms. After drain-

TABLE 1

Rate of slime formation on cottage cheese held at various temperatures and changes in pH after 5 days at the holding temperature

Culture No.	Trial No.	Days required to produce slime at			pH of cheese[a] after 5 days at		
		4.4° C.	10° C.	21° C.	4.4° C.	10° C.	21° C.
16	1	11	6	2	4.75	4.97	5.37
	2	11	6	2	4.80	4.94	5.37
17	1	11	6	4	4.73	4.85	6.02
	2	10	5	2	4.83	5.38	5.37
18	1	10	5	2	4.78	4.98	4.80
	2	10	5	4	4.75	4.97	5.01
25	1	9	6	4	4.75	4.83	5.88
	2	8	5	4	4.78	5.03	5.30
28	1	11	6	2	4.73	4.98	4.99
	2	11	6	2	4.73	4.81	4.94
33	1	[b]	[b]	4	4.74	4.78	4.90
	2	[b]	[b]	2	4.75	4.77	4.91
34	1	10	6	2	4.73	5.00	4.09
	2	10	6	4	4.83	4.92	5.10
36	1	[b]	[b]	1	4.75	4.77	4.96
	2	[b]	[b]	1	4.76	4.76	4.93
42	1	8	6	2	4.71	4.98	5.06
	2	7	7	2	4.76	4.78	5.11
Control	1	[b]	[b]	[b]	4.71	4.70	4.56
	2	[b]	[b]	[b]	4.75	4.31	4.31

[a] Initial pH of cheese 4.77.
[b] No evidence of slime in 14 days.

ing, the curd washed with a particular culture was divided among three sterile glass jars and held at 4.4, 10, and 21° C. Results of the experiment are presented in Table 1.

Of the nine cultures used in this experiment, seven produced slime on cottage cheese held at 4.4° C. in 7 to 11 days. The same cultures produced slime at 10° C. in a period of 5 to 7 days. All of the test cultures produced slime on cottage cheese held at 21° C. in 1 to 4 days.

The pH determinations were made on the cottage cheese 5 days after inoculation of the samples with the test organisms. Samples held at 4.4° C. showed little change in pH, and those held at 10° C. either showed no change or increased in pH. At 21° C. all the inoculated samples showed a definite increase in pH, although the extent of the increase varied with the particular test culture.

The pH values of different areas of slimy cottage cheese. The slimy defect of cottage cheese appears first on the surface. As the amount of slime increases, it gradually extends to the interior of the sample. During early stages of the defect, the top of the sample may show slime while the bottom portion appears normal. Samples were taken for pH determination in different areas of the same lot to determine how uniformly the acid was distributed. Data obtained in this experiment are presented in Table 2.

TABLE 2

pH values of different areas of slimy cottage cheese

Culture No.	pH values[a] of cottage cheese incubated at 21° C. for			
	2 days		5 days	
	Lower area	Slimy area	Lower area	Slimy area
16	4.26	5.00	4.72	6.05
28	4.17	4.46	4.10	5.09
33	4.15	4.98	4.16	5.24
36	4.18	4.54	4.00	5.37
49	4.24	4.85	4.17	5.32
50	4.31	4.66	4.35	5.13

[a] Initial pH of cheese 4.77.

Cottage cheese inoculated with slime-forming bacteria and held at 21° C. showed marked differences in pH at the top and bottom of the sample. After holding for 2 days, the lower area decreased in pH while the top portion showed both increases and decreases. However, the lower area was always lower in pH than the top or slimy area. After holding for 5 days, the lower area was still lower in pH than the original cheese while the top or slimy area was considerably higher. Variations were encountered with different test cultures.

Number of bacteria added to cottage cheese curd by washing with contaminated water. Since several of the cultures capable of producing slimy cottage cheese belonged to the genus *Aerobacter*, the ease with which this group is determined made it seem desirable to determine the number of bacteria that would be added to cottage cheese curd by washing with contaminated water. In this experiment, 15-oz. portions of country-style cottage cheese were washed with 100 ml. of

bacterial suspensions containing 2,000,000 to 120,000,000 cells per milliliter. The number of bacteria retained by the curd varied with the culture employed and also with different trials and ranged from 0.0093 to 0.40% *Aerobacter* cultures which produced a ropy slime were retained more readily by cottage cheese curd than cultures which were nonropy.

Influence of the number of bacteria in wash water and temperature of incubation on the rate of slime formation. Suspensions containing different amounts of slime-producing bacteria were used to wash cottage cheese curd. After being washed with contaminated water, the cheese was drained and held at 10 and 21° C. The three cultures for which data are given (Table 3) belong to different

TABLE 3

Influence of the number of bacteria in wash water and temperature of incubation on the rate of slime formation on cottage cheeese[a]

Incubation Temperature	Days held		Culture No. 47		Culture No. 50		Culture No. 25	
			Bacteria per ml. of wash water *(millions)*					
			37.2	3.72	43.7	4.37	42.0	4.20
10° C.	1	Defect	none	none	none	none	none	none
		pH	4.73	4.74	4.77	4.71	4.79	4.72
	2	Defect	none	none	none	none	none	none
		pH	4.74	4.73	4.75	4.69	4.74	4.73
	5	Defect	none	none	none	none	none	none
		pH	4.68	4.64	4.73	4.74	4.94	4.79
21° C.	1	Defect	putrid	none	none	none	none	none
		pH	4.95	4.82	4.73	4.70	4.76	4.72
	2	Defect	slime	none	slime	none	none	none
		pH	4.27	4.16	4.38	4.33	4.13	4.17
	5	Defect	slime	slime	slime	slime	slime	none
		pH	4.85	4.45	4.67	4.96	4.09	4.04

[a] Initial pH of cheese 4.76

genera and the extent of contamination is about the same. The results show that the slimy defect did not occur in 5 days at 10° C., even though the cheese was washed with water containing large numbers of spoilage bacteria. Cheese contaminated to the same extent and held at 21° C. developed defects in 1 to 5 days, depending on the culture employed and extent of contamination. All of the samples washed with suspensions containing the larger number of spoilage bacteria produced defects in a shorter time than those washed with a smaller number of bacteria. The results further emphasize the importance of temperature in the production of slimy cottage cheese, since the defect occurred at 21° C. but not at 10° C. during the 5-day holding period. Slime formation took place at less than pH 5 in all trials.

Heat resistance of slime-forming bacteria. Fifteen cultures capable of forming slime on cottage cheese were grown in skimmilk and tested for their ability to survive various exposure times at 62.8° C. The data presented in Table 4

TABLE 4

Heat resistance of bacteria associated with the slime defect of cottage cheese

Culture No.	Minutes exposure to 62.8° C.						
	0	0.25	0.5	1.0	1.5	2.0	2.5
16	+	−	−	−	−	−	−
17	+	+	−	−	−	−	−
18	+	+	+	−	−	−	−
25	+	+	+	−	−	−	−
28	+	−	−	−	−	−	−
33	+	+	+	+	+	+	−
34	+	−	−	−	−	−	−
36	+	+	+	+	−	−	−
42	+	+	+	−	−	−	−
47	+	+	+	−	−	−	−
49	+	+	+	+	+	−	−
50	+	+	+	+	+	−	−
65	+	−	−	−	−	−	−
66	+	+	−	−	−	−	−
67	+	−	−	−	−	−	−

+ growth
− no growth

show that all of the slime-forming bacteria are destroyed by short exposures to 62.8° C. The most resistant culture survived a 2-minute but not a 2.5-minute exposure. Five of the 15 cultures did not survive a 0.25-minute exposure. Certain cultures belonging to the genus *Aerobacter* were the most heat resistant.

Chlorine resistance of slime-forming bacteria. Cultures of slime-forming bacteria washed from agar slants and standardized to contain from 1 to 5 million cells per milliliter were used in this experiment. The cultures were checked for their ability to survive exposures of 5, 10, 25, 45, and 60 seconds to chlorine concentrations of 5, 25, 50, and 100 p.p.m.

Table 5 shows that seven of the 15 cultures survived a 60-second exposure to

TABLE 5

Minimum exposure to various chlorine concentrations required to destroy cultures of slime-producing bacteria using exposure times of 5, 10, 25, 45, and 60 sec.

Culture No.	p.p.m. chlorine			
	5	25	50	100
	Exposure necessary to destroy culture (seconds)			
16	45	< 5	< 5	< 5
17	25	< 5	< 5	< 5
18	> 60	< 5	< 5	< 5
25	> 60	> 60	> 60	45
28	60	10	< 5	< 5
33	> 60	> 60	45	25
34	> 60	45	45	10
36	60	< 5	< 5	< 5
42	> 60	25	10	< 5
47	60	45	< 5	< 5
49	45	25	25	10
50	25	10	< 5	< 5
65	45	10	< 5	< 5
66	> 60	> 60	> 60	45
67	> 60	< 5	< 5	< 5

5 p.p.m. chlorine; but the least resistant cultures did not survive a 25-second exposure. Three cultures survived a 60-second exposure to 25 p.p.m. and two cultures a 60-second exposure to 50 p.p.m. None of the cultures survived a 60-second exposure to 100 p.p.m. chlorine, the two most resistant cultures surviving a 25- but not a 45-second exposure.

Other experiments were conducted to determine whether hypochlorites or chloramine T compounds added to the wash water would destroy slime-forming bacteria present on the surface of the curd particles. In these experiments cottage cheese curd was washed with suspensions of slime-forming bacteria, drained, divided into equal portions, and washed with solutions containing definite concentrations of chlorine. A portion of each lot was treated as follows: *(a)* a single wash with 5 p.p.m. chlorine using hypochlorite or chloramine T, *(b)* a single wash with 100 p.p.m. chlorine using hypochlorite or chloramine T, and *(c)* a double wash with 100 p.p.m. chlorine using hypochlorite or chloramine T. Each wash was allowed to remain in contact with the curd for 10 minutes. The curd was held at 21° C. and observed for slime formation.

The results of these experiments indicate that it is not possible to destroy slime-forming bacteria on cottage cheese curd by washing with hypochlorite or chloramine T solutions even when the curd is given two 10-minute washes with solutions containing 100 p.p.m. available chlorine. In all trials, the cottage cheese curd washed with chlorine solutions developed slime just as rapidly as the control cheese which received no chlorine treatment. In some trials, the organisms appeared to be stimulated by the chlorine treatment.

Hypochlorite solutions containing 100 p.p.m. available chlorine contained from 3.5 to 7.0 p.p.m. residual chlorine after being used to wash cottage cheese; chloramine T solutions used in the same manner contained 3.5 p.p.m. residual chlorine or less. When cottage cheese curd was washed a second time with 100 p.p.m. of a hypochlorite solution, the amount of residual chlorine in the solution

TABLE 6

Appearance of the slime produced and generic classification of bacteria capable of forming slime on cottage cheese

Culture No.	Type of slime produced	Generic classification
16	Brownish watery slime	*Proteus*
17	Brownish watery slime	*Proteus*
18	White slime	*Pseudomonas*
25	White slime	*Pseudomonas*
28	Deep yellow slime	*Aerobacter*
33	White, ropy slime	*Aerobacter*
34	White slime	*Pseudomonas*
36	White, ropy slime	*Aerobacter*
42	Greenish brown slime	*Pseudomonas*
47	White slime	*Achromobacter*
49	White, ropy slime	*Aerobacter*
50	White slime	*Aerobacter*
65	Brownish, ropy slime	*Proteus*
66	Brown, watery slime	*Alcaligenes*
67	Brown, watery slime	*Alcaligenes*

ranged from 5 to 10 p.p.m.; chloramine T solutions used similarly contained from 3.5 to 7 p.p.m. residual chlorine.

Appearance of the slime formed and generic classification of the bacteria employed in these studies. Table 6 presents information concerning the type of slime produced by cultures employed in the experiments and the generic classification of the bacteria. Cultures 16, 17, and 65 were classified as belonging to the genus *Proteus* and all produced a brownish colored slime. Cultures 16 and 17 were of the same species. Cultures 18, 25, 34, and 42 were *Pseudomonas* types; Cultures 18 and 34 were the same species. Cultures 18, 25, and 34 produced a white slime and Culture 42 produced a greenish-brown slime. Cultures 28, 33, 36, 49, and 50 belonged to the genus *Aerobacter*. Culture 28 formed a deep yellow slime; all other *Aerobacter* types formed a white slime that was ropy with all cultures except No. 50. Cultures 66 and 67 belonged to the genus *Alcaligenes* and Culture 47 to the genus *Achromobacter*. The organisms were classified according to descriptions given in *Bergey's Manual (1)* and by Skerman*(5)*.

DISCUSSION

The results obtained in this study emphasize the importance of temperature in the formation of slime on cottage cheese. Even though cottage cheese curd was extensively contaminated with slime-forming bacteria, slime failed to develop on some samples held at 4.4° C. for 14 days and was not evident on any sample until after 7 days. The rapidity with which the defect develops at 21° C. suggests the use of this temperature for keeping-quality tests. It is the practice in some plants to hold one or more packages of cottage cheese from each lot at a constant temperature to determine the approximate shelf life of the product. Some manufacturers conduct a keeping-quality test at the storage temperature used in the plant and fail to take into account the fact that cottage cheese frequently is held at higher temperatures on retail routes, in household refrigerators, and in retail stores.

The cottage cheese employed in the experiments with slime-forming bacteria had a pH of approximately 4.7. This pH appears to be about the average for cottage cheese manufactured in this area. Experiments were not carried out to determine whether slime was produced faster in cottage cheese of higher pH; the results of Parker *et al. (3)* indicate that it is produced more rapidly at a higher pH. It was noted, however, that addition of cream to cottage cheese increased the pH 0.26 to 0.33 of a pH unit, but washing the curd with a 0.1% sodium carbonate solution increased the pH 0.04 to 0.08 of one pH unit. Extensive growth of the slime-forming bacteria on cottage cheese always resulted in an increase in pH, especially in the area where the slime was concentrated. Generally, the first evidence of slime occurred at a lower pH when the cheese was held at 10° C. than when it was held at 21° C. Slime formation on cottage cheese increased rapidly after its first appearance. The rapid formation may be due to the fact that the pH was made more favorable by bacterial growth.

The extremely low heat resistance of the bacteria associated with the slimy defect of cottage cheese suggests that the organisms result from post-pasturiza-

fion contamination, as pointed out by Elliker *(2)*. Contaminated equipment or water supplies appear to be likely sources of the organisms.

Bacteria capable of producing slimy cottage cheese were isolated from the water supplies of two plants. Although the results presented in Table 5 indicate that some of the slime-forming bacteria are relatively resistant to chlorine, it should be emphasized that the bacterial populations used in these trials were greater than may be expected in water supplies. However, such populations may be present on equipment and containers reused for storage and transportation of bulk curd. Relatively low concentrations of chlorine have been effective in eliminating spoilage organisms from the water supply, particularly in instances where the chlorine was injected into the water supply in a manner that permitted sufficient time for destruction of organisms. Chlorination of a water supply at the point of entering the vat or chlorination in the vat could not be expected to accomplish the desired result.

Bacteria representing five genera were capable of producing slime on cottage cheese. Since the samples from which these organisms were isolated came from a rather small area, it is probable that other types also are capable of producing the defect.

SUMMARY

The rate of slime formation on cottage cheese inoculated with bacteria capable of producing the defect was related to the temperature of holding. Some cultures failed to produce slime at 4.4 or 10° C. in 14 days, whereas others produced it in 7 to 11 days at 4.4° C., and in 5 to 7 days at 10° C. All cultures produced slime on cottage cheese held at 21° C. in 1 to 4 days.

Cottage cheese having a pH of 4.7 readily developed slime when the temperature was favorable. As slime formed on a cottage cheese sample, the pH of the slimy area increased. Formation of slime was rapid after its initial appearance.

Cottage cheese curd washed with suspensions containing 2,000,000 to 120,000,000 bacteria per milliliter retained from 0.0093 to 0.40% of the organisms. Ropy cultures were retained more readily than nonropy cultures. The temperature of incubation had greater effect on the rate of slime formation than did the number of bacteria in the wash water.

The bacterial cultures capable of forming slime on cottage cheese employed in this study were destroyed by a 2.5-minute exposure to 62.8° C. Seven of the 15 slime-forming cultures survived a 1-minute exposure to 5 p.p.m. chlorine; three survived a 1-minute exposure to 25 p.p.m. chlorine; and two survived a 1-minute exposure to 50 p.p.m. chlorine. The two most resistant cultures survived a 25- but not a 45-second exposure to 100 p.p.m. chlorine.

Cultures capable of producing slime on cottage cheese were classified as belonging to the following genera: *Proteus* (three cultures), *Pseudomonas* (four cultures), *Aerobacter* (five cultures), *Alcaligenes* (two cultures), and *Achromobacter* (one culture).

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A DETAILED STUDY OF LEVELS OF CERTAIN BLOOD CONSTITUENTS IN NORMALLY CALVING DAIRY COWS AND IN DAIRY COWS WITH PARTURIENT PARESIS [1, 2, 3]

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Considerable information is available in the scientific literature concerning the levels of organic and inorganic constituents in the blood of normal parturient dairy cows and of cows afflicted with parturient paresis (milk fever). It is well established that levels of many of these constituents change as a result of milk fever; however, how rapidly the changes occur prior to the appearance of symptoms is not known.

Increased blood levels of magnesium, lactic and pyruvic acids, and glucose and lowered blood citric acid have been reported in cows afflicted with parturient paresis *(8, 14)*. The low blood levels of calcium and phosphorus in cows with this disorder have been recognized for many years.

One of the most detailed studies on levels of blood constituents in cows with milk fever was made by Fish *(4)*. This study, as is true of nearly all studies of this type, was made during the posttreatment period when the cow was recovering from milk fever symptoms. This investigator reported increased blood glucose and lactose levels after insufflation therapy. The levels of these constituents were determined at frequent intervals until about 40 hours after insufflation. The same author *(5)* also studied blood calcium and inorganic and acid soluble phosphorus after udder insufflation. Levels of these constituents were low at the time of treatment and returned to normal 10 to 18 hours after treatment. Blood sugar levels, already rather high prior to treatment, continued to increase after inflation of the udder, after which they declined to normal levels within 20 to 30 hours.

Niedermeier and Smith *(11)* also have reported a study in which blood samples were drawn from cows with milk fever immediately pretreatment and 0.5, 1.5, 3.0, 5.0, 8.0, 11.0, 14.0, 17.0, 20.0, 36.0, and 48.0 hours postinflation. After studying calcium, phosphorus, and magnesium levels under these conditions, these authors suggest that relative levels of calcium, magnesium, and phosphorus may be more important to symptomatology than the level of any one constituent.

Ward *et al. (14)* determined the levels of calcium, sodium, potassium, chlorides, pyruvic acid, lactic acid, citric acid, and hematocrit in cows with milk

Received for publication August 13, 1953.

[1] Scientific Paper No. 1248, Washington Agricultural Experiment Stations, Pullman. Projects 920 and 1053.

[2] This investigation was supported in part by funds provided for biological and medical research by the State of Washington Initiative Measure No. 171.

[3] These data were taken in part from a thesis presented by the senior author to the faculty of the State College of Washington in partial fulfillment of the requirements for the Master of Science degree.

fever before treatment and at 5, 10, 15, and 30 days posttreatment. However, these investigators did not report any extensive study of the constituents prior to the development of symptoms.

The purpose of this experiment was to make a detailed study of the changes in blood levels of various organic and inorganic constituents in the period immediately prior to and subsequent to parturition or the development of milk fever. This study was designed to discover when these changes occur as related to parturition and the development of milk fever symptoms. In order that this might be done, blood samples were drawn at frequent intervals before, during, and after parturition in both normal and milk-fever cows.

EXPERIMENTAL METHODS

Two groups of cows were chosen for this experiment. One group of four cows had a history of recurrent milk fever, and the other group of four cows had no previous history of milk fever. More specifically, cow 2116, a mature Jersey and cows 237, 243, and 259, mature Holsteins, had prior histories of milk fever. These cows varied in age from 7½ to 11 years. The other four cows in this study were 309 and 311, 3-year-old Holsteins, and 3063 and 3067, 3- and 4-year-old Guernseys, respectively. The plan was to take blood samples from these cows at 30, 15, and 10 days prior to calving, and daily beginning 5 days prior to calving. When a cow was thought to be within 12 hours of parturition, samples were taken every 4 hours until parturition, after which time they were taken every 3 hours for 24 hours.

Blood was drawn for analysis every 6 hours for the succeeding period of 24 hours, after which samples were taken daily until 5 days postpartum and subsequently at 10, 15, and 30 days postpartum. The blood samples were taken to a chemical laboratory for analysis. The above procedure was found to be reasonably satisfactory. However, difficulty was experienced in accurately predicting the day and hour of calving. This resulted, in some cases, in a paucity of prepartum data.

Blood samples were drawn from the external jugular vein at the specified times. Two 15-ml. centrifuge tubes were filled at each collection. In one of the tubes, heparin was used as an anticoagulant; the blood in the other tube was allowed to coagulate. From the heparinized blood a Folin-Wu (Tungstic acid) protein-free filtrate and a trichloroacetic acid filtrate were prepared. The Folin-Wu filtrate was used for the glucose analyses and the trichloroacetic acid filtrate for the pyruvic acid analyses. A portion of the heparinized blood was used for the hematocrit determination. The remainder was centrifuged to obtain the plasma for the phosphorus determination.

The tube in which the blood was allowed to coagulate was centrifuged and the serum was removed for calcium determination. The samples were refrigerated within 1 hour after taking. For the blood glucose and pyruvic acid, the determinations were carried out within a few hours after the samples were drawn. The methods of analyses used were, for calcium, that of Clark and Collip *(3)*;

for phosphorus, that of Fiske and Subbarow *(6)*; for glucose, that of Benedict *(1)*; for pyruvic acid, that of Friedman and Haugen *(7)*; and for hematocrit, that of Wintrobe and Landsberg *(15)*. Statistical analyses were made according to Snedecor *(12)*.

RESULTS AND DISCUSSION

Data are given on eight cows—two Guernseys, five Holsteins, and one Jersey. Blood levels of various blood constituents were determined pre- and postpartum, according to the proposed plan. The blood data by constituents are given for each cow in Figures 1 to 5. The data are presented in this way in order to facilitate comparison of normal and milk-fever cows for any single constituent studied.

Cow 2116, a Jersey, was observed through two parturitions. She exhibited symptoms and was treated for milk fever at both parturitions. All of the other cows were observed through only one parturition. Cow 237, a Holstein, exhibited symptoms and was treated for milk fever. Thus, three cases of milk fever were available for this study. The other cows did not show any clinical symptoms of milk fever and appeared to be normal. However, cows 243 and 259 were put in

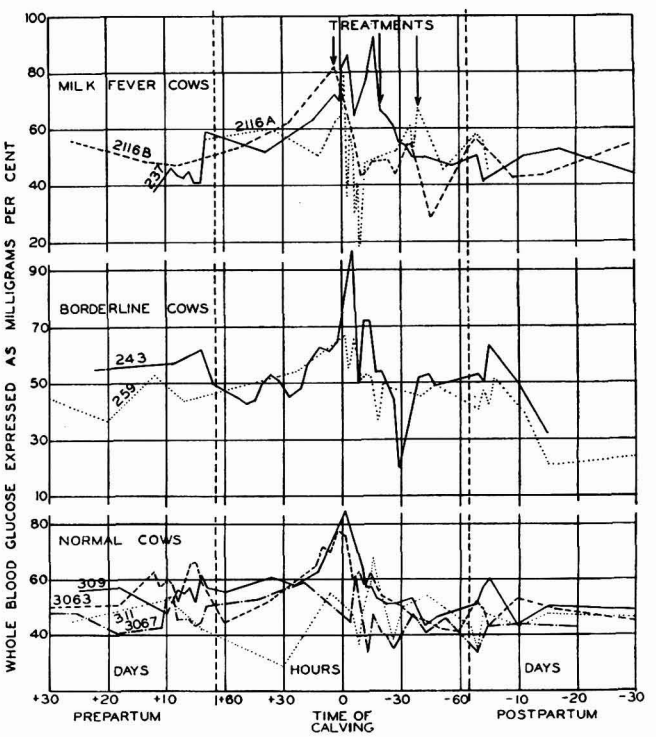

FIG. 1. Changes in blood levels of glucose in milk fever, borderline, and normal cows during the period near parturition.

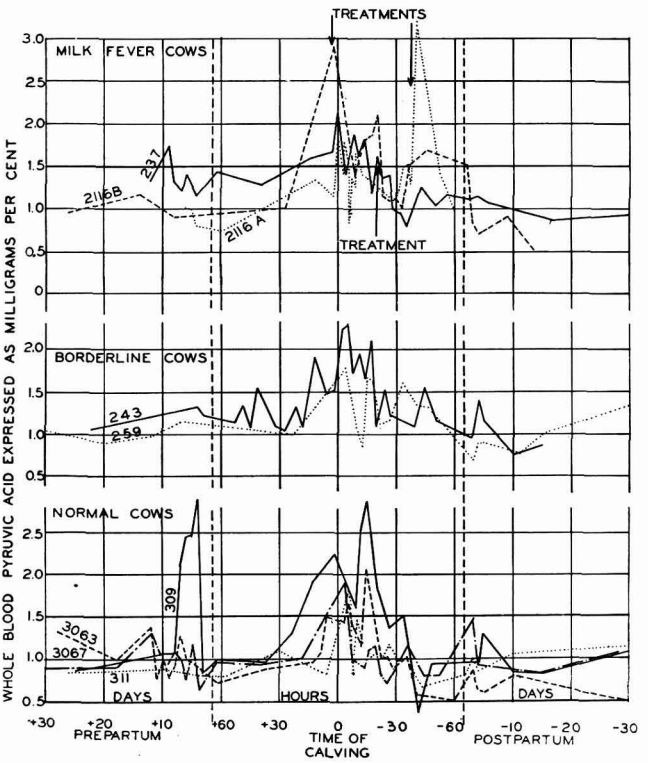

FIG. 2. Changes in blood levels of pyruvic acid in milk fever, borderline, and normal cows during the period near parturition.

a separate group in analyzing the data, since the drop in their blood calcium and phosphorus was much more marked than that of the other normal cows. The procedure of designating such animals as "borderline" has been used by other investigators *(2)*. Both the borderline cows and the cows showing milk fever symptoms in this experiment had previous histories of milk fever. None of the four normal cows had such a history.

Cow 2116, in her first lactation studied (designated as 2116A), became unsteady and depressed about 4 hours after parturition and 3 hours later appeared very weak. However, 1 hour later she had apparently recovered and was eating and behaving normally. At 28 hours postpartum this cow went down in a semi-comatose condition. At this time the udder was inflated and a partial recovery occurred, but 5 hours later the cow had relapsed and could not rise. A calcium borogluconate injection was then given, and a prompt and complete recovery resulted. In the second parturition studied (designated as 2116B), symptoms of milk fever developed prepartum, and after treatment with calcium borogluconate, the cow rapidly recovered and calved normally.

Cow 237 began to show some weakness 13 hours postpartum. By 17 hours post-

partum the cow was unable to rise. Three and one-half hours later the cow was treated with calcium borogluconate, and complete recovery ensued.

Figure 1 shows trends in whole blood glucose associated with parturition. In cows 2116B and 237, separate peaks in blood glucose occurred, which were associated with the development of milk fever. In these cows the glucose was the highest at the onset of the symptoms and remained at a high level, declining as recovery occurred after treatment. However, in cow 2116A and the borderline cows, 243 and 259, the blood glucose reached a high level after parturition and declined precipitously to a very low value and then rose again. In cow 2116A, this was associated with the spontaneous recovery from milk fever symptoms. As has been mentioned, however, this cow later relapsed. In cow 243, the total decline in blood glucose level in 24 hours was 76 mg. %. Possibly this pattern may be indicative of some response to the condition of impending milk fever whereby that condition is avoided.

A very marked rise in pyruvic acid (Figure 2), which was generally associated with the rise in glucose, was noted in all cows. The rise in blood pyruvic acid began about 24 hours prepartum. Blood pyruvic acid levels were considerably

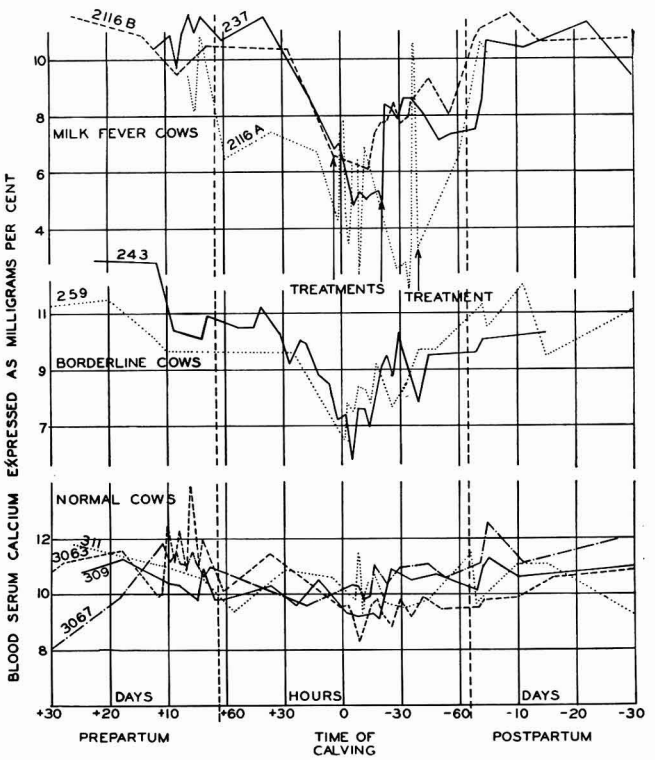

FIG. 3. Changes in blood serum levels of calcium in milk fever, borderline, and normal cows during the period near parturition.

higher in the cows exhibiting symptoms of milk fever than in normally calving cows. This is in agreement with the findings of Ward *et al. (14)*. However, the levels found in normal cows in this study seemed to be somewhat higher than those reported by Ward *et al.*

Between the 7th and 4th days prepartum, cow 309 showed an unexpectedly high level of blood pyruvic acid. This is difficult to explain, since from outward appearances the cow was not abnormal. It is perhaps worthy of note, however, that this cow had a foreign body operation about 4 months after calving. It is possible that this cow was suffering from the foreign body near the time of parturition and that the pressures arising as a result of pregnancy caused some temporary discomfort. Furthermore, this cow was difficult to bleed during the first few days in which samples were taken daily. The excitement associated with bleeding may have contributed to the elevated pyruvic acid levels.

Figures 3 and 4 show, respectively, blood levels of calcium and phosphorus in the cows studied. The most noticeable difference between the cows with milk fever and the borderline cows as compared with the normal animals was the progressive and rapid decline in blood calcium and phosphorus in the former group from approximately 30 hours prepartum until a few hours postpartum,

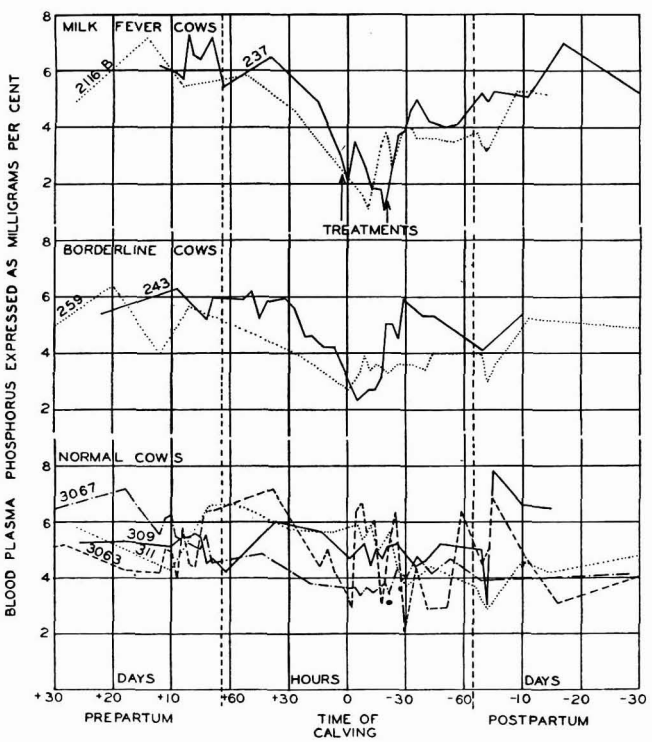

FIG. 4. Changes in blood plasma levels of phosphorus in milk fever, borderline, and normal cows during the period near parturition.

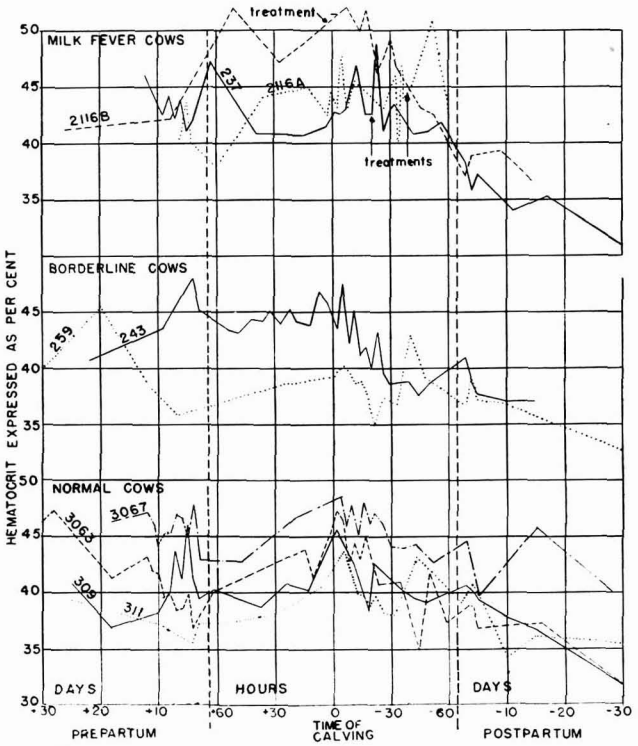

FIG. 5. Changes in hematocrit percentage in milk fever, borderline, and normal cows during the period near parturition.

or until the development of milk fever symptoms. A great deal of detailed data were secured from cow 2116A, for at that parturition blood samples were drawn hourly from 2 hours prepartum until 9 hours postpartum. This cow was not treated until 34.5 hours postpartum, but for the few hours near parturition she apparently was warding off an impending attack of milk fever. No blood plasma phosphorus determinations were run on this cow. It is apparent from studying blood calcium levels of 2116A in Figure 3 that this cow seemed to "bounce" down to her low levels of calcium. Blood glucose levels (see Figure 1) also showed very erratic behavior during this period.

In order to emphasize the differences in blood levels of certain constituents between cows with milk fever and normal cows, correlations between glucose and pyruvic acid, glucose and phosphorus, and phosphorus and pyruvic acid were calculated. In making these calculations, the data were divided so that analyses of blood from normally calving cows were in one segment of the data and those from milk-fever and borderline cows in another. Only data for samples taken from parturition to 48 hours postpartum were used in the calculations.

In the data for cows with milk fever and borderline cows, when the between-cow differences were removed, the correlations were, for glucose and pyruvic

acid, +0.47; for glucose and phosphorus, —0.84; and for phosphorus and pyruvic acid, —0.59. All of these correlations were significant at the 1% level of probability. In the normal group contrasting correlations were obtained. All of the correlations were positive and only one of them, that between glucose and pyruvic acid (+0.39), was significant, and that only at the 5% level of probability. The correlations between pyruvic acid and phosphorus (+0.24) and glucose and phosphorus (+0.11) were of lower order and not significant.

Interpretation of these differences is difficult. It seems, however, that blood pyruvic acid parallels blood glucose in both milk-fever and normally calving cows. This situation is not true in ketosis, where the blood glucose is low and the blood pyruvic acid is high.

The lower order and nonsignificant correlations of glucose and pyruvic acid with phosphorus seem to indicate that in normal cows the inorganic plasma phosphorus moves more or less independently of these two organic constituents. However, the significant negative correlations between these constituents in cows with milk fever illustrate the consistency with which these constituents change in relation to one another.

The hematocrit data are presented in Figure 5. In most of the cows studied, the level of hematocrit was higher during parturition and later declined, so that by 10 to 30 days postpartum, values were lower than at any other time in the study. There were no important differences between milk-fever, borderline, or normal cows in the levels of hematocrit. The high hematocrit values at parturition might be explained on the basis that during the short period near the time of parturition water intake may be low. Furthermore, Ward *et al. (13)* have indicated an increased volume of urine on the day of parturition. This may enhance the hemoconcentration. It is a well known fact that lactating cows consume more water than nonlactating cows. This increase in water consumption may account for the decline in hematocrit in the period following parturition.

In view of the high blood glucose and pyruvic acid and also the glycosuria *(10)* and increased nitrogen excretion reported in milk fever *(9)*, the similarity to the effect of the injection of adrenal hormones suggests an adrenal involvement probably as a response to the stress condition occurring. Thus, one could hypothesize that at the time of parturition there is an increased level of corticoid hormones in the body as a result of stress. This in turn would create the hyperglycemia, glycosuria, and increased nitrogen excretion also found when adrenal cortical hormones are injected.

The elevated blood pyruvic acid demonstrated here both in normally calving cows and in cows with milk fever has been reported to occur also in cows with ketosis and also in humans and experimental animals under varied stress conditions such as physical exercise, diabetes mellitus, and thiamin deficiency.

Although certain of the conditions occurring in parturient paresis can be explained on the basis of adrenal activity, no research has been reported to relate the adrenals directly to calcium and phosphorus metabolism. Thus, the influence of the adrenals on the occurrence of milk fever in parturient cows is not known.

SUMMARY

Experimental results have been presented on eight cows in nine parturitions in a study of the changes that occur in levels of whole blood glucose and pyruvic acid, serum calcium, plasma phosphorus, and hematocrit, during normal and abnormal parturitions.

A markedly increased blood glucose and blood pyruvic acid were noted in all parturient cows. Increases associated with the development of milk fever in three cows also were noted. The level of pyruvate associated with milk fever was considerably higher than that associated with parturition. Erratic declines in blood plasma phosphorus and serum calcium were noted in cows that exhibited symptoms of milk fever. Smaller declines were noted in two borderline cows. Normal cows showed slight declines in these constituents.

Correlations between blood glucose and blood plasma phosphorus and between blood pyruvic acid and blood plasma phosphorus were negative and very highly significant in the milk fever and borderline cows. These same correlations were positive and not significant in normal cows. The correlations between blood glucose and blood pyruvic acid were positive and highly significant for both milk fever and borderline cows and for normal cows.

An increase in the level of hematocrit was noted in all cows at the time of parturition with a gradual decline in the postpartum period. Generally the hematocrit level was lower in all cows at 15 to 30 days postpartum than at any other time in the study. No important differences were noted in the hematocrit data between normal, borderline, or milk-fever cows other than the expected individual differences.

ACKNOWLEDGMENTS

The assistance of G. M. Ward and J. B. Crilly with the collection and analysis of some of the samples is gratefully acknowledged.

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THE EFFECT ON MILK AND FAT PRODUCTION OF INJECTIONS OF OXYTOCIN AT ALTERNATE 14-DAY PERIODS DURING LACTATION[1]

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An injection of oxytocin after a thorough normal milking causes a more complete evacuation of the mammary gland *(1, 2, 3, 6, 7, 8, 11)*. Milk retained in the udder after a normal milking is known as residual milk, and in normal cows it represents 12 to 20% of the total yield *(1, 2, 3, 7)*.

The amount of residual milk is proportional to the yield and thus varies directly with the stage of lactation *(6, 7, 11)*. A difference has been noted between breeds *(11)* in the amount of residual milk. The amount of milk obtained at the first subsequent normal milking after an injection of oxytocin is reduced by approximately the same amount as the additional milk obtained at the previous milking *(3, 7)*. Thus, it appears that a rather constant amount of milk is retained in the udder after a normal milking, and evidence presented by Adams and Allen *(2)* indicates that the fat not obtained at one milking is removed at the subsequent milking.

The amount of residual milk is directly influenced by the completeness of response to the milking stimulus. If the stimulus is inadequate, then the response is incomplete. Any circumstance at the time of milking that distracts the cow results in a less complete emptying of the udder, with a concomitant increase in the amount of residual milk *(4, 12)*. Some workers have reported that cows which chronically fail to respond adequately to a normal milking stimulus lack in persistency *(8, 9)*. Since increased intraalveolar pressures have an inhibiting effect on the synthesis of milk *(5, 10)*, lack of persistency has been explained on the basis that residual milk contributes to higher pressures, thereby inhibiting milk secretion *(8)*.

The purpose of this study was to ascertain the effect on milk and fat production of injections of oxytocin during alternate 2-week intervals throughout lactation with cows that previously had showed lack of persistency and with normal cows.

Received for publication August 13, 1953.

[1] Published with the approval of the Director of the Wisconsin Agricultural Experiment Station. A contribution from the Wisconsin Agricultural Experiment Station as a collaborator under the North Central Region cooperative project entitled "Improvement of Dairy Cattle Through Breeding—NC2," conducted in cooperation with the Bureau of Dairy Industry, U.S.D.A.

[2] Agent of the Bureau of Dairy Industry.

EXPERIMENTAL PROCEDURE

Seven cows in the Emmons Blaine, Jr., Experimental Farm herd at Lake Mills, Wisconsin, were used in this experiment. Two of the cows, No. 55 and 56, half-sisters, had demonstrated a lack of persistency of production in the second lactation by drying off spontaneously at 277 and 265 days, respectively. The first three lactations of cow 102 were characterized by low production. The other four cows were relatively high producers with records consistently above herd average. Each cow had a preliminary period prior to being subjected to a control or experimental period. Four of the cows were subjected to a control period before an experimental period, and the other three were subjected to treatment of an experimental period before a control period. Each cow was milked through ten experimental and ten control periods. The length of the preliminary period was dependent on the relation of the time of calving to the beginning of a period and rapidity of recovery from calving. Ten I.U. of oxytocin were injected intrajugularly immediately prior to each milking during experimental periods. The normal milking procedure practiced during control periods consisted of washing and massaging the udders several minutes before attachment of the machines. The cows were milked twice daily and machine stripped. The first day of each period was considered transitional. Single samples were taken from each milking on the transition days, day 2 and day 14. A composite for each period was made up of an aliquot sample from each milking on days 7, 8, and 9. All samples were tested for milk fat by the Babcock method.

RESULTS

Mean milk production by 13-day periods (the day of transition was omitted) for each cow is presented in Table 1. A composite mean for each period, each lactation, and composite lactation also are presented. Without exception, the over-all mean milk production was higher for experimental periods than for previous or subsequent control periods. The mean differences between experimental and control periods for individual cows ranged from 0.7 to 7.0 lb., with a composite mean of 3.6 lb. per day per cow higher for the experimental periods.

In Table 2 are presented the mean milk and fat production and the butterfat percentage on specific days of the experimental and control periods throughout the lactation. There was an increase in mean milk production from the transition or first day of control periods through the 14th day of the experimental periods. The mean difference in milk production between the 14th day of the experimental periods and the transition days or first days of control was 7.8 lb. This marked difference was apparently the result of the retention of milk by the udder.

The mean per cent of fat was lowest on the day of transition from experimental to control and highest on the day of transition from control to experimental. Mean fat production followed the same pattern as the per cent of fat.

In Table 3 is presented the weighted mean per cent of fat for the control and experimental periods. These values were obtained by multiplying the per cent of fat of the aliquot samples taken on days 7, 8, and 9 of each period by the

TABLE 1

Mean daily milk production for preliminary, experimental and control periods

Cow No.	Preliminary period		Treatment	Number of period 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	Total	Mean	Difference
	(days)	*(lb.)*		*(lb.)*	*(lb.)*	*(lb.)*	*(lb.)*	*(lb.)*	*(lb.)*	*(lb.)*	*(lb.)*	*(lb.)*	*(lb.)*	*(lb.)*	*(lb.)*	*(lb.)*	*(lb.)*	*(lb.)*	*(lb.)*	*(lb.)*	*(lb.)*	*(lb.)*	*(lb.)*	*(lb.)*	*(lb.)*	*(lb.)*
3	17	51.9	Exp.		69.6		69.5		59.2		48.0		43.9		40.3		36.8		33.0		28.8		19.9	449.0	44.9	
			Control	63.9		62.5		54.4		43.3		43.8		39.1		35.1		30.1		25.8		26.5		424.5	42.5	2.4
34	17	53.6	Exp.	62.3		58.8		52.5		47.4		42.1		34.7		39.8		38.4		38.6		37.1		451.7	45.2	
			Control		57.8		50.7		45.3		39.8		35.8		35.1		36.4		35.9		35.9		32.5	405.2	40.5	4.7
56	10	44.5	Exp.		54.0		58.4		57.0		50.1		45.7		42.8		35.6		26.1		12.3		5.6	387.6	38.8	
			Control	48.6		54.5		54.5		49.8		43.0		41.4		36.0		31.0		16.3		6.0		381.1	38.1	0.7
55	10	49.9	Exp.	59.5		70.0		67.2		59.8		53.2		46.6		41.0		32.3		25.2		16.0		470.8	47.1	
			Control		64.9		66.1		59.5		48.9		46.6		41.0		32.8		25.3		17.2		6.8	409.1	41.0	6.1
65	7	55.3	Exp.		66.1		64.9		57.9		50.9		41.8		42.2		39.7		38.2		36.2		34.8	472.7	47.3	
			Control	62.0		61.9		56.4		47.1		42.6		40.7		40.3		35.4		33.3		28.7		448.4	44.8	2.5
102	8	49.8	Exp.	58.5		71.3		69.9		60.5		51.4		50.6		43.3		34.9		29.0		25.4		494.8	49.5	
			Control		69.0		65.6		56.3		45.5		46.8		40.9		31.4		29.1		25.1		15.1	424.8	42.5	7.0
97	6	50.5	Exp.		64.8		63.1		59.2		51.6		48.6		49.5		46.6		46.6		42.1		32.4	504.5	50.5	
			Control	57.8		63.0		56.7		53.2		45.4		45.7		44.2		42.6		43.7		31.1		483.4	48.3	2.2
Total	75	355.5	Exp.	180.3	254.5	200.1	255.9	189.6	233.3	167.7	200.6	146.7	180.0	131.3	174.8	124.1	158.7	105.6	143.9	92.8	119.4	78.5	92.7	3231.1		
Mean	10.5	50.7		60.1	63.6	66.7	63.9	63.2	58.3	55.9	50.1	48.9	45.0	43.7	43.7	41.4	39.7	35.2	35.9	30.9	29.9	26.2	23.2	46.1		
Total			Control	232.3	191.7	241.9	182.4	221.8	161.1	193.4	134.2	174.8	129.2	166.9	117.0	155.6	100.6	139.1	90.3	119.1	78.2	92.3	54.4	2976.5		3.6
Mean				58.1	63.9	60.5	60.8	55.4	53.7	48.4	44.7	43.7	43.1	41.7	39.0	38.9	33.5	34.8	30.1	29.8	26.1	23.1	18.1	42.5		

TABLE 2

Mean milk, B.F. percentage, and fat production on specific days of the experimental and control periods

Cow No.	Transition 1st day of control			2nd day of control			14th day of control			Transition 1st day of exp.			2nd day of exp.			14th day of exp.		
	Milk	Fat		Milk	Fat		Milk	Fat		Milk	Fat		Milk	Fat		Milk	Fat	
	(lb.)	*(%)*	*(lb.)*	*(lb.)*	*(%)*	*(lb.)*	*(lb.)*	*(%)*	*(lb.)*	*(lb.)*	*(%)*	*(lb.)*	*(lb.)*	*(%)*	*(lb.)*	*(lb.)*	*(%)*	*(lb.)*
3	36.0	1.84	0.66	38.9	2.83	1.10	41.1	3.18	1.31	42.4	3.72	1.58	43.0	3.19	1.37	45.0	3.19	1.43
34	35.6	2.56	0.91	40.3	3.77	1.51	41.8	3.81	1.60	42.4	4.20	1.78	42.8	3.82	1.63	42.9	3.94	1.69
55	36.9	2.10	0.77	38.8	2.77	1.07	39.5	3.04	1.20	40.4	3.35	1.35	40.7	3.00	1.22	43.3	3.07	1.33
56	31.4	2.47	0.78	35.0	3.01	1.05	35.6	3.35	1.20	36.8	3.53	1.30	36.3	3.24	1.18	38.0	3.41	1.29
65	37.3	2.55	0.95	41.1	3.65	1.50	42.8	3.51	1.50	42.3	3.93	1.66	43.8	3.65	1.60	45.0	3.83	1.72
97	38.0	2.10	0.80	43.4	3.31	1.43	44.9	3.40	1.52	45.7	3.71	1.69	47.1	3.39	1.60	46.5	3.53	1.64
102	37.1	1.36	0.50	41.0	2.13	0.88	42.1	2.70	1.14	44.8	3.36	1.50	45.2	2.78	1.30	45.7	2.93	1.33
Total	252.3		5.37	278.5		8.54	287.8		9.47	294.8		10.86	298.9		9.90	306.4		10.43
Mean	36.0	2.12	0.77	39.8	3.07	1.22	41.1	3.29	1.35	42.1	3.68	1.55	42.7	3.31	1.41	43.8	3.41	1.49

amount of milk produced on those days. The per cent of fat was 0.063 higher for the experimental periods. However, the difference was not statistically significant ($P > 0.05, < 0.10$).

TABLE 3

Weighted mean per cent of fat for control and experimental periods

Cow No.	Experimental	Control
	(%)	*(%)*
3	3.27	3.25
34	3.92	3.89
55	3.14	3.01
56	3.51	3.34
65	3.80	3.84
97	3.46	3.42
102	3.16	3.07

TABLE 4

Fat production records of cows used in the experiment adjusted to a M.E. basis

Cow No.	Production for experimental lactation		Production for preceding lactation		Mean production of all other lactations	
	(lb.)	*(%)*	*(lb.)*	*(%)*	*(lb.)*	*(%)*
3	417	3.2	368	3.2	427	3.4
34	522	3.9	524	3.9	510	4.0
55	412	3.0	301	3.1	298	3.2
56	417	3.5	331	3.3	366	3.4
65	506	3.7	475	3.7	455	3.8
97	522	3.4	496	3.4	486	3.5
102	371	2.7	327	2.8	299	2.9
Mean	452	3.34	403	3.36	406	3.48

The 305-day lactation records for the cows in the experiment are presented in Table 4. The amount of butterfat produced while the cows were on this experiment is compared with the fat production of the preceding lactation, as well as with the mean fat production of all previous records. All records were adjusted to a mature equivalent basis using the Holstein-Friesian factors given in the 1953 report of the Dairy Cattle Breeding Committee at the 48th annual meeting of A.D.S.A. The mean difference between the records made on experiment and in the preceding lactation was 49 lb., whereas the difference between the mean of all previous records of these cows and the record made on the experiment was 46 lb. Both of these differences are statistically significant.

Cows 55, 56, and 102 were those whose previous history indicated poor persistency, and they have shown the largest increases in production. It may be argued that since they were low producers in earlier lactations, some regression to the herd average would be expected. On the other hand, if regression was important in the case of the above three cows, one might expect cows 34, 65, and 97 also to regress to the herd average. They actually showed an increase of 18 lb. over their preceding records and 33 lb. more than the mean of all their previous records.

The mean number of services required per conception prior to the experimental lactation was 1.80. For the experimental lactation, 1.43 services were required per conception, which resulted in a calving interval of 13.0 months. Thus, oxytocin as administered in this study appeared to have no effect on conception.

DISCUSSION

It is difficult to determine the effect of a treatment when the only standard of comparison is a previous lactation, since production is affected by age and environment. Consequently, this experiment was designed with relatively short alternate experimental and control periods within a lactation. However, even in a lactation the effect of stage of lactation has to be taken into account. Those cows, No. 34, 55, and 102, with the greatest mean difference in milk production between experimental and control periods had an experimental period, with natural relatively high production, prior to a control period. Furthermore, the last control period of a cow that commenced with an experimental period occurred 2 weeks after the last experimental period at a stage of lactation when production normally falls off rapidly. Thus, there is a production advantage to whichever period occurs first in a lactation. Consequently, the mean difference in milk production of 3.6 lb. per cow per day between experimental and control periods is probably conservative, since four of the seven cows started their lactations with control periods.

In order to compare the effect of oxytocin injections on persistency, the mean production at biweekly intervals of all lactations for all cows was plotted. Injections of oxytocin did not appear to affect persistency as judged by these comparisons. The actual level of production for cows 55, 56, and 102 for the experimental lactation was considerably higher than for previous lactations, but the rate of decline was not appreciably different. Oxytocin injections maintained these three cows at a higher plane of production without affecting persistency.

The mechanism responsible for the increased milk production when a cow is injected with oxytocin is not known. Perhaps oxytocin has a favorable stimulating effect on the physiological processes involved in lactation, or the effect may be purely physical. The more complete evacuation of the udder by use of oxytocin may be the stimulating factor.

SUMMARY

The effect of an intravenous oxytocin injection at alternate 14-day periods on milk and fat production of seven Holstein-Friesian cows during a lactation has been studied. Mean daily production per cow during the injection periods was 3.6 lb. higher than during control periods when the transition day was omitted from each period. The per cent of fat in the milk was not significantly different between the experimental and control periods.

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A MODIFIED PEROXIDE TEST FOR DETECTION OF LIPID OXIDATION IN DAIRY PRODUCTS

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Oxidation of milk lipids is of major importance to the dairy industry. A simple objective method of measurement would facilitate control procedures. The modified ferric thiocyanate method of Hills and Thiel *(3)* for estimating fat peroxides yields very satisfactory results on butter oil, but obtaining an oil sample from homogenized fluid products is very difficult, if not impossible, and even churning of unhomogenized cream is a laborious procedure. Furthermore, it is not possible to secure a sufficiently high concentration of oil in the benzene-methanol solvent proposed for the extraction of dry milk. This is especially true of powders of low peroxide content.

Various surface-active agents have been successfully employed to liberate fat from milk and cream *(1, 2, 4, 5, 6, 7)*. The work reported herein was undertaken to investigate the possibility of utilizing such an agent to secure butter oil not only from fluid milk but also from reconstituted dry milk for the estimation of fat peroxides by the ferric thiocyanate method.

APPARATUS

Centrifuge. A Babcock machine is satisfactory, or any centrifuge that will receive the cream test bottles employed in the procedure may be used.

Boiling water bath. A metal container holding water at a depth of about 5½ in. and heated on a gas plate is satisfactory.

Constant temperature water bath. The bath should be equipped with a stirrer and should be thermostatically held at 50 ± 0.2° C.

Spectrophotometer. A Coleman Model 11 Universal Spectrophotometer and 13 mm. square cuvettes were used.

Glassware. Standard 9-g., 50% cream test bottles, 0.5-ml. Ostwald-Folin pipettes and 10-ml. glass-stoppered volumetric flasks. All glassware must be free of iron or oxidized fat. Thorough washing with a detergent such as "Sepco," rinsing with distilled water, and oven drying just prior to use yield the most satisfactory results. Additional glassware is required for the preparation of the standard curve.

REAGENTS

BDI Reagent (5). Thirty g. of Triton X-100 (a nonionic surface-active agent manufactured by the Rohm and Haas Company, Philadelphia, Pa.) and 70 g. of sodium tetraphosphate are made up to a volume of 1 l. with distilled water.

Received for publication August 13, 1953.

Paper 3039, Scientific Journal Series, Minnesota Agricultural Experiment Station.

Peroxide Reagents (3).

a) Benzene-methanol solvent. A mixture of 70 volumes of thiophene-free benzene and 30 volumes of C. P. methanol is employed. The benzene is redistilled and the methanol is dried by refluxing for 4 hours with magnesium ribbon (5 g. per liter) followed by distillation.

b) Ferrous chloride solution (approximately 0.014 *M*). Hydrated barium chloride (0.4 g.) dissolved in 50 ml. water is added slowly with stirring to a solution of 0.5 g. of hydrated ferrous sulfate in 50 ml. water. Finally, 2 ml. of 10*N* hydrochloric acid is added. The precipitated barium sulfate is allowed to settle, and the clear solution is decanted into a brown glass bottle and stored in a refrigerator. All reagents should be as free as possible of ferric iron.

c) Standard ferric iron solution. Iron wire (0.250 g.) is dissolved in 25 ml. of 10*N* hydrochloric acid and oxidized with hydrogen peroxide. The excess peroxide is boiled off and the solution diluted to 250 ml. with water.

d) Ammonium thiocyanate solution. Thirty g. of this salt is dissolved in water and made to a volume of 100 ml.

PREPARATION OF THE STANDARD CURVE

For the preparation of the standard curve, appropriate aliquots of the standard ferric iron solution (containing 1 mg. of ferric iron per milliliter) are first diluted to 100 ml. including sufficient 10*N* HCl to bring the concentration of the acid to approximately that used in the ferrous chloride solution. One-half ml. portions of the diluted standard iron solutions are added to 50 ml. volumetric flasks and made up to the mark with the benzene-methanol solvent as indicated by the details in Table 1. After vigorous mixing, a 10-ml. volumetric flask

TABLE 1
Preparation of standard curve

Aliquot of stock Fe^{+++} soln. diluted to 100 ml.	Volume of 10N HCl included in diluted Fe^{+++} soln.	Concn. of Fe^{+++} per 10 ml. of solvent
(ml.)	*(ml.)*	*(γ)*
2	1.8	2
4	1.6	4
6	1.4	6
8	1.2	8
10	1.0	10
15	0.5	15

is filled to the mark with the iron-solvent mixture and one drop of ammonium thiocyanate reagent is added. After closing with a glass stopper, the flask is vigorously shaken and immediately placed in a water bath at 50° C. for exactly 2 minutes for color development. The temperature of the colored standard is lowered to approximately room temperature during about 2 minutes in an ice bath. Finally, the mixture is transferred to a cuvette and the light transmission is determined at 505 mμ with the instrument adjusted to 100% transmittance

with the benzene-methanol solvent. When the logarithm of the per cent transmittance is plotted against the micrograms of Fe^{+++} per 10 ml. of solvent, a straight line is obtained, indicating close adherence to Beer's law for the concentrations used.

PROCEDURE

A. *Isolation of the fat.*

1. The proper amount of sample is added to a 9-g. 50% cream test bottle. The amount of product required varies, of course, with its fat content, but the following have proved satisfactory:

a. Fluid whole (including homogenized) milk: 25 ml.
b. Cream: 9 ml.
c. Condensed whole milk: 9 ml.
d. Dry whole milk: 8 g. powder dispersed in 15 ml. distilled water.

2. BDI reagent is next added in sufficient quantity to bring the contents to within about ¼ in. of the base of the neck. The sample and reagent are well mixed.

3. The bottle is placed in a gently boiling water bath for 4 minutes. During this period the sample will show signs of oiling off. More BDI reagent is then added until the graduated portion of the neck is approximately half full. The bottle is then replaced in the bath for 3 more minutes, after which it is centrifuged for 1 minute in a Babcock centrifuge.

4. The oil is tempered by returning the bottle to the water bath for 3 minutes.

B. *Determination of peroxide value.* The method employed in determining the peroxide value of the isolated milk fat is essentially that of Hills and Thiel *(3)*.

1. A 0.5-ml. sample of the tempered butter oil is removed from the cream test bottle with a 0.5-ml. Ostwald-Folin pipette and placed in a 10-ml. standard taper volumetric flask. After the addition of benzene-methanol to the mark, the stoppered flask is inverted several times to dissolve the fat. This mixing must not be postponed and combined with the next step in the procedure.

2. One drop of ferrous chloride, followed by one drop of ammonium thiocyanate reagent, is added to the mixture in the flask and the flask shaken vigorously to disperse the reagents.

3. The color is developed and the light transmission determined as indicated for the preparation of the standard curve.

4. A fat blank should be run on the sample of the milk fat. A 0.5-ml. sample of oil is handled in precisely the same manner except that no ferrous chloride reagent is added.

5. A reagent blank, determined by the above procedure and omitting the milk fat addition in Step 1, should be run periodically to check for possible deterioration of the reagent. The ferrous chloride reagent should be discarded

when the transmittance on the reagent blank decreases appreciably below that of freshly prepared reagent.

6. It is convenient to express the peroxide value in terms of milli-equivalents of oxygen per kilogram of fat. In making this calculation, the per cent transmittance for the blanks must first be converted to micrograms of iron per 10 ml. solvent by means of the standard curve. The net value for the unknown in terms of micrograms of iron per 10 ml. of solvent is then calculated by subtraction of the sum of the fat and reagent blanks.

Then:

$$\text{Peroxide value (as m. eq. } O_2\text{/kg. fat)} = \frac{\text{Net } \gamma \text{ Fe per 10 ml.}}{\text{g. of fat used} \times 55.84}$$

EXPERIMENTAL

Selection of the de-emulsifier. The reagent recently proposed by Sager and Sanders *(5)* for determining the fat content of milk was chosen to prepare the milk fat, since it was the only reagent of the various materials tested that would effectively de-emulsify both fluid milk and reconstituted dry whole milk.

Effect of BDI reagent on peroxide value. The use of this reagent for securing the fat sample apparently has little effect on the resulting peroxide value when compared to the churning and oiling-off technique for obtaining a sample of fat. This is indicated in Table 2.

TABLE 2

Comparison of the peroxide values of oil secured from cream by churning and by de-emulsification with BDI reagent

	Peroxide value *(m. eq. of oxygen per kg. of fat)*			
Sample No.	I.	II.	III.	IV.
Fat from butter	0.214	0.220	0.247	0.180
Fat from BDI reagent-de-emulsified cream.	0.196	0.209	0.238	0.192

Oiling-off time. The time that the sample and de-emulsifying reagent remain in the boiling water bath is not critical since, as shown in Table 3, the limits of 5 and 15 minutes in the bath produced similar peroxide values. An oiling-off time of 7 minutes was found to be adequate for every type of sample tested and was selected to standardize the procedure.

TABLE 3

Effect of "oiling-off" time in the bath on the peroxide value of fresh cream

Time in bath *(minutes)*	Peroxide value *(m. eq. oxygen per kg. fat)*
5	0.160
15	0.160
30	0.183

Measurement of the oil by means of a pipette. If the centrifuged oil is first tempered for 3 minutes in the boiling water bath, excellent accuracy is obtained by measuring the fat sample by means of an Ostwald-Folin pipette rather than by weighing it. One pipette will suffice for an unlimited number of determinations if it is thoroughly rinsed between samples with acetone, followed by drying with an aspirator. Five successive 0.5-ml. aliquots of oil were found to deviate less than 0.2% from the average value of 0.4285 g. This results in a considerable saving of time when a large number of samples are to be analyzed.

Reproducibility. The method has good reproducibility, as illustrated by the data in Table 4. Determinations were made on four separate aliquots of oxidized whole milk by each of three operators. The best results were obtained by Operator 1, who had considerable previous experience with the method, but there are are only two cases in which the individual values deviate from the mean by more than 3%.

TABLE 4

Reproducibility of the proposed procedure for determining fat peroxides

Aliquot	Peroxide values *(m. eq. oxygen per kg. fat)*		
	Operator I	Operator II	Operator III
1	0.282	0.292	0.289
2	0.286	0.284	0.293
3	0.287	0.274	0.287
4	0.283	0.280	0.282
Av. each operator	0.285	0.283	0.288
Av. all aliquots	0.285		

Effect of concentration of ferrous chloride reagent. Hills and Thiel *(3)* suggested dilution of the ferrous chloride solution to 20% of its original concentration for use with milk fat low in peroxide content. Although such dilution may increase the sensitivity somewhat, it was observed that a single sample of fat gave different peroxide values if aliquots were analyzed with diluted and undiluted reagent. In order to ascertain the effect of the ferrous iron concentration on the peroxide value, solutions were prepared containing 20, 100, 200, and 300% of the ferrous iron concentration of the original ferrous chloride reagent (approximately 0.014 *M*). The concentration of hydrochloric acid was held constant in all of these solutions. Results obtained with these reagents are shown in Table 5.

Since there was little difference in the peroxide value on Cream Sample I, an excellent quality product, when using diluted or full-strength ferrous chloride, it seemed advisable to use the full-strength solution on all samples analyzed. Increasing the concentration of the iron beyond that recommended for the full-strength iron solution gave no values sufficiently higher to justify their use. It was also noted that the concentrated ferrous chloride reagents deteriorated more rapidly than did the recommended concentration.

Application of proposed method to dry milk. There is a definite increase in the peroxide content of milk fat during the manufacture of dry whole milk, as

TABLE 5

The effect of concentration of the ferrous chloride reagent on the peroxide values of milk fat (using 0.5 ml. oil per 10 ml. solvent)

Concentraton of ferrous chloride reagent as per cent of Hills & Thiel reagent[a]	Peroxide values *(m. eq. oxygen per kg. fat)*			
	Fluid milk	Cream		Dry milk
		I	II	
20	0.195	0.080	0.146	0.408
100	0.224	0.084	0.180	0.685
200	0.259	0.096	0.176	0.692
300	0.244	0.088	0.184	0.700

[a] Approximately 0.014 *M*.

TABLE 6

The increase in fat peroxides during the manufacture of dry whole milk

Sample No.	Peroxide value[a]		Sample No.	Peroxide value[a]	
	Raw milk	Dry milk		Raw milk	Dry milk
30	0.132	0.360	40	0.142	0.188
31	0.132	0.209	41	0.142	0.172
32	0.132	0.163	43	0.142	0.176
33	0.142	0.205	44	0.142	0.151
34	0.142	0.172	45	0.159	0.246

[a] As m. eq. of oxygen per kg. of fat.

shown by the data in Table 6 for 10 lots of dry milk. Furthermore, preliminary results indicate that most, if not all, of this lipid oxidation occurs during the drying step in the manufacturing process.

SUMMARY

The use of a nonionic detergent (BDI reagent) for breaking the emulsion has been found a satisfactory means of obtaining fat for estimation of peroxide value by the ferric thiocyanate method. The procedure is applicable to milk, cream, and condensed and dried milk products. It appears to be very satisfactory for routine control purposes. Its reproducibility and sensitivity are excellent.

ACKNOWLEDGMENTS

This paper reports research undertaken in cooperation with the Quartermaster Food and Container Institute for the Armed Forces and has been assigned No. 445 in the series of papers approved for publication. The views or conclusions in this report are those of the authors. They are not to be construed as necessarily reflecting the views or endorsement of the Department of the Army.

The support of the Minnesota Institute of Research also is gratefully acknowledged.

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GROWTH CHARACTERISTICS OF STREPTOCOCCAL PHAGES IN RELATION TO CHEESE MANUFACTURE

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A group of pure strains of *Streptococcus cremoris* and *Streptococcus lactis* selected for their lack of interrelationship in phage reactions has been in use in over 200 commercial cheese factories in New Zealand for about 8 years *(8)*.

It has been found in commercial practice, even when all practicable measures to exclude phage infection are taken, that some cultures cannot be used for more than 1 or 2 days in succession without serious risk of failure through phage infection either *(a)* of the starter culture during the course of its preparation or *(b)* of the starter during its growth in the cheese curd in the vat. Some cultures, however, are much less liable to infection and may (with the appropriate safeguards) be used daily over long periods without a similar risk of failure. Gradually it has become clear that these apparent differences in susceptibility to infection are due to the differences in the levels of air-borne phage infection, which normally becomes established in commercial factories for the different cultures. These levels are in turn related to the concentrations of the phage which appears in cheese whey even when cheese manufacture proceeds normally. A consideration of all the data led to the hypothesis that the concentration of phage developed in cheese whey under conditions of minimal initial infection depends on the two main growth characteristics of a phage race, viz., *(a)* its latent period or minimum length of time from infection of bacterial cell to lysis and *(b)* its burst size or average yield of phage particles per infected bacterial cell *(1, 2)*.

The experiments to be described in the present paper were designed to test this hypothesis, which, if true, would provide a rational explanation for the behavior of the various cultures in cheese-making practice and serve as a guide in the search for cultures less liable to infection than the majority under cheese factory conditions.

EXPERIMENTAL MATERIAL

Cultures. The cultures used comprised seven strains of *S. cremoris,* most of which have been in use as cheese starters in New Zealand for many years. They were selected in the first place for their high acid-producing activity in milk, for their capacity to withstand exposure to a temperature of 37° C. with a minimum of damage *(9)*, and for their lack of relationship to one another with respect to a series of lytic phage races. The strains were maintained in daily

Received for publication August 24, 1953.

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subculture at 22° C. in sterilized skimmilk. Throughout the experiments the initial inoculum (unless otherwise indicated) consisted of 0.1 ml. of the 24-hour clotted milk culture per 9 ml. of medium.

Phages. Six distinct phage races corresponding to six of the streptococcal strains were used in the experiments. The phages were originally isolated from cheese whey taken from vats in which the cultures were in use as starters. For the seventh culture, ML_5, three distinct phage races (easily distinguishable on the basis of plaque size) were available. The phages were prepared in the form of whey filtrates and stored in the refrigerator at 5° C. (For convenience in description in this paper the phages are named after the strains they attack, e.g., *hp* is the phage which attacks strain *HP*. But this is not to be taken as implying that the phage races have a unique relationship with the organisms, each of which may be susceptible to attack by several different phages, as is ML_5.)

PART I.

Method used for determination of rate of growth of phage. In order to obtain a general picture of the characteristics of the different phages under conditions similar to those which exist in a cheese vat, the following experimental method was used. A series of six tubes (9 ml.) of sterilized skimmilk was inoculated with culture at the rate of 0.1 ml. per tube (giving a bacterial infection of the order of 20 millions per milliliter). To each tube was added 1 ml. of a dilution of the specific phage containing a relatively small number of phage particles, not exceeding 3,000 in any experiment. The tubes were incubated at 30° C. At hourly intervals one of the cultures was taken for assay of its content of phage and bacteria. A direct count of the bacteria under the microscope was made on suitable dilutions of the culture. For the phage assay a set of serial 1-in-10 dilutions in water was prepared. Drops taken with a standard loop (0.004 ml.) from these dilution tubes were placed on marked areas on the surface of an agar plate which had just previously been spread with a mat of the appropriate organism. The plate was incubated overnight at 30° C. Clear areas or isolated plaques appeared on the bacterial mat where the phage had acted. The results were recorded diagrammatically, giving a picture of the rate and extent of phage development over a period of 7 hours. This experimental method was designed to give phage-growth results similar to those which might occur in a cheese vat where a strain of streptococcus in use as a starter became infected, at the beginning of the process, with a small amount of a phage capable of attacking the organism. Such an infection is in fact liable to occur regularly in practice, e.g., from the air or from incompletely sterilized equipment, wherever a given culture is in daily or even intermittent use in a factory over long periods.

RESULTS

The results obtained when small amounts of phage were added to cultures of six strains of streptococci in milk are shown in diagrammatic form in Figures 1 and 2, the dark areas corresponding to areas of lysis or to plaques. The right-

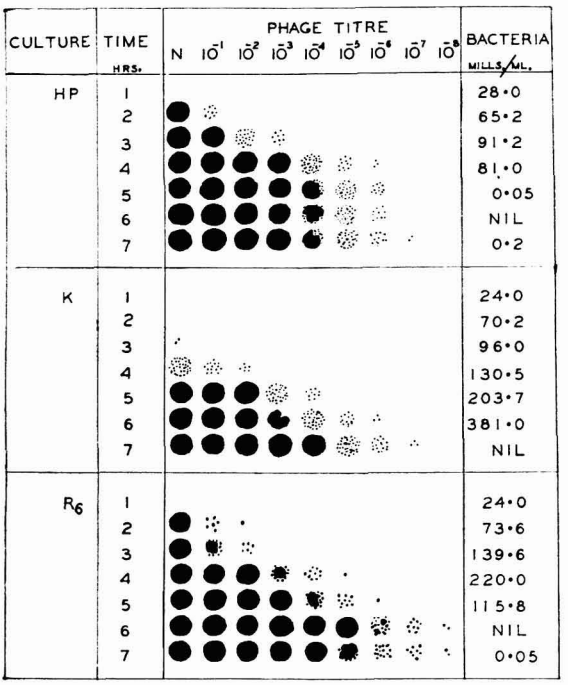

FIG. 1. Phage development in milk cultures of *S. cremoris*.

hand column gives the number of bacteria (in millions per milliliter) in the cultures as determined by direct count under the microscope. The following conclusions may be drawn.

(a) The several phage races showed marked differences in rate of development. Phage ml_6 was by far the most rapid in growth, reaching its maximum titre in 3-4 hours, whereas phage us_3 was still increasing in titre at 7 hours. The other races were intermediate between these two in their rate of development.

(b) Both slow- and fast-developing phages reached final titres of the same order since the fast phages (e.g., ml_6) lysed all the bacteria in the culture within 3 or 4 hours and hence could not develop further, while the slow phages (e.g., us_3) continued to develop on the bacteria for over 7 hours.

(c) With phages *hp* and r_6 there was evidence towards the end of the experiment of the multiplication of bacteria resistant to the phage.

The general characteristics of the phages as disclosed in the experiments, tended to correspond with the impressions gained during the use of the cultures as cheese starters. Cheesemakers had found that some of the cultures were more liable than others to phage infection during the course of starter preparation or manufacturing procedure and hence were more difficult to handle in commercial practice. In particular, as an extreme example, culture ML_6 had proved so "unreliable" that its use as a commercial starter had been discontinued.

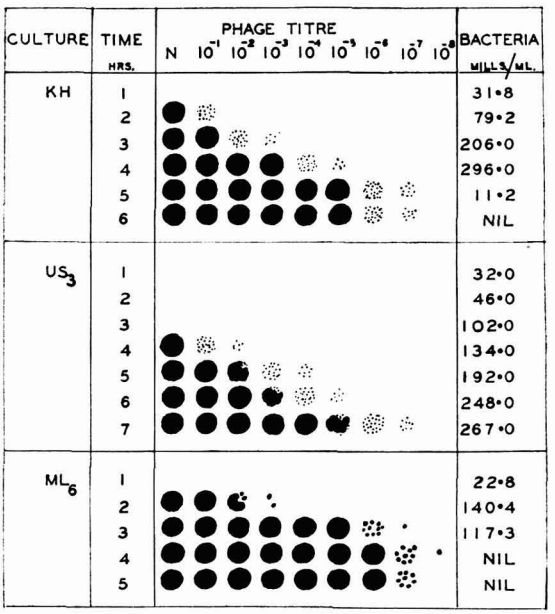

FIG. 2. Phage development in milk cultures of *S. cremoris*.

Culture R_6 also was known as an unreliable starter whereas K and US_3 were less liable to infection and consequent failure. So far as could be judged, therefore, making allowance for the impossibility of stating in definite terms the general experience of the cheesemaker, the level of "reliability" of the six cultures could be correlated, at least to some extent, with their behavior in milk in the presence of trace of a specific phage.

So far only a broad picture of the characteristics of the various phage races had been obtained. There was a multiplicity of factors operating under the experimental conditions used. The large excess in number of bacteria over number of phage particles at the beginning of an experiment meant that the processes of infection, multiplication of phage within the bacteria, lysis with liberation of a crop of phage particles, and reinfection of further bacteria, could be repeated until all the bacteria in the culture had been infected. The rate of development of phage would depend partly on the length of the latent period between infection and lysis, and partly on the burst size or average number of particles liberated from each lysing organism. But the supply of bacteria to serve as a substrate for phage growth would depend on the rate of multiplication of the organisms. A slow-growing strain would soon be "overtaken" by the phage, whereas a fast-growing strain would survive longer and thus yield a higher final concentration of phage. Multiple infection of some of the bacteria in the later stages of the process might reduce slightly the yield of phage.

The results so far described give, therefore, merely a laboratory parallel to, and a rough quatitative measure of, the complicated sequence of events following

infection of a starter with phage in the cheese vat. It then became necessary to undertake a more detailed investigation of phage growth in an attempt to disclose the precise reasons for differences in behavior between phage races.

PART II.

Method used for determination of latent period and burst size. Several of the variable factors in the foregoing experiments were eliminated in the technique used for determination of latent period and burst size of a phage. The essential features of the technique were: *(a)* infection of a few of the bacteria in a culture with one phage particle to each bacterium, and *(b)* dilution of the infected culture to such an extent that fresh phage particles produced when the bacteria burst were unlikely immediately to infect a further group of bacteria. In so far as it was possible to attain this state of affairs, estimation of *(a)* the minimum time from infection to lysis of the bacteria and *(b)* the average "crop" of phage particles from a single bacteria, was possible. Hence the relative parts played by latent period and burst size in determining the rate of development of a phage race could be defined.

The basis of the technique used was taken from Adams *(1)*. The procedure was as follows:

(a) A tube of lactose-yeast-phosphate (L.Y.P.) broth *(1)* was inoculated from the stock skimmilk culture of the appropriate streptococcal strain and incubated at 30° C. for 7 hours.

(b) One ml. of a dilution of the phage preparation in saline was added to the 7-hour broth culture, and an adsorption period of exactly 10 minutes at 30° C. was allowed.

(c) The tube of culture was spun in the centrifuge at 4,000 r.p.m. for 10 minutes. The supernatant, containing unadsorbed free phage, was poured off, and the mixture of infected and noninfected bacteria was resuspended in 9 ml. of sterile broth.

(d) A series of 1-in-10 dilutions of the phage-infected bacterial suspension was made in sterile skimmilk. The dilutions were held at 30° C. in a water bath. At precise intervals, timed from the moment of original infection with phage, drops (0.02 ml. by calibrated dropping pipette) were taken from one or more of the dilutions, mixed on the surface of an agar plate with a small amount of a heavy broth emulsion of streptococci, and spread over the agar surface. The plate also had previously been spread with a drop of milk culture of the streptococcus. The plate was incubated overnight at 30° C.

(e) The phage plaques which appeared on the bacterial mat on the plate were counted. From the counts, the latent period and burst size of the phage race were calculated.

During the course of each experiment, plaque counts were made to determine the total number of phage particles added to the adsorption tube and the number of particles remaining unadsorbed in the supernatant fluid after centrifugation. The number of individual bacterial cells and of chains of streptococci in

the adsorption tube was determined by direct count under the microscope. From these figures estimates were made of *(a)* the percentage of the phage particles adsorbed on the bacteria, *(b)* the multiplicity of infection of the bacteria and of the chains (i.e., the ratio of number of adsorbed phage particles to numbers of individual organisms and of chains), and *(c)* the probable percentage of bacteria and of chains infected by more than one phage particle.

In settling the experimental details for determination of burst size, the difficulty had to be faced that streptococci occur as chains, the length of chain varying with the strain. This characteristic of the organisms meant that when the relative numbers of bacteria and phage particles to be used in a reaction mixture were being considered, it was desirable to avoid not merely multiple infection of individual organisms but multiple infection of chains. Two infected organisms in a chain would yield at the start only one plaque in the plate count, whereas after a burst, double the expected number of plaques would appear, thus giving a false result for the burst size. In all the experiments, therefore, the number of chains of streptococci in the culture was counted and approximately one tenth of this number of phage particles was added to the adsorption tube. With all the cultures investigated, an adsorption period of 10 minutes at 30° C. resulted in adsorption of over 90% of the phage, and, according to a Poisson distribution, approximately only 5% of the chains was infected with more than one phage particle. Serial dilution of adsorption mixture (after removal of the unadsorbed phage by centrifugation), usually to 10^{-3}, 10^{-4}, and 10^{-5}, gave dilution tubes from which plaque counts were made at intervals. There was evidence in some of the experiments of a second crop of phage particles after the first burst. This may have been another consequence of the spatial arrangement characteristic of streptococci, since it is evident that no matter how far a streptococcal culture is diluted, the organisms in a chain remain in close proximity to one another, and particles liberated from a burst of one organism in the chain are more likely to infect the other organisms than they would be if the organisms were individually dispersed throughout the fluid.

In spite of these limiting factors, it was found possible, over a long series of experiments, to obtain repeatable results which indicated that the various phage races had characteristic latent periods and burst sizes.

RESULTS

The results obtained in representative experiments are given in Figure 3. The results for the latent periods were accurate to within 5 minutes on either side of the values quoted. The burst sizes were calculated from eight to ten values given by samples taken from the dilution tubes during the latent period and a corresponding eight to ten values given by samples taken during the steady 15 to 20 minutes period after lysis of the infected bacteria was complete. The standard error of the average burst sizes is indicated in the figure. The burst size values are subject to the reservation that plaque counts may be low because of failure of some phage particles to develop. The precise details of the plating technique are known to affect the numbers of plaques which develop from a

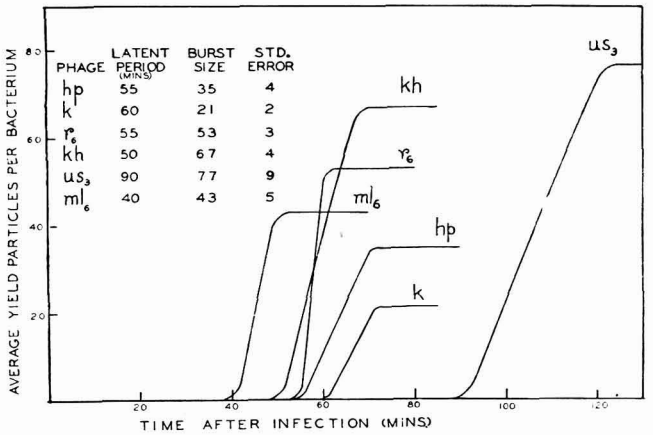

FIG. 3. Latent periods and burst sizes of phage races active against lactic streptococci.

given number of phage particles (the "efficiency of plating") *(2, 7)*. The method used in the present work was adopted after a series of preliminary trials had shown that, of several modifications, it gave the highest values.

From the curves it is clear that among the six-organism-phage combinations there were significant differences in both latent period and burst size. The extremes in latent period were 40 minutes for ml_6 and 90 minutes for us_3. The burst sizes ranged from 21 for *k* to 77 for us_3.

Influence of temperature on latent period and burst size. All the above experiments were carried out at a temperature of 30° C. In order to determine whether temperature of incubation influenced either burst size or latent period,

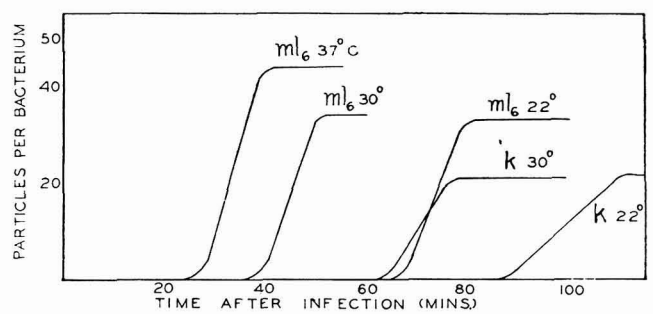

FIG. 4. Effect of incubation temperature on latent period and burst size.

some experiments were carried out with strains ML_6 and *K* and their corresponding phages at three temperatures, viz., 22°, 30°, and 37° C. Figure 4 gives some representative results.

In agreement with the findings of Ellis and Delbruck *(2)*, the results indicate that the latent period is significantly reduced by rise in incubation temperature within the range 22° to 37° C. At 37° C. phage *k* failed to give any burst in

the dilution tubes. Hunter *(3)* also observed that several phages for the lactic streptococci failed to grow at 37° C., although the bacteria were capable of growth at that temperature. A test on the supernatant fluid in an adsorption tube containing strain *K* and its phage after a 10-minute adsorption period at 37° C. indicated that the phage was not adsorbed to a significant extent. Strain ML_6 in common with many other strains of *S. cremoris (3, 5)*, was found to be somewhat inhibited in growth and acid production at 37° C. Hence the shortening of the latent period of phage ml_6 with rise in temperature between 30° C. and 37° C. cannot be directly connected with the bacterial growth rate but is an indication that the phage has an optimum temperature different from that of the substrate organisms.

The burst sizes were not significantly influenced by change in incubation temperature. The higher value for ml_6 at 37° C. is not outside the range of standard error.

Action of different phage races on one bacterial strain. In the later stages of the work there happened to become available three distinct phage races which were all capable of attacking strain ML_5. The three races could easily be differentiated on the basis of plaque size. One of the races formed pin-point plaques

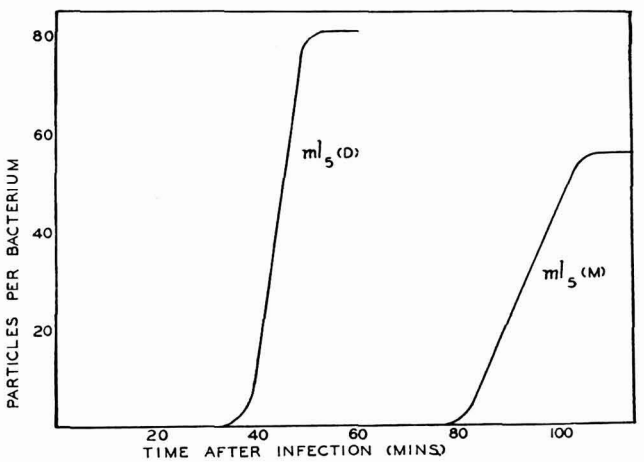

FIG. 5. Latent periods and burst sizes of two phage races active against one streptococcal strain.

which were too small to enable plaque counts to be made under the conditions of burst size experiments. The curves in Figure 5 drawn from the results of typical experiments on the other two races indicate clearly that both latent period and burst size are characteristics of the streptococcal phage race and not of the substrate bacterium.

DISCUSSION

The original object of the work was to find a complete explanation of the fact that some strains of *S. cremoris*, used as starters in commercial cheese fac-

tories, were more liable to phage infection and consequent failure than were other apparently similar strains. The primary explanation was already known, viz.,

(a) Where strains of streptococci are in regular use in factory cheese vats, phage races capable of attacking them are always present in the cheese whey. Normally, if adequate precautions are taken, the phage concentration is not high enough to cause failure of the starter.

(b) Under such conditions the phage races for the more "sensitive" or "unreliable" strains usually reach a higher titre in the vat whey than that reached by phages for less sensitive strains.

(c) A higher phage titre in the whey results in a higher air-borne infection and thus there is greater probability that the starter culture will become infected on subsequent usage.

The present work has shown that the different phage races have characteristic rates of development in milk cultures. The laboratory results gave a rough quantitative measure of the rates of development of the phages and explained in a general way the differences between cultures in sensitivity to phage infection in commercial cheese-making practice.

Determination of latent periods and burst sizes of the phage races appeared to provide a reasonable fundamental explanation for the differences observed in rates of phage development. A shorter latent period or a higher burst size necessarily leads to a more rapid propagation of phage and to a higher final titre, provided that there is an adequate number of bacteria on which the phage may act. For the six main phage races investigated, latent periods (at 30° C.) ranged from 40 minutes to 90 minutes and burst sizes from 21 to 77. A simple calculation shows that within these ranges of values, latent period has more influence on rate of phage propagation than has burst size. Thus although us_3 has a high burst size, its long latent period makes it one of the more slowly developing races. The rapid rate of development of ml_6 is due mainly to its short latent period; its burst size is not so high as those of r_6 and *kh*. The two factors, latent period and burst size, can, of course, act in a compensating manner to give two phages, with quite different characteristics, a similar rate of development. Thus *k* and us_3 give a similar final result in milk cultures, the shorter latent period of *k* apparently compensating for its lower burst size. In summary, therefore, a consideration of latent period and burst size, with due regard for the greater effect of latent period, enables one to predict the rate of development of different streptococcal phages under cheese vat conditions.

The isolation from cheese whey from different sources of three apparently distinct races of phage for strain ML_5 raises the interesting point that a phage race isolated in a cheese factory does not necessarily bear a unique relationship to the strain of organism on which it acts. Although in this paper the races have been designated *hp*, *k*, etc., as a matter of convenience, it must be remembered that there may be many phages capable of attacking each strain. Hunter *(6)* reported that in commercial practice strain *HP* was generally attacked by one particular phage race which seemed to have a preferential relationship with the strain; but the three different races which attacked strain ML_5 were all isolated

from cheese whey obtained from widely separated sources. Evidently there is no special relationship of one phage with ML_5. The very different characteristics of the two races which were investigated indicate that the "reliability" of ML_5 as a starter would depend on which of the two races became established in a given factory.

A phage race capable of attacking a streptococcal strain often can be isolated from cheese whey very soon after the culture has been introduced into a factory and before any phage-failure of the starter has occurred. Under such circumstances a determination of the latent period and burst size of the phage should (presuming our hypothesis is correct) enable a prediction to be made of the relative ease of maintenance of the starter culture under commercial conditions. There are several qualities which have to be considered in the selection of cultures for use in Cheddar cheese manufacture, but for ease of maintenance of the culture it seems desirable that the phage race or races which may attack it should have long latent period, and if possible, low burst size.

SUMMARY

Under the conditions of commercial cheese manufacture some strains of *S. cremoris* were more liable than others to phage infection. This greater chance of infection appeared to be connected with a more rapid development of certain phage races, leading to a higher concentration of phage in the cheese whey and hence to a higher degree of air-borne phage infection. The observed characteristics of the phage races could be reasonably explained on the basis of latent period and burst size. Short latent period or high burst size, or both, were characteristic of phage races which developed rapidly and reached a high concentration in cheese whey. Within the limits of the values found, latent period was more important than burst size in determining rate of phage development, but both had an influence and could reinforce or compensate one another. Increase in incubation temperature brought about a decrease in latent period but had no significant effect on burst size. The change in latent period with rise in temperature did not depend directly on the changed rate of growth of the bacterium. Two phage races which were capable of acting on the one strain of streptococcus differed both in latent period and burst size.

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DILUTERS FOR BOVINE SEMEN. II. EFFECT OF MILK PROTEINS UPON SPERMATOZOAN LIVABILITY [1]

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Since Thacker and Almquist *(22)* showed that heated milk maintains satisfactory motility and fertility of bovine spermatozoa, heated fresh homogenized milk or skimmilk has been shown by these workers *(23)* and Dreher and Webb *(4)* to give fertility results equivalent to, or better than, those obtained with the conventional yolk-buffer diluters. However, studies with reconstituted nonfat dry milk solids, canned skimmilk, and evaporated whole milk have not yielded as encouraging results to date. Marion and Olson *(16)* and Bennett *(2)* found that nonfat dry milk solids prepared at low temperatures gave extremely poor results unless heated after reconstitution, whereas reconstituted high temperature powders, without additional heating, gave results more nearly approaching those obtained with heated fluid milk and yolk-citrate. Jacquet and Cassou *(9)* have questioned the use of canned skimmilk after observing wide variations in fertility between bulls. Collins *(3)* obtained fair livability but poor fertility results with semen diluted in evaporated milk reconstituted with an equal volume of distilled water. Thacker and Almquist *(23)* reported very poor spermatozoan livability in unheated fresh milks, as well as a reduction in motility survival when the milk was heated for an extended time in an uncovered vessel.

From these reports it is apparent that bovine spermatozoa are sensitive to products developed in milk upon excessive heating, as well as to chemical or physical factors present in unheated milk.

Milk albumin and globulin were found by Rowland *(20)* to be completely denatured when heated at 95° C. for 10-15 minutes or at 100° for 5 to 10 minutes. No change was observed in the nonprotein nitrogen content of milk on heating at temperatures up to 100° C.; on continued heating at 95 and 100° C., extremely small amounts of proteose were produced by hydrolysis of the whey proteins. In milk heated at 115-120° C. the denaturation of albumin and globulin was accompanied by appreciable hydrolysis of protein. Harland *et al. (6)* studied the time and temperature relationships determining the extent of denaturation of serum proteins in fluid skimmilk at temperatures from 145 to 175° F. They found a ten-fold decrease in the time required for a given percentage denaturation of the serum proteins for each 13.5° F. increase in temperature.

These reports indicate that changes take place in the whey proteins at roughly the same temperatures which convert fluid milk to a satisfactory semen diluent.

Received for publication August 26, 1953.

[1] Published with the approval of The Pennsylvania Agricultural Experiment Station as Journal Article No. 1814.

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It appeared, therefore, that the milk proteins would be the logical location of the toxic factor of unheated milk. The present investigation was undertaken to determine if the toxicity of unheated milk was associated with any particular fraction of the milk proteins and to determine the effect of the heating of these protein fractions upon their spermicidal properties.

EXPERIMENTAL AND RESULTS

I. Casein—heated skimmilk. Casein and whey were prepared and tested to establish whether the spermicidal entity of unheated milk was associated with the casein or the whey. Casein was precipitated from fresh raw skimmilk by acidifying to pH 4.6 with 10% acetic acid. The casein was filtered off, redissolved in 6% ammonium hydroxide, and reprecipitated with acetic acid. The casein was dispersed and precipitated once more, then washed with alcohol and with ether, and dried. For testing as a diluent, the dried casein was dispersed in water with the aid of sodium bicarbonate, and an aliquot was removed and heated at 92° C. for 10 minutes, and cooled.

The acid whey was adjusted to pH 6.3 with sodium bicarbonate, and an aliquot was removed, heated at 92° C. for 10 minutes, and cooled. Heated and unheated aliquots of the casein and whey preparations were then added to the skimmilk, which had been previously heated at 92° C. for 10 minutes and subsequently cooled, at the rate of one volume of casein or whey to 24 volumes of skimmilk. The 1:24 ratio was adopted as a result of preliminary trials which revealed that the addition of one part of unheated skimmilk usually was toxic to bovine spermatozoa within a 4-day storage period at 4° C. The control diluter consisted of skimmilk heated and cooled as described above. All semen samples were diluted at the rate of one part semen to 50 parts diluter. Diluted samples were stored at 4° C. for 12 days, and motility of the spermatozoa was estimated at 37° C. by using a microscope equipped with a thermostage. The mean motility data for 10 ejaculates in each treatment are shown in Table 1. That the treatments used did not detract materially from their value as diluents is indicated in a comparison of spermatozoan survival in heated casein or whey as compared to the control, heated skimmilk. Maintenance of motility in unheated preparations indicated that the toxicity was associated almost exclusively with the whey. Subsequent studies dealt with fractions of the whey in an effort to further characterize the toxic factor.

II. *Whey proteins—heated skimmilk.* Whey protein fractions were prepared by the procedure of Polis *et al. (19)*, slightly modified by Hutton and Patton *(8)*. In order to avoid denaturation as completely as possible, the protein fractions were not dried completely but were dialyzed until free of salt and dissolved in 0.1 *N* sodium chloride. Concentrations, based upon Kjeldahl nitrogen determinations, were adjusted to roughly the level of each fraction found in milk. Moieties of each fraction were heat treated as previously described. Heated and unheated aliquots of the protein fractions were mixed into heated skimmilk at the rate of one volume of fraction to 24 volumes of skimmilk, and semen was added at the

TABLE 1

Livability of spermatozoa in boiled skimmilk containing additions of casein and whey (mean of 10 ejaculates)

Diluter		% motile spermatozoa after storage at 4° C. for							
		1 day	2 days	3 days	4 days	6 days	8 days	10 days	12 days
Casein	u	58	48	44	35	31	18	13	7
	h	61	55	50	45	41	26	21	10
Whey	u	54	54	25	11	3	0		
	h	59	55	48	40	34	23	18	10
Skimmilk	h	62	59	51	46	37	23	22	13

u = unheated
h = heated

rate of one volume of semen to 50 volumes of diluter. The mean motility observations for 10 ejaculates in each treatment are presented in Table 2. It is obvious from these data that the skimmilk used in the preparation of the serum protein fractions was relatively nontoxic to spermatozoa, as motility was maintained for 6 days in the unheated skimmilk. Normally, spermatozoa become immotile in unheated skimmilk within 1 to 2 days. Since the toxic principle was absent from the source material, it could not be identified with any of the fractions in this trial. The data do indicate, however, that more than one factor affecting sperm livability is present in the protein fractions, for in all fractions except Fraction III spermatozoan motility in unheated samples tended to be higher than motility in heated samples. Thus, in the absence of the toxic principle, heating of the fractions appears to have an adverse effect upon sperm livability.

Analysis of variance *(21)* showed a significant difference ($P = < 0.05$) between treatments (fractions). Treatments were then allocated to three groups: fractions containing the albumins, fractions containing the globulins, and the control. With this classification, the between-groups differences were highly signifi-

TABLE 2

Livability of spermatozoa in boiled skimmilk containing protein fractions of the whey proteins (mean of 10 ejaculates)

Diluter		% motile spermatozoa after storage at 4° C. for							
		1 day	2 days	3 days	4 days	6 days	8 days	10 days	12 days
Fraction I	u	47	42	37	29	2	0		
	h	48	42	27	17	3	0		
Fraction II	u	55	48	37	26	17	9	4	1
	h	61	48	37	22	5	1	0	
Fraction III	u	53	46	34	23	16	11	5	1
	h	55	48	39	28	7	2	0	
β-lactoglobulin	u	55	46	34	29	21	14	3	1
	h	50	45	30	20	4	1	0	
Pseudoglobulin	u	57	54	51	45	35	24	16	10
	h	51	47	42	27	20	13	7	4
Euglobulin	u	54	55	52	42	33	25	15	10
	h	55	47	36	27	18	13	6	3
Skimmilk	u	57	50	40	13	5	1	0	
	h	55	52	46	34	21	14	7	7

u = unheated
h = heated

cant; among-globulins differences, significant; and among-albumins differences, not significant.

III. *Whey proteins—protein-free milk serum.* In the preceding trial, fractions were added to heated skimmilk prior to testing as a semen diluent. In order to eliminate a number of milk proteins from the system, protein-free milk serum was prepared by placing 1 l. of distilled water in Visking membrane and suspending in 2 gal. of raw skimmilk at 3 to 4° C. Three changes of skimmilk at 24-hour intervals were employed. Protein-free milk serum thus prepared was found to support sperm motility for 8 to 10 days at 4° C.

Fresh protein fractions were prepared as previously described. Each fraction was added to the protein-free milk serum at the rate of one volume of fraction to 24 volumes of serum. Each preparation was then divided in half, and one portion was heated at 92° C. for 10 minutes while the other portion received no heat treatment. Since bacterial growth had been observed in the later stages of preceding trials, 5,000 units each of penicillin and streptomycin were added per milliliter of diluter in this trial. Semen was added to a dilution of 1:50. Mean motility observations are recorded in Table 3. The skimmilk used in preparing these fractions was definitely spermicidal, no motility being observed after 1 day of storage in the unheated skimmilk. Of the fractions, Fraction III was decidedly spermicidal and Fraction I exhibited evidence of toxicity. Analysis of variance showed a significant difference between treatments, and the interaction between treatments and heating or not heating was highly significant. The detrimental effect of heating observed in the preceding trial apparently was masked in most cases by the spermicidal effect of the unheated fractions but is evident in the pseudoglobulin and euglobulin fractions.

TABLE 3

Livability of spermatozoa in protein-free milk serum with added whey protein fractions (mean of 10 ejaculates)

Diluter		% motile spermatozoa after storage at 4° C. for							
		1 day	2 days	3 days	4 days	6 days	8 days	10 days	12 days
Fraction I	u	34	24	15	5	1	1	0	
	h	38	29	21	10	5	3	1	1
Fraction II	u	42	35	26	13	9	4	2	0
	h	44	33	22	12	3	0		
Fraction III	u	10	6	4	2	1	0		
	h	43	31	23	12	3	1	0	
β-lactoglobulin	u	33	27	20	15	10	3	1	0
	h	37	26	20	10	3	1	0	
Pseudoglobulin	u	42	31	24	15	6	3	0	
	h	31	23	19	7	3	1	0	
Euglobulin	u	44	36	28	20	10	2	0	
	h	37	29	19	7	1	0		
Protein-free milk serum	u	41	32	22	11	5	1	0	
	h	38	27	20	9	3	1	1	0
Skimmilk	u	0	0						
	h	54	52	44	34	24	13	6	3

u = unheated
h = heated

Samples of the lyophilized fractions prepared by Hutton and Patton *(8)* were obtained for comparison. Unfortunately, samples of euglobulin and pseudoglobulin were not available. These fractions were added to protein-free milk serum at the rate (weight per volume) of 0.5% for Fractions I, II, and III, and 0.12% for β-lactoglobulin. After reconstitution, each preparation was divided into two portions, one moiety being used after heat treatment and the other being used unheated. Penicillin and streptomycin were added to inhibit bacterial growth. The mean motility estimations for 10 ejaculates are presented in Table 4. Sperm livability was significantly improved by heating Fractions I, II, and III. Results in this trial differ from those in the preceding trial principally in that β-lactoglobulin, both heated and unheated, gave very poor results. This may have been due to deterioration during storage, alteration during lyophilization, or some other factor.

TABLE 4

Livability of spermatozoa in protein-free milk serum with added protein fractions at levels normal to skimmilk (mean of 10 ejaculates)

Diluter		% motile spermatozoa after storage at 4° C. for							
		1 day	2 days	3 days	4 days	6 days	8 days	10 days	12 days
Fraction I	u	18	13	11	7	4	1	0	
	h	42	32	27	18	9	4	3	1
Fraction II	u	18	8	8	6	5	1	1	1
	h	49	41	32	20	14	4	3	2
Fraction III	u	22	12	9	6	4	1	2	1
	h	41	34	29	22	11	6	2	1
β-lactoglobulin	u	16	9	4	3	2	2	2	1
	h	39	29	12	5	1	1	0	
Protein-free milk serum	u	34	24	19	12	5	1	0	
	h	32	27	16	8	0			
Skimmilk	u	2	0	0					
	h	47	46	42	35	28	20	16	10

u = unheated
h = heated

DISCUSSION

In this series of experiments, only occasionally did any of the prepared fractions approach heated skimmilk in maintaining the motility of bovine spermatozoa during storage at 4° C. This was due in part at least to the use of the simplified system of protein-free milk serum plus the tested fraction, for evidence is presented in Table 2 indicating that the addition of unheated pseudoglobulin or euglobulin to heated skimmilk results in improved livability over that obtained in heated skimmilk alone. This observation leads to the postulation that as heat is applied to the milk or milk constituent, two opposing groups of forces interact. On the one hand, a factor (or factors) detrimental to sperm motility is inactivated or destroyed; on the other hand, a factor (or factors) detrimental to sperm motility is activated or formed. In the intact skimmilk system, the former appears to dominate the latter, and harmful effects are noticed only after prolonged heating *(23)*. However, when the fractions are heated individu-

ally, the activation of detrimental principles often predominates. This interaction makes the elucidation of the toxic factor(s) present in unheated skimmilk somewhat more difficult than would otherwise be true. The interaction also dims the prospect of simplifying the milk diluter to a stable item without critical storage conditions, for the product, whether it be canned milk *(9)*, evaporated milk *(3)*, nonfat dry milk solids *(2)*, or some other product, must be processed under rather exacting conditions if it is to be used successfully as a diluter.

Although the several trials included in this report are not in absolute agreement, certain deductions with regard to the toxic factor(s) which is destroyed by the heat treatment of skimmilk appear to be justified. Certainly the factor(s) is not united with the casein, or closely linked with any of the globulin fractions. It does appear to be associated with the albumin fractions, and in these trials it was predominantly found in connection with Fraction III. Its variable activity in Fractions I and II may have been due to slightly different fractionations of the proteins dependent upon small procedural variations which may have occurred from one trial to another. Fraction III, according to Hutton and Patton *(8)*, is composed of β-lactoglobulin, true milk albumin *(19)*, and component C *(17)*. Whether the factor(s) is albumin per se, or an agglutination factor, enzyme, or enzyme inactivator associated with the albuminous fraction remains to be determined.

Enzymatic activity could well account for the spermicidal effect of unheated milk, but the removal of fat and casein removes many of the enzymes of milk *(17)*. The enzymes associated with milk serum proteins probably are inactivated by a considerably milder heat treatment than is necessary to convert milk to a satisfactory semen diluent, with the possible exception of lactoperoxidase.

MacLeod *(15)* has observed that certain substances which have an affinity for sulfhydryl groups inhibit the motility of spermatozoa and that this inhibitory effect may be prevented by adding sulfhydryl compounds to the system of sperm and inhibitor. Since reactive sulfhydryl groups may be released from albumin and other proteins by heat denaturation *(1, 11, 13)*, they could conceivably be responsible for the favorable action of heated milk as a diluter. Other forms of sulfur also have been implicated in regulating the metabolism of bovine spermatozoa *(5, 10, 14)*. Although the release of sulfhydryl groups offers a specious explanation for the results obtained, it also has its limitations. β-lactoglobulin constitutes 55 to 60% of the milk serum proteins *(17, 18)* and accounts for nearly all of the sulfhydryl groups in normal skimmilk *(7, 12, 13)*. In view of the results obtained with the β-lactoglobulin fraction, i.e., the absence of toxicity in the unheated fraction and the failure of heating to materially affect results with this fraction, it appears unlikely that sulfhydryl groups account for the differences obtained in sperm livability in heated and unheated skimmilk. Theoretically, denaturation and liberation of sulfhydryl groups from albumin could occur at a temperature too low to promote release of sulfhydryl groups from β-lactoglobulin. At the temperature employed in these trials (92° C.), however, considerable denaturation of β-lactoglobulin should take place *(13)*.

SUMMARY

In attempts to identify a factor in unheated skimmilk which is toxic to spermatozoa, casein and protein-free milk serum were prepared and found devoid of the toxic factor.

Milk serum protein fractions prepared by ammonium sulfate additions and pH adjustments were added to heated skimmilk and to protein-free milk serum in order to test for spermicidal activity. The toxicity was associated only with the albumin-containing fractions. Heating these fractions at 92° C. for 10 minutes eliminated the toxicity.

Heating of euglobulin and pseudoglobulin fractions resulted in a decrease in sperm livability when compared to similar fractions unheated.

ACKNOWLEDGMENT

The authors are indebted to Stuart Patton for providing the lyophilized serum protein fractions employed in one phase of the experiment and for helpful counsel throughout the investigation.

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METABOLIC RESPONSES OF BOVINE SPERMATOZOA TO ANTIBACTERIAL AGENTS[1]

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Sulfanilamide and the antibiotics, penicillin and streptomycin, are now being used singly or in various combinations almost universally in the diluted semen processed and shipped from bull studs throughout this country. The widespread use of these agents has resulted from research and observations which showed that these agents control bacteria *(3, 4, 5, 11)*, prolong livability *(3, 4, 5, 11, 12)*, and improve fertility *(1, 2, 16, 22)* of bovine spermatozoa. The improvement in fertility has been more marked for semen from bulls with moderate and low fertility levels than for semen from bulls with relatively high fertility *(1, 2, 16)*.

The beneficial effects of these agents on livability and fertility of bovine spermatozoa generally have been ascribed to their control of the bacterial flora of the semen. The results of the metabolic studies by Almquist *et al. (5)* and Knodt and Salisbury *(12)* of penicillin and sulfanilamide treated semen, respectively, indicate that some of the beneficial effects may be due to the effects of these agents on spermatozoan metabolism. The metabolic studies with bacteria subjected to penicillin *(25)* or streptomycin *(7, 9, 23)* tend to substantiate this belief. However, no data on the effect of streptomycin, or combinations of sulfanilamide, penicillin, and streptomycin, on spermatozoan metabolism have been reported.

In view of this lack of data, the investigations reported herein were undertaken. The purpose of these investigations was to study the livability and metabolic responses of bovine spermatozoa to no antibacterial agents and to combinations of sulfanilamide, penicillin, and streptomycin in egg yolk–citrate diluent.

EXPERIMENTAL

Semen used. Two experiments, using ten ejaculates for each, were conducted. Each ejaculate met the following minimum standards: initial motility, 50%, and spermatozoan concentration, 500×10^6 per milliliter. Initial motility and motility after storage were estimated microscopically as described by Branton *et al. (8)*. Spermatozoan concentration was determined by means of a calibrated Klett-Summerson photoelectric colorimeter *(19, 26)*.

Immediately after evaluation, each ejaculate was divided and diluted with the respective diluents at the 1:4 ratio. The final volume of diluted semen used

Received for publication August 27, 1953.

[1] A portion of the data for this paper is from a thesis to be presented by the junior author to the Graduate School, Louisiana State University, in partial completion of the requirements for the degree of Master of Science.

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for each diluent or treatment was 3.0 ml. in a 10 × 75 mm. test tube. The test tubes of diluted semen were placed in beakers of water at 27° C. and allowed to cool gradually to 4.5° C. They then were stored at 4.5° C. for 10 days.

Diluents. The diluents used in the first experiment were as follows:

EYC = egg yolk–citrate (control)
EYCS = egg yolk–citrate–sulfanilamide (300 mg. per 100 ml.)
EYCSt = egg yolk–citrate–streptomycin (500 units per ml.)
EYCP = egg yolk–citrate–penicillin (500 units per ml.)
EYCSStP = egg yolk–citrate–sulfanilamide (300 mg. per 100 ml.), streptomycin (500 units per ml.), and penicillin (500 units per ml.)

In the second experiment an egg yolk–citrate–streptomycin (500 units per milliliter)–penicillin (500 units per milliliter), or EYCStP, diluent was used in addition to those above. In these diluters streptomycin and penicillin are expressed as units of streptomycin sulfate and penicillin-G (crystalline-potassium) per milliliter of diluted semen; whereas sulfanilamide is expressed as milligrams of sulfanilamide (U.S.P.) per 100 ml. of diluent. These levels and combinations of antibacterial agents were chosen for study because they represent routine practices at many bull studs. In each of the above diluents the egg yolk and the citrate buffers were mixed at the 1:3 ratio.

Livability and chemical determinations. The levels of fructose and lactic acid in the diluted semen samples were determined initially and at 4 and 10 days of storage in the first experiment. Fructose was determined by a modification of the method described by Roe *(17)* and Mann *(13)*. The lactic acid levels were determined by the method of Barker and Summerson *(6)*.

During the course of the first experiment reported herein Vantienhoven *et al.* *(24)* and Mann *(14)* reported that under anaerobic conditions bull spermatozoa utilize glucose when it is available in preference to fructose. Vantienhoven *et al.* also found that egg yolk has a sparing effect on fructose utilization by bull spermatozoa during anaerobic incubation at 37 or 46.5° C. They explained this sparing effect by the occurrence of glucose in egg yolk as reported by Romanoff and Romanoff *(18)* and by the utilization of the glucose in preference to fructose by bull spermatozoa. Therefore, in the second experiment reported in this paper total reducing substances, fructose, lactic acid, and motility determinations were made on each semen sample initially and at 4 and 10 days of storage at 4.5° C. The method outlined by Nelson *(15)* was used for the total reducing substances.

The statistical analyses of all data were carried out according to the methods described by Snedecor *(21)*.

RESULTS

Experiment 1. A summary of the mean values for ten ejaculates for fructose utilization and lactic acid production by 10^9 spermatozoa as affected by the various diluents is presented in Table 1. It will be noted that the average fructose losses in the control diluent (EYC) were significantly greater ($P < 0.05$) at

TABLE 1

Fructose utilization and lactic acid production by bull semen as affected by antibacterial agents (Bull semen diluted 1:4 with the appropriate diluter and stored at 4.5° C. for 10 days) Experiment 1

Diluent	Fructose loss		Lactic acid gain	
	4 days	10 days	4 days	10 days
	(mg./10⁹ cells)			
EYC	1.06	2.07	1.16	1.80
EYCS	0.77	1.54	0.93	1.51
EYCSt	1.00	1.79	1.05	1.71
EYCP	0.97	1.76	1.00	1.57
EYCSStP	0.77	1.43	0.85	1.49
Least significant differences:				
P = 0.01	0.21	0.30	0.19	0.19
P = 0.05	0.15	0.22	0.14	0.14

4 days of storage than only those in the two diluents containing sulfanilamide (EYCS and EYCSStP). At 10 days of storage, however, fructose losses in the control diluent were significantly greater ($P < 0.01$) than the losses in each of the diluents except the one containing streptomycin (EYCSt). The difference between losses of fructose in the control diluent and in the diluent containing streptomycin alone was significant at the 5.0% level of probability. It also will be observed in Table 1 that lactic acid production by the spermatozoa in the different diluents followed the same general pattern of the fructose losses.

Experiment 2. The egg yolk–citrate diluents contained an average of 29.8 mg. of total reducing substances per 100 ml. and no fructose or substances with fructose activity. The average initial levels of total reducing substances and fructose in the diluted semen samples on a milligrams per 100 ml. basis were 135 and 114, respectively. By subtracting the average fructose value from that for total reducing substances a value of 21 mg. per 100 ml. of diluted semen is obtained for glucose. Thus, fructose accounted for approximately 85% of the total reducing substances in the diluted semen samples.

Table 2 presents the average values for ten diluted semen samples for total reducing substances (TRS) and fructose utilization and lactic acid production. Initial levels of these also are given. It will be observed that the sulfanilamide diluents (EYCS and EYCSStP) again had the most marked effects on spermatozoan metabolism. Next in order was the diluent containing a combination of streptomycin and penicillin (EYCStP). When used alone, penicillin had more effect than streptomycin on metabolism of the spermatozoa. The least significant differences required for significance at the 1.0 and 5.0% levels of probability are given in the bottom portion of Table 2.

If the fructose losses in Table 2 are substracted from those for total reducing substances (TRS) at 4 or 10 days of storage, approximate values for glucose utilization can be obtained. The effects of the antibacterial agents on the utilization of glucose by the spermatozoa were essentially the same as those for fructose utilization.

The average motilities of the diluted semen samples initially and at 4 and 10

TABLE 2

Total reducing substances and fructose utilization and lactic acid production by bull semen as affected by antibacterial agents (Bull semen diluted 1:4 with the appropriate diluter and stored at 4.5° C. for 10 days)
Experiment 2

Diluent	TRS			Fructose			Lactic acid		
		Loss			Loss			Gain	
	Initial level	4 days	10 days	Initial level	4 days	10 days	Initial level	4 days	10 days
	(mg./10^9 cells)								
EYC	5.81	2.08	2.87	4.85	0.85	1.62	0.36	1.00	2.00
EYCS	5.57	1.34	1.80	4.77	0.54	1.05	0.41	0.78	1.53
EYCSt	5.56	1.85	2.34	4.78	0.77	1.46	0.40	0.95	1.71
EYCP	5.54	1.54	2.00	4.72	0.73	1.35	0.40	0.90	1.66
EYCStP	5.54	1.50	1.96	4.77	0.56	1.30	0.40	0.73	1.39
EYCSStP	5.52	1.16	1.70	4.74	0.54	1.05	0.42	0.65	1.38
Least significant differences:									
P = 0.01		0.52	0.38	.....	0.27	0.26	.....	0.19	0.42
P = 0.05		0.39	0.29	.....	0.20	0.19	.....	0.14	0.31

TABLE 3

Motility of bull semen as affected by antibacterial agents initially and at 4 and 10 days of storage at 4.5° C. (Bull semen diluted 1:4 with the appropriate diluter)
Experiment 2

Diluent	Motility		
	Initial	4 days	10 days
	(%)	(%)	(%)
EYC	62	47	29
EYCS	62	45	22
EYCSt	62	52	37
EYCP	62	56	37
EYCStP	62	58	41
EYCSStP	62	53	29
Least significant differences:			
P = 0.01		5.51	11.33
P = 0.05		4.13	8.50

days of storage at 4.5° C. are shown in Table 3. It will be noted that the motility of the spermatozoa in the sulfanilamide diluent (EYCS) was significantly ($P < 0.01$) lower than that in the diluents containing streptomycin (EYCSt), penicillin (EYCP), or a combination of the antibiotics (EYCStP) at 4 and 10 days of storage. However, the motility was not significantly lower ($P < 0.05$) in the sulfanilamide diluent than in the control diluent (EYC). It appears, nevertheless, that sulfanilamide had a detrimental effect on the livability of the spermatozoa and that streptomycin and penicillin each had beneficial effects.

DISCUSSION

The data for fructose losses (Tables 1 and 2) and those for the utilization of total reducing substances and fructose as affected by sulfanilamide (Table 2) are in agreement with the findings of Knodt and Salisbury *(12)* and Vantienhoven

et al. (24). Sulfanilamide depressed fructolysis and the utilization of total reducing substances or glucose by the bull spermatozoa. Also, sulfanilamide increased the percentage of total reducing substances losses recovered as lactic acid at 4 and 10 days of storage.

It appears that some of the depression of glucose and fructose utilization caused by sulfanilamide in these studies can be explained by the lowered livability of the spermatozoa in the sulfanilamide diluent as compared with that in the control diluent. These livability results are contradictory to those reported by Knodt and Salisbury *(12)*, who found that the addition of 300 mg. of sulfanilamide per 100 ml. of egg yolk–citrate diluent gave a significant ($P < 0.01$) improvement in the livability of ejaculated bull spermatozoa over a 20-day storage period at 5° C. Perhaps differences in experimental conditions, such as the quality of semen and the proportions of egg yolk and citrate buffer in diluents, account for these contrasting livability results. However, in the investigations reported herein and in routine observations in our laboratory sulfanilamide had a definite detrimental effect on livability. This effect became noticeable at 2 days of storage at 5° C. and was more marked at 4 and 10 days of storage. In this connection, it is of interest to note that Dunn *et al. (10)* recently reported that they attributed a reduction in viability of spermatozoa ranging from 29 to 43% to the presence of 0.3% sulfanilamide in samples stored at −75° C. Furthermore, they suggested that sulfanilamide should not be included in egg yolk–citrate diluents to be frozen.

From the data in Table 2 it appears that the spermatozoa used glucose in preference to fructose, particularly through 4 days of storage. This confirms the results reported by Vantienhoven *et al. (24)*. However, if the values at 10 days of storage in Table 2 are considered, it will be found that fructose was used in preference to glucose. This, no doubt, was due to the relative proportions of these sugars remaining after 4 days of storage *(24)*.

The utilization of total reducing substances by the spermatozoa in the presence of penicillin (EYCP) was depressed, as shown in Table 2. These results agree with those reported by Almquist *et al. (5)*. On the other hand, the lactic acid production data as shown in Tables 1 and 2 are contradictory to the results obtained by Almquist *et al.* They found that the amounts of lactic acid which were accumulated were not significantly affected by penicillin. Studies by Wilkowske *et al. (25)* of the influence of penicillin in skimmilk on the lactic acid-producing ability of *Lactobacillus acidophilus, Lactobacillus bulgaricus*, and *Lactobacillus casei* tend to substantiate results reported in this paper.

The effects of streptomycin on the utilization of total reducing substances and fructose and on the production of lactic acid by spermatozoa were similar to those for penicillin, though they were not as large (Tables 1 and 2). Perhaps streptomycin affects the carbohydrate metabolism of bovine spermatozoa and certain bacteria similarly. Streptomycin apparently inhibits some terminal step in both aerobic and anaerobic carbohydrate metabolism of these bacteria *(7, 9, 23)*.

The diluent containing a combination of penicillin and streptomycin

(EYCStP) gave better livability than any of the other diluents (Table 3) and had almost as much effect on spermatozoan metabolism as did the sulfanilamide diluents (Table 2). These results and those in the literature indicate that the egg yolk–citrate diluent containing a combination of penicillin and streptomycin (EYCStP) is a better diluent for widespread use in artificial breeding than is either of the diluents containing sulfanilamide (EYCS or EYCSStP).

SUMMARY

Two experiments were conducted to determine the effects of antibacterial agents in egg yolk–citrate diluents upon livability and metabolism of bovine spermatozoa at 4 and 10 days of storage at 4.5° C.

It was found that streptomycin and penicillin, either singly or in combination, had beneficial effects on spermatozoan livability, whereas sulfanilamide apparently had a detrimental effect on viability of the spermatozoa.

Sulfanilamide, streptomycin, and penicillin each depressed the utilization of total reducing substances and fructose and the production of lactic acid by the spermatozoa. Sulfanilamide gave the most marked metabolic effects. A combination of streptomycin and penicillin in the egg yolk–citrate diluent depressed spermatozoan metabolism almost as much as sulfanilamide and gave the best livability in comparison with the other five diluents.

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PEOPLE and EVENTS
in the Dairy Science World

Borden Award Winners for 1953

The American Chemical Society—ROBERT JENNESS, Univ. of Minnesota

The American Dairy Science Association—
Dairy Production—R. B. BECKER, Univ. of Florida
Dairy Manufacturing—F. E. NELSON, Iowa State College

The American Home Economics Association—RUTH M. LEVERTON, Univ. of Nebraska

The Association of American Medical Colleges—JEAN OLIVER, State Univ. of New York College of Medicine

The American Institute of Nutrition—H. H. WILLIAMS, Cornell Univ.

The American Academy of Pediatrics—LAWSON WILKINS, Johns Hopkins Univ. School of Medicine

The Poultry Science Association—H. R. BIRD, Univ. of Wisconsin

The American Veterinary Medical Association—G. H. HART, Univ. of California

Distribution of Consumer's Evaporated Milk Dollar

Economists of the National Grange and Grocery Manufacturers of America recently released the results of a study of 1952 retail prices of evaporated milk. A summary of these results is as follows:

		Distribution of price paid	
To farmers: (price received at the condensery for the 2.05 lb. of raw 3.5% butterfat milk required for a 14½ oz. can of "evap.")		7.8¢	51.3%
Manufacturers' gross margin:		5.8¢	38.2%
From this was paid:			
Packaging materials and supplies	2.1¢		
Wages and salaries (except executive)	1.0		
Executive salaries	(a)		
Outbound transportation	0.8		
Advertising	0.3		
Other business expenses	1.0		
Federal and state income and payroll taxes	0.4		
Net profits	0.2		
Supermarkets' gross margin:		1.6¢	10.5%
From this was paid:			
Wages and salaries (except executive)	0.7¢		
Executive salaries	0.1		
Supplies	0.1		
Advertising	0.1		
Other business expenses	0.2		
Federal, state, and local taxes	0.2		
Net profit	0.1		
Price to consumers:		15.2¢	100%

(a) Less than 1/10 of a cent.

Raw milk prices in 1952 were below 1948 by about 3/10 of a cent per can. However, wholesale and retail prices of evaporated milk were up by about the same amount. The factors responsible for the increased spread were—higher wage, packaging, and transportation costs and taxes. Net profit rates of manufacturers and super markets were lower in 1952 than in 1948.

Harry S. Fielder Dies

HARRY S. FIELDER, Director of the New Products Planning Group of Cherry-Burrell Corp., died after a short illness Dec. 16, 1953. A member of the U. of I. class of 1912, Fielder started with the A. H. Barber Creamery Supply Co. in 1913. This company later merged with the Cherry-Burrell Corp. Since 1946 he has served as Chairman of the D.I.S.A. Technical Committee and was active in the Sanitary Standards Committee. He is survived by his wife and two children.

Oregon State Holds Industry Meeting

The 43rd Annual Convention and Short Course of the Oregon Dairy Industries was held February 15-18 at Corvallis with G. H. WILSTER in charge. Among the out of state speakers were G. M. TROUT of Michigan State, C. F. WEINREICH of the Cherry-Burrell Corp., and M. W. HALES of the Chris Hansen Laboratories, Milwaukee, Wis.

California Milk Hearings

A series of hearings was held in the different milk market areas in California during the month of December. These were in conformance with the State Agricultural Code, which was enacted in 1935, and amended later, presently Chapter 13, "Stabilization and Marketing of Fluid Milk and Cream."

The purposes of this chapter are to provide funds for administration and enforcement of the Act, to authorize and enable the Director of Agriculture to prescribe marketing areas and to formulate stabilization and marketing plans, to enable the dairy industry with the aid of the state to correct existing evils, to develop and maintain satisfactory marketing conditions, to bring about a reasonable amount of stability and prosperity in the production and marketing of fluid milk and cream, and to provide a means for carrying on essential educational activities.

As a result of the hearings, where both producers and processors voiced opinions, producer minimums were cut 46 cents per hundredweight on 3.8% test Class 1 usage in most of the areas, enabling consumers to realize a one cent per quart reduction in the minimum retail price of milk. According to reports, Bureau Chief DON M. WEINLAND said, "This is the second consecutive milk price reduction in California within the past eight months, despite rising costs of other food commodities."

The present minimum retail price of milk in 19 northern and central California areas varies from 20 cents to 21½ cents per quart, the greatest differential between store price and home delivered price being 1½ cents. In southern California and in one extreme northern area, the minimum price is generally about one cent higher.

There is always considerable disagreement at the hearings, as the subject is highly controversial. Many producers want higher prices, claiming the methods used by the Bureau of Milk Control in determining feed, labor, and management cost are inaccurate. These costs are determined by the Bureau auditors, who go into an area and select a limited number of farms for study.

According to news reports, certain distributors are also dissatisfied with the minimum store prices, one chain organization claiming they could sell in their stores for 2 cents less than the minimum price, and another chain organization maintaining they could lower the price 3 cents.

It seems to be generally agreed that the present surplus of Grade A milk was the determining factor in the general over-all reduction in the price of milk.

Study Made of Bulk Milk Handling in Wisconsin

About 600 farmers now use bulk milk cooling tanks in Wisconsin—or about 1% of the total number of milk producers in the state, according to ARTHUR H. MILLER, Univ. of Wisconsin economist. The plants using bulk handling methods are widely scattered, with most of them in the southern half of the state. Generally, these plants are handling milk destined for fluid milk markets.

Miller's studies have shown that the greatest opportunity to cut costs through bulk milk handling rests with the small plant with a few large patrons. In areas where dairy herds are large, it is entirely feasible for cheese factories to adopt bulk handling.

According to Miller, bulk handling could bring about almost revolutionary changes in the marketing of Wisconsin milk. Receiving costs, especially in the small plants, could be radically reduced through complete conversion to bulk handling by as much as 25 cents per hundredweight of milk. Larger operations will have smaller savings, and very large plants with many small patrons may have savings completely absorbed in added hauling, field service, and quality test expenses.

Bauman Joins Pillsbury Mills

HOWARD BAUMAN has joined Pillsbury Mills, Inc., as Head of the Microbiological Section of the Research and Development Dept. in Minneapolis.

Dr. Bauman received his B.S. degree from the Univ. of Wisconsin in 1949, his M.S. degree in 1951, and his Ph.D. in 1953, majoring in Bacteriology and minoring in Dairy and Food Industry.

News from the Golden State

E. L. JACK, Chairman of the Dept. of Dairy Industry, returned January 1 from a 6-month sabbatical leave abroad. After attending the Intern. Dairy Congress as an official delegate of the State Department, he traveled in several countries on the continent and in the British Isles.

Short courses have been revived after a number of years. A 2-week short course in market milk was given Nov. 3-13, 1953, with an enrollment of 15. A 2-week short course in ice cream was given Jan. 25 to Feb. 5. The annual Dairy Industry Conference was held Feb. 8, 9, 10.

New members of the Dept. of Dairy Industry staff are WALTER G. JENNINGS, who is working in the field of dairy chemistry, and LEON A. KELLEY, a specialist in market milk. Jennings is a graduate of the Univ. of California and Kelley of the Univ. of Wisconsin.

Dairy Institute to Study Methods and Techniques

Dairy fieldmen, inspectors, salesmen, educators, administrators, and health officers had an opportunity to learn the latest in dairy marketing and health and production programs at the Univ. of Tennessee, Feb. 25-26. Visual aids, personal contacts, group meetings, and unified program approaches to dairy problems were discussed.

Cherry-Burrell Named Distributor for Laboratory Centrifuge

Cherry-Burrell Corp. has been named distributor for the Westfalia clarifier, a versatile laboratory centrifuge that can be used for clarification, mixing and emulsifying, solvent extraction, separation, and concentration of solids. Being only 32 in. high and weighing only 163 lb., it is well suited to installation on a laboratory work bench for use in research, pilot plant operations, or quality control. Capacity is from 12 to 140 gal. per hour.

Course in Artificial Insemination Given for Foreign Visitors

The Dept. of Dairy Science at the Univ. of Illinois in cooperation with the Foreign Operations Admin. sponsored a short course in artificial insemination for leaders in this field from various foreign countries. The 13 participants represented Norway, The Netherlands, Yugoslavia, Turkey, Iran, Bolivia, and Brazil. R. L. HAYS spent 8 weeks with the group acting as a technical consultant. The program included a 4-week short course at the Univ. of Illinois supervised by N. L. VANDEMARK and G. W. SALISBURY and taught by the staff of the Dept. of Dairy Science and the Veterinary College. Also included in the program were 2 weeks spent at Washington, D. C., 1 week at the Univ. of Massachusetts, 3 days at the Univ. of Maryland, and 2 days at Cornell Univ.

Coulter Warns Against Research Curb

At the annual meeting of the Illinois Dairy Products Association in December, S. T. COULTER of the Univ. of Minnesota Dairy Dept. made a strong appeal for freedom in academic research. He cited as an example of lack of such freedom the ouster of a college professor from a Midwestern university for publishing material construed as favorable to margarine in comparison with butter. He also warned the ice cream people not to make the mistake made by the butter industry in their fight against margarine and advised them to adopt a reasonable attitude toward competitive products, such as the soft-served ice milk and ice cream.

Dr. Coulter strongly urged support for expanded research in the dairy field and cited as an example of how the industry can be benefited through basic research the recent development of "Nu World" cheese by the cooperative work of scientists at the Universities of Wisconsin and Minnesota. Coulter advised the members of the industry to be more progressive and warned them of the dangers of holding to outmoded standards. He stated that progress usually brings about changes in the things we do and how we do them.

Carman Esmond Serves in New Capacity

CARMAN W. ESMOND, who served for 20 years with the G. P. Gundlach Co. as a dairy sales promotion specialist, has become Supervisor of Activities of the Parish for Christ Church in Cincinnati, Ohio. Esmond, a cofounder of the Gundlach Co., retired from active service in the business in 1952 because of illness.

Iowa State News

The Dairy Husbandry Extension staff has moved into new quarters in Curtiss Hall (formerly Ag. Hall).

KARL WESTER has resigned, effective Feb. 18, as Assistant Professor of Dairy Industry Extension to become associated with the Grading Service of PMA, Dairy Branch. His headquarters will be Banon, Wis.

The annual Dairy Industry Short Course will be held March 23 at Ames.

Bureau of Dairy Industry Reorganized

The reorganization of the Agricultural Research Service of the U. S. Department of Agriculture, which became effective Jan. 2, 1954, was carried out on a functional rather than a commodity basis. Accordingly, the work formerly done by the Bureau of Dairy Industry is divided into categories of production and utilization research. The Dairy Products Research Labs., dealing with the processing and utilization of milk and milk products, become a section of the Washington Utilization Research Branch. G. E. HOLM remains as head of this section.

The Bureau's production work will continue to be conducted by the Sections (formerly Divisions) of Breeding, Feeding and Management; Nutrition and Physiology; and Dairy Herd Improvement Investigations, under the new Dairy Husbandry Research Branch. M. H. FOHRMAN, L. A. MOORE, and J. F. KENDRICK remain as the respective heads of the three sections. R. E. HODGSON has been made chief of the Branch.

O. E. REED, chief of the former Bureau of Dairy Industry, has been made Director of Livestock Research and is responsible for the research programs of the Dairy Husb., Animal and Poultry Husb., and Animal Disease and Parasite Research Branches of the Agricultural Research Service.

Virginia Polytechnic Institute Holds Series of Courses

To train laboratory workers for the industry, V.P.I. held a course for beginners Feb. 8-13, which was followed by a 1-week advanced course. The ice cream conference was held Feb. 24-25. D. V. JOSEPHSON of Pennsylvania State Univ. was the guest speaker and scored

the clinic samples. A cottage cheese and buttermilk conference will be held at V.P.I. Mar. 17-18. Practical demonstrations of the manufacture of cottage cheese and cultured milk will be made.

Completed Theses

M.S. Degree:

CECIL G. FORTNEY—Cleaning sanitary pipe lines in place. Iowa State College.

ALBERT E. FREEMAN—Genetic analysis of the components of type conformation and production in Ayrshire cows. West Virginia Univ.

Ph.D. Degree:

DEE M. GRAHAM—Selective chemical antagonism of lactic streptococcus bacteriophage. Iowa State College.

HOWARD J. LARSEN—Digestion and absorption of carbohydrates in the young bovine. Iowa State College.

New General Biochemicals Catalogue Published

Over 600 items of interest to research workers in the fields of biology, microbiology, bacteriology, biochemistry and nutrition are listed in the new 1954 issue of the GBI catalogue. The following types of products are included: amino acids, peptides, carbohydrates, nucleates, purines, pyrimidines, enzymes, pH indicators, microbiological and bacteriological media, complete animal test diets, test diet ingredients, and miscellaneous research biochemicals.

Michigan State Dairy Engineering Conference

The annual Dairy Engineering Conference sponsored by the Departments of Agricultural Engineering and Dairy Husbandry at Michigan State College will be held March 3 and 4. Topics to be discussed include:

Engineering design and operation of bulk milk coolers.
Effect of bulk handling on plant operations.
Water conditioning for the dairy plant.
Engineering the modern receiving room.
Installation and use of conveyors.
Maintenance of refrigerator systems.
Application of time-motion studies to dairy plant operations.
Dairy plant paint problems.
In-place cleaning installations for specialized equipment.
Floors for dairy plants.

West Virginia University News

A. E. FREEMAN has gone to Cornell Univ. to study animal breeding under C. R. HENDERSON.

The West Virginia Dairy Products Association has announced that it will offer three scholarships valued at $250 each for the 1954-55 school year. The awards are given to sophomores. The recipients of the 1953-54 awards were DAVID KING and CHARLES RHODES.

Borden Chooses Ice Cream Operations Chief

HARRY L. ARCHER has been made General Manager of the Borden Co. ice cream operations in the United States. He has been associated with Borden's since 1926. He is also a member of the executive committee of the Intern. Assoc. of Ice Cream Mnfrs.

Johnson Made Head of Oakdale Laboratory

ARNOLD H. JOHNSON has been elected president of the National Dairy Research Labs., Inc., Oakdale, N. Y. He has served the company since 1930 and has been vice-president and research director since 1950.

Oregon Approves Vegetable Fat in Frozen Dessert

Beginning Jan. 22, it is legal to sell Mellorine, a frozen dessert containing vegetable fat, in the state of Oregon. All final-delivery consumer packages must be labeled "Mellorine," and no words which would associate such products with ice cream can be used on the label or in advertising. The new regulation is a ruling of the Department of Agriculture.

U. of T. Reports on Graduates

Univ. of Tennessee graduates in dairying are doing well in their professional careers, with approximately 90% of them holding good positions in the dairying industry and allied fields. That conclusion was made by C. E. WYLIE of the U-T Department of Dairying after conducting a survey of U-T dairying alumni.

Professor Wylie received answers from 198 alumni in the survey and found that all but 20 of them (not counting 15 in military service) were engaged in work connected with dairying and dairy manufacturing, holding a wide range of positions. The survey also showed that 136 alumni (about 70%) were located in Tennessee, the others in 21 other states, the District of Columbia, and three foreign countries.

Thirty of the graduates are dairy farm operators, breeders, and herdsmen. This was the largest single classification of activities. The next largest group, 28, are in extension work—as county agents and specialists. Third largest, 25, are in administrative work connected with the dairy industry. Twenty are dairy fieldmen; 20, teaching in colleges and schools; 19, in dairy manufacturing; 10, in sales work; 6, health inspectors; and 5, research workers.

Dunn Joins Staff of New York Breeders Cooperative

HENRY O. DUNN has accepted a position at the New York Artificial Breeders Cooperative at Ithaca. His research work will have to do with artificial breeding and the application of dairy cattle genetics to selection for higher milk production. He will work cooperatively with C. R. HENDERSON and R. W. BRATTON of Cornell University.

The Nominating Committee Solicits Your Help

The nominating committee needs help in selecting nominees for ASDA election of officers in 1954. Please help by promptly mailing suggestions for candidates for the office of Vice President from among members affiliated with the Manufacturing Section and for Directors representing both the Extension and Production Sections to committee chairman R. Whitaker, National Dairy, Oakdale, N. Y.

Future of Dairying May Depend Upon A.D.A.

A Guest Editorial

One of the dairy industry's most important experiments of all time is now in progress in this country. I have in mind the efforts of our dairy farmers and dairy processors to increase the consumption of dairy products through the recently accelerated research and advertising campaign of the American Dairy Association. The results of this experiment and the solution of the surplus problem confronting the dairy industry may have a lasting impact on the number of dairy farmers in this country, the type of teaching and research required by the dairy industry, and the number of persons seeking training for the dairy industry.

J. B. Fitch

There may not be proper controls to justify calling this effort of A.D.A. an experiment and there may be certain factors that will bias the results, but the agencies conducting the campaign are using the best means available to conduct the campaign in a carefully controlled manner and are endeavoring to establish means of measuring the results.

Large numbers of our dairy farmers have come to realize the seriousness of the inroads that other food products and beverages have made on the eating habits of our people. The problem has become so acute that it is not so much a matter of increasing the average consumption of dairy products as it is of maintaining the present consumption level.

The per capita consumption of fluid milk was lower in 1951 and '52 than in the ten previous years. The total per capita consumption of butterfat in 1952 is the lowest on record by the Bureau of Agricultural Economics, U.S.D.A., as given in the Sept.-Oct. 1953 Dairy Situation Report and is 5.5 lb. less than it was in 1942. The consumption of nonfat solids was slightly higher in 1952 than in 1951 but is still under the amount consumed in the years 1945 to 1947. The increase in 1952 is small in view of reported increases in the consumption of dried nonfat and sales of fluid nonfat in stores and on milk routes. The consumption of cheese and ice cream is in a more favorable position.

The dairy industry has been too late and has done too little in taking a realistic attitude toward the use of research and promotional agencies in expanding the use of dairy products. It is not enough to know that milk is the most nearly perfect food. Consumers must be told this fact time after time. Most of the urge behind the present campaign is directed toward persons twenty years old or older. We have neglected directing facts about the food value of dairy products to adults. We have failed to emphasize that older people can be benefited by the use of more milk and dairy products. There is also the fact that milk and dairy products can be used in a diet by those who want to reduce their weight. This does not mean any let-up on selling the younger groups but an added emphasis on adults. The promoters of this campaign hope that the consumers of dairy products will develop a "king-size" demand for dairy products rather than the "regular" demand as featured with another product.

The American Dairy Association year-round check-off plan for dairy promotion will be developed on a national basis as soon as it is estimated that the producers of 60% of the total milk produced are cooperating. On the basis of collecting two cents per 100 lb. of milk or one-half cent per pound of butterfat for the annual production of the dairy farmers under agreement, A.D.A. hopes to raise six million dollars annually. As this is written, more than 50% of the total volume of milk produced in the United States has been signed up, and the national campaign to approve the annual check-off has been under way less than a year.

It is well to remember in connection with this producer-sponsored program that the processors and distributors of dairy products have annually spent a large amount of money in promoting the sale of dairy products.

In general, dairy farmers are becoming more willing to participate in the A.D.A. program. In Minnesota, it has been easier to collect

$400,000 in 1953 than it was to collect $50,000 in 1940. The large surplus of dairy products and the fact that support prices on dairy products have placed us at a disadvantage on the export market have created an awareness of the seriousness of our problems at the producer level.

The surplus of dairy products has gotten the headlines, but it should not be overlooked that several other agricultural products also are in surplus. The history of production and consumption of flour products is quite similar to that of butter, but the millers have met the situation by curtailing production. In 1900 the per capita consumption of flour products was 225 lb. and in 1940 it was 154 lb.—a decrease of 31% in 40 years. Since 1940, the per capita consumption has declined to 130 lb., or an additional 15%. There has been a sharp decline in butter consumption during this same period, dropping from 17 lb. per capita in 1940 to about 9 lb. in 1952, a decline of 47%.

The economic status of a large segment of our population is such that food products from all over the world have been attracted to the easily available and well advertised domestic markets. This has given the consumer a wider variety of choices for a limited storage space in the human stomach.

In some markets, the cost of processing and distributing fluid milk has been such as to almost price it out of the market. Marketing agreements in some areas have created high retail prices for milk, which has resulted in more milk being diverted into dairy products that were already in surplus. The Government is purchasing high quality butter to go into storage, leaving the lower grades to be sold to the consumer. Research and education on the value of milk fat in proper nutrition and good eating should help to absorb some of this surplus.

The results of the A.D.A. program to promote the consumption of milk and its products will help determine which products are in greatest demand in the market place and will form a basis for future research and advertising programs. The outcome of these efforts will determine the type of farming pursued by many farmers in the future. This will be particularly true of the marginal producer.

J. B. Fitch, Head
Div. of Dairy Husbandry
Univ. of Minnesota

LETTERS TO THE EDITOR

Likes Changes in Journal

In reading recent issues of the *Journal of Dairy Science*, I have been very much impressed with its excellent get-up and appearance and especially with its completeness.

It is my opinion that the inclusion of the sections captioned People and Events, Letters to the Editor, and Our Industry Today contribute very much to making the Journal more comprehensive and of greater value to those of us who like to keep in touch with the industry across the board.

Such an excellent job is thus being accomplished that I feel I would like to pass my compliments to yourself as Editor, and to the several members of your Editorial Board and the members of the Committee on Journal Management.

F. S. Board
Chicago

Dairymen Should Set an Example

I have attended many dairy banquets and luncheons, and I have eaten out with a number of dairy people, including dairy equipment salesmen, and I am amazed at the lack of enthusiasm that these people have for their own products. I recently attended an annual meeting of a state dairy association at which practically everyone drank coffee—much of it black; there was no cottage cheese on the table, no ripened cheeses were served, the butter had acquired a surface taint from being stored in the refrigerator after being cut, and for dessert we had pie. This I have seen happen time after time.

At dairy short courses and conferences the faculty sometimes kindly arrange for a break in the middle of the morning and afternoon—for what? Usually coffee, not even hot chocolate.

In the hotel rooms at dairy conventions great hospitality is shown the guests by the supply people. Some serve cheese and crackers, but a high percentage serves liquor. No one ever offers you a glass of milk or buttermilk. One company at a recent meeting did offer hot chocolate along with the liquor, a commendable move on their part.

Even dairy farmers emphasize coffee at their dinner meetings rather than the product that is their livelihood and which is in jeopardy at the present time because of the inroads being made by competitive food products. We render a great deal of lip service to the cause of using more milk and milk products, yet we certainly do not do our share of promoting their use by setting an example to others when we eat in public places. Instead of boosting sales for liquor, coffee, and other competitive beverages, let us show the world we believe in our own

products by serving complete lines of high quality dairy products at our association meetings, by establishing milk or hot chocolate hours in place of coffee hours, and by encouraging our employees to drink milk at their rest periods instead of soft drinks. How can we expect other people to increase their milk intake if we don't show them the way?

J. W. HEIZER
New York City

Suggests Addition of Courses in Public Health

I notice with much interest the report of the Curriculum Committee which appeared in the December issue of the *Journal of Dairy Science*.

To one who has spent many years in public health, it seems to me that the Land-Grant colleges have neglected to include either sufficient or well-rounded courses in public health for students in both the dairy and food technology sciences.

For basic science, a combination of sanitary and food bacteriology is a recommendation for manufacturing majors only. Under Dairy Production, animal health and sanitation are grouped together once again as a recommended elective. For Dairy Manufacturing majors, there is not a single full-time course devoted to public health.

While it is true that many of the courses given do include a smattering of sanitation and public health in relation to the particular subject matter, it appears that the colleges and universities do not have a full understanding of the importance of public health to food technology.

The basis for most of the laws and regulations of public health is environmental sanitation. Prevention of outbreaks of disease, whether they be directly or indirectly traceable to food, has its basis in a properly controlled environment. An understanding of communicable diseases, the mode of transmission, the sources of infection, their prevalence, and the methods of control should be a basic part of food technology.

One might advance the theory that a course in bacteriology would suffice, but it has been my experience that the average graduate has a pitiful understanding of the importance of public health to the dairy and food industries.

HAROLD WAINESS
Chicago

People Should Eat More Cheese

The editor of the *Pacific Foods Review* in the January 1954 issue makes the significant statement: "On the present basis of population increase in the United States the current surplus could be wiped out if each family of four would spend just $2.50 more a year for cheese. High protein cheese dishes make inexpensive, nutritious meals. One new dish made with cheese 'sold' to every consumer in the United States could wipe out surpluses too!"

Here is a challenge to the dairy industry!

The per capita consumption of cheese, not including cottage, in the United States increased from 4.4 lb. in 1932 to 7.7 lb. in 1952. There has been a tremendous increase in the production of Cheddar, Swiss, Italian, and Blue Cheese during this 20-year period. Of the 1,170,404,000 lb. of cheese made in 1952, 375,181,000 lb. were of varieties other than Cheddar. In 1952, a total of 439,000,000 lb. of creamed cottage cheese was made, with California producing 20% of the total.

I believe there is an opportunity to further increase the per capita consumption of cheese in the United States. When I visited Denmark in 1949 I was impressed with the attractive displays of many varieties of cheese in specialty stores. The quality of the cheese was excellent. The most common varieties were: Samsoe (Swiss-type), Fynbo (Gouda), Molbo (Edam), Danablu (Blue), Taffel (Tybo), Esrom (Port du Salut), and Danbo (Steppe). Cheese was served even for breakfast. Yes, why not? A fine breakfast that the writer enjoyed at restaurants was "Kaffe Complet." It consisted of boiled egg, several kinds of sliced bread, a dish with balls of tasty butter, several kinds of delicious cheese nicely arranged on a tray, and coffee with cream. For the evening meal, which generally consisted of open-faced sandwiches, butter and cheese were used generously. This small country (only one-sixth the size of Oregon) produced 215,000,000 lb. of cheese in 1951. A large percentage was exported. Domestic consumption was 55,000,000 lb. Cheese consumption in several other European countries is also high.

Cheese is one of God's finest gifts to man. We should serve it more often.

G. H. WILSTER
Oregon State College

Likes New Department of Journal

Since my student days at the Manitoba Agricultural College over a third of a century ago, I have been a reader of the *Journal of Dairy Science*. I have been interested in following its evolution over the years and wish to take this opportunity to express to President Price, the Editor, and the Journal Management Committee my appreciation for the improvements that have been made in the Journal during the past year. Formerly, my Journal went unopened for some time after it arrived. Now I look forward to receiving it, and the first thing I turn to is your "People and Events" column, where I like to read what is happening in the various parts of the country.

I particularly enjoy your tributes to our

dairy leaders, and the guest editorials. I also like very much the department on completed M.S. and Ph.D. theses. I would like to see you develop the People and Events column even more. I suggest you get more news about industry developments in the different states in addition to the events happening at universities. I have wondered too why you don't have more news about what is happening at Washington, D. C., that people in the dairy industry would like to know about. I also suggest you include more news about recent developments in laboratory and plant equipment.

I find your "Industry Today" articles very much worth while. In my position as quality supervisor for our company, I frequently have to contact farmers. I have long since learned the value of being able to talk to them about something they are interested in and in a nontechnical language. The article by C. R. Hoglund on "How Efficient Can the Dairy Cow Become" in the December issue was tops in my opinion. It gave the essential facts without frills that I need when I talk to farmers on milk production problems. Let's have more such articles.

So, once again, congratulations on a more interesting and more useful journal. Keep up the good work.

MORRIS BARON
Chicago

OUR INDUSTRY TODAY

Brief Reviews of Current Topics

MANUFACTURE OF QUALITY CREAM CHEESE

A Means of Utilizing Some of Our Excess Milk Fat

ERIK LUNDSTEDT
Consulting Dairy Chemist
Miami, Fla.

Cream cheese occupies a relatively small but important place among the many varieties of cheeses made in the United States. The annual per capita consumption is about one-half pound. During the last hundred years a large amount of technical skill and knowledge has been accumulated. Many firms and individuals have contributed to the development of this product. Some of their names are remembered, but many others have been forgotten.

In a discussion of the manufacture of cream cheese it is necessary to review its legal background in order to better understand the reasons for the present-day methods of manufacture.

First Standards Established in 1921

The first advisory standard of identity for cream cheese was issued in 1921 by the Secretary of Agriculture, who at that time administered the Federal Food and Drug Act which stated that, "Cream cheese is the unripened cheese made by the Neufchatel process from whole milk enriched with cream. It contains in the water-free substance not less than sixty-five per cent (65%) of milk fat." A product containing 26% fat and 60% moisture had been made commercially from 7% fat mixes for many years prior to this time. Today, however, such a product would be considered as a high-fat Neufchatel cheese. In the 10-year period following the issuing of the 1921 standard, competition caused manufacturers to raise the fat content of cream cheeses to over 33%. The sweetness and smoothness of the higher fat cheese was far superior to that of the old type "Neufchatel Cream" cheese and resulted in increased sales.

During the next decade the cream cheese industry was in a state of confusion. The hot pack method of manufacture was developed in the late twenties, using a heated mixture of carob bean gum, cream, and cold pack curd. A big advantage of this type of cheese was that it could be discharged hot directly into foil lined boxes or containers. It was soon found by practical cheese makers that a satisfactory cheese could be made containing 24% fat and 68% moisture. This product was well over the 1921 standard since it contained 75% fat on the dry matter basis. The industry now had two alternatives, either to lower the fat of their cream cheeses to meet the competition of the new product made by the hot process or to have laws promulgated which took into consideration different limits of fat and moisture.

It was not until 1939 that the Secretary of Agriculture notified the industry that a public hearing would be held in Washington to consider new standards for cream cheese. Fortunately, the majority of the industry had continued to produce cheese with a minimum of 33% fat and a maximum of 56% moisture. After 3 years of study and argumentation, an order was published in the Federal Register, December 23, 1942, but due to judicial reviews it did not become effective before March 1943. An amendment to the order was signed by the Federal Security Administrator September 13, 1948 (9/17/48 FR 13-5422-5424).

These standards apply to cream cheeses shipped in interstate commerce or to that made in states which have adopted the federal standards. They state that cream cheese must have not less than 33% fat and not more than 55% moisture; the raw materials used may be skimmilk, milk, cream, condensed milk or condensed skimmilk or nonfat dry milk solids. The order further specified that the cheese must not contain more than 0.5% of a water-binding substance, such as Karaya, gum tragacanth, carob bean gum, gelatin, and algin, and the label must bear a statement to that effect.

In the United States both the cold and hot pack types of cream cheese are produced today, and many different methods are employed to make these two types.

Making Cream Cheese by the Cold Pack Method

One of the best methods used by the industry in producing a cold pack cream cheese with a fat content higher than the federal standard is as follows:

1. Standardize milk or skimmilk with fresh or frozen cream to a fat content of about 12%.
2. Pasteurize at 150° F. for 30 minutes.
3. Cool to 120° F. and homogenize single stage at 1800 lb. pressure.

4. Cool to 72-75° F. and inoculate with starter.
5. Ripen for 16 to 18 hours to a pH of 4.6 or lower.
6. Heat the coagulated mix in coil vats to about 130° F. or until a proper "break" is obtained between the curd and the whey.
7. Cool to 90° F., add 1% of salt, and further cool to 40° F.
8. Drain in bags, ice, and press over night to a yield of 33%.

Resulting cheese will contain about 36% fat and 53% moisture. To produce a cheese with 33.5% fat and 54% moisture an 11% fat mix should be used. Some of the older school prefer to use 18% mixes, producing a cheese with 40% fat and 51% moisture.

Factors determining quality of cold pack cheese. The keeping quality of cold pack cream cheese is about 3 weeks or less, depending upon the efficiency of the plant and the methods of distribution. Manufacturers using the cold pack method are primarily concerned about the taste, flavor, body, and texture of their cheese. The taste and flavor, or aroma, depend upon the raw material and the quality of the starter used. The body and texture are regulated by a series of factors which are more complicated and therefore need discussion.

The size, shape, arrangement, and degree of hardness of the solid particles determine the body and texture characteristic of the cheese. A desirable cheese is one that is smooth and soft and has good spreading qualities even at refrigerator temperatures. The physical state of the milk solids can be controlled by proper manipulation of the cheese making process. The more important factors affecting body and texture in the finished cheese are as follows:

The source of the butterfat.
The percentage of fat and milk solid not fat.
The pasteurizing temperature.
The pressure and temperature of homogenization.
Whether or not salt is added before cooking the mix.
The pH of the mix at the time of cooking.
The cooking temperature.
The amount of agitation during cooking.
The extent of cooling before homogenization.
The amount of water added after the mix is cooked.
The extent to which the acid is reduced after cooking.
The temperature at which the mix is drawn.
The amount of pressure to which the curd is subjected.
Mechanical treatment given the finished curd.

In choosing the percentage of fat for mixes, it is not correct to base calculations on the percentage of fat and solids-not-fat alone, as the casein in the solids-not-fat does not have a constant value. This solid varies in different parts of the country from 23 to 30% of the milk solids-not-fat. The importance of the composition of the solids-not-fat in milk in cream cheese making will be discussed under the hot pack method of manufacture.

Proper pH and cooking temperature necessary. The best cheese is obtained at a pH of 4.6 using a cooking temperature of 130° F. With a higher pH value (less acidity) and higher cooking temperature the resulting curd will become dry, crumbly, and rubbery; but with a decrease in pH and cooking temperature the curd will become smooth, soft, sticky, and undrainable. If for some reason it is desirable to use a higher cooking temperature, the mix should have a pH lower than 4.6 in order to maintain a desirable body and texture. Because of possible variations in milk solids-not-fat and CO_2 present in the mixes, the titrable acidity test is not a satisfactory guide in making cream cheese.

Establishing proper drainage conditions. An experienced cheese maker can predict the texture and body of the resulting cream cheese curd by observing the rate of drainage of whey from the bags. The proper breaking point for whey separation depends on the pH and temperatures, and by means of a stop watch and filter paper it can be demonstrated that this point is reached at a pH of 4.6 at 110° F., but for good drainage an additional 20 degrees of heat is necessary. The drainage is slowed down by high pasteurizing temperatures, high homogenizing pressures, high fat content mixes, high acidities, low "cooking" temperatures, and the addition of salt and neutralizers before "cooking." Fast drainage is obtained by minimum pasteurizing temperatures, low homogenizing pressures, low fat content mixes, low acidities, gentle agitation, high cooking temperatures, and the addition of at least 25% water to the mix. In general, the faster the drainage the coarser the cheese, and the slower the drainage the smoother the cheese.

Source of the fat important. The texture of the cheese to a certain extent also is influenced by the type of product used to supply the butterfat in the mixes. The texture varies from coarse to smooth in order of the following types of fat sources employed—butter oil, butter, frozen cream (3 months or more), plastic cream, and fresh cream. Butter oil cannot be used to the extent of more than 30% of the total amount of butterfat required without influencing the taste as well as the body of the finished product.

Some manufacturers fortify their mixes by the addition of concentrated skimmilk or low heat milk powders. It is possible to increase the output of a plant by 20% or more by the addition of extra solids and fat.

Making Cream Cheese by the Hot Pack Method

The base for hot pack cream cheese is curd manufactured according to the principles laid down for the cold pack process. The best and most economical hot pack method is the combination of whole milk curd and cream, since the curd can be used both for Neufchatel and cream cheese. Further, a more uniform cheese can be produced at the legal limits with a firm, spreadable, and smooth texture and a flavor and taste surpassing the straight curd method.

After the publication in 1927 of the Dahlberg method *(J. Dairy Sci.,* **10**: 106) of packaging cream cheese directly from the homogenizer into foil lined boxes, a vast amount of experimentation took place. Secrecies surrounded the industry since all foresaw an era similar to the one created by the late J. L. Kraft in the processed cheese field. Patents were taken out on the addition of gums and combination of gums and pectins, on the use of cottage cheese curd and plastic cream, and on all sorts of mechanical devices. However, it was almost 20 years before a new process of significance was created in the hot pack cream cheese industry. At this time the centrifugal curd concentrator was developed by the DeLaval Separator Co. and the Kraft Foods Co.

In order to control the texture and body of this type of curd, it is necessary to use a combination of a low pH mix and a high cooking temperature already described under the cold pack method. If mixes are "cooked" at temperatures over 165° F., the finished cheese will lose the delicate flavor and aroma obtained with lower temperatures of "cooking."

The use of high casein milk may result in economy. It is difficult for the uninitiated to understand that two hot pack cheeses of apparently widely different compositions can have the same firmness of body.[1] For example, a Neufchatel cheese with 20% fat and 65% moisture may have the same firmness of body as a cream cheese with 33% fat and 55% moisture. From the casein content of the mixes it can be calculated that both cheeses on a fat-free basis contain the same amount of casein and moisture, i.e., 10.5% casein and 82% moisture.

Therefore, if the cream cheese contains 21 lb. of casein per 100 lb. fat the Neufchatel cheese will have twice as much casein, or 42 lb. per 100 lb. of fat. The proportion of casein to the fat in cream cheese mixes ranging from 10 to 13% fat decreases only 2 lb. per 100 lb. of fat in the cream cheese for each per cent increase of the fat in the mixes. This certainly is important from a standpoint of cost. If a manufacturer has a choice of milk supplies, it is to his advantage to know how much casein there is in the solids-not-fat of the milk, because the higher the percentage, the lower the cost of his finished product.

pH must be controlled. Another important factor in the production of hot pack cream cheese is the control of the pH in the finished cheese. A whole milk curd with a pH of 4.7 will by the addition of sweet cream have its acidity reduced to a point where the finished cheese will be soupy or sticky. It is therefore desirable to have on hand ripened cream to use for this purpose. The proper proportion of sweet or ripened cream and whole milk curd can be determined by a pH determination of a small amount prior to making the factory batch. Each manufacturer should determine for himself what the pH should be in order to produce a cheese with the type of body the market in his area demands.

If a market requires a very sweet but not sticky cheese of a pH of 4.7 to 4.8, the milk mix should be cooked at a pH of 4.6 and the finished cheese should contain 22 lb. of casein per 100 lb. of fat when making cream cheese and 46 lb. casein per 100 lb. fat when making Neufchatel cheese.

There Is a Future for the Cream Cheese Industry

The cream cheese industry has a very interesting background and a promising future, and it is to be hoped that the increased interest in this type of cheese will absorb part of our growing surplus of butterfat for the benefit of all concerned.

[1] Some homogenizers, regardless of type and pressure, will not produce a firm-bodied hot pack cheese because they are unable to properly disperse the fat in the casein.

APPRAISING THE TRANSMITTING ABILITY OF SIRES PLACED IN ARTIFICIAL SERVICE AS YOUNG BULLS

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The cooperative artificial breeding program in Illinois was started and functions for the purpose of improving the efficiency, production, type, and value of Illinois dairy cattle. Consequently, keeping an adequate number of bulls in service that have the ability to transmit the desired characteristics to their offspring is one of the most important problems in connection with this project.

The number of bulls adequately and favorably proven for production and type that are available for use in artificial breeding associations falls far short of supplying the demand. Some bulls proven satisfactory for production transmit undesirable type. Bulls proved on the basis of a few dam and daughter pairs in one

herd may show up differently when final appraisal is made on a large number of artificially sired daughters. With this situation in mind, the practice of carefully selecting and making special matings to produce young sires to be sparingly used until proved on the basis of artificially sired daughters was started soon after the program got under way in Illinois in 1940.

When this type of breeding program is followed, making an early appraisal on the transmitting ability of young bulls is of major importance. Early in 1953, an organized plan was outlined by the author to meet this need. The plan consists of the following major phases:

1. Arrangements should be made with 100 or more cooperators who are members of dairy herd improvement associations to breed two or more cows to each young bull as he is placed in service. Heifers resulting from these matings should be retained until they have finished a lactation record.

2. The association should keep a separate record of the location of the first two or three hundred cows bred to each young bull. More complete information will be made available and time saved if this procedure is followed.

3. Technicians should be asked to report the location of heifer calves.

4. Twenty to 50 daughters of each sire should be checked for type and uniformity when they are 6 to 12 months old. At that time, a report card should be made out for each calf. This record should show the number of the calf, her sire and dam's number, date of birth, owner, and notations relative to the type of the calf and feeding and management practices. At this time the dam should be classified (unofficially) and the rating listed on the card. The dam's record should also be recorded. It is important to list the type classification of the dam at this time, as she may not be available when her daughter comes into milk.

5. An early check on 20 or more daughters should be made before first-calf lactation records are finished. At this time, the report card is completed by listing the calving date, days in milk, production of milk and butterfat to date, and the unofficial type rating.

6. The production and type data are analyzed.

7. If the inheritance picture is favorable, the cooperator, fieldmen, and technicians should be notified and the bull placed in heavier service. If the data on a bull indicate strong transmitting ability for certain characteristics, this fact also should be made known to the abovementioned group. Any faults should be noted and kept in mind when matings are made.

8. If an early and incomplete appraisal of a bull raises some question as to his transmitting ability, he, of course, should be taken out of even limited service until a final decision can be made on the basis of more complete information.

Following a program of this kind gives an early appraisal of the transmitting ability of young bulls and makes possible a wiser selection of sires to be used in individual herds and on individual cows.

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JOURNAL OF DAIRY SCIENCE

ABSTRACTS OF LITERATURE

W. O. Nelson, Abstract Editor

ANIMAL DISEASES

237. Dilator for clogged teats. V. GARIEPY. U. S. Patent 2,664,894. 1 claim. Jan. 5, 1954. Official Gaz. U. S. Pat. Office, **678**, 1: 146. 1954.

A round instrument, surrounded by a solid medicament, for inserting in and treating injured or diseased teats. R. Whitaker

238. Comparative study of the content of starch and sugars of Tribulus terrestris, lucerne, some Gramineae, and Pentzia incana under different meteorological, edaphic, and physiological conditions. II. Carbohydrate nutrition (bloating and dikkop sickness). M. HENRICI, Veld Reserve, Fauresmith, S. Africa. Onderstepoort J. Vet. Research, **25**, 45. 1952. (Chem. Abstr., **48**: 302e. 1954.)

S. Patton

BUTTER

239. Automatic individual butter server. V. PALAZZOLO. U. S. Patent 2,663,932. 4 claims. Dec. 29, 1953. Official Gaz. U. S. Pat. Office, **677**, 5: 1208. 1953.

Sticks of butter held in vertical channels are sliced by a knife blade into single individual servings by pressing a level. The butter is held in servable condition by cracked ice in an adjacent chamber. R. Whitaker

240. Forsök med bitning av färsksmör (Experiments in the printing of fresh butter). English summary. K. E. THOMÉ, E. G. SAMUELSON, and N. MATTSON, Dairy Dept. of The Alnarp Inst., Sweden. Report 39. 1953.

The most important factors influencing the quality of printed butter were storage time and temperature prior to printing. Butter stored at room temperature as well as cooler temperature was inferior with regard to flavor and general appearance 10 d. after printing. Deterioration was due to an increase in the size of the serum droplets resulting in a wavy, mottled butter and an increase in the number of bacteria. The churn seemed to have little influence on the quality of the butter, although prolonged working in a wooden churn increased the number of bacteria as compared to normal working in a wooden churn, and normal and prolonged working in a stainless steel churn.

The use of regular salt or a special salt (buffer) did not influence the quality of the butter. T. Kristoffersen

CHEESE

241. Quality cheese and government standards. H. L. WILSON, Kraft Foods Co., Chicago. Southern Dairy Products J., **54**, 5: 133. 1953.

The three important items in the manufacture of cheddar cheese in order of importance are quality milk, a dependable starter and satisfactory cheese making procedure. Low-grade milk can cause defects in the cheese in spite of pasteurization. A competent grader in the receiving room usually can pick out milk of poor quality by smell and thus reduce the amount of low-grade cheese. The methylene blue test has served a purpose in the past but its value in grading milk to be pasteurized for cheese making is questionable. The Wisconsin curd test is considered superior but may be no better than a competent grader by smell. Starters should be handled carefully by an approved procedure. The details of good cheese making procedure are reviewed. Holding the cheese at 50-65° F. for the first 60 days is recommended for cheese of good quality. Reference should be made to government standards, which may be obtained from the Production and Marketing Administration, in order to meet the requirements as to composition and characteristics of the cheese. F. W. Bennett

242. Cheese and process of preparing the same. S. G. KNIGHT (assignor to Wisconsin Alumni Research Foundation). U. S. Patent 2,665,990. 2 claims. Jan. 12, 1954. Official Gaz. U. S. Pat. Office, **678**, 2: 526. 1954.

By using a white mutant obtained by irradiation of blue-green *Penicillium roqueforti*, a Roquefort type of cheese is made which is free from the blue-veins, characteristic of such cheese. R. Whitaker

243. Cheese curd cutter. B. T. HENSGEN (assignor to Swift & Company). U. S. Patent

2,663,081. 1 claim. Dec. 22, 1953. Official Gaz. U. S. Pat. Office, **677**, 4: 910. 1953.

A cheese curd cutter for a vertical cylindrical shaped cheese vat, consisting of a fixed shaft mounted in the center, to which one cutter is attached, and around which another cutter is made to rotate. The fixed cutter holds the curd from rotating. R. Whitaker

244. Open texture or late gas defect in ripening cheese. W. V. PRICE, Univ. of Wis., Madison. Milk Products J., **45**, 1: 22. 1954.

Cheesemakers and dealers have observed excessive openings, gas holes, "sweet-holes" or splits developing in cheddar cheese during ripening. The defect may appear as early as seven d. to two wk. in cheese with moisture approximating 40% or more, or it may appear in Cheddar cheese after three mo. of curing at 55° F.

The difficulty generally is attributed to the presence in the cheese of anaerobic gas forming microorganisms which can survive pasteurizing temperatures. The control measures that will minimize the development of late gas in cheese are summarized. J. J. Janzen

245. New investigations on the development of "bank"-red in cheese. H. H. HANNI, Milchwirtsch. Anstalt Liebefeld. Schweiz. Milchztg., **79**: 343. 1953. (Chem. Abstr., **48**: 300b. 1954.) S. Patton

CONDENSED AND DRIED MILKS; BY-PRODUCTS

246. Preparation of milk powder. F. F. HANSEN. U. S. Patent 2,663,644. 6 claims. Dec. 22, 1953. Official Gaz. U. S. Pat. Office, **677**, 4: 1067. 1953.

Dry skimmilk powder, having a minimum particle size of 200 mesh, is exposed to ultraviolet light of 2,500 to 3,000 Å for 20 to 40 min. to neutralize the sulfhydryl (–S–H) radicals, for the purpose of improving the oven-spring of bread dough containing the powder. R. Whitaker

247. Milk powder and its preparation. F. F. HANSEN. U. S. Patent 2,663,643. 4 claims. Dec. 22, 1953. Official Gaz. U. S. Pat. Office, **677**, 4: 1067. 1953.

The oven-spring of bread dough made with dry skimmilk is improved if the reducing action of the sulfhydryl (–S–H) radicals of the powder has been neutralized by chlorination. R. Whitaker

248. Process of concentrating milk and product. R. WHITAKER and A. C. HERRO (assignors to National Dairy Research Laboratories, Inc.). U. S. Patent 2,663,642. 22 claims. Dec. 22, 1953. Official Gaz. U. S. Pat. Office, **677**, 4: 1067. 1953.

A process is described for producing a concentrated pasteurized milk which, on reconstitution with water, gives a whole milk with a flavor indistinguishable from fresh milk. Critical ranges are given for the temp. and duration of heating during pasteurization and concentration, so as to avoid the formation of a cooked milk flavor. R. Whitaker

249. Milk protein food product and process. H. W. HOWARD, R. J. BLOCK, and H. E. SEVALL (assignors to The Borden Co.). U. S. Patent 2,665,989. 3 claims. Jan. 12, 1954. Official Gaz. U. S. Pat. Office, **678**, 2: 526. 1954.

Casein and lactalbumin are simultaneously precipitated from skimmilk at 85-95° by adding the amount of acid required to bring the pH to the isoelectric point. Prepared in this way, the proteins are readily dispersible in water at the neutral point. R. Whitaker

250. Combined can puncturing and can holder device. J. O. KEENEY. U. S. Patent 2,663,460. 2 claims. Dec. 22, 1953. Official Gaz. U. S. Pat. Office, **677**, 4: 1016. 1953.

A can of evaporated milk is placed in this device which has a can puncturing attachment, which also serves as a pouring spout. R. Whitaker

DAIRY BACTERIOLOGY

251. Psychrophilic bacteria, effect on finished product. G. H. WATROUS, JR., Penn. State Coll., State College. Southern Dairy Products J., **54**, 5: 102. 1953.

Seventy-five raw milk samples were collected from milk receiving platforms. Some portions were laboratory pasteurized. All were stored at 5° C. for 20 d. The average standard plate count of the raw milk increased from 29,000 to 88,000,000/ml. The average psychrophilic count increased from 690 to 84,000,000/ml. The standard plate count of the pasteurized milk decreased from 730 to 510/ml. and no psychrophils were found. Three types of variability of bacteria counts were obtained during the storage of commercially pasteurized milk samples. In one type the standard psychrophilic and coliform counts all increased rapidly. In a second type there was no increase in the standard count, psychrophils reached 1/ml. only after 6 d. and then increased rapidly and no coliforms were found. In a third type there was no increase in the standard count, psychrophils reached 1/ml. first after 3 d. and increased rapidly thereafter and coliforms reached 1/ml. first after 12 d. and then increased rapidly. Changes in counts when plates were incubated at 25° C. for 3 d. followed somewhat the same pattern as the changes in psychrophilic counts. Psychrophils appeared not to withstand pasteurization but were found in pasteurized milk as a result of post-pasteurization contamination. F. W. Bennett

252. Testing quaternary ammonium germicides. G. R. GOETCHIUS and H. GRINSFELDER, Rohm and Haas Co., Philadelphia, Pa. Appl. Microbiol., **1**, 6: 271. 1953.

An improved procedure has been suggested for evaluating the bactericidal properties of alkyl (C_9–C_{15}) totyl methyl trimethyl ammonium chloride (50%). A comparative study on the bactericidal efficiency of phenol and the quaternary ammonium compound tested over a wide variation in concentrations showed that these substances are not similar in their behavior, and therefore it is believed to be incorrect to speak of a phenol coefficient value for quaternaries. H. H. Weiser

253. The nutrition of Brucella melitensis. T. H. SANDERS, K. HIGUCHI, and C. R. BREWER, Camp Detrick, Frederick, Md. J. Bacteriol., **66**: 294. 1953. (Chem. Abstr., **47**: 12511i. 1953.) B. L. Larson

254. Some properties of the hexokinase of Pseudomonas putrefaciens. H. P. KLEIN. J. Bacteriol., **66**: 650. 1953. F. E. Nelson

255. The differentiation of Aerobacter aerogenes and Aerobacter cloacae. M. S. BROOKE. J. Bacteriol., **66**: 721. 1953. F. E. Nelson

256. Synthesis of citrovorum factor by Leuconostoc citrovorum: potentiation by ascorbic acid. (Note) G. E. FOLEY and E. C. HALEY. J. Bacteriol., **66**: 727. 1953. F. E. Nelson

DAIRY CHEMISTRY

257. Improved method of determining densities of viscous fluids with Babcock bottles. R. S. CASS, J. B. GREGORY, and E. LEVENS, Frederick S. Bacon Laboratories, Watertown, Mass. Analyt. Chem., **25**, 11: 1773. 1953.

Babcock milk test bottles were used as pycnometers for determining densities of viscous liquids. A formula was developed to calculate density at a known temperature when the bottle neck reading and the weight of the fluid in the bottle were known. A precision of ± 0.0002 gram per ml. was obtained with viscosities of 1000 to 2000 cp. and temperatures of 160° to 180° F. B. H. Webb

258. Studies on casein. VII. Identity of γ-casein isolated from casein or paracasein. E. CHERBULIEZ and B. WOLF, Univ. Geneva, Switz. Helv. Chim. Acta, **36**: 1174. 1953 (in French). (Chem. Abstr., **47**: 12433f. 1953.) B. L. Larson

259. The terminal amino groups of α- and β-caseins. E. F. MELLON, A. H. KORN, and S. R. HOOVER, Eastern Regional Research Lab., Philadelphia, Pa. J. Am. Chem. Soc., **75**: 1675. 1953. (Chem. Abstr., **47**: 12461a. 1953.) B. L. Larson

260. Amino acid composition of γ-casein. WM. G. GORDON, WM. F. SEMMETT, and M. BENDER, Eastern Regional Research Lab., Philadelphia, Pa. J. Am. Chem. Soc., **75**: 1678. 1953. (Chem. Abstr., **47**: 12461b. 1953.) B. L. Larson

261. Pyruvic acid and acetaldehyde in human milk according to different Arakawa reactions. A. SATO and T. ARAKAWA, Tohoku Univ., Sendai. Tohoku J. Exptl. Med., **55**: 176. 1952 (in English). (Chem. Abstr., **47**: 12496a. 1953.) B. L. Larson

262. Nicotinic acid content of human milk. G. NICHELE, Univ. Rome, and G. Rovelli. Arch. Ital. Pediat e puericult, **15**: 281. 1951. (Chem. Abstr., **47**: 12555b. 1953.) B. L. Larson

263. Hydrolysis of casein. E. SELLES and J. JARA. Galenica Acta (Madrid), **5**: 143. 1952. (Chem. Abstr., **47**: 12474f. 1953.) B. L. Larson

264. Composition of kangaroo milk (wallaroo, Macropus robustus). A. BOLLIGER and J. V. PASCOE, Univ. Sydney. Australian J. Sci., **15**: 215. 1953. (Chem. Abstr., **47**: 12569e. 1953.) B. L. Larson

265. The influence of the ration of dairy cows on the amino acid composition of milk. P. V. KUGENEV. Izvest. Timiryaz ev. Sel'skokhoz. Akad., Moscow, **1**, 2: 187. 1953. (Chem. Abstr., **48**: 296a. 1954.) S. Patton

266. The fat-albumin correlation and its significance in practice. O. STÜBER and J. GÖBELT. Milchwiss. Ber., 3: **1952**. (Chem. Abstr., **48**: 298d. 1954.) S. Patton

267. Relationship of lactose to chlorides in milk. M. MEAD, Dairy Farmers' Co-op Milk Co., Sydney. Australian J. Dairy Tech., **7**: 77. 1952. (Chem. Abstr., **48**: 298e. 1954.) S. Patton

268. The changes of some physical constants of milk produced by the addition of water or urine. J. F. VALE SERRANO, F. TAVARES, and F. TEIXEIRA, Centro Estudos Farmacol., Porto, Portugal. Anais fac. farm. Porto, **12**: 35. 1952. (Chem. Abstr., **48**: 298e. 1954.) S. Patton

269. Determination of vitamin E in food and fodder. S. NOBILE and H. MOOR, F. Hoffmann-LaRoche & Co., A.-G., Basel, Switz. Mitt. Gebiete Lebensm. u. Hyg., **44**: 396. 1953. (Chem. Abstr., **48**: 302d. 1954.) S. Patton

DAIRY ENGINEERING

270. HTST timing with homogenizers. H. L. MITTEN, JR., and D. C. ROAHEN, Creamery

Package Manufacturing Co., Fort Atkinson, Wis. Milk Dealer, **43**, 1: 52. 1953.

Many HTST pasteurizer installations use an homogenizer as the timing or metering pump. This eliminates the usual metering pump thus lowering the initial cost for both equipment and installation work. One pump less may also mean that clean-up cost is less, maintenance requirements are lower, and depreciation is reduced. *Diagrams showing the installation of* homogenizers as timing pumps are given and the design and engineering of the installation is discussed.

C. J. Babcock

271. Pumping mechanism. R. HORTON, N. E. SPIESS, JR., and K. R. WEAVER (assignors to National Dairy Research Laboratories, Inc.). U. S. Patent 2,644,829. 6 claims. Jan. 5, 1954. Official Gaz. U. S. Pat. Office, **678**, 1: 127. 1954.

An air actuated, electrically controlled sanitary type pump for moving liquids with minimum agitation, with no moving parts other than inlet and outlet valves. Of particular value in pumping such products as cream, buttermilk, etc., the physical properties of which are impaired by agitation.

R. Whitaker

272. Bending pipe cuts installation costs 75%. W. R. SCHUH, E. D. Wesley Co., Milwaukee, Wis. Milk Products J., **45**, 1: 24. 1954.

The savings involved when using bent pipe instead of welded ells are discussed. This type *of installation has possibilities* in any dairy plant as it is applicable to refrigeration, water, steam or fuel line construction.

The efficiency of such an installation is still another phase where bends prove their superiority. The smooth walls allow flow with lower resistance than ells, helping maintain the pressure.

J. J. Janzen

DAIRY PLANT MANAGEMENT AND ECONOMICS

273. A system of payment for milk. E. O. WHITTIER, Bureau of Dairy Industry, USDA, Washington, D. C. Milk Products J., **45**, 1: 27. 1954.

The present system of payment for milk based on a differential for each tenth of a per cent fat above or below the standard percentage needs re-evaluation. Greater emphasis should be placed on the solids-not-fat content of the product.

The method presented here is based on a consideration of the relative value of the components of milk to the consumer in terms of nutrition and in terms of prices of competing nutrients.

The basic formula employed is $Af + Bs = P$, in which f is the number of pounds of fat in 100 lbs. of milk, A is the value assigned to the fat in dollars per lb., s is the number of lbs. of total solids in 100 lbs. of milk (approx. four times the number of lbs. of protein), B is the value assigned to the total solids in dollars per lb. (approx. ¼ the value of the protein per lb.), and P is the price of 100 lbs. of milk. Values of A, B, and P may be established by assuming values for any two, but preferably by setting *a base price for 3.5% milk of average solids* content and assuming a definite ratio of A to B.

Three tables are included which show the changes involved in 'price paid to farmer' using different base prices.

J. J. Janzen

274. Milk sales with bulk flow dispensers. ANON. Milk Dealer, **43**, 1: 56. 1953.

Milk dealers are installing dispensers in special outlets such as restaurants, drug stores, drive-ins, schools and hospitals, where milk is used in large quantities and where quick service is an important factor. A survey of the use of these dispensers shows that they enable the milk dealer to sell more milk with a lower distributing cost.

C. J. Babcock

275. Sample with quarts. WM. SKINNER, The Milk Shed, Inc., Moultrie, Ga. Milk Plant Monthly, **42**, 10: 25. 1953.

The Milk Shed, Inc. of Moultrie, Georgia reports spectacular results from chocolate-selling by the Johnson Company "set-off" contest plan. The plan involves placing at each retail consumer's doorstep a qt. of the dairy's chocolate drink and a message from routemen. If the housewife does not want the drink, it may be set back out for pickup on the next delivery date. It is not a free sample, and if not set back out is billed at regular price. A few days later the routeman leaves literature urging the housewife to order chocolate drink regularly. The route which won the contest had a sales base of 6 qt. per d. retail and 286 wholesale. During the contest, this route totaled 52 qt. retail and 521½ qt. wholesale. Two wk. after the contest, the route was selling 33 qt. retail and 443 qt. wholesale.

C. J. Babcock

276. Students learn by doing in cooperatives milk plant. ANON. Milk Dealer, **43**, 2: 54. 1953.

As an aid to teaching high school agricultural *students about cooperatives* the Miami Valley Milk Producers Association in Dayton, Ohio employed 9 members of the Beavercreek High School Future Farmers of America Chapter for a day's work in its plant and offices. The basic aim was a better understanding of dairy manufacturing and marketing operations. The preparations prior to the day in the plant and the activities in the plant are discussed. The event should be held in late spring when students are thinking about the type of work they want to *do after graduation.* Public relations programs

of dairy manufacturers can easily be coordinated with opportunities offered by groups such as the Junior Chamber of Commerce. Furnishing information to high school seniors about jobs in the dairy industry would add to the interest of students for the work as well as increasing understanding of the work done by industry. C. J. Babcock

277. Sell dairy chocolate to restaurants. C. O. DAVIS, JR., Editor, Milk Plant Monthly, **42**, 10: 19. 1953.

Tests concerning restaurants as outlets for hot chocolate, and the equipment required for the sale of restaurant chocolate are discussed. Factors causing increased sales are listed.
C. J. Babcock

278. Mejeridriftens Organisation. 1. Glasmjölk och Osttillverkning. (Organization problems in the dairy plant. 1. Production of bottled milk and cheese). English summary. E. EMILSON, Dairy Dept. of The Alnarp Inst., Sweden. Report 40. 1953.

Studies were carried out on choice of technic and capacity in the Swedish dairy industry. Recommendations were made for a bottling plant handling 40,000 bottles a day and a cheese factory making cheese of 10,000 litres of milk a day. T. Kristoffersen

FEEDS AND FEEDING

279. Fedtfattigt og fedtrigere kraftfoder til malkeköer (Concentrates with different fat content for dairy cows). English summary. H. W. ESKEDAL, Exp. Laboratory, Copenhagen. Bulletin, 265. 1953.

Rations containing linseed cake, linseed meal and linseed were given to 3 different groups of dairy cows. The more fat in the ration the higher was the yield of milk. Rape oil and soybean oil had the same effect with soybean oil being somewhat better. All 3 vegetable fats lowered the iodine no. of the butterfat resulting in a better consistency of winter butter.

Animal fats, lard, and ox-tallow also resulted in higher yields of milk when given to cows. The iodine no. was also lowered but butter made from such milk was considered less desirable.
T. Kristoffersen

280. Subnormal butterfat tests affected by roughage supply. R. B. BECKER and P. T. DIX ARNOLD, Fla. Agr. Expt. Sta., Gainesville. Guernsey Breeders' J., **90**, 1: 16. 1954.

Instances are given of subnormal butterfat tests occurring when normal roughages are lacking in the dairy cow rations. These include finely ground and pelleted feeds, chopped straw and molasses, corn or sorghum silages with limited pasture and inadequate pasture. Other cases of subnormal butterfat tests have been reported when cows were pastured on young, succulent oats.

Few instances of butterfat tests below standard were found when cows had adequate pasture, hay or silage. Where the herds with subnormal butterfat tests were fed hay the fat tests soon began a gradual increase.
A. R. Porter

281. The influence of feeding tocopherol to dairy cows on the yield of milk and milk-fat and on the tocopherol content and keeping quality of the butter. J. NIELSEN, Natl. Research Inst. of Animal Husbandry, Copenhagen, A. N. FISKER and A. H. PEDERSEN, Gov't. Research Dairy, Hillerød, and L. PRANGE, E. SØNDERGOARD, and H. DAM, Dept. of Biochem. and Nutrition, Polytechnic Inst., Copenhagen. J. Dairy Research, **20**, 3: 333. 1953.

Two groups of nine cows were used in three periods in an experiment to determine the effect of feeding α-tocopherol acetate. One group remained on a control winter type diet, while the experimental group first was observed in a preliminary 42 d. period without supplement and then under supplementation with 2.0 g. of tocopherol daily for 63 d. There was a further observation period of 35 d. following cessation of feeding the tocopherol.

Tocopherol feeding did not significantly influence milk yield.

There was an indication that the feeding of 2.0 g. α-tocopherol acetate daily to cows caused butter made from their milk to develop an oxidized oily flavor.

A second experiment on supplemental feeding at the same rate used two groups of seven cows for three periods of 35 d. each. The difference in butter quality was less definite but in the same direction on butters stored for 3 months at –12° C.

It was found in this group of butters that feeding tocopherols did raise the tocopherol content of butter to about twice that of butter from the unsupplemented group. The higher levels returned to normal in 2 weeks of the final period. The cows were then turned to pasture and tocopherols rose to about what the supplemented group had been (25 μg./g.).

The butters were tested on chicks to determine the tendency toward producing encephalomalacia. In neither group did the disease appear when butterfat was incorporated at a 30% level of the diet. In the group receiving butter from cows not supplemented, white striations appeared in 5 of 10 chicks while in the other group, 1 of 10. Thus, an increased protection was afforded chicks by the increased content of the tocopherol in the butter.

The supplementation of a winter ration with 2.0 g. daily of α-tocopherol acetate did raise the tocopherol content of the resulting butter. Milk yield and fat % were not influenced. Butter keeping quality was not materially changed but was slightly decreased. J. D. Donker

282. The effects of feeding iodinated casein and L-thyroxine upon the health, reproduction and yield of a dual-purpose breed of cattle, and a purely dairy breed. J. N. AITKEN, A. W. BOYNE, and J. A. CRICHTON, Rowett Research Inst., Bucksburn, Aberdeenshire. J. Dairy Research, **20**, 3: 291. 1953.

Ten Red Poll and ten Ayrshire cows were treated with iodinated casein or an equivalent amount of L-thyroxine following the peaks of production in several consecutive lactations. Their herd health record, reproductive performance and milk yields were compared to a control group of cows of similar genetic make-up. The iodinated casein was given for 15 weeks beginning at the 17th week of each lactation in the amount of 20 g. daily (80 mg. L-thyroxine) for the first 12 weeks, 15 for the next week, 10 the next and 5 the final week.

The incidence of disease over four lactations was not different between groups. There were for the control and experimental herds respectively 1.85 and 1.73 services per conception and 440 and 446 days between calvings, these figures not being significantly different. The treated Ayrshires lost weight during the first 12 weeks of feeding iodinated casein (18.6 lbs.) while the controls gained (46.2). Both groups of Red Polls gained; 68.2 lbs. for controls and 13.2 for experimental.

The control group of Ayrshire cattle gave 600 lbs. more milk per cow per lactation in the preexperimental period. The Red Polls were more evenly matched. Increased productivity reached maximum proportions 4-6 weeks after the start of feeding the drug. This increase became progressively less until the control animals were giving more milk before the end of the feeding periods. Consequently the lengths of the lactations averaged approximately 5 weeks less overall. It was only if daily average production for the experimental period was calculated that one of the experimentally fed groups produced significantly better than the control groups. This benefit appeared only in the first lactation of the Red Polls and may have been the result of their heavy fleshing prior to the start of the feeding of iodinated casein. There appeared to be no difference in butterfat % of the milk from the experimental group. J. D. Donker

283. The suitability and utilization of winter forages for dairy cattle. M. E. MCCULLOUGH, W. E. NEVILLE, JR., and O. E. SELL, Ga. Agr. Expt. Sta., Athens. Mimeo. Series, 62. 1953.

Types and nutritional value of winter pastures are discussed. R. W. Hunt

284. The nutritive value of tobacco seed extraction salvage in milk cows and suckling sheep. G. COMBERG and W. ROSENHAHN, Univ. Halle, Ger. Arch. Tierernahr, **2**: 376. 1952. (Chem. Abstr., **47**: 12541g. 1953.)
B. L. Larson

285. The animal protein factor, vitamin B_{12} and antibiotics in the feeding of domestic animals. R. FERRANDO and J. PHILIPPE, Ecole veterinaire, Lyons, France. Rev. fermentations et inds. aliment, **7**: 79. 1952. (Chem. Abstr., **47**: 12557a. 1953.) B. L. Larson

286. The nutritive value of the indigenous grasses of Assam. VI. The grass Joy-Joha (Ischaemum rugosum) as a cattle feed. B. K. DAS and N. C. MUKHERJEE, Animal Nutrition Research Scheme, Gauhati. Indian J. Vet. Sci., **22**: 239. 1952. (Chem. Abstr., **47**: 12548d. 1953.) B. L. Larson

287. The use of vitamin concentrates and salts of cobalt in feeding cattle. A. M. POPOV. Latvijas PSR Zinatnu Akad. Vèstis, **1951**: 421. (In Russian with Latvian summary, 423). (Chem. Abstr., **47**: 12550h. 1953.)
B. L. Larson

288. Contents of carotene and vitamin A in natural, cultivated, and prepared cattle feed in Latvian S.S.R. E. TAUCINS, Inst. Zootech. & Zoohyg., Acad. Sci. Latv. S.S.R. Latvijas PSR Zinātnu Akad. Vēstis, **10**: 40. 1949. (Chem. Abstr., **48**: 303d. 1954.)
S. Patton

289. Needles of coniferous trees as a vitamin-rich food for domestic animals. K. BRENCIS, Inst. Zootech. & Zoohyg., Acad. Sci. Latv. S.S.R. Latvijas PSR Zinātnu Akad. Vēstis **5**, 59. 1950. (Chem. Abstr., **48**: 303c. 1954.)
S. Patton

GENETICS AND BREEDING

290. A study of variation in twin cattle. I. General description. H. P. DONALD, Agr. Research Council, Animal Breeding Research Organization, Edinburgh. J. Dairy Research, **20**, 3: 355. Oct. 1953.

Forty-five pairs of sister animals were obtained equally distributed among identical twins, fraternal twins and half sibs. Pairs were observed as to variances arising within members and this compared to variances between pairs to arrive at heritability estimates of various characteristics. Management was within normal farm routine with care taken to hold environmental variation to a minimum. Hay was fed *ad lib* in winter. Free access was had to pasture in summer. Grain and silage were rationed according to body weight and milk production. The feed available was considered to be of such quantity and quality as to not be a limiting thing in the observed performance. Each animal was weighed every second week. The heifers were to be bred at 15 months to members of identical twins.

It was stated that diagnosis of one-egg twins on subjective characteristics are accurate to the

extent that week-old calves are properly diagnosed in 90% of the cases.

The data on which recordings were to be made include growth, milk and butter-fat production, body dimensions, fertility and pattern of tooth growth. J. D. Donker

291. A study of variation in twin cattle. II. Fertility. H. P. Donald and D. Anderson. Agr. Research Council, Animal Breeding Research Organization, Edinburgh. J. Dairy Research, **20**, 3: 361. 1953.

An effort was made to determine the effects of heredity on factors affecting reproduction by using data obtained from twin animals. If the variance observed between one-egg twins was less than that observed between two-egg twins this is support that genetic influences are manifest in the trait under study.

A study was made within a herd consisting of 15 pairs of one-egg twins and 15 pairs of two-egg twins, along with 15 pairs of half sibs for age at first heat, intervals between heat, services per conception, gestation period, sex and twinning in the progeny. Although a trend was evident, the statistical significance of the analysis used failed to prove that differences in age of first heat arose from a genetic basis within this group of animals. Similar analysis failed to show a genetic bearing upon length of estrus cycle. Services required for first service showed no genetic influences to distinguish the variations observed. Although a difference in the variance between pairs compared to within pairs existed in one-egg twins, the differences were not of great enough magnitude to assume mathematical significance.

Of 122 matings of one-egg twins to one-egg twins there was only one occurrence of twins (two-egg). Thus, there is no evidence to support the contention that occurrence of identical twins is associated with a recessive gene. Since the heritabilities of these characteristics are not assured at a high level the author concludes there is little justification for using identical twins for this type of study. J. D. Donker

HERD MANAGEMENT

292. Effect of two systems of management on milk yield and body temperature of dairy cattle. K. A. Alim. Nature, **172**, 4391: 1195. 1953.

Native Egyptian cows were either kept indoors and provided with barseem or taken to the fields for 10 hrs. daily and fed the same ration. There was no significant difference between the two systems on either the milk yield or rectal temp. Barn temp. ranged from 13.4 to 21.5° C. and atmospheric temps. from 12.5 to 19.7° C. R. Whitaker

293. Vacuum tank with pipe line milking. T. D. Roland, Roland Bros., Pocatello, Idaho. Milk Plant Monthly, **42**, 11: 14. 1953.

Construction details and diagram are given of a farm vacuum tank for use with bulk milk pick-up systems. Air agitation of milk in the tank is accomplished by vacuum instead of by air pressure. The same vacuum pump is used to operate milking machines and to agitate the milk in the tank. It takes about 1½ min. to build 14 in. of vacuum in the tank, but as soon as this reserve has been built up, it improves the efficiency of the entire milking system. This tank is particularly adapted for climates where the milk pump on the tanker may freeze during the winter months. C. J. Babcock

294. The deterioration of milking rubbers. III. The effect of farm treatment. J. H. Cooper and E. R. Gardner, Avon India Rubber Co. Ltd., Melksham, Wilts. J. Dairy Research, **20**, 3: 340. 1953.

A study of factors affecting the life of rubber teat cup liners and the pattern of fat accumulation in the liners has shown that fat accumulates mainly at the mouth piece and along the inner surface in contact with the teat. Rubber under stretch absorbs more fat than relaxed material and this condition results in the rubber becoming supersaturated and later exudating fat in relation to its concentration. It has been estimated that 3.0 cc. of fat become absorbed during the milking of 3000 cows by virtue of the liner only absorbing the fat from ½ drop of milk per cow.

When fat becomes absorbed the rubber swells and becomes more susceptible to oxidation which causes further swelling. The source of fat to be absorbed is from teat skin exudates, ointments and milk fats. The life of the rubber is directly proportional to the care received in protection from prolonged contact with these sources. Fat absorption in the liner increases linearly with milkings performed. Dry or sour milk caused a rapid deterioration. Atmospheric ozone caused cracking of rubber, this being increased if the rubber was under partial tension such as where hoses were slipped on to metal tubes or where they were kept in a bent position. While modern cleaning agents do not harm rubber they generally do not remove absorbed fat except under prolonged soaking as the fat diffuses slowly out of the rubber. J. D. Donker

295. Animal operated drinking bowl. A. E. Anderson. U. S. Patent 2,664,069. 4 claims. Dec. 29, 1953. Official Gaz. U. S. Pat. Office, **677**, 5: 1246. 1953.

A drinking fountain for cattle, consisting of a bowl to which water is admitted from a pipe by a valve operated by a plate which the animal depresses with its nose. R. Whitaker

296. Stock waterer. C. H. Nelson. U. S. Patent 2,664,070. 11 claims. Dec. 29, 1953.

Official Gaz. U. S. Pat. Office, **677**, 5: 1246. 1953.

A cattle drinking fountain in which the water level is maintained by a float valve.

R. Whitaker

297. Valved coupling for milking system. A. E. ANDERSON. U. S. Patent 2,664,104. 16 claims. Dec. 29, 1953. Official Gaz. U. S. Pat. Office, **677**, 5: 1255. 1953.

A coupling for attaching a milking machine to a fixed vacuum outfit. A built-in valve closes when the milking machine is disconnected.

R. Whitaker

298. Milk strainer with removable baffle and centering device. W. H. HARSTICK (assignor to International Harvester Co.). U. S. Patent 2,665,009. 4 claims. Jan. 5, 1954. Official Gaz. U. S. Pat. Office, **678**, 1: 180. 1954.

A design for a milk strainer, so constructed that the filter pad is centered and rigidly held in place on the strainer. R. Whitaker

299. Livestock feeder. A. M. BEST (assignor to Sperry Corp.). U. S. Patent 2,665,015. 16 claims. Jan. 5, 1954. Official Gaz. U. S. Pat. Office, **678**, 1: 182. 1954.

A conical shaped scraper is installed in the bottom of a silo. Pressure of the silage actuates a motor which rotates the scraper and delivers silage to feeding outlets as consumed.

R. Whitaker

300. Electric automatic stock and chicken waterer. W. F. CLEVELAND. U. S. Patent 2,665,366. 1 claim. Jan. 5, 1954. Official Gaz. U. S. Pat. Office, **678**, 1: 275. 1954.

A watering trough suitable for cows, chickens, etc., having a float valve to maintain a given level and a thermostatically controlled electric heater. R. Whitaker

301. Pneumatic pulsator. S. J. ERLING (assignor to Aktiebolaget Separator). U. S. Patent 2,665,703. 3 claims. Jan. 12, 1954. Official Gaz. U. S. Pat. Office, **678**, 2: 442. 1954.

Details are presented covering the construction of a pneumatic type milker pulsator.

R. Whitaker

302. Pulsator. E. RAWSON (assignor to Package Machinery Co.). U. S. Patent 2,665,702. 18 claims. Jan. 12, 1954. Official Gaz. U. S. Pat. Office, **678**, 2: 442. 1954.

Construction details are given for a vacuum type milker pulsator. R. Whitaker

303. Suspended milker. E. RAWSON (assignor to Package Machinery Co.). U. S. Patent 2,665,663. 8 claims. Jan. 12, 1954. Official Gaz. U. S. Pat. Office, **678**, 2: 430. 1954.

A cylindrical shaped portable milker, having a detachable pulsator and connections to a vacuum supply and lines to teat cups.

R. Whitaker

ICE CREAM

304. Trends that will influence sales — 12 months of vegetable oils and ice cream. W. A. WENTWORTH, Borden Co., New York. Southern Dairy Products J., **54**, 5: 42. 1953.

Ice cream gallonage in 4 midwestern ice cream plants which also made vegetable-oil frozen desserts (Group 1) was down 9.3% and in 3 plants which did not make these new products (Group 2) it was down 4.1% during June 1952-May 1953 as compared with the past 12 mo. The overall gallonage of frozen desserts increased 11.5% in Group 1 plants but decreased 1.3% in Group 2 plants. Gains in the utilization of serum solids were 1.4% and less than 1% respectively. F. W. Bennett

305. Liquid sugar—what it is. M. F. HUGHES, Director, Research Laboratory, Refined Syrup and Sugars Inc., Yonkers, New York. Ice Cream Rev., **37**, 5: 44. 1953.

Liquid sugar is a 67% sugar syrup containing varying amounts of impurities depending upon the method and degree of purification carried out by the refinery. High quality liquid sugar is virtually free of color and it has no flavor other than sweetness. It has a low ash and invert sugar content, is practically neutral in reaction and is comparatively free of colloidal material.

Means of protecting liquid sugar from possible deterioration, methods for measuring liquid sugar into a mix, and the advantages claimed for the use of liquid sugar are discussed.

The author indicates that the marketing branch of the U.S.D.A. is studying the question of formulating standard grades for liquid sugar products. In setting up standards for the industry, the need for uniform procedures in evaluating the various factors which determine quality in liquid sugar products is stressed.

W. J. Caulfield

306. Consumer satisfaction from packaged ice cream. R. T. SMITH, Robert T. Smith and Associates, Scranton, Pa. Ice Cream Rev., **37**, 5: 49. 1953.

The opinions of 1500 representative consumers were obtained in an effort to learn what factors are important in consumer acceptance of packaged ice cream.

Summarization of these opinions indicates that: (1) availability, (2) good assortment of flavors, (3) storage of the ice cream in a special cabinet so as to keep the packages firm, and (4) providing insulated bags are factors which are important to 75% or more of the consumers in the purchase of packaged ice cream. Numerous other data relative to the sale of ice cream are discussed. W. J. Caulfield

307. Report on saving the half-gallon container through standardization. F. M. Skelton, General Ice Cream Corp., Schenectady, N. Y. Ice Cream Trade J., **49**, 11: 36. 1953.

The simplified practice committee of the IAICM reports that two standard ½-gallon packages have been developed with the following dimensions:

No. 1 squat 3½″ x 4-13/16″ x 6-47/64″
No. 2 tall 3″ x 5″ x 7-19/32″

Restraining forms for use with the two styles of cartons have the following dimensions:

No. 1 3-39/64″ x 4-59/64″ x 4″
No. 2 3-7/64″ x 5-7/64″ x 6″

W. H. Martin

308. Keeping ice cream consumers loyal. G. P. Gundlach and C. W. Esmond. Ice Cream Trade J., **49**, 11: 54. 1953.

A survey made by Univ. of Ore. to find out why and how often consumers changed brands of various kinds of merchandise showed that 3,755 customers made 24,401 changes over a 6-yr. period. 5,422 changes were due to dissatisfaction with the product, 3,751 because the price was too high, 3,323 changes resulted from other product advertising, 2,946 were caused by recommendation of a friend, 2,886 due to recommendation of sales people, 2,064 because they did not like the style of the product, and 1,561 changed because they did not like the dealer who supplied the former brand.

W. H. Martin

309. Trends in ice cream costs. Paul Beck, IAICM, Washington, D. C. Ice Cream Trade J., **49**, 11: 30. 1953.

Based on 1952 operations, the weighed average cost for ice cream per gallon in the U. S. was $1.595, or 9¢ more than in 1951, 22.7¢ more than 1950, 25.5¢ more than 1949, and 74¢ more than was reported for 1939.

In 1952 percentage costs were: ingredients, 48.99%; manufacturing, 20.17%; selling and advertising, 9.56%; delivery and customer service, 15.43%; and administrative costs, 5.85%.

W. H. Martin

310. A tabulation of routine coliform and standard plate counts on frozen desserts. J. L. Courtney, Public Health Laboratory, Oak Ridge, Tenn. Ice Cream Rev., **37**, 5: 47. 1953.

Examination of 669 samples of frozen desserts from 15 different processors over a period of 47 mo. revealed that 20% of the samples were negative for coliform bacteria and that 48% contained not more than 10 coliform bacteria per ml. It was found that 69% of the samples had standard plate counts of not more than 50,000 bacteria per ml. The bacteriological quality of novelties was found to be inferior to that of ice cream. The 85 samples of counter freezer products examined had lower coliform counts but higher average standard plate counts than either ice cream or novelties.

W. J. Caulfield

311. Stabilized ice cream compositions. J. Kamlet (assignor to The B. F. Goodrich Co.). U. S. Patent 2,665,216. 3 claims. Jan. 5, 1954. Official Gaz. U. S. Pat. Office, **678**, 1: 241. 1954.

An ice cream stabilizer consisting of ammonium and alkali metal salts of polyacrylic acid.

R. Whitaker

312. Thickening and stabilizing agents. C. T. Roland (assignor to Calgon, Inc.). U. S. Patent 2,665,212. 17 claims. Jan. 5, 1954. Official Gaz. U. S. Pat. Office, **678**, 1: 241. 1954.

A body building material or thickening agent, consisting of milk protein dispersed in an aqueous solution of a mixture of Na and K metaphosphates and a solubilizing agent for the metaphosphate. R. Whitaker

313. Automatic ice cream filler. P. Gilberty (assignor to Dairymat Corp.). U. S. Patent 2,663,482. 14 claims. Dec. 22, 1953. Official Gaz. U. S. Pat. Office, **677**, 4: 1023. 1953.

A can filling head is described, which is mounted over a conveyor. Means is provided for holding the can under the head until almost full and then releasing it, so that when it leaves the filling head, it is level full. R. Whitaker

MILK AND CREAM

314. Origin of sunlight flavor in milk. S. Patton and D. B. Josephson, Pa. Agr. Expt. Sta., State College. Milk Dealer, **43**, 1: 70. 1953.

Exposure of milk in conventional glass bottles to daylight for about ½ hr. produces a defect commonly known as sunlight or activated flavor. Experiments have indicated that the flavor substance has its origin in methionine and that flavor production is dependent, in large measure, on the presence of riboflavin. When distilled water solutions of methionine (20 mg./qt.) and riboflavin (1.5 mg./qt.) were exposed, the sample containing methionine developed a slight sunlight flavor, while that containing methionine and riboflavin developed the flavor to an extreme degree. Flavor defects did not develop in the sample containing only riboflavin. Samples containing added cysteine and cystine exhibited about the same degree of sunlight flavor as the control. The flavor was greatly intensified in the sample containing methionine. C. J. Babcock

315. Refrigerated cream dispensing cabinet. R. W. Walker. U. S. Patent 2,665,563. 2 claims. Jan. 12, 1954. Official Gaz. U. S. Pat. Office, **678**, 2: 401. 1954.

A small cabinet for holding a container of cream fitted into a chamber surrounded by ice

or other refrigerant. The cabinet also provides refrigerated storage space for a number of small individual containers. R. Whitaker

316. Formulas for pricing milk to producers in Ohio. E. F. BAUMER and C. G. MCBRIDE, Ohio Agr. Expt. Sta., Wooster. Research Bull., 739. 1954.

In 1946, a meeting of marketing economists was called to explore the feasibility of expanding and refining the methods of pricing milk in Ohio city markets. The participants were from several states, the P.M.A., and from every federal order market in Ohio. As research progressed, it was recognized that the problem was to build a formula based on economic factors. The Tri-State market was the first Ohio market to consider incorporating economic factors into their formula. Feed and labor, wholesale price index and retail sales index were to be used. After considerable deliberation, the Dairy Branch did not approve the use of this new formula. In 1949, the Akron Milk Producers requested that the Dept. of Agr. Econ. work on a formula using the following factors: cost of production, condensory prices, wholesale price index and retail sales index. Comparison of actual prices vs. prices calculated according to economic factors reveals that generally the calculated price would have been higher than the actual price paid. It is believed that neither of the formulas is being used.

R. W. Hunt

MILK SECRETION

317. Automatic apparatus for drawing the milk ejection curves of dairy cows under controlled milking conditions. W. G. WHITTLESTONE and D. S. M. PHILLIPS, Ruakura Animal Research Sta., Dept. of Agriculture, New Zealand. J. Dairy Research, **20**, 3: 319. 1953.

The authors describe in detail a fully automatic milking rate and yield recording device which needs no special attention from anyone other than the regular milker and in no way interferes with his routine work. The timing and recording mechanism is actuated by a switch within the teat cups when they are placed upon the cow. It automatically stops recording when the rate of milking falls below a prescribed level (0.5 lb./min.) at which time the milker may machine strip the cow. The recorder automatically zeros itself when the milk container is emptied. There is included a description of an improved type experimental magnetic pulsator for the milking machine to be used in studying how pulsation stimulation affects the cow's response. J. D. Donker

318. Pulsation and milking rate. P. A. CLOUGH, F. H. DODD, and E. W. HUGHES, Natl. Inst. Research Dairying, Univ. of Reading. J. Dairy Research, **20**, 3: 375. 1953.

The effect of varying the pulsation rate at 20, 50 and 80 cycles per min. was examined as to effect on milking rate. Three groups of three cows were used. Each group contained a slow, medium and fast milking animal. Each group received each treatment of pulsation rates. The groups were rotated at weekly intervals and average milking rates were compounded from the last four days in each period.

It was found that rate of milking in first min. increased 1.92 lbs. when going from 20 to 80 pulsations per min., peak flow increased 1.39 lb. per min. and overall rate 0.34 lbs./min. while total duration of milking decreased 1.26 min.; all effects being significant at the 5% level. There appeared to be no effect on total yield or stripping yield.

An experiment was set up to determine whether the observed effects were due to a stimulatory effect on the milk ejection mechanism or due to mechanical conditions in the teat cup assembly. This was done by milking two halves of the same cow at two pulsation rates simultaneously thus eliminating the cow effect. Five cows with a wide range of milking rates were chosen (1.95 to 7.03 lb./min.). The experiment was set up so that each cow was subjected to all treatments on both udder halves. The milk obtained expressed as a percentage of the 50 cycles per min. peak rate was 109.72% for 80 cycles and 81.10% for the 20 cycles/min.

It appears from these experiments that milking times may be reduced by about 10% when milking cows at a pulsation rate of 80 cycles per min. compared to a usual of 50 cycles/min.

J. D. Donker

319. Normal variations in the rate of machine milking. F. H. DODD, Natl. Inst. Research Dairying, Univ. of Reading. J. Dairy Research, **20**, 3: 301. 1953.

Three hundred twenty-six records of milking rates from 141 cows were gathered from 1946 to 1952 from Shorthorn, Guernsey, Friesian and Ayrshire cows. The records were made between the 5th and 6th weeks of each lactation for three consecutive morning milkings. In addition ten cows had records taken daily for two weeks and another ten cows had weekly records made over a lactation.

The lactation trends showed that as yield increased or decreased so did yield in first minute, mid-milking rate, peak flow, machine rate and overall rate. The duration of milking decreased due to the fact that yield dropped faster than milking rate. Of the regression coefficients calculated for the ten cows of morning yield to rate in first minute, mid-milking rate, peak flow, machine rate, and machine time. All are positive and 38 of 50 are significant at the 1% level and 3 at the 5% level. Stripping yield on milk yield showed no relationship. There is an indication from partial regression coefficients that as the time from calving increases independent of yield that peak flow

and machine rate decrease while machine time increased. Calculation showed that the fast milking cows had a greater absolute and relative fall in milking rate associated with decreased yield than the slower milking cows.

Total time of milking also increases as the yield increases and to a greater extent than rate. The trend is gradual and the statistical significance of the differences between lactations is usually above 5%. Partial regression coefficients show that while peak flow is not effected by increased age, the duration of milking time increases, probably because of the effect of age increase on increased yield of strippings and machine rate.

Statistical analysis showed that total duration of milking was influenced in the following ratio by milk yield, stripping yield and peak flow; 3:1:6. A good measure of machine rate is the % yield in the first two minutes of milking. The correlation coefficient between this datum and machine rate is .869 and significant at the 1% level.

While the anatomy of the teat orifice is the main controlling factor in rate of milking there are other minor changes associated with yield, stage of lactation and age. J. D. Donker

NUTRITIVE VALUE OF DAIRY PRODUCTS

320. Multiple sclerosis in rural Norway. Its geographical and occupational incidence in relation to nutrition. R. I. Swank, O. Lerstad, A. Strøm, and J. Backer, McGill Univ., Montreal, Can. New Engl. J. Med., **246**: 721. 1952. (Chem. Abstr., **47**: 12540g. 1953.) B. L. Larson

321. Chick-growth stimulation by alfalfa juice, casein, and aureomycin. C. H. Hill, R. L. Borchers, C. W. Ackerson, and F. E. Mussehl, Univ. Nebraska, Lincoln. Poultry Sci., **32**: 775. 1953. (Chem. Abstr., **47**: 12543b. 1953.) B. L. Larson

322. Milk diet, p-aminobenzoic acid, and malaria (Plasmodium berghei). F. Hawking, Natl. Inst. for Med. Research, London. Brit. Med. J., **I**: 1201. 1953. (Chem. Abstr., **47**: 12626a. 1953.) B. L. Larson

PHYSIOLOGY AND ENDOCRINOLOGY

323. Characteristics of testicular hyaluronidase. H. Malmgren, Inst. Biochem., Upsala, Sweden. Biochim. et Biophys. Acta, **11**: 524. 1953. (Chem. Abstr., **47**: 12454i. 1953.) B. L. Larson

324. Biosynthesis of fatty acids from carboxy-C^{14}-acetate by soluble enzyme system prepared from rat mammary-gland homogenates. G. Popjak and A. Tietz, Natl. Inst. Med. Research, London. Biochim. et Biophys. Acta, **11**: 587. 1953. (Chem. Abstr., **47**: 12456c. 1953.) B. L. Larson

325. Methods of studying cow urine. A. P. Gorbacheva and T. K. Rzhevskaya. Doklady Vsesoyuz. Akad. Sel'skokhoz. Nauk im. V. I. Lenina, **18**: 23. 1953. (Chem. Abstr., **47**: 12483a. 1953.) B. L. Larson

326. Effects of diet and antibiotics on utilization of nonprotein nitrogen by rumen microorganisms. J. M. Prescott, Texas Agr. Expt. Sta., College Station. J. Agr. Food Chem., **1**: 894. 1953. (Chem. Abstr., **47**: 12545d. 1953.) B. L. Larson

327. Placental and mammary transfer of vitamin A and carotene by beef cows. F. H. Baker, R. MacVicar, L. S. Pope, and C. K. Whitehair, Oklahoma A. & M. Coll., Stillwater. Proc. Soc. Exptl. Biol. Med., **83**: 571. 1953. (Chem. Abstr., **47**: 12546g. 1953.) B. L. Larson

328. Biosynthesis of milk proteins in the fasting goat. B. A. Askonas and P. N. Campbell, Natl. Inst. Med. Research, London. Biochim. et Biophys. Acta, **11**: 304. 1953. (Chem. Abstr., **47**: 12552f. 1953.) B. L. Larson

329. Mineral metabolism in growing calves. II. Ahmad Tremazi, Agr. Coll. Research Inst., Lyallpur. Pakistan J. Sci. Research, **4**: 77. 1952. (in Pakistan J. Sci., **4**: 3). (Chem. Abstr., **47**: 12555h. 1953.) B. L. Larson

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SANITATION AND CLEANSING

337. Cleaning glass lines in place. J. J. Sheuring and T. R. Folds, Univ. of Ga., Athens. Milk Plant Monthly, **42**, 11: 48. 1953.

This article is a discussion of the experimental procedure and experimental data on cleaning glass lines in place. The following conclusions are emphasized: (1) Velocities of 1, 2, 3, and 4 ft. per sec. of a cleaning solution resulted in a line that is visibly bright and shiny; (2) A medium strength acid cleanser followed by a medium strength alkaline cleanser is the most effective cleansing method for removing organisms from the line; (3) The higher the velocity the more effective the removal of bacteria from the line in the cleansing process; (4) Hot water is more effective than chlorine solution in sanitizing a glass line; (5) A velocity of 3 ft. per sec. of 190° F. water or 200 p.p.m. chlorine solution will satisfactorily sanitize a glass line, and (6) Hot water (190° F.) or a 200 p.p.m. chlorine solution will destroy coliform and thermophilic bacteria in a glass line. C. J. Babcock

338. Fly control in dairy barns. H. King, G. Guyer, and H. P. Ralston, Mich. Agr. Expt. Sta., East Lansing. Quart. Bull., **36**, 2: 179. 1953.

A project involving 10 barns and 15 treatments was conducted during the summer of 1953. The following conclusions were obtained: (1) In conventional dairy barns, residual wall sprays of malathion or diazinon were satisfactory; (2) In conventional barns, floor treatments of malathion, lindane-TEPP, or Bayer L 13/59 were satisfactory; (3) In loose housing, direct spraying of the animals was the only effective method of control; (4) In milk parlors, bags sprinkled with malathion bait mixture gave satisfactory control; (5) In the 1952 season, lindane vaporizers were ineffective.
R. W. Hunt

339. A method for assessing the cleaning efficiency of detergents. M. Mead and J. V. Pascoe, Dairy Farmers Co-op Milk Co., Sidney. Australian J. Dairy Technol., **7**, 114. 1952. (Chem. Abstr., **48**: 389g. 1954.)
S. Patton

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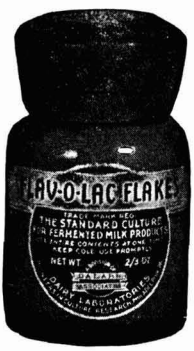

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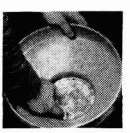

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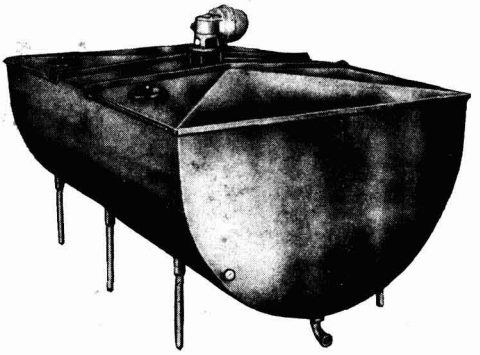

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