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Contents

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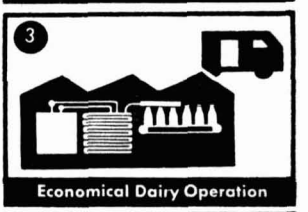

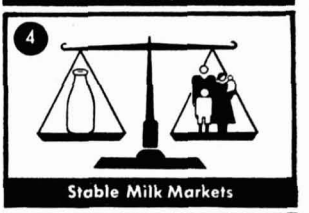

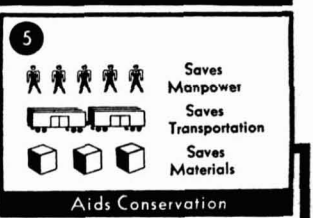

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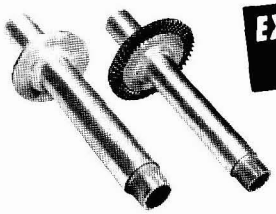

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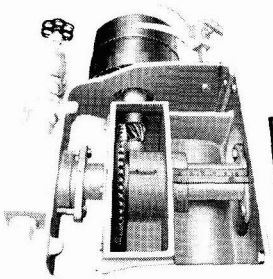

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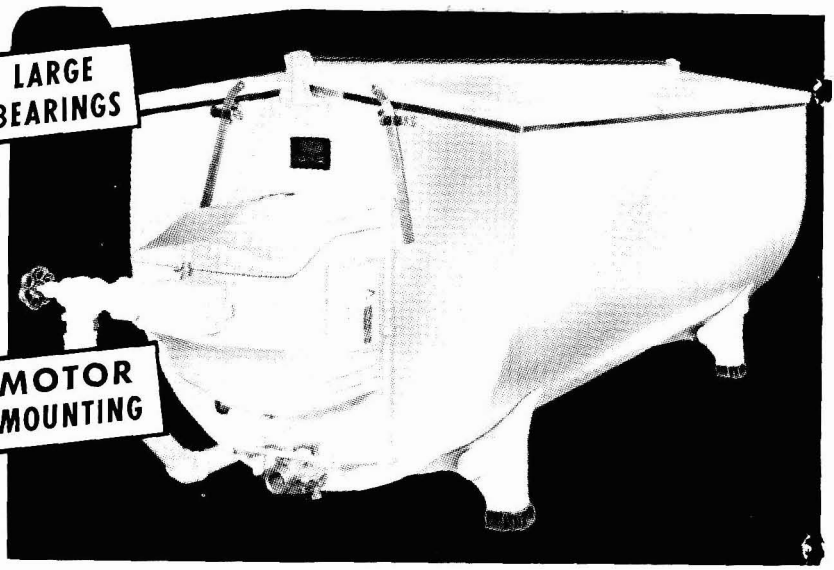

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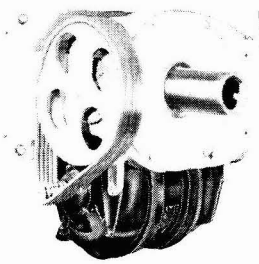

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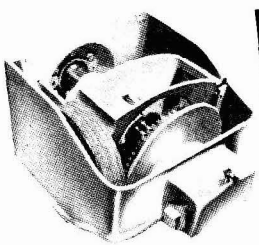

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THE LACTIC ACID FERMENTATION OF STREPTOCOCCI UNDER AEROBIC CONDITIONS

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From the works of Suzuki, Hastings and Hart (8) with *Streptococcus lactis,* Friedemann (3, 4) and Hewitt (6) with pathogenic streptococci, and Smith and Sherman (7) with representatives of various groups and species of the genus, it is known that the true streptococci are homofermentative in their metabolism of glucose, lactic acid usually making up around ninety per cent or more of the products yielded in the fermentation of the sugar. This is true in growing cultures or with so-called "resting cells," under strictly anaerobic conditions, or without anaerobic precautions. (This of course is not the case with the heterofermentative, carbon dioxide-producing streptococci, the *Streptococcus citrovorus* and *Streptococcus paracitrovorus* of Hammer, *Streptococcus kefir,* etc., which organisms are frequently classified as a separate genus, *Betacoccus* or *Leuconostoc.*)

Although the yield of lactic acid produced by the homofermentative streptococci is not modified under the conditions of ordinary "aerobic" culture, the situation under conditions of strong aeration is not clear. Thus, Hewitt and others have obtained similar results under aerobic and anaerobic conditions, but Friedemann (5) has shown, with the pneumococcus, that if the cultures are grown in a thin layer of medium and rotated to give highly aerobic conditions, the yield of lactic acid from the sugar consumed is only about forty-seven per cent. Davis and Rogers (1), using the resting-cell technique, have reported that the enterococci produce only about fifty per cent lactic acid from the sugar fermented under aerobic conditions, whereas *Streptococcus lactis* and some other streptococci are homofermentative under these conditions.

The present investigation was conducted in order to determine whether or not the metabolism of the streptococci is markedly influenced under strictly aerobic conditions, and, if so, whether the same is true of representatives of the different important groups of the genus.

METHODS

Before using for lactic acid production, the cultures were transferred at least three times in a medium containing, per liter:

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Glucose	1 gm.
Tryptose (Difco)	10 gm.
Meat infusion	500 ml.
Tomato juice	25 ml.
Water to volume	
pH 7.0	

After this preliminary culture, 1 ml. of an 8-hour culture was inoculated into a 300-ml. flask containing 100 ml. of a medium consisting of, per liter:

Glucose	5 gm.
Tryptose (Difco)	5 gm.
Yeast extract	2 gm.
Calcium carbonate	6.25 gm.
Water to volume	

The flasks for anaerobic fermentation were stoppered and placed in a 37° C. incubator for 48 hours. The flasks for aerobic fermentation were fitted with sterile aeration tubes and aerated vigorously for 48 hours either by a water pump or an air pressure system. The air was filtered through sterile cotton and was washed and saturated with water at the temperature of incubation (37° C.). The whole apparatus was assembled inside a small electric incubator kept at 37° C.

At the end of the incubation period the cultures were checked for purity, and the samples were adjusted to pH 1 with sulphuric acid to stop fermentation. The samples were diluted to a definite volume and stored in the cold until analyzed. Glucose was determined by the colorimetric method of Folin and Malmros (2). Lactic acid was determined by the short distillation method of Troy and Sharp (9).

RESULTS

In preliminary experiments in which the aeration was not vigorous and complete we found, as have others, that there may be no significant lowering of the percentage of lactic acid produced under such apparently "aerobic" conditions. However, under strictly aerobic conditions, although extremely variable results were obtained in the percentage of lactic acid produced from the glucose utilized in individual tests, the results (table 1) were consistent in showing a lower yield of lactic acid under strong aeration.

Most of the results for the individual streptococci in table 1 represent only one determination and the differences shown between species, especially under aerobic conditions, are not significant. With vigorous aeration growth and fermentation were very erratic. In some tests, the fermentations were feeble and the analytical results are doubtless not very significant, but are nevertheless adequate to show the substantial difference between aerobic and anaerobic conditions. In the case of the enterococci, the figures in table 1 represent the averages of a number of determinations; the entero-

cocci grew more vigorously under extreme aeration than did the other streptococci and gave more constant results.

Especial attention was given to *Streptococcus lactis* because of its practical significance and also because under ordinary conditions it is perhaps the most strictly "homofermentative" of all the streptococci, usually yielding from 95 to 99 per cent lactic acid from the glucose utilized. Four strains of this organism were used. Unless the aeration was very vigorous and complete, results were obtained comparable to those under anaerobic conditions with a yield of 95 per cent or more of lactic acid. On the other hand, with very complete aeration growth and fermentation were frequently too feeble to allow accurate determination of the lactic acid. With one culture, although three attempts were made, we did not succeed in getting sufficient fermentation for analytical purposes under conditions of extreme aeration. However, five satisfactory fermentations were obtained with the other three cultures under strictly aerobic conditions, with resulting yields of lactic acid ranging from only 11 to 63 per cent; the anaerobic controls in each case giving more than 95 per cent lactic acid from the glucose fermented. These results indicate rather definitely that the metabolism of this organism is actually modified under truly aerobic conditions. On the other hand, it is obvious that it is not likely that such conditions ever actually exist in those places where this organism grows under natural conditions, certainly not in milk or in milk products.

TABLE 1

Lactic acid production from glucose by streptococci (Per cent lactic acid per unit of glucose fermented)

Group or species	Aerobic	Anaerobic
Pyogenic streptococci		
Group A (S. pyogenes)	76.4	91.1
Group B (S. mastitidis)	72.2	95.3
Group C ("animal")	66.0	86.9
Group C ("human")	75.6	98.5
Group E	22.2	83.7
Group F	49.8	87.7
Group G ("minute")	28.6	86.2
Viridans streptococci		
Streptococcus salivarius	71.2	94.4
Streptococcus bovis	82.9	87.3
Lactic streptococci		
Streptococcus lactis	43.2	99.4
Enterococci (Group D)		
Streptococcus fecalis	76.0	93.8
Streptococcus liquefaciens	77.7	92.3
Streptococcus durans	78.8	91.7
Streptococcus zymogenes	76.2	92.3

SUMMARY

Under strictly aerobic conditions, obtained by vigorous and complete aeration, the percentage of lactic acid produced per unit of glucose

fermented by streptococci is substantially lower than under anaerobic conditions.

This appears to be true of strains representing the various important divisions of the streptococci and does not seem to be limited to any particular group or species.

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AN EVALUATION OF THE VISUAL MOLD TEST FOR CREAM*

F. E. NELSON, W. H. MARTIN, R. W. MORRISON AND W. J. CAULFIELD
Kansas Agricultural Experiment Station

For a number of years the Federal Food and Drug Administration has sought a method suitable for determining whether butter entering into interstate commerce has been manufactured from cream which has undergone extensive microbial deterioration. Since June, 1940, the Wildman microscopic method (14) for detection of mold fragments in butter has been used as a basis of condemnation of butter. Such butter is considered as "consisting in whole or in part of a filthy, putrid or decomposed substance." Studies by various investigators (1, 4, 11, 12, 13) have shown that some relationship exists between the mold content of butter and quality of cream used for its manufacture. Numerous investigators have shown that a relationship exists between the mold content of cream and some of the criteria of cream quality which have been in use for some time (1, 3, 4, 6, 9, 10, 11, 12, 13, 14, 15). The Parsons modification (9, 10) of the original Wildman methylene blue-borax visual method (14) has been employed extensively by the butter industry to determine by visual methods the amount of mold in cream, principally as it is delivered to the creamery or cream station by the producer. The merits of these methods for evaluating the quality of cream or butter by means of mold content depend upon the basic assumption that when conditions have been such as to permit the development of molds, other microorganisms also may have been able to develop. Although the molds are not important from the standpoint of production of undesirable flavors and aromas, the other microorganisms which develop under the same conditions may be responsible for pronounced and undesirable changes. Only if both the molds and the organisms responsible for undesirable changes develop simultaneously can the mold content be used as a satisfactory criterion of quality of cream or butter.

The studies herein reported were undertaken in an attempt to evaluate under Kansas conditions the visual mold test as a means of determining the quality of cream for buttermaking. The results of the methylene blue-borax visual mold test on cream have been compared with (a) the plate count of molds, (b) the plate count of yeasts, (c) the grade of the cream, (d) the titratable acidity of the cream and (e) the age of the cream. Also the yeast plate count has been compared with the grade of the cream and the mold plate count.

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* Contribution No. 215, Dept. of Bacteriology and Contribution No. 147, Dept. of Dairy Husbandry.

METHODS

All cream samples were obtained from deliveries made to two Manhattan, Kans., cream buyers. Most of the samples were from a group of 131 producers chosen upon the basis of diversity of production conditions, although a number were taken at random from other deliveries. Samples were obtained in late 1940 and early 1941 during periods of two or three weeks in (a) late September and early October, (b) mid-December, (c) February, (d) late April and very early May, (e) late May and (f) late June and very early July. During these collection periods weather conditions of great diversity were encountered. Samples for this study were taken at the time those for butterfat testing were obtained and were refrigerated until examined in the laboratory.

Mold determinations were made by the Parsons modification (9, 10) of the original Wildman methylene blue-borax method for cream (14). A

TABLE 1

Comparison of visual mold standards used in this study with those proposed by the American Butter Institute

Class or quality of cream	Standards used in this study	Corresponding American Butter Institute standards
Good	1	1
	2	.....
Fair	3	2
Doubtful	4	3
	5	4
Excessive	6	.....
	7	.....

motor-driven stirring device was employed to obtain uniform agitation. Seven mold score classes were used instead of the four standard classes of the American Butter Institute, making possible more accurate evaluation of the test. The relation of the mold score classes used in this study to the usual standards is shown in table 1. The yeast and mold plate counts were determined by the methods for butter outlined in Standard Methods for the Examination of Dairy Products (2). The titratable acidity was determined on a 9-gram sample diluted with 9 milliliters of distilled water, using tenth-normal alkali for titration, with phenolphthalein as the indicator.

Each sample of cream was graded independently by two or more experienced individuals and designated as first, second or third grade according to the definitions as given in the Kansas dairy law (7).

Statistical analysis of tables 2, 3, and 4 was made by the chi-square method. Since the number of degrees of freedom was large, the function

$$\sqrt{2\chi^2} - \sqrt{2n-1} = U$$

was used as the normal deviate with unit variance (5). Classes in the tables were combined in a few instances to minimize the presence of cells in which no samples were found. The critical value for 5 per cent probability was used as the criterion for determining whether the distributions obtained could be accounted for upon the basis of experimental variation.

RESULTS

The mold score by the visual method and the mold count by the plate method were compared on 1235 lots of cream to determine whether the visual method accurately measured the mold content of the cream samples examined. The results are shown in table 2.

The data indicate close agreement between the amount of mold as determined by the visual mold test and the mold count as determined by the plate

TABLE 2

Relationship of the visual mold score and the mold plate count

Mold plate count, thousands per ml.	No. of samples in each mold plate count class with a visual mold score of							Total
	1	2	3	4	5	6	7	
Less than 10	560	88	21	9	2	1	.....	681
10 to 29	41	41	24	8	.....	1	1	116
30 to 99	11	46	43	18	3	.....	.....	121
100 to 199	1	15	49	20	3	1	1	90
200 to 499	1	13	36	28	8	1	.....	87
500 to 999	.....	2	14	26	13	4	5	64
1000 to 1900	.....	.....	4	10	18	8	7	47
Over 2000	.....	2	2	6	6	7	6	29
Total	614	207	193	125	53	23	20	1235

method. Exact agreement was not obtained in all individual comparisons, the distribution being moderately wide in several classes, whether the class in question was based upon mold plate count or upon visual mold test.

The value of U far exceeded the critical value of 1.96, which is the value for 5 per cent probability that the distribution obtained was due to random sampling variation. This was true whether all of the table, all of the table except mold class 1, or all of the table except mold class 1 and the samples with yeast counts of less than 10,000 per ml. was used as the basis of calculation. The results furnish statistical evidence that a high degree of probability exists that the distribution obtained is due to a relationship between visual mold score and mold plate count. Udder infection and advanced lactation, as observed by Garrison and Gholson (5) in the case of gravity-separated cream, probably were not factors of importance in causing lack of complete agreement between the two methods of mold estimation, since nearly all of the cream had been obtained by centrifugal separation. Incomplete clumping of the mold hyphae, inclusion of material other than

mold on the disc and variations in the degree of fragmentation and conidia formation which result in varying numbers of mold colonies developing per unit of mold mass are some of the factors which may contribute to the relatively small number of cases of discrepancy between the results of the two methods. The visual mold test apparently is a satisfactory method of evaluating the mold content of cream. Although the results of the plate count may be subject to certain inaccuracies, the occasional case in which visual mold test and mold plate count do not agree would indicate that caution probably should be used in the interpretation of the results of the visual mold test.

If the visual mold score is to be used as a criterion of the development of organisms other than mold, a relationship should exist between the visual mold score and the numbers of other organisms such as yeasts in the cream. The visual mold scores and the yeast plate counts of 1241 lots of cream are compared in table 3.

TABLE 3

Relationships of visual mold score and yeast plate count

Yeast plate count, thousands per ml.	No. of samples in each yeast count class with visual mold score of							Total
	1	2	3	4	5	6	7	
Less than 10	339	62	53	24	9	6	5	498
10 to 29	89	31	17	13	8	3	2	163
30 to 99	71	34	31	22	10	2	2	172
100 to 199	31	15	12	14	5	4	2	83
200 to 490	32	28	24	16	4	2	3	109
500 to 990	11	16	23	15	5	3	1	74
1000 to 1900	17	13	24	14	6	4	3	81
2000 to 5000	14	9	8	5	7	1	0	44
Over 5000	5	2	5	3	1	0	1	17
Total No. of samples	609	210	197	126	55	25	19	1241

The value of U, using all portions of the table but combining visual mold classes 6 and 7 to avoid cells containing no samples, was 11.58, far in excess of the critical value of 1.96 for 5 per cent probability that the distribution obtained was due to sampling distribution, rather than to agreement between the two values whose relationship is being tested. However, this result is misleading, for the value of U drops to 0.37 when the samples in visual mold class 1 are not included in the calculations. This indicates that distribution of the large number of samples in visual mold class 1 has had a distorting effect upon the interpretation of the significance of the data in the remainder of the table. Treatment of the results on all samples except those having a yeast plate count of less than 10,000 per ml. gave a normal deviate of 4.40, indicating a distribution among these samples which cannot be attributed to sampling error. Even in visual mold class 1, which accounts for virtually all of the relationship between visual mold score and yeast plate count, 110

of the 609 samples had yeast plate counts in excess of 100,000 per ml. Thus, even where the relationship apparently is best, one sample out of six was found to contain a large number of yeasts despite its low mold content. Among the other samples of this table the relationship between visual mold content and yeast plate count was considerably less satisfactory.

The data presented in table 4 show that the relationship between mold plate counts and yeast plate counts was not appreciably different from that found when visual mold test results and yeast plate counts were compared.

The value of U for all samples, for all samples except those with mold plate counts of less than 10,000 per ml. and for those samples with yeast plate counts below 10,000 per ml. were 13.56, 1.28 and 5.11, respectively.

TABLE 4

Relationship of mold plate count to yeast plate count

Yeast count, thousands per ml.	No. of samples in each yeast count class with mold counts in thousands per ml. of									Total No. of samples
	Less than 10	10 to 29	30 to 99	100 to 190	200 to 490	500 to 990	1000 to 1900	2000 to 3000	Over 3000	
Less than 10	379	25	23	22	16	10	11	3	2	491
10 to 29	98	18	12	10	8	8	8	5	.....	167
30 to 99	77	21	21	14	15	9	4	3	2	166
100 to 190	35	14	13	7	5	7	2	.....	3	86
200 to 490	44	13	17	15	7	9	7	3	.....	115
500 to 990	17	10	4	10	13	9	6	1	1	71
1000 to 1900	18	9	13	9	18	3	10	2	1	83
2000 to 3000	14	3	3	.....	3	5	1	.....	.....	29
Over 3000 ...	10	.....	9	3	3	3	1	1	2	32
Total No. of samples	692	113	115	90	88	63	50	18	11	1240

Only the second of these values is indicative of a distribution which might be attributed to random sampling. This indicates again that any relationship between the two values in question (the mold content and the yeast content of cream) is attributable to the distribution of the large number of samples with low mold content, rather than to the distribution of the samples with higher mold content. The data indicate that when the mold content gets beyond minimal values its relationship to yeast content could be due to experimental variation. The absence of a close relationship between yeast plate count and either visual mold score or mold plate count except in the lowest range of mold content indicates that yeast and mold developments frequently are not parallel. The observation made in other studies that bacteria in cream reach a maximum population and then decrease in numbers while yeasts and molds continue to increase (8) is evidence that the numbers of molds cannot be expected to parallel the population of bacteria in cream which has been held for some time. Apparently the populations of bacteria, of yeasts and of molds are capable of considerable independent

variation, and the numbers of one type may be no criterion of the numbers of another type. Thus the visual mold test cannot be expected to serve at all times as an index of the microbiological population of cream. The test unquestionably has some value in this respect, since conditions favorable to the development of one group of micro-organisms frequently are favorable for the growth of others, but results of this type must be interpreted with caution because of the obvious opportunities for discrepancies.

Another possible means of evaluating the visual mold test is in terms of the relationship between mold content and amount of change caused in the cream by development of microorganisms. The data indicating the degree of relationship between visual mold score and cream grade are presented in table 5. Much larger numbers of the samples of first grade cream than of second grade cream were placed in class one on the basis of visual mold score. Much larger numbers of second than of first grade cream deliveries are found in visual mold classes three or poorer. These data indi-

TABLE 5

Relationship of visual mold score and organoleptically determined grade

Grade of cream	No. of deliveries in each cream grade with a mold score of							Total deliveries
	1	2	3	4	5	6	7	
1	488	122	100	59	12	0	4	785
2	186	95	122	99	45	27	18	592
Total samples	674	217	222	158	57	27	22	1377

cate a tendency for first grade cream to contain less mold than does second grade cream. However, this trend is misleading in some respect. For example, of the 217 deliveries of cream having a visual mold score of two, only 122 were first grade on the basis of organoleptic examination. A somewhat similar division occurs among samples with a visual mold score of 3. Of the 158 deliveries with visual mold score of four, 59 were classed as first grade, although extensive development of mold had occurred. The relationship between grade and mold score is somewhat better in visual mold score classes 5, 6 and 7 than in cream samples containing less mold. Although some relationship between visual mold score and organoleptically determined grade was apparent, the inability of the visual mold test to separate cream samples according to grade with any degree of accuracy, especially in visual mold classes 2, 3, and 4, detracts from its general applicability as an aid in the grading of cream.

Data presented in table 6 indicate that the yeast plate count is no more closely related to organoleptic grade than is the mold content of the cream. The lack of close relationship between either yeast or mold count and organoleptic grade of cream indicates that the factors which control mold and yeast

TABLE 6

Relationship of yeast plate count to organoleptically determined grade of cream

Grade of cream	No. of samples in each grade class with yeast count in thousands per ml. of									Total No. of samples
	Less than 10	10 to 29	30 to 99	100 to 190	200 to 490	500 to 990	1000 to 1900	2000 to 3000	Over 3000	
1	347	100	96	51	58	27	32	10	7	728
2	142	68	72	34	51	47	47	21	25	507
Total No. of samples	489	168	168	85	109	74	79	31	32	1235

count frequently are not predominantly responsible for determining the grade of cream for butter-making purposes.

The data showing the degree of relationship between visual mold score and titratable acidity (calculated as per cent lactic acid) on 1369 deliveries of cream are summarized in table 7. More than half of all deliveries were in the 0.50 to 0.69 per cent titratable acidity class in which no relataionship between mold score and titratable acidity was apparent. In the 0.20 to 0.49 per cent acidity class, the percentage of the samples that fell in this acidity range decreased regularly as the mold score increased from one to six, indicating that some relationship between the results of these two criteria of cream quality existed in this range. In passing from mold score 1 to 7 the percentage of deliveries in each mold class that fell in the acidity range of 0.70 to 0.89 per cent increased quite regularly. The results in the 0.90 to 1.09 per cent and the 1.10 and more per cent acidity ranges were much less regular, undoubtedly because of the comparatively small number of samples with acidities within those ranges. The data show some relation-

TABLE 7

Relationship of titratable acidity of cream to visual mold score

Visual mold score	No. and per cent of deliveries in each mold class with a titratable acidity (per cent lactic acid) of												Total
	Less than 0.20		0.20 to 0.49		0.50 to 0.69		0.70 to 0.89		0.90 to 1.09		1.10 or more		
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	
1	10	1.5	299	44.3	343	50.8	19	2.8	4	0.6	.....	.....	675
2	.....	.....	52	23.0	147	65.1	19	8.4	5	2.2	3	1.3	226
3	.....	.....	33	15.2	138	63.6	39	18.0	3	1.4	4	1.8	217
4	.....	.....	15	10.1	95	64.1	33	22.3	4	2.7	1	0.7	148
5	.....	.....	3	5.1	40	67.8	15	22.4	1	1.7	.....	.....	59
6	.....	.....	1	4.3	13	56.5	5	21.7	4	17.4	.....	.....	23
7	.....	.....	2	9.5	6	28.5	8	38.1	4	19.0	1	4.8	21
Deliveries in each acidity class	10	.....	405	.....	782	.....	138	.....	25	.....	9	.....	1369

ship between visual mold score and titratable acidity. However, so much overlapping of classes was found that any one cream sample could not be classified with any degree of certainty with respect to acidity as the result of mold test score.

The data relative to the relationship of age of cream to visual mold score are presented in table 8. As the age of the cream at delivery increased the percentage of deliveries with a mold score of 1 progressively decreased. The percentage of samples in mold score group 2 seemed relatively constant for all age groups. The percentage of deliveries with mold score of 3 was low on cream one day old, but the percentage having this mold score increased somewhat on the second day and reached essentially a constant level on the third day. The percentage of each age group in mold class 4 increased from

TABLE 8

Relationship of visual mold score to age of cream

Age at delivery (days)	Per cent of deliveries in each age class with a visual mold score of							Deliveries in each age group
	1	2	3	4	5	6	7	
1	73.8	19.0	4.8	2.4	.....	.....	.....	42
2	66.7	17.9	9.6	5.8	.....	.....	.....	156
3	53.2	20.0	16.6	6.4	1.7	1.7	0.4	235
4	56.9	15.6	16.0	9.6	1.4	.....	0.5	218
5	50.8	14.8	18.8	10.9	3.1	0.8	0.8	128
6	44.8	19.4	11.9	13.4	3.0	3.0	4.5	67
7	40.2	13.1	17.8	15.2	7.9	3.0	2.8	433
8	34.4	34.4	15.6	15.6	.....	.....	.....	32
9	27.8	11.1	22.2	11.1	16.7	11.1	.....	18
10	22.2	11.1	22.2	27.8	.....	11.1	5.6	18
Over 10	17.4	17.4	17.4	17.4	17.4	4.3	8.7	23
Deliveries in each mold score group	677	225	217	151	54	25	21	1370

a low level of 2.4 on deliveries one day old to 15.2 on the seventh day. No deliveries with mold scores of five or above were encountered when the cream was one or two days old, but as the age increased up to seven days, an increasing percentage of high mold scores was found. Among the deliveries more than seven days old, the numbers of deliveries with mold score of five or above were so small that percentage figures probably are without significance. The data indicate that age of the cream is an important factor in determining the mold content, but other factors also are operative and keep the relationship from being as close as might be desired.

DISCUSSION

The lack of close relationship between visual mold score and several of the other criteria of cream quality should not be taken to indicate that the test has no value as a method for grading cream. The results merely

emphasize the complexity of the term "quality" as applied to cream and serve to indicate the futility of applying but one criterion in the estimation of cream quality. Microbial changes are but one of the aspects of the problem, and each different type of microbial change has its own significance. To separate one microbial type from the several which may influence cream quality and say that the one chosen is the key to the grading situation probably is not possible. The visual mold test does have a place in the grading of cream as an additional criterion which may be used to best advantage on samples of questionable quality. If the cream has been held under such conditions that mold has developed, more undesirable microorganisms may also have developed, and the cream is at least potentially undesirable. On the other hand, if mold has not developed, that does not necessarily mean that undesirable changes have not occurred.

The visual mold test probably is at its best when used in the cream station on questionable and poor deliveries of cream where some tangible evidence, understood by the producer, is desired as a basis for rejection or warning. The psychological value of the mold disc unquestionably is as great as are the abilities of the method to segregate microbiologically poor cream. Employment as a demonstration method apparently is the most valuable use of the test.

SUMMARY AND CONCLUSIONS

A total of 1380 samples of cream were collected from two Kansas cream stations and examined for visual mold score, titratable acidity and organoleptic grade. Mold and yeast plate counts were determined on the majority of the samples. The results of the visual mold test were compared with results of other criteria of cream quality.

A fairly close relationship existed between visual mold score and mold plate count, indicating that the visual mold test usually is a good index of the mold content of a cream sample. Visual mold score and organoleptic grade were related to some degree, but numerous exceptions to the general relationship were encountered. A tendency for low mold content to be accompanied by low yeast count was observed, but for many of the samples no close relationship between mold content and yeast count could be discerned. This would indicate that the development of one type of microorganism is not necessarily accompanied by a parallel development of other types. Visual mold score and titratable acidity were related in that low acidities frequently were accompanied by low mold scores and high acidities by high mold scores; but the majority of the samples were in the 0.50 to 0.69 per cent titratable acidity range in which acidity and mold score showed no apparent relationship. Some relationship between visual mold score and age of the cream was apparent.

The field of applicability of the visual mold test seems to be that of an additional criterion of cream quality, a test which can be made easily at

the cream station or at the creamery receiving dock and which has value under some circumstances in showing the producer in terms he can understand that his cream is unsatisfactory. As a definite criterion of microbial deterioration in Kansas cream the test leaves much to be desired.

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THE LIPOLYTIC ACTIVITY OF BOVINE MAMMARY GLAND TISSUE*

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It seems generally accepted that the neutral fat of the blood is the most important precursor of milk fat. It is little known how it is transported, or the changes which take place from the time it leaves the circulatory system till it emerges as a synthesized fat.

A previous study, Kelly and Petersen (3), indicated the presence of significant amounts of free fatty acid in the secretory tissue of the bovine mammary gland which had been developed by pregnancy. Since this might indicate the presence of an active lipase, the work to be reported is concerned with evidence regarding the presence or absence of lipase in the mammary gland in various stages of development together with any possible function which it might have in milk fat synthesis.

A review of the literature shows meager and conflicting evidence for an active lipase in mammary gland tissue, though there is ample evidence of the presence of small amounts of lipase in milk. The literature on the lipase content of milk has recently been reviewed by Roahen and Sommer (8). Grimmer (2) reports evidence for a monobutyrinase in glycerine extracts of the secretory tissue of bovine mammary glands although the activity he obtained per gram of tissue extracted was extremely small. Bradley (1) obtained such a small amount of activity from similar analyses he concluded there was no significant lipase activity from this material. He did, however, obtain more lipolytic activity from mammary tissue than from blood. In contrast Loevenhart (7), using an aqueous extract of the mammary glands of a dog, obtained large amounts of lipase activity from which he concluded that mammary gland tissue is one of the most important sources of this enzyme. Species differences may have accounted for the differing results obtained in his experiments from those of other workers. Virtanen (10), again using a glycerine extract of bovine mammary gland tissue, obtained some evidence of lipase activity although he, like Grimmer, raised the question regarding the original source of the lipase which is present. He points out the possibility of the small amount of activity being due to blood lipase since blood is always present in the samples which are analyzed. His extracts, like those of Grimmer, were very weak in activity. Kleiner and Tauber (6) extracted bovine mammary gland tissue for four weeks with

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glycerine, but failed to obtain any lipolytic activity from their extracts. Kelly (4) in a preliminary paper reported positive evidence of lipase activity from three bovine mammary glands when ethyl-butyrate was used as the substrate and toluene was used as the preservative.

Lipolytic Activity of Mammary Gland Tissue

METHODS

Bovine mammary glands in different stages of activity and development were obtained from a local slaughter-house for use in this study.

When fresh tissue was used it was either ground as finely as possible in a meat grinder and used immediately or frozen and kept in that condition till needed. Definite weights of the tissue were used. Either an ethyl-ester or a triglyceride was used as the substrate. Four preservatives, toluene, sucrose, glycerine, and formalin were used in the trials. When toluene was used, it was added in sufficient amounts to cover the tissue. The sucrose was used as a 60 per cent aqueous solution, but proved unsatisfactory both because of high bacteria counts and because of difficulties in extraction. When glycerine was used, it was mixed with an equal volume of water and added to the tissue at the rate of five parts of the solution to one part of tissue. The formalin was used in concentrations of 0.5, 1.0, and 1.5 per cent aqueous solution in the same proportions to the tissue as when glycerine was used. Since the higher concentrations of this preservative did not cause any noticeable effect on lipase activity, all of the later experiments were carried out with the 1.5 per cent solution. The controls were treated in a similar manner and heated to over 80° C. in a water bath to kill enzyme action. The samples were incubated at 37° C. Plate counts were made at the end of the experimental period whenever there was any question regarding the effectiveness of the preservative. At the end of the incubation period the samples were dried with five times their weight of plaster of Paris and extracted in a continuous extractor with ethyl ether. Ethyl alcohol was added and the extract was titrated against an aqueous solution of sodium hydroxide using phenolphthalein as the indicator.

In some of the work reported, dried, defatted tissue was used. The tissue was ground in the usual manner and dried by a modified method similar to that of Willstatter and Waldschmidt-Leitz (11). Five pounds of the tissue were extracted at room temperature with 3-liter portions of acetone, followed by an equal volume of acetone-ether, and then by two portions of ether. After drying at room temperature, this material was ground as finely as possible in a coffee mill, extracted two or more times with ether till there was no trace of color in the extract and stored at a temperature just above freezing. With the dried tissue from Glands 113, 116, and 117, the final grinding was done in a Wiley mill to make for better sampling.

RESULTS

Twenty-nine bovine mammary glands in various stages of development were used in this group of studies. The data on three of these are given in a preliminary report, Kelly (4). Because of differences in the techniques used and the objectives of the studies carried out, the data on only 19 glands

TABLE 1

Relation of the stage of development of the gland to the lipolytic activity of its secretory tissue

Gland number	Preservative	Incubated	Tissue used per sample	Number samples used	Titration*	Condition of gland
		days	*gm.*		*ml. n/10 NaOH*	
Original, undried tissue						
10	Formalin	26	14.0	1	1.95	Not developed by pregnancy
11	Formalin	22	20.0	1	0.16	Not developed by pregnancy
100	Formalin	5	20.0	1	0.34	Not developed by pregnancy
101	Formalin	5	20.0	1	0.00	Not developed by pregnancy
7	Toluene	5	20.0	11	16.71	Milked out several pounds just previous to slaughter
117	Formalin	7	4.0	1	6.72	Large, in active secretion
6	Toluene	5	20.0	7	16.12	Small, dry
8	Toluene	12	15.0	3	11.00	At end of lactation
	Glycerine	12	15.0	3	15.61	
	Formalin	12	15.0	3	24.38	
104	Formalin	20	4.0	2	5.65	Dry
110	Formalin	15	4.0	3	7.26	Dry
112	Formalin	6	10.0	2	6.65	Dry
Dried, defatted tissue						
102	Formalin	19	2.0	4	16.67	Large, active secretion
103	Formalin	19	2.0	4	14.23	Large, non-lactating
110	Formalin	19	1.4	3	14.08	Dry one month
111	Formalin	21	1.0	1	3.66	Small, dry
113	Formalin	11	1.0	3	1.82	Small, dry
114	Formalin	17	1.0	5	0.31	From an old Shorthorn cow, very small, contained very high percentage of adipose tissue
115	Formalin	8	1.0	6	2.29	Small, dry
116	Formalin	8	1.0	5	3.30	In active lactation
117	Formalin	2	1.0	5	0.81	Large, in active secretion

* Titrations calculated as increases above the blank analyses.

When the original, undried tissue was used, the substrates were mammary gland fat and tributyrin. With the dried, defatted tissue, tributyrin was the substrate.

are presented here. However, with the exception of the four reported in table 1 which had not been developed by pregnancy, every gland had essentially the same lipase activity. Since the glands studied were obtained from a local slaughterhouse, their lactation histories were unknown except for the observations which could be made at the time of slaughter. In the data

shown in table 1 tributyrin and the tissue fat present were the substrates for the gland tissue which had not been dried and defatted. In the data for the extracted tissue tributyrin only was the substrate.

A study of the data in table 1 shows that the tissue from glands undeveloped by pregnancy contained only a trace of lipolytic activity. Since the large amount of adipose tissue chacteristic of such glands might be the cause of these low results, a portion of Gland 101 was dried with acetone and ether. A five-gram sample still gave no hydrolysis of tributyrin after a 15-day incubation period. While the data are not strictly comparable because of differences in incubation periods and different preservatives used, the actively secreting glands and the non-lactating glands both show sig-

TABLE 2

A comparison of the lipolytic activity of the original tissue with that dried and extracted with acetone and ether

Gland number	Incubated	Titration per gm. tissue		Titration dried tissue calculated per gram on original tissue*
		Original	Dried	
	days	*ml. n/10 NaOH*	*ml. n/10 NaOH*	*ml. n/10 NaOH*
102	21	1.28		
	19		8.34	1.59
103	19	1.10		
	19		7.12	1.36
110	21	1.08		
	19		9.84	1.87
111	21	0.74		
	21		3.66	0.69
117	2	0.28†		
	2		0.81	0.15

* Calculated as 1 gm. dried extracted tissue equivalent to 5.25 gms. original tissue.
† Calculated as $\frac{2}{3}$ of the 3-day analyses which had an average of 0.42.
When the original tissue was used, the substrates were mammary gland fat and tributyrin. With the dried, defatted tissue tributyrin was the substrate.

nificant amounts of lipolytic activity. The number analyzed, however, is too small to make an accurate comparison between the two conditions.

The samples which were dried and extracted with acetone and ether represent approximately 18 to 20 per cent of their original weight. Material treated in this manner proved much more satisfactory for several reasons. More uniform samples could be obtained which together with its greater activity allowed the use of much smaller samples. The dried tissue could be stored in a cool room in a tightly stoppered bottle for several months without noticeable deterioration. These samples have the additional advantage that when pure substrates are used the results are not complicated by the hydrolysis of the gland fat which would always take place with the samples which had not been defatted. In order to compare their lipolytic activity experiments were carried out with both undried and the dried de-

fatted tissue from the same glands. Table 2 shows the results of these comparisons calculated on the basis of a gram of tissue used and also on the basis of a gram of the original undried tissue. The results shown represent the average of several individual analyses.

The data in table 2 show that while there is some variation, partly due to the tissue fat serving as an additional substrate in the unextracted samples, the extracted samples have retained a very high percentage of their original lipolytic activity.

Due to some definitely varying results in the trials with Gland 8 some data were obtained on the effects of various preservatives on lipolytic action. Data on the use of preservatives are shown in tables 3 and 4. The method used in obtaining the data presented in table 3 comparing the use of formalin and glycerine is slightly different than that carried out in the other trials except for Gland 8. In the others, the 20-gram samples of the unex-

TABLE 3

The effect of glycerol and formalin when used as the preservative on lipolytic activity when tributyrin is the substrate

Gland number	Incubated	Tissue used	Glycerine	Formalin
	days	*gm.*	*ml. n/10 NaOH*	*ml. n/10 NaOH*
8	12	15	15.61	24.38
102	43	20	53.66	57.96
103	41	20	75.50	65.63
104	37	20	33.24	56.54
105	44	20	49.14	68.09
110	49	20	59.56	57.34

tracted tissue were mixed with either 50 ml. of the glycerine-water solution or 100 ml. of a 1.5 per cent aqueous formalin solution. One and five-tenths ml. of tributyrin was added to each. At intervals 20-ml. aliquots were extracted in the usual manner and at the end of the experimental period the remaining tissue and liquid were also extracted. Similar samples which had been heated were used as blanks. The total of these titrations of aliquots plus the final titrations minus the blank titrations are reported. Bacterial counts as well as lipolytic bacterial counts were carried out, but since only one count in the unheated samples was over 300 per ml. at the end of the experimental period, it is assumed the preservatives were used in sufficiently large concentrations to be effective.

Since there seemed to be no significant difference between the effects of glycerine and formalin, the formalin was used with the short chain fatty acid substrates because it was much easier to use in carrying out the fat extractions.

Table 4 shows a comparison between the use of formalin and toluene as a preservative in which the various substrates are used. In this table the calculated weights of the various acids hydrolized are also given.

TABLE 4

Lipolytic activity of mammary gland tissue on various substrates with toluene or formalin as preservatives

Gland No.	Incubated	No. samples	Preservative	Substrate	Size of sample	Type of tissue	Ave. increase in titration over blank	Calculated	
								As acid	Wt.
	days				*gm.*		*ml. n/10 NaOH*		*gm.*
12	10	1	Toluene	Ethyl-butyrate	20	Original undried	7.32	Butyric	0.064
	10	1	Toluene	Ethyl-oleate	20	Original undried	4.02	Oleic	0.113
113	10–14	15	Formalin	Olive oil	1	Dried	0.30	Oleic	0.008
	10–14	11	Formalin	Tributyrin	1	Dried	1.82	Butyric	0.016
115	8–9	6	Formalin	Ethyl-oleate	1	Dried	0.26	Oleic	0.007
	8–9	9	Formalin	Tributyrin	1	Dried	2.29	Butyric	0.020
116	9–11	4	Toluene	Tripalmitin	1	Dried	0.47	Palmitic	0.012
	9–11	10	Toluene	Tributyrin	1	Dried	0.73*	Butyric	0.006
117	3	4	Toluene	Tributyrin + gland fat	4	Original undried	0.98	Butyric	0.009
	3	4	Toluene	Tricaproin + gland fat	4	Original undried	0.59	Caproic	0.068
	3	4	Toluene	Tripalmitin + gland fat	4	Original undried	0.43	Palmitic	0.011
	3	6	Formalin	Mammary gland fat	4	Original undried	0.65	Oleic	0.018
	3	4	Formalin	Tributyrin + gland fat	4	Original undried	1.26	Butyric oleic	0.024

* In Table 1 where formalin was used as the preservative an 8-day incubation period with one-gram samples of tissue from Gland No. 116 showed an average increase in titration of 3.3 ml. over the blank analyses.

The data show a considerable difference in the effectiveness of the two preservatives depending in a large measure on the type of substrate used. When the shorter chain fats are used in the trials, the aqueous formalin solutions show the greatest activity. When the longer chain fats were used which show greater insolubility in water, the toluene samples show the greater weight of fatty acid hydrolysis.

Most of the experiments reported in the literature use tributyrin or ethyl-butyrate as the substrate. This is due to less difficulty with solubility as well as greater titration for a given weight of fat hydrolized. If the lipase was only active on a type of substrate which did not occur commonly in milk fat it could not be important in milk fat synthesis. These data were obtained to learn the effects of the lipase being studied on the fats normally found in milk fat. The data show this lipase to have as great an effect when the longer chain fats are used as when the short chain fats are used based on the weight of fatty acids hydrolized.

TABLE 5

A comparison of two fractions of the dried tissue from gland number 113

Incubated	Activity per gram fine dried tissue*	Activity per gram coarse dried tissue†
days	*ml. n/10 NaOH*	*ml. n/10 NaOH*
0	0.02	0.02
5	1.63	2.31
	2.07	1.87
7	1.60	2.06
8	2.32	.....
9	1.66	2.07
	.....	1.89
	.....	1.79
10	1.87	.....
11	1.49	1.63
	1.64	.....
	2.34	.....
12	1.59	2.22
	.....	2.07
Average	1.82	1.99

* Fine tissue refers to that portion which passed through a 1 mm. mesh sieve.
† Coarse tissue to that which was too large to pass through the sieve openings.

When the dried tissue was ground in the coffee mill, a considerable difference was noticed in the material. Some of the tissue was present in the form of fine particles while some of it contained a considerable amount of connective tissue. It was thought possible that by separating these through a 1-mm. sieve the finer particles might consist largely of alveoli while the coarser portions would consist more of the connective tissue normally surrounding the alveoli and the blood capillaries. Table 5 shows the results of a study where this was tried in which no significant differences were noted though the data did show slightly higher titrations when the coarser material was used.

With present methods, it is never possible to completely separate the blood from the mammary gland tissue used in these trials. Since the blood might be the source of the lipolytic activity as is suggested by Grimmer (2) and Virtanen (10), some analyses were made to compare the activity of cow's blood under similar conditions of analyses. The data in table 6 show the results with blood serum taken from 5 cows in active secretion compared to typical analyses of tissue from mammary glands analyzed. Formalin was used as the preservative except for Glands 6 and 7 when toluene was used. Undried tissue was used in this trial.

While these data show some very interesting variations in the lipase content of cow's blood, they also show there was greater lipase activity in the gland tissue than in the blood serum of the five cows analyzed. Even if

TABLE 6

A comparison of the lipolytic action of similar amounts of blood serum from cows in active lactation and of mammary gland tissue

	Incubated	Increase in titration over blank for one ml. of serum or one gm. of undried tissue
	days	*ml. n/10 NaOH*
Number of blood sample		
1	14	0.44
2	14	0.54
3	14	0.28
4	14	0.84
5	14	0.25
Number of mammary gland		
8	15	1.45
112	16	0.89
110	14	1.08
6	5	0.80
7	5	0.83

the blood lipase had shown equal activity, the tissue would have demonstrated some activity since only a small portion of the tissue sample would have been composed of blood serum.

The curves for lipolytic activity over a period of several days show a great deal of difference between the tissue of the various glands. Figure 1 illustrates some of these data together with a description of the stage of development of the glands used.

In the data shown in the tables only two groups of figures have been used, the blank analyses at the start of the trials and those taken on a given day or a few days after the incubation was started. Actually the entire picture is not shown by any two comparative periods because of differences in the speed of hydrolysis over a period of days. The reason for these differences is not known. The data in figure 1 show curves obtained from tissue which had been ground without further processing. Figure 2 shows titrations obtained from tissue which had been dried and defatted before use.

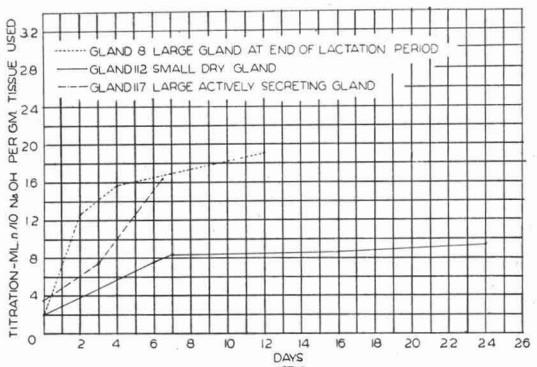

FIG. 1. Activity of hydrolysis of original mammary gland tissue. Tributyrin was used as the substrate. Formalin was the preservative.

The condition of the gland is described in each instance. There is a great deal of difference in the speed of hydrolysis when tissue of different glands is used. With Gland 111 there was a five-day period before the effects of the hydrolysis are shown. With Gland 116 there was an increase followed by a decrease in titration before equilibrium was reached. All of the others show a fairly rapid hydrolysis during the first 4 to 6 days followed by more gradual increases in titration.

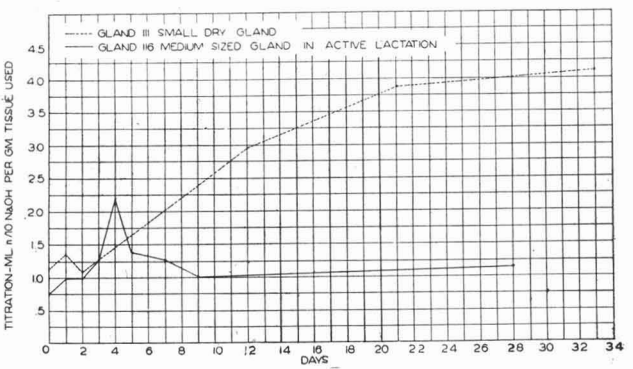

FIG. 2. Activity of hydrolysis of dried, defatted mammary gland tissue. Tributyrin was the substrate. Formalin was the preservative.

TISSUE RELATIONSHIP TO HYDROGEN ION CONCENTRATION

If under *in vivo* conditions, a hydrolizing lipolytic enzyme were active there would be some free fatty acid content in the secretory areas. This might bring about changes in the pH which could be detected. In previous work, Kelly and Petersen (3), it was noted that when the stain, Neutral Red, was used as a histological stain, the secretory tissue always showed a red

color. Since this stain is an indicator which turns red at a pH of 6.8, there was a possibility that the secretory portions of the gland were more acid than the remaining cells. Other compounds might cause this reaction, but it is logical to assume that if the free fatty acid were present in any significant amounts they would cause a sufficient change to be detected. A preliminary paper, Kelly (5), has been presented on this portion of the study.

METHODS

Two methods were used in these trials. The first was to use a glass electrode with a calomel half cell on slices of tissue excised from the gland as soon as possible after slaughter. The second was to use colorimetric indicators as stains on slices of tissue which had been cut on a freezing microtome. These were examined with a microscope. Several stains were used with the tissue of each gland, both because more significant results would be obtained and because a more accurate idea of the actual pH's studied could be shown. The two methods of study were used to learn if essentially the same results would be obtained.

TABLE 7

The pH of mammary gland tissue as measured with a glass electrode

Gland No.	Interval between slaughter and time of reading	Glass electrode readings	Condition of gland	Remarks
	min.	*pH*		
1	45	5.85 to 6.45	Contained slight amount of milk	
2	45	5.98 to 6.18	Dry	
3	45	5.78 to 6.59	Dry	
4	45	6.40 to 6.89	Dry	
5	45	6.07 to 6.75	Dry	
6	45	6.02 to 6.23	Dry	
7	7	6.18 to 6.20	Lactating	Connective tissue showed reading of 7.08
8	60	5.85 to 6.47	Lactating	Gland at end of lactation period
9	60	6.05 to 6.80	Dry	
10	10	6.90 to 7.10	Not developed by pregnancy	
11	10	6.35 to 6.45	From about a 3-yr.-old heifer never pregnant	Adipose layer gave a reading of 7.0 to 7.1
12	10	6.65	Not developed by pregnancy	Adipose layer gave a reading of 7.2
13	10	6.80	Not developed by pregnancy	Adipose layer gave a reading of 7.2

RESULTS

Thirteen glands were studied with the glass electrode method and the data are presented in table 7. Eleven glands were studied with colorimetric indicators with the results of this study shown in table 8.

TABLE 8

The colorimetric study of the pH of mammary gland tissue

Gland No.	Interval between slaughter and reading	Condition of gland	Histochemical analyses					Estimated pH of ST* tissue
			Bogan's Universal	Brom-Cresyl Green	Brom-Phenol Blue	Phenol-Red	Brom-Cresyl Purple	
			Colorimetric changes with pH					
			Orange 4.0 Yellow 6.0	Yellow 3.8 Blue 5.4	Yellow 3.0 Blue 4.6	Yellow 6.8 Red 8.4	Yellow 5.2 Purple 6.8	
	hrs.							
3– 1	4½	Lactating	ST* orange-yellow	CT† blue ST slightly yellow			CT purple ST yellow	4.0
4– 5	1¼	Lactating	ST orange-yellow	CT blue ST definitely yellow	CT blue ST slightly yellow			3.8
3–30	4	Lactation small gland at end of lactation	ST orange-yellow	CT blue ST slight tinge of yellow				4 plus
4–19	4	Lactation		CT blue ST definitely yellow	CT blue ST definitely yellow	No differentiation	CT blue ST yellow definite differentiation	3.0–4.0
4–19 1–10	4	Lactation	ST orange-yellow	CT blue ST definitely yellow	CT blue ST slightly yellow	CT yellow	CT purple ST yellow	3.5
4–10	3½	Dry	ST orange-yellow	CT blue ST not stained	Very slight differentiation	CT red ST yellow		4.5
4–10 2– 6	3½	Dry	ST yellow	CT blue ST not colored		CT red ST yellow		4.5–5.0
4–10 3– 7	3½	Dry	ST yellow with slight orange	No differentiation	CT blue ST blue			5.5–6.0
4–10 4– 8	3½	Dry	ST orange-yellow	No differentiation	CT blue ST blue			5.0–5.5
4–10 5– 9	3½	Dry	ST yellow-orange	No differentiation	CT blue ST blue			5.0–5.5
3– 1 1– 1	4½	Dry	ST orange-yellow	CT blue ST blue			Slight differentiation	5.2–5.5

* ST refers to secretory cell areas.
† CT refers to connective tissue cell areas.

In table 7, the results usually show some variations between readings. This seems quite logical because several slices of tissue were taken from various parts of the gland in making the readings and might, therefore, contain different amounts of alveoli and perhaps different degrees of activity. The data show a considerable amount of variation between glands. No differences were detected between the glands which are listed as dry and those which are listed as milking perhaps because no very active glands were obtained. It will be noted, however, that there was a difference between those which had been developed by pregnancy and those which had not, with the undeveloped glands much nearer to neutrality. If the lipase activity were the cause or the partial cause for this change in pH this would be as expected since the lipase apparently becomes active with pregnancy.

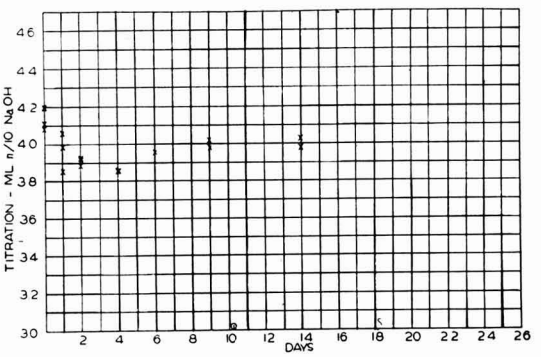

FIG. 3. A series of experiments with gland tissue from Gland No. 116 in which the dried, defatted gland tissue was added to a solution of toluene and oleic acid and placed on a shaker in an incubator at 37° C. Five-ml. aliquots were titrated at stated intervals.

① Ten drops of glycerol added on tenth day.

A study of the results, table 8, show a significant difference in pH between the secretory cells and the connective tissue cells. The connective tissue cells were at approximate neutrality with the secretory cells more acid. This was especially true for the glands in active secretion which were very definitely more acid than those in the non-lactating glands studied.

FAT SYNTHESIS BY LIPOLYTIC ENZYMES

If enzyme synthesis is an important process in the transport and formation of fat by the secretory cells it would be necessary to have some form of resynthesis in order to recombine the free fatty acids hydrolized. Virtanen (10) was able to obtain some recombination of free fatty acids by means of his glycerol extracts.

METHODS

To the samples composed of the dried tissue and toluene, one of several substrates were added. Butyric, oleic, and palmitic acids were used as the

substrates. Glycerine was sometimes added to note the effect its addition would have on the speed of the reaction. In some of these trials ethyl-alcohol was also added to the samples, but since the results were all negative these data are not shown. In studies in which hydrolysis was attempted it was found that this either completely inhibited or seriously retarded lipolytic activity.

RESULTS

Three separate series of analyses are reported for Gland 116. Data for the first are shown in figure 2, where the titration of the one-gm. samples of

TABLE 9

The recombination of free fatty acid by enzymes of mammary gland tissue

Gland No.	Incubated	Substrate	Decrease in titration*		Procedure
			Ave. titration at start	Ave. final titration	
	hrs.	*acid*	*ml. n/10 NaOH*	*ml. n/10 NaOH*	
116	216	Oleic	6.56	5.87	1 gm. samples dried, defatted tissue added to 10 drops glycerol plus 5 ml. solution Oleic acid in Toluene. Dried with $CaSO_4$, extracted, alcohol added to the extract and titrated.
103	216	Oleic	6.81	6.09	" "
113	216	Oleic	5.90	6.25	" "
117	22	Butyric	2.06	1.98	25 gm. original, undried tissue plus 100 ml. toluene and butyric acid. Five ml. aliquots to which alcohol added used for titration.
117	48	Butyric	1.99	1.66	1 gm. of dried, defatted tissue added to 10 ml. of solution of toluene plus substrate. Two ml. aliquots taken for titration.

* When the tissue samples were heated these changes in titration did not occur.

dried tissue showed increases in titration for a time and then a decrease in titration indicating that a recombination of the free fatty acids formed was taking place. The data for the second series are shown in figure 3 where a 10-gm. sample of the dried tissue was added to 50 ml. of a toluene and ethyl-oleate solution to which 1.5 ml. of formalin was added. This was placed in the shaker and incubated at 37 degrees C. At intervals 5-ml. aliquots were taken, ethyl alcohol was added, and titrations were made with sodium hydroxide. Ten drops of glycerol were added on the tenth day but the last titrations did not show any change in values. The remainder of the data obtained in this study are presented in table 9.

All of the series which used the tissue from Glands 116 and 103 showed the same definite indications of a recombination of free fatty acids. Using

similar procedures the tissue from Gland 113 gave negative results. When the gland fat was present in the trials on Gland 117, it is assumed that hydrolysis of this fat took place. The decrease in titration when butyric acid was used as the second substrate indicates that both hydrolysis and a recombination of free fatty acids was taking place. When dried tissue samples of this gland were used there was a recombination when tributyrin was used but not when oleic or palmitic acids were the substrates.

DISCUSSION

The evidence obtained indicates the tissue of the bovine mammary gland possesses the power to hydrolyze neutral fats if it first has been developed by pregnancy. Whether this is important in milk fat synthesis is not known, though it seems logical to assume that if this power is present, it must be of importance in the normal functioning of the gland. While the lipase content is not large per gram of tissue, it must be remembered that according to Swett and coworkers (9), the average grade bovine mammary gland weighs 29 pounds, indicating that when the activity of the entire gland is taken into consideration the lipolytic power of the tissue under living condidions might be a very considerable force to be reckoned with in working out the processes of milk fat synthesis.

Data are also reported which indicate the pH of actively secreting mammary gland tissue is definitely on the acid side with the histochemical analyses showing the actual secretory cell areas to be much more acid than the connective tissue cell areas in slices of the tissue. This was especially true for the actively secreting glands and least true for glands not developed by pregnancy. An active lipase *in vivo* in mammary gland tissue might be expected to cause such a reaction.

Evidence was also presented in which a recombination of free fatty acids also took place due to enzyme action indicating that mammary gland tissue contained enzymes which could both hydrolyze the neutral fats from the blood and recombine the free fatty acids as they crossed the secretory cell tissue. However, there were exceptions to this recombination in the trials carried out. The tissue of Glands 113 and 117 did not act in a similar manner to that of Glands 103 and 116.

SUMMARY

Data have been presented which show the tissue from bovine mammary glands which have not been developed by pregnancy contain only traces of an active lipase.

Tissue which has been developed by pregnancy contains significant amounts of this enzyme.

Data have been reported indicating the actively secreting gland tissue has an acid reaction with the secretory cell areas of the lactating glands showing the highest hydrogen ion concentration.

Some experiments which show positive evidence for a recombination of free fatty acids by means of lipolytic tissue enzymes of the bovine mammary gland have also been reported.

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EVIDENCE OF THE SYNTHESIS OF VITAMIN C BY DAIRY COWS*

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The results of early investigations (4, 11, 12) indicated that it was unnecessary to provide a food source of vitamin C in the ration of dairy cows. Consequently little attention was paid to this factor when the experimental rations used in our rather extensive studies of vitamin D in dairy cattle nutrition were initiated. Among other symptoms which developed on the experimental ration were atrophy of the gum tissue and looseness of the teeth which suggested a possible vitamin C deficiency. Both incisors and molars were affected. In one case the incisors were extremely loose and could be moved back and forth so the outer ends covered a distance of an inch and a half or so. Upon checking the ration it was found that it was very low in vitamin C as well as in vitamin D. As many of the cows were being kept on this ration for three or four years it seemed advisable to make sure that vitamin C deficiency symptoms were not being encountered as a complicating factor in the vitamin D studies. This possibility was investigated using two different approaches to the problem.

Experiment I

METHODS

The first part of the investigation was set up to compare the vitamin C in the blood plasma and milk of the experimental cows with that of other groups receiving increasingly larger amounts of vitamin C. The concentration of vitamin C in blood plasma is generally considered to be a good index of the vitamin C reserves and an indication of the state of vitamin C nutrition. A normal concentration of vitamin C in the blood plasma of these experimental cows comparable to that in cows under ordinary herd management would constitute good evidence that they were not suffering from a vitamin C deficiency. Furthermore, if the amount of vitamin C in their milk was similar to that in the milk from cows on good herd rations including pasture it would add further evidence as to the sufficiency of the vitamin C available to the experimental cows.

The cows used for this purpose were grouped as follows: Group 1—Five grade Holstein experimental cows on the ration low in vitamin C. This ration consisted of molasses beet pulp for roughage, and a grain mix made up of ground yellow corn 100 pounds, ground oats 100 pounds, corn gluten

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meal 50 pounds, bone meal 4 pounds, and salt 3 pounds. Additional bone meal was added as a daily supplement when necessary to meet the mineral requirement. A carotene concentrate was fed to prevent vitamin A deficiency. Group 2—Two cows fed alfalfa hay and a grain ration continuously. Group 3—Six herd cows receiving alfalfa hay, silage and grain. Group 4—Five herd cows on pasture and receiving a grain supplement.

Observations extended over two summer seasons and included the intervening winter period. Blood plasma samples were taken twice monthly and an A.M. milk sample and a P.M. milk sample were collected monthly from each cow. There was a total of 249 blood plasma samples, 98 A.M. milk samples and 100 P.M. milk samples.

Vitamin C determinations were made chemically, using the 2,6-dichlorophenolindophenol oxidation-reduction indicator. The essential features of

TABLE 1

The average amount of vitamin C in the blood plasma and milk of groups of cows receiving various amounts of vitamin C in the ration

Material	Group 1 Ration low in vitamin C	Group 2 Alfalfa hay, grain	Group 3 Alfalfa hay, corn silage, grain	Group 4 Pasture, grain
Vitamin C in blood plasma (mgm./100 ml.)	0.335	0.432	0.338	0.270
Vitamin C in milk (mgm./100 ml.)				
A.M. samples	2.039	2.092	1.816	1.746
P.M. samples	1.983	2.116	1.829	1.764
All samples	2.011	2.104	1.823	1.755

the Farmer and Abt (3) method were used in making the blood plasma determinations, while the procedure reported by Knight, Dutcher, and Guerrant (6) was used for making the determinations on milk.

RESULTS

The significant information obtained in these observations is shown in table 1. The detailed month-to-month results are not shown as it seemed unnecessary to do so to bring out the essential information. The figures shown represent the average of all the cows in the group for the item concerned. To illustrate the method of figuring let us take the value of 0.335 mgm. of vitamin C per 100 ml. of blood plasma as shown for Group 1 on the experimental ration low in vitamin C. The average value for each cow in the group was first obtained by averaging the results of all of the vitamin C determinations made on the blood plasma for that cow. The average values for the individual cows in the group were then averaged to get the figure which represents the group average. All other figures in table 1 were obtained in the same manner.

There is some variation in the average amount of vitamin C in the blood plasma of the different groups of cows but it does not vary consistently with the vitamin C intake. Group 1 on the low vitamin C intake has almost exactly the same concentration of vitamin C in the blood plasma as Group 3 getting alfalfa hay, corn silage and grain, and somewhat more than Group 4 getting an abundant source of vitamin C on summer pasture. The highest concentration is in Group 2 getting only a limited amount of vitamin C in alfalfa hay and grain.

The figures for the vitamin C in the milk show that for Group 1 the values for the A.M. samples averaged slightly higher than for the P.M. samples but for the other three groups the reverse condition was true. The differences in all cases are extremely small and the average for all the A.M. samples and for all the P.M. samples is exactly the same, namely, 1.923 mgm. per 100 ml. of milk. Under the conditions of this experiment, then, there was no difference in the vitamin C content of the A.M. and the P.M. milk.

The averages for all milk samples by groups show only moderate differences, however, the cows getting the lower intakes had slightly more vitamin C in their milk. In fact, the cows in Group 4, getting an abundance of vitamin C on pasture showed the lowest concentration in the milk, while those in Group 1 getting a very low vitamin C intake in the experimental ration showed next to the highest amount.

A consideration of these results fails to give any evidence that the experimental cows were suffering from a deficiency of vitamin C even though they had been on this vitamin-C-low ration for 3 to 4 years in some cases. On the other hand, the fact that these experimental cows showed a normal concentration of vitamin C in their blood plasma after secreting considerable amounts in their milk over an extended period of time lends support to the view that dairy cows are able to synthesize this factor.

EXPERIMENT II

METHODS

To get further evidence on the probability of an active vitamin C synthesis several vitamin-C-balance trials were run on three experimental animals which had been on the experimental ration low in vitamin C for a considerable time. One of these cows was dry, one was giving about 25 pounds of milk daily, and the other about 50 pounds daily. Two separate 24-hour vitamin-C-balance trials were run on each cow. Attendants were on the job continuously for collecting urine and feces samples. Each voiding was weighed immediately. A small portion was placed in a preservative for analysis as quickly as possible after weighing. These samples were then carried immediately to the laboratory where a chemist was continuously at work to make immediate vitamin C determinations. The difficulty of arranging for the immediate analysis of each voiding of urine and feces

accounts for the comparatively short length of time for the trials. The intake in the feed and the outgo in milk, feces, and urine were determined for the purposes of these balance trials.

The milk samples taken during the balance trials were analyzed for vitamin C as already described in Expt. I. The feed, feces, and urine samples were analyzed by the Department of Experiment Station Chemistry using the photoelectric colorimeter and 2,6-dichlorophenolindophenol dye. The urine samples were placed immediately in 5 per cent glacial acetic acid at the time of voiding. These were brought to the laboratory for immediate analysis using the methods of Evelyn, Malloy, and Rosen (2). A feces sample of about 5 grams was placed in a weighed 200-cc. Erlenmeyer flask containing a 3 per cent metaphosphoric acid solution at the time of voiding and was then immediately analyzed, using the essential features of the

TABLE 2

Twenty-four-hour vitamin-C-balance trials on cows receiving a ration low in vitamin C

Cow	Milk production	Vitamin C					
		Intake	Outgo				Balance
			Milk	Feces	Urine	Total	
	lbs.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
4 E—1st trial	Dry	74.8	.....	498.4	5.5	503.9	(−) 429.1
2nd trial	Dry	00.0	.....	266.9	12.4	279.4	(−) 279.4
14 E—1st trial	26.9	54.1	257.5	962.6	28.6	1248.7	(−)1194.6
2nd trial	24.5	00.0	265.6	667.3	12.3	945.2	(−) 945.2
417—1st trial	49.5	73.1	525.1	702.7	19.0	1246.8	(−)1173.7
2nd trial	49.5	12.7	574.9	779.2	19.0	1373.1	(−)1360.4

method described by Bessey (1) with modifications by Dr. H. D. Anderson of the Experiment Station Chemistry Department to minimize the errors likely to result from turbidity of the sample and the presence of other reducing substances in the feces. The feed samples were analyzed by a method similar to that used for the feces.

RESULTS

A summary of the results of the six 24-hour vitamin C balance trials is shown in table 2.

The data in table 2 show that in every case the outgo of vitamin C far exceeded the intake of this factor in the feed. As to the avenue of outgo it is notable that only a very small portion was accounted for in the urine and that the heaviest outgo was in the feces. The outgo in the milk depended of course on the amount of milk being produced and varied from nothing in the case of the dry cow to around 40 per cent of the total outgo for the cow producing 50 pounds of milk daily. The excess of outgo of vitamin C over

the intake increased, as would be expected, with an increase in milk production.

DISCUSSION

The evidence accumulated in these experiments indicates that these experimental dairy cows were quite independent of a food source of vitamin C and were not suffering from a lack of this factor even though in some cases they had been maintained on rations low in vitamin C for periods up to three or four years.

As shown in table 1, the concentration of vitamin C in the blood plasma, which is generally considered to be a good index of the vitamin C reserves, is as high for the experimental cows on the ration low in vitamin C as for the main herd cows getting alfalfa hay, corn silage, and a grain mix, and it even exceeds that of Group 4 getting an abundance of vitamin C on pasture. Likewise, the concentration of vitamin C in the milk was fully as high, or even a little higher, for the experimental cows (Group 1) receiving a ration supplying only a small amount of this factor as it was for Group 4 on a ration supplying vitamin C in abundance. These results with milk agree in general with the observations of other investigators (4, 7, 9, 10, 13) who have also found that the vitamin C concentration in the milk remains fairly uniform regardless of the amount fed. The rapid destruction of vitamin C in the rumen (5) undoubtedly explains why the feeding of large amounts of vitamin C fails to increase appreciably the vitamin C content in the blood plasma and milk of dairy cows.

According to the findings of this investigation, the average vitamin C content of the morning and evening milk was practically identical for all groups. Furthermore, the average for all A.M. samples and all P.M. samples was exactly the same, namely, 1.923 mgm. per 100 ml. of milk. This varies slightly from the results of Whitnah and Riddell (13) who found the evening milk to contain 2.0 ± 0.7 mgm. per liter more vitamin C than the morning milk.

The results of the vitamin-C-balance trials shown in table 2 suggest some interesting implications. It is recognized that the necessarily short length of time involved introduces opportunities for rather wide variations and also that the chemical analysis for vitamin C on some of these materials is difficult to perform with accuracy. It still seems, however, that certain broad generalizations may be valid. It would seem from the work of Knight, *et al.* (5) that the analyses of the urine should be quite accurate as rather extensive tests by these authors indicated that all of the ascorbic acid in the urine was in the reduced form when voided and could be measured quite accurately by the indophenol titration method if the analyses were carried out at once on the fresh samples. This procedure was followed in our investigation. For the feces analysis, Dr. Anderson of the Experiment Sta-

tion Chemistry Department modified the usual photoelectric procedure so that the dye was added rapidly in successive 2-ml. portions with mixing of sample and reading the galvanometer following as rapidly as possible after each addition of the dye. Additions were made until the galvanometer swung lower on the scale with each addition. As the dye is decolorized by vitamin C the first points represent a dilution curve with the galvanometer readings increasing proportionately. As soon as the end point is reached the galvanometer readings decrease approximately proportional to the increasing concentration of the dye. The point of intersection of these two lines locating the break in the curve gives a reasonably accurate end point. The rapidity of this process reduces materially any error likely to be introduced by the presence in feces of other reducing substances acting at a slower rate on the indophenol dye.

Even after allowing for sizable errors the data show that these dairy cows after long periods on a ration decidedly low in vitamin C were excreting an amount of this factor far in excess of their intake which gives strong support to the hypothesis that somewhere the dairy cow maintains an active vitamin C synthesis. These findings are in harmony with the results of the early investigations (4, 11, 12) already mentioned and also with the more recent work of Ray, Chand, and Rau (8). After feeding two calves on a heat-treated ration low in vitamin C for 9 months Ray, Chand, and Rau (8) concluded that calves were apparently able to synthesize sufficient vitamin C for their normal growth and activity. They used the ascorbic acid content of the blood plasma as their criterion. Although the ration used in the present investigation may not have been quite as low in vitamin C, the period of time covered was much longer (up to 4 years for one of the animals) than that used by Ray, Chand, and Rau (8) which to that extent makes this investigation a more critical test of the animals' independence of a food source of vitamin C. Since cows were used in the present study, information is supplied on mature individuals which were also secreting very appreciable amounts of this factor in their milk as an added drain on body supplies. A daily outgo of anything like the amount of vitamin C shown by the vitamin-C-balance trials would soon deplete such vitamin C reserves as might be expected from the reported vitamin C values of bovine tissues, so the accumulated evidence points strongly to the conclusion that the dairy cow is able to synthesize vitamin C.

The site of this probable synthesis still seems to be rather obscure. The fact that there is such a small outgo in the urine as compared with the rather large outgo in the feces suggests that the synthesis might take place along the gastro-intestinal tract somewhere beyond the rumen where it has been demonstrated that vitamin C is destroyed. If that were the case, the fact that the milk contained a relatively large portion indicates that the vitamin C must have been absorbed into the blood stream from the gastro-intestinal

tract. Of course, it would be possible for the synthesis to take place at some other point followed by excretion through the gut but the fact that intravenous injections of large doses of vitamin C were found by Knight *et al.* (5) to be excreted largely in the urine argues against this possibility. While the present data seem to favor the idea of a synthesis along the gastro-intestinal tract, the work of Ray, Chand, and Rau (8) is more or less opposed to such an hypothesis. These investigators cultured bacteria taken from various organs of the gastro-intestinal tract in media prepared from the contents of the organ from which it was isolated. Although good growths were obtained very little or no vitamin C was found to be present. The available evidence, therefore, seems insufficient to indicate conclusively just where in the body the probable synthesis of vitamin C takes place.

SUMMARY

1. Dairy cows are not dependent upon a food source of vitamin C. Animals maintained up to three or four years on an experimental ration low in vitamin C showed a concentration of this factor in the blood plasma and milk equal to, or even somewhat higher than, that of some cows receiving larger amounts in normal herd rations which included silage and other cows on pasture receiving an abundance of this factor.

2. Six twenty-four-hour vitamin-C-balance trials run on three cows receiving the experimental ration low in vitamin C showed that the outgo of this factor far exceeded the intake even after long periods of time on this ration. The negative balances were greater for cows in heavier milk production. The outgo was relatively large in the feces, next highest in the milk for cows in production, and extremely small in the urine.

3. The presence of normal concentrations of vitamin C in the blood plasma and milk of dairy cows after three to four years on experimental rations low in vitamin C, and on which the outgo of this factor has undoubtedly been far in excess of the intake as evidenced by the results of six vitamin-C-balance trials, lends strong support to the hypothesis that dairy cows synthesize vitamin C.

4. While the site of the probable synthesis of vitamin C has not been conclusively demonstrated these data suggest that it may be along the gastro-intestinal tract.

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OXIDATION OF BUTTER OIL AS INFLUENCED BY PREVIOUS HEAT TREATMENT OF THE OIL, BUTTER, OR CREAM[1,2]

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The modern trend in the processing of cream for buttermaking is toward higher pasteurization temperatures, with certain deodorizing procedures involving temperatures of 93° C. to 149° C. (7). This high temperature trend raises certain questions as to the influence of such treatment upon the oxidative tendencies of the resulting butter and butter oil; questions which have to some extent, at least, remained unanswered. In addition, the findings of Gould and Sommer (5), Gould (4), and Josephson and Doan (8), which showed that high heat treatment of milk stabilized the milk against oxidized flavor development due to the production of reduced sulfur compounds, have not been specifically applied to the buttermaking field nor to the stabilization of butter oil against oxidation by heat treatment of the cream.

The objects of this particular experiment were, therefore, mainly threefold: (a) to secure additional information on the influence of high temperature heat treatment of butter and butter oil upon the oxidation tendencies of the butter oil itself; (b) to determine the influence of various heat treatments of cream upon the stability of the resulting butter oil; (c) to ascertain the ability of various heat treatments of cream to stabilize the resulting butter oil against metallic-induced oxidation as produced by adding copper salts to the cream before or after the heat treatment.

The influence of temperatures above boiling upon the oxidation of butter and butterfat has been given attention by Patil and Hammer (2), Ritter and Nussbaumer (15) and Kochling and Taufel (9). Patil and Hammer observed ghee heated to 130°–140° C. to have superior keeping quality to that heated to 110°–120° C. Ritter and Nussbaumer found that fat from cooked butter exhibited slower peroxide increases than fat from non-heated butter.

Kochling and Taufel (9) and Ritter and Nussbaumer (15) noted that heating butter oil to temperatures of 150° C. and 250° C. respectively reduced the induction period markedly. The latter workers also found that when tallowy fat was heated to 250° C. and then returned to normal temperatures, the peroxide value was decreased, due likely to the destruction of the peroxides by the heat. Furthermore, the shortening of the induction

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period of fat by high heat treatment was attributed to the destruction of certain antioxidant properties.

Several workers have studied the influence of pasteurization temperatures of cream upon the keeping quality of the resulting butter. In the majority of cases, however, the keeping quality was considered from the angle of general flavor changes without specific attention being given to those involving oxidation. Mortenson (11) and Guthrie, *et al.* (6) used temperatures of 76.6° C. and 73.9° C., respectively, for cream pasteurization and found the butter made therefrom to have superior keeping quality to that made from cream pasteurized at 62.8° C. However, White and Campbell (18) found no appreciable difference in the keeping quality of butter made from cream pasteurized at 62.8° C. and 73.9° C.

"Vacreation" of cream, which involves temperatures of 88°–96° C. has been reported to give butter with keeping quality superior to that made from vat pasteurized cream (3, 21). Roberts, Coulter, and Combs (16) observed no appreciable difference in the keeping quality of butter made from cream flashed at 126.6° C. or vat pasteurized at 71.1° C. for 30 minutes. Wilson, *et al.* (20), studied the Reid flash, Cooney flash, and vat pasteurizers and found the higher-temperature methods produced butter of superior keeping quality. Platon and Olsson (13) found butter made from cream pasteurized at 80° to 83° C. to be inferior in keeping quality to that processed at 90° to 96° C.

The influence of pasteurization temperatures upon oxidative spoilage of butter has been given specific attention by Wiley (19) and Ritter (14). Wiley flashed pasteurized cream at 66.1° C. (150° F.), 79.4° C. (175° F.), and 93.3° C. (200° F.) and found the higher temperatures to reduce the rate of oxidation in the butter. However, the changes in acid degree of the fat in the sample pasteurized at 66° C. indicated that a certain degree of lipolysis had occurred during storage which may have influenced the rate of spoilage. The protective action of heat against oxidation was more striking in the case of unsalted acidified butter than with unsalted ripened, salted ripened, or salted acidified butter.

Ritter (14) reports that butter made from cream flash pasteurized at 78° C., 80° C., or 82° C., developed a fishy flavor on storage. However, fishiness seldom occurred when the pasteurization temperature was 86° C. and practically never appeared when the processing temperature was 90° C. The heat influence was attributed to chemical and not bacteriological effects. Wiley (19) does not concur in this belief, but instead suggests that an oxidizing system and not an anti-oxidant is involved at 90° C., and that the oxidizing enzyme is favored by low pH and high salt concentration.

EXPERIMENTAL PROCEDURE

Cream used in this study was secured from milk produced by the College Dairy herd under controlled conditions to eliminate metal contamination.

When the cream was processed at temperatures below boiling, the heating was accomplished in a round-bottom glass flask suspended in a boiling water bath. A mechanical glass agitator stirred the cream during the heating period. The time required to attain temperatures of 85°–95° C. was about 15 minutes, whereas considerably less time was required for cooling.

To obtain temperatures above 100° C., the cream, butter, or butter oil was placed in Erlenmeyer flasks and heated in the autoclave for 30 minutes at the desired steam pressure. Cooling was then accomplished by submerging the samples in cold water. In every case, the cream was aged over night in the same container in which it was heated before churning.

When copper was utilized to catalyze oxidation, it was added in the form of a weak solution of copper sulfate at the rate of 5 ppm.

Churning was accomplished in well tinned, two-gallon churns. After churning, the butter granules were washed thoroughly with distilled water. If butter was desired, the granules were worked into a homogeneous mass by means of a wooden paddle. However, if only the butter oil was desired, the granules were melted in a water bath at 50° C., the water and curd siphoned off, and the butter oil centrifuged and filtered. Unless otherwise specified, filtering was accomplished in an incubator held at 40°–50° C. In case the oil was not to be utilized immediately it was refrigerated at 3° C.

The stability of the butter oil to oxidation was determined by incubating 25 ml. samples in 100 ml. beakers in an oven at 100° C. This acceleration method was previously thoroughly studied (2), and it was found that when the method was properly used the induction periods of duplicates agreed within about 7 per cent. Thirty-two samples of butter oil constituted one series, and duplicate samples were removed from the oven for examination at the desired intervals. At least three trials were conducted in each experiment.

Special precautions were taken in the cleansing of all glassware. The glassware was first washed thoroughly in a solution of tri-sodium phosphate, rinsed with water, and placed in a chromic acid bath for at least 12 hours. The chromic acid was removed by rinsing with hot water and then the glassware was rinsed with six or more changes of distilled water, each lot of water being allowed to remain over the glassware for 12 hours or more. This thorough rinsing is in line with the findings of Laug (10) which showed that several successive leachings with distilled water are necessary to remove chromic acid from glass. Before use, the glassware was thoroughly dried in a hot air oven.

The peroxide determination was conducted by Wheeler's technique (17). Results are reported as peroxide values, *i.e.*, the millimoles of peroxides per kg. of fat.

A peroxide value of five was arbitrarily established as representing the end of the induction period and the attempt was made to remove the samples

from the oven at suitable intervals so as to adequately cover the induction period.

EXPERIMENTAL RESULTS

Butter and butter oil. Trials were conducted in which butter and butter oil, secured from cream previously pasteurized at 63° C., were heated in an autoclave at 22 pounds pressure (127° C.) for 30 minutes. The butter oil secured was then oxidized in the 100° C. oven and samples taken at various periods for the peroxide determinations. The results of this experiment are shown by figure 1.

This figure shows that heating either butter or butter oil definitely hastens the rate and extent of oxidation. On the basis of hours, the induc-

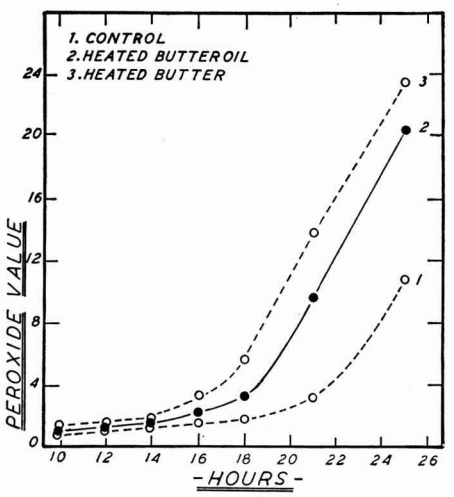

FIG. 1. Effect of heating butter oil and butter at 22 lbs. pressure (127° C.) upon the rate of oxidation of the butter oil.

tion period for the heated samples were approximately 2–4 hours shorter than for the control samples. There was little difference between the butter and butter oil in this connection. Mention should be made, however, that in these trials the temperature of filtering the butter following heating was 80°–90° C., which may have some influence upon the results. In other studies, when the heated butter was filtered at 45° C., the resulting butter oil was decidedly more stable to oxidation than the heated butter oil. However, these latter studies were too limited to warrant the drawing of definite conclusions.

Additional trials dealing with the influence of heat upon the oxidation of butter oil were conducted. In these trials, butter oil was subjected to 6 pounds (109.8° C.) and 22 pounds (127° C.) pressure for 30 minutes.

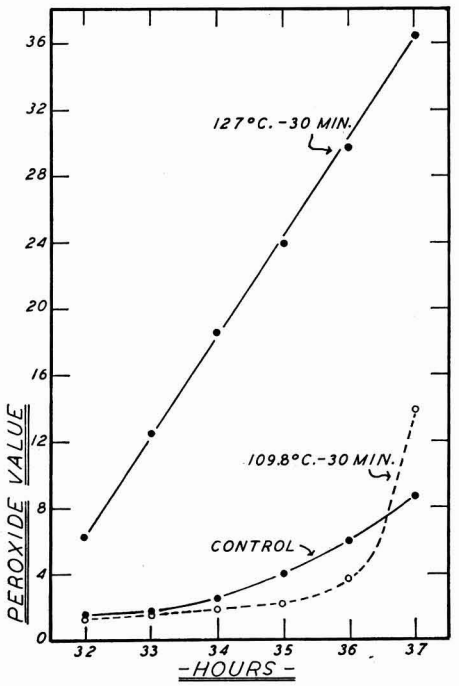

FIG. 2. Effect of heating butter oil at 6 lbs. pressure (109.8° C.) and 22 lbs. pressure (127° C.) upon its rate of oxidation.

The control sample was secured from cream previously pasteurized at 63° C. The results of a representative trial are shown in figure 2.

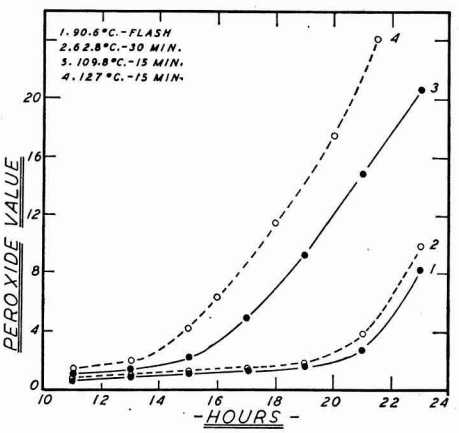

FIG. 3. Effect of various temperatures of processing cream upon the rate of oxidation of the butter oil.

These results reveal that the fat processed at 6 pounds pressure did not oxidize appreciably different than the control; however, its extent of oxidation was somewhat more at the close of the experiment. Those fat samples heated to 127° C. oxidized at a much greater rate and extent than the other lots. Results of the various individual trials indicate that the induction period of the fat heated to 127° C. was on the average about five hours less than in the case of the control lots, a reduction of approximately 15 per cent.

Cream. To determine the influence of heating cream to various temperatures upon the stability of the butter oil to oxidation, cream was heated to 62.8° C. for 30 minutes, to 90.6° C. flash, or to 109.8° C. and 127° C. for 15 minutes. The cream was cooled and held overnight before churning. The stability of the butter oil is illustrated by figure 3.

There was no distinguishable difference in the oxidative stability of the fat when the cream was processed at 62.8° C. and 90.6° C. However,

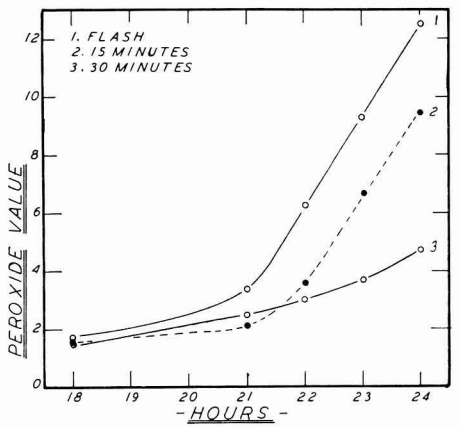

FIG. 4. Effect of prolongation of the holding period for cream at 90.6° C. upon the rate of oxidation of the butter oil.

processing the cream at 109.8° C. and 127° C. decreased the stability of the fat. In this particular series, the induction period of the control fat was about 22 hours. The heat treatment at 109.8° C. shortened this induction period to 17 hours and the higher temperature of 127° C. resulted in an induction period of about 15 hours.

Increasing the length of the holding period for cream to a reasonable extent at temperatures below boiling apparently has no harmful effect upon the stability of the fat. This is illustrated by figure 4, in which cream was processed at 90.6° C. for 0, 15, and 30 minutes. In fact, the longer processing times had a tendency to increase the stability of the fat but the difference in induction period was slight, amounting to 1–2 hours for induction periods of 23–28 hours.

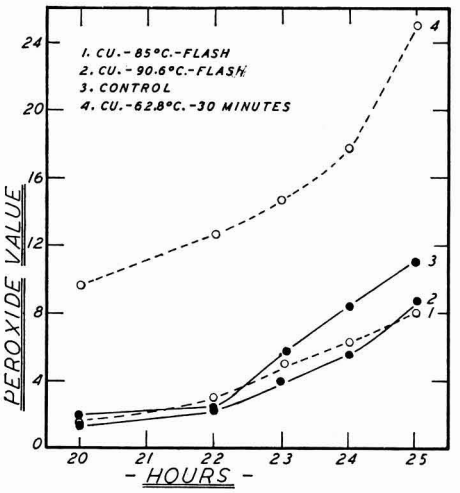

FIG. 5. Effect of different heat treatments of cream upon the rate of oxidation of the butter oil when copper contamination occurred before heating.

Cream and copper. In this portion of the experiment, trials were conducted to determine the role heat may play in stabilizing butter oil against oxidation as catalyzed by contamination of the cream with an active oxidizing metal. The first series of trials were conducted in which cream was contaminated with 5 ppm. copper before being subjected to the heat treatment. Following the heat treatment, butter oil was secured and its comparative stability noted. Temperatures of processing the cream were 62.8° C.—30 min., 85° C.—flash, and 90.6° C.—flash. The control was pasteur-

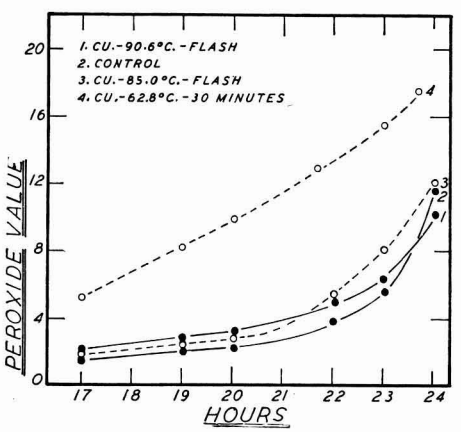

FIG. 6. Effect of different heat treatments of cream upon the rate of oxidation of the butter oil when copper contamination occurred after heating.

ized at 62.8° C. and contained no added copper. Results are portrayed in figure 5.

This graph shows temperatures of 85° C. and 90.6° C. to markedly stabilize the fat against metallic-induced oxidation; in fact, fat from these samples were at least as stable as the control sample. This is in marked contrast to the rapid oxidation that occurred in the lot containing copper and pasteurized at 62.8° C. Results on individual trials showed the higher temperatures to prolong the induction period by approximately 20–25 per cent in contrast to the samples containing copper and heated to 62.8° C.

In another series, similar conditions of processing the cream were used but copper contamination occurred subsequent to pasteurization. Results of this experiment are shown in figure 6, and are practically identical with those secured when copper contamination occurred before heating.

In these series of trials involving copper catalysis (figures 5 and 6), the butter oil from cream which contained added copper and was pasteurized at 62.8° C. possessed relatively high peroxide values when the first samples were removed from the oven. These high peroxide values resulted because the butter oil had already undergone appreciable oxidation before samples were removed for examination, and are not the result of any influence of copper itself upon the peroxide determination. That the copper itself was not involved in creating errors in the peroxide method was indicated by the fact that peroxide determinations upon all samples at the 0-hour period gave negligible values. Furthermore, other trials were conducted in which as many as 10 ppm. of copper were added to cream and the peroxide values of the butter oil ascertained immediately. The results did not indicate any influence by the copper upon the peroxide determinations. One point to consider in this connection is that the actual amount of copper retained by the fat likely represents only a small portion of that which was in the cream. Consequently, with the concentrations of copper used in these experiments, the error created by the action of copper on the peroxide method may be expected to be negligible.

DISCUSSION

The shortening of the induction period of butter fat by subjecting it to high temperatures is in line with the finds of Kochling and Taufel (9) and Ritter and Nussbaumer (15). This destabilization is apparently not due to hydrolysis of the fat since titration of the fat for free fatty acids did not show any appreciable increase due to the heat. Conceivably, certain natural antioxidants of the butter oil are destroyed as suggested by Ritter and Nussbaumer (15), or, at least, undergo changes due to the heat influence.

Wide practical applications may be made of the findings on the influence of heat on cream. Temperatures above boiling for abnormal periods of time should be avoided, but apparently some laxity in the time-temperature relationship is permitted in the lower temperature range without appreciably affecting the oxidative-stability of the fat.

The results of the experiments with metal contamination of the cream are especially applicable to the buttermaking industry where the equipment often may contaminate cream with copper. The beneficial effect of heating cream to temperatures of approximately 85°–90° C. on metallic-induced oxidation is likely due to the production of reduced sulfur compounds at these temperatures (4, 5, 8). Doubtless, the antioxidant effect is created by the inactivation of the copper by the sulfur compounds rather than to any additional stability influence created in the butter oil itself. Consequently, the efficiency of the stabilization influence of these temperatures would be expected to be affected by (a) the quantity of antioxidant formed (governed by temperature and time), (b) the quantity of copper contamination, and (c) whether the metal contamination occurs before or after the heat treatment. Studies on milk have revealed that the heat influence is much greater if metal contamination occurs before pasteurization (1, 4, 5). Similar results would likely have occurred in this study if higher copper contamination had been utilized.

SUMMARY AND CONCLUSIONS

Heating either butter or butter oil to 127° C. for 30 minutes hastens the oxidation of the butter oil. A temperature of 109.8° C. did not appreciably influence the subsequent oxidation of the butter oil.

When cream was heated to 62.8° C.—30 minutes, 90.6° C.—flash, and 109.8° C. or 127° C.—15 minutes, the two higher processing temperatures shortened the induction period of the resulting butter oil.

Butter oil secured from cream pasteurized at 90.6° C. for 0, 15, and 30 minutes was not adversely affected by the longer heating periods but instead appeared to be stabilized to a slight degree.

Cream containing 5 ppm. of added copper and pasteurized at 85° C. flash and 90.6° C. flash, produced butter oil of stability equal to that of a control pasteurized at 62.8° C.—30 minutes and containing no added copper. Cream pasteurized at 62.8° C.—30 minutes and containing added copper oxidized extremely rapidly in comparison to the other lots.

The stabilization influence of temperatures of 85° C. and 90.6° C. was about equal regardless as to the time of metal contamination, *i.e.*, whether before or after heating. This heat influence is doubtless due to the formation of hydrogen sulfide and sulfhydryl groups which inactivate the copper sufficiently to prevent its full catalytic action.

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RELATION OF DEGREE OF SATURATION OF MILK FAT TO DEVELOPMENT OF OXIDIZED FLAVOR*

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Various investigators have suggested that the degree of saturation of the milk fat is related to the oxidation of the fat and occurrence of oxidized flavor. Henderson and Roadhouse (4) found that cows on submaintenance rations produce a more unsaturated fat which is more susceptible to oxidation than cows on regular rations. Stebnitz and Sommer (8) have reported that butterfat produced in the summer was more unsaturated and more readily oxidized than winter fat, although it is known that winter milk is much more likely to develop an oxidized flavor than summer milk.

Hilditch and Sleightholme (5) observed that addition of fats to the ration of dairy cows gave definite but slight changes in the composition of the butterfat. These investigators also observed that changing the herd from indoor to outdoor conditions, and from dry feed to pasture, markedly affected the composition of the fat. The iodine number of the butterfat was found to change from a high of 42.2 in November to a low of 36.5 in February while on winter feed and to a high of 44.2 in June when the cows were turned on pasture. This would suggest that atmospheric conditions as well as type of roughage are important factors in so far as composition of the butterfat is concerned. The above authors state "the appearance of a more saturated fatty acid mixture in butterfat under cold conditions is, of course, in harmony in that the body reserves of fat may be drawn upon under cold conditions and presumably the more unsaturated portions will be more readily consumed and later the more saturated fats."

Sutton, Brown and Johnson (9) observed that the inclusion of one pound of corn oil in the daily ration caused an increase in the unsaturated fatty acids and in the higher molecular weight fatty acids, but a decrease in volatile soluble fatty acids. The feeding of this amount of corn oil did not influence the rate of milk secretion or percentage of butterfat. Bender and Maynard (1) reported the effect of feeding rations of different fat levels on the composition of the fat of goat's milk. They found that a low fat ration and the ration in which coconut oil was included resulted in the secretion of a fat which was much more saturated and lower in molecular weight than was that produced on the high fat (7 per cent) ration or the linseed oil ration.

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* The data presented in this paper are from a thesis submitted by the senior author to the Graduate School of the University of Illinois in partial fulfillment of the requirements for the degree of Doctor of Philosophy, 1942.

[1] Resigned January 1, 1942.

Maynard, McKay and Madsen (7) fed cows alternate rations containing ground flaxseed (unsaturated fat) and coconut oil (saturated fat). They observed that inclusion of unsaturated fats in the ration produced a higher proportion of unsaturated fatty acids in the milk, while cows fed the more saturated fats gave milk containing a more saturated butterfat. The changes occurred in the milk fat within 18 to 24 hours after the flaxseed or coconut oil was fed. Bratton, Epple, Wilbur, and Hilton (2) observed that feeding soybean oil increased the percentage of fat in the milk and produced a more unsaturated fat. The oleic and linoleic acid content was increased.

Hill and Palmer (6) in a study of the effect of feeds on the composition of butterfat noted a decline in iodine number (4.49) and Reichert-Meissl number (1.01) and an increase in the melting point and hardness of the butterfat when cows were changed from the herd ration to an experimental ration of barley, bran and hay. The fat constants were not significantly changed when timothy hay, alfalfa hay and beet pulp were fed. When 85 per cent of the nutrients in the ration were supplied by alfalfa hay the iodine value was significantly increased, the Reichert-Meissl number was increased and the hardness of the butterfat was decreased. Changing the cows from the herd ration to pasture increased the iodine value markedly (3.17). These authors were of the opinion that the composition of butterfat is directly related to the type of oil present in the feeds in the ration. The feeding of coconut or cottonseed oil was found to produce a more saturated butterfat. On the other hand, the inclusion in the ration of corn or linseed oil gave a more unsaturated fat. Feeding butterfat or tallow did not influence the composition of the milk.

EXPERIMENTAL PROCEDURE

Six cows were selected from the University herd for this study. To aid in their selection, milk samples from individual cows in the entire University herd were first studied for flavor changes. The cows were selected on the basis of susceptibility of the milk to oxidized flavor development, stage of lactation, milk yield, and breed. It was desired to have cows whose milk varied in its susceptibility to oxidized flavor, to have cows giving a normal or average amount of milk, and cows in the mid-part of their lactation period. Six cows whose milk was the most constant concerning the development of oxidized flavor were finally selected. Data related to these six cows are given in table 1.

The cows were divided into two groups with three animals in each group. Each group consisted of one cow which gave "spontaneous susceptible" milk, one whose milk was very readily oxidized upon the addition of 0.66 part per million copper, and one whose milk was only slightly oxidized by the addition of copper.

The cows were fed on the regular ration until the susceptibility of the milk to oxidized flavor was determined, after which time the oils were included in the ration. The cows were fed one-third pound oil daily for three days, then two-thirds pound daily for three days, after which they were fed one pound daily for six days. The cows were then put back on the regular ration for 10 days and the groups reversed and fed the same as outlined above. It was necessary to omit cow No. 616 after the first trial (because of nearness to parturition) and substitute cow No. 642.

The milk was handled in such a manner as to prevent metal contamination as much as possible. The cows were hand milked into stainless steel buckets and the milk transferred into glass bottles in which it was pasteurized at 143° F. for 30 minutes, after which the milk was cooled by placing

TABLE 1

Data related to cows used in feeding experiments

Cow No.	Age		Breed	Month of lactation	Per cent fat, average†	Lbs. milk daily, average†	Susceptibility of milk to oxid. flavor	
	Yr.	Mo.					No Cu added	0.66 ppm. Cu added
616*	6	2	Guernsey	9.0	5.7	22	non-sus.	sus.
642*	4	3	Guernsey	5.0	4.9	17	non-sus.	sus.
675	4	7	Jersey	5.0	5.9	23	non-sus.	sl. sus.
693	3	10	Holstein	4.5	3.4	50	non-sus.	sus.
751	3	1	Holstein	4.0	3.5	50	sus.	sus.
753	2	11	Guernsey	5.0	4.9	21	sus.	sus.
755	2	10	B. Swiss	3.5	4.5	26	non-sus.	sus.

* Cow 642 was substituted for 616 for second part of experiment.

† The fat averages were computed from weekly tests and the average milk yield was computed from daily milk yields for period during which this experiment was made.

the bottles in cold water. To part of the milk copper (0.66 ppm.) was added in a 1 per cent copper sulphate solution. The milk was judged for oxidized flavor each day for three days. One quart bottle of each milk was let stand undisturbed until the fat rose to the top. The top cream was pipetted off and churned with a malted milk mixer. The fat was purified and the Hanus iodine number and refractive index were determined.

EXPERIMENTAL RESULTS

Inclusion of the oils in the ration did not have a noticeable effect on the daily milk yield or fat content of the milk. The data in table 2 show the effect of the oils fed on the degree of saturation of the milk fat. One cow on the coconut oil ration in each trial went off-feed for a few days, during which time the iodine number and refractive index of the milk fat increased markedly.

In trial 1 for the group of cows fed corn oil the iodine number of the milk fat was increased from an average of 32.61 while on the control ration

to an average of 48.55 when one pound of corn oil was fed daily. In trial 2 the iodine number was increased from an average of 29.56 while on the control ration to 44.64 when one pound of corn oil was included in the ration. Feeding coconut oil decreased the iodine number of the milk fat.

TABLE 2

Effect of feeding oils on the saturation of the milk fat

Date of sampling	Amt. oil fed daily	Days oil fed	Group 1				Group 2			
			616*	751	755	Ave.	675	693	753	Ave.
	lbs.		Fed corn oil				Fed coconut oil			
			Iodine number							
Feb. 8	0	...	36.55	29.18	30.60	32.11	27.32	28.45	34.65	30.14
Feb. 23	0	...	37.45	29.07	32.10	32.87	27.87	26.00	30.65	28.17
Feb. 26	0	...	36.55	31.20	32.55	33.43	28.92	26.52	28.72	28.09
Feb. 29	⅓	3	40.65	33.47	37.05	37.05	26.75	28.72	28.25	27.90
Mar. 3	⅔	3	45.85	42.50	43.55	43.97	25.67	25.65	26.17	25.83
Mar. 6	1	3	51.12	45.77	47.90	48.26	24.36	26.21	28.13	27.23
Mar. 9	1	6	52.50	44.40	48.75	48.55	24.37	25.45	36.42†	24.91
			Refractive index‡							
Feb. 8			35.3	27.5	29.0	30.6	21.5	27.3	34.5	27.8
Feb. 23			36.5	28.0	34.0	32.8	25.0	24.5	27.5	25.7
Feb. 26			36.0	31.0	30.5	32.5	24.0	23.5	24.8	24.1
Feb. 29			42.5	32.7	40.0	38.4	20.0	26.5	25.5	24.0
Mar. 3			51.0	.....	51.0	51.0	20.0	20.0	20.0	20.0
Mar. 6			63.5	53.0	49.5	58.7	17.0	21.5	23.0	20.5
Mar. 9			66.0	50.5	63.0	59.8	19.0	22.0	37.0†	20.5
			Fed coconut oil				Fed corn oil			
			Iodine number							
Mar. 19	0	...	34.30	32.35	30.70	32.52	30.10	28.25	30.32	29.56
Mar. 22	⅓	3	32.40	29.25	37.40§	30.55	31.70	32.57	37.70	33.99
Mar. 25	⅔	3	30.00	29.00	31.97	30.32	38.10	37.92	43.25	39.76
Mar. 28	1	3	30.50	29.65	36.47†	30.07	40.25	39.12	47.80	42.39
Mar. 31	1	6	31.35	28.60	31.52	30.49	42.70	43.72	47.50	44.64
			Refractive index‡							
Mar. 19			35.2	32.0	37.5	34.9	28.0	26.0	29.0	27.7
Mar. 22			29.5	25.5	40.0	27.5	29.5	32.2	41.0	34.2
Mar. 25			27.5	27.0	32.2	28.9	42.0	43.5	42.2	42.6
Mar. 28			29.5	27.0	26.0	28.3	43.0	45.0	56.0	48.0
Mar. 31			30.0	25.0	29.0	28.0	48.5	53.0	57.0	52.8

* Cow 642 substituted for cow 616 in second period.
† Cow went off-feed.
‡ Refractive index—1.4517 to 1.4563.
§ Ovaries received medical attention on March 21, 1940.

In trial 1 the average iodine number of the milk fat from the cows on the control ration was 28.89 and was decreased to 24.91 when one pound coconut oil was fed. In trial 2 the average iodine number of the milk fat was 33.78 and 30.49 while on the control ration and coconut oil ration respectively.

The effect of the oils on the iodine number is shown graphically in figure 1. The correlation between iodine number and refractive index is shown in figure 2. Except for one or two exceptions the refractive index was very closely correlated with the iodine number. The coefficient of correlation was r = .9697.

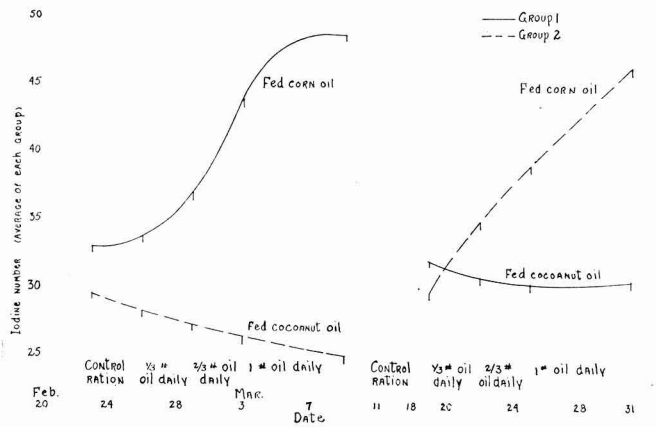

FIG. 1. Effect of Feeding Oils on Iodine Number of the Milk Fat.

Table 3 gives the degree of oxidized flavor in the metal-free milk at the end of three days' storage. The susceptibility of the milk to oxidized flavor development was not materially changed by inclusion of the oil in the ration, although the saturation of the milk fat was appreciably changed.

Table 4 gives the degree of oxidized flavor in milk containing 0.66 ppm. of added copper after 1 and 2 days' storage. As observed in the table the inclusion of either vegetable oil in the ration had only a slight effect on the development of the oxidized flavor in copper contaminated milk. The coefficient of correlation between iodine number of the milk fat and oxidized flavor scores was $r_{xy} = 0.3032$ and $r_{xy} = 0.344$ for one- and two-day storage periods respectively. This relationship is further shown in figures 3 and 4.

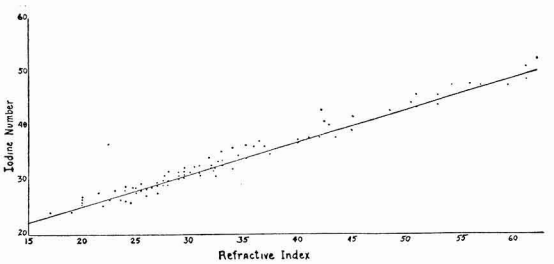

FIG. 2. Relation between Iodine Number and Refractive Index.

TABLE 3

Degree of oxidized flavor in metal-free milk at end of three days

Date	Amount oil fed daily	Days fed	Degree of oxidized flavor*					
			616†	751	755	675	693	753
			Fed corn oil			Fed coconut oil		
Feb. 8	0	...	0	2	0	0	0	2
Feb. 23	0	...	0	2	0	0	0	2
Feb. 26	0	...	0	3	0	0	0	2
Feb. 29	½	3	0	3	0	0	0	3
Mar. 3	½	3	0	3	0	0	0	3
Mar. 6	1	3	0	3	0	0	0	3
Mar. 9	1	6	0	3	0	0	0	0
			Fed coconut oil			Fed corn oil		
Mar. 16	0	...	0	0	0	0	0	0
Mar. 19	0	...	0	2	0	0	0	1
Mar. 22	½	3	0	4	0	0	0	3
Mar. 25	½	3	0	2	0	0	0	3
Mar. 28	1	3	0	0	0	0	0	5
Mar. 31	1	6	0	4	0	0	0	4

* The degree of oxidized flavor is represented by number, 0 meaning no off flavor was detectable.

† Cow 642 substituted for cow 616 in second period.

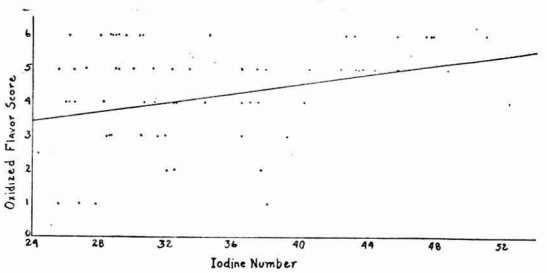

FIG. 3. Relation between Iodine Number and Oxidized Flavor Score of Copper Contaminated Milk (0.66 ppm. Cu) after 24 Hours' Storage.

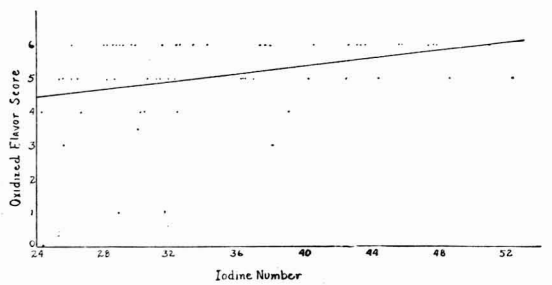

FIG. 4. Relation between Iodine Number and Oxidized Flavor Score of Copper Contaminated Milk (0.66 ppm. Cu) after 2 Days' Storage.

It can be seen from the figure that the most noticeable relation between iodine number and development of oxidized flavor appears to be where the iodine number is very high, in which cases the oxidized flavor scores were also very high, but the reverse was not true. It is evident that a low iodine

TABLE 4

Degree of oxidized flavor in milk containing 0.66 ppm. of added copper

Date	Amount oil fed daily	Days fed	Degree of oxidized flavor*							
			616†	751	755	Ave.	675	693	733	Ave.
Part I. After 24 hours' storage										
	lbs.		Fed corn oil				Fed coconut oil			
Feb. 8	0	...	5	5	6	5⅓	5	3	6	4⅔
Feb. 23	0	...	4	6	2	4	1	4	6	3⅔
Feb. 26	0	...	4	5	2	3⅔	0	4	3	2⅓
Feb. 29	⅓	3	5	5	4	4⅓	1	5	3	3⅓
Mar. 3	⅔	3	5	5	5	5	1	5	4	4⅓
Mar. 6	1	3	6	6	6	6	2½	6	6	4⅔
Mar. 9	1	6	4	5	5	4⅔	0	5	0	1⅔
			Fed coconut oil				Fed corn oil			
Mar. 16	0	...	5	5	5	5	0	4	5	3
Mar. 19	0	...	4	5	4	4⅓	0	4	2	2
Mar. 22	⅓	3	4	5	5	4⅔	0	4	5	3
Mar. 25	⅔	3	5	6	3	4⅔	1	5	6	4
Mar. 28	1	3	3	6	3	4	4	3	6	4⅓
Mar. 31	1	6	4	6	3	4⅓	6	5	6	5⅔
Part II. After 2 days' storage										
			Fed corn oil				Fed coconut oil			
Feb. 26	0	...	5	5	4	4⅔	1	5	5	3⅔
Feb. 29	⅓	3	6	6	5	5⅔	4	6	6	5⅓
Mar. 3	⅔	3	6	5	6	5⅔	3	5	5	4⅓
Mar. 6	1	3	6	6	6	6	4	6	6	5⅓
Mar. 9	1	6	5	5	5	5	1	5	5	3⅔
			Fed coconut oil				Fed corn oil			
Mar. 16	0	...	5	5	5	5	4	5	5	4⅔
Mar. 19	0	...	6	5	5	5⅓	3½	5	4	4
Mar. 22	⅓	3	6	6	6	6	1	6	6	4⅓
Mar. 25	⅔	3	6	6	5	5⅔	3	6	6	5
Mar. 28	1	3	4	6	5	5	5	4	6	5
Mar. 31	1	6	5	6	6	5⅔	6	6	6	6

* The degree of oxidized flavor is represented by number, 0 meaning no off flavor was detectable.

† Cow 642 substituted for cow 616 in second period.

number does not necessarily mean that the milk will not develop a strong oxidized flavor. This is further shown by a summary of the data in tables 2 and 3.

Throughout the iodine number range of 24 to 49 the average oxidized flavor values were practically the same but increased somewhat in the case

of the milks having iodine numbers above 40. These differences were slightly greater in the milk judged after a 24-hour storage period than that held 2 days. It is likely that these differences are too slight to be of any practical significance. Since this study was completed Brown, Dustman and Weakley (3) report that in feeding trials in which coconut oil, expeller soybean oil, crude soybean oil and hydrogenated soybean oil were fed that the feeding of coconut oil decreased slightly the iodine number of the milk fat and the susceptibility of milk to copper induced oxidized flavor. Feeding of crude- and expeller-soybean oil markedly increased the iodine number of the milk fat and increased the susceptibility of the milk to copper-induced oxidized flavor. However, an examination of their data show that

TABLE 5

Relationship of oxidized flavor development and iodine number

Iodine number range	Average oxidized flavor development in milk containing 0.66 ppm. of added copper			
	1 day		2 days	
	Number observations	Average oxidized flavor	Number observations	Average oxidized flavor
24.00–28	11	3.50	9	4.10
28.01–32	24	4.08	21	4.88
32.01–36	9	4.00	6	5.50
36.01–40	10	3.60	9	5.00
40.01–44	8	5.12	7	5.70
44.01–48	7	5.71	6	5.83
48.01–52	3	5.33	2	5.50

the differences in susceptibility of the milk to oxidized flavor development as a result of feeding the various oils are rather small and probably not significant.

SUMMARY

A marked variation in the iodine number of the milk fat produced by six individual cows fed a control ration of alfalfa hay, corn silage and grain mixture was observed. Differences in the susceptibility of the milk to become oxidized were noted, yet there was very little correlation between the iodine number of the fat and the tendency of the milk to develop an oxidized flavor. When the degree of saturation of the milk fat was varied by the addition of either corn oil or coconut oil to the ration the iodine number of the milk fat was altered markedly, yet there was only a slight change in the susceptibility of the milk to oxidized flavor development. The susceptibility of milk to development of oxidized flavor was not correlated to the iodine number in the range of 24 to 40. However, milk with an iodine number greater than 40 developed a slightly greater degree of oxidized flavor than milk having a lower iodine number. The differences,

however, do not appear to be significant. Although the duration of the feeding trials covered a relatively short period of time, it is likely that if any important relation existed between saturation of the milk fat and oxidized flavor development it would have been evident within the period studied. It seems reasonable to conclude that the seasonable nature of the occurrence of oxidized flavor cannot be explained on the basis of changes in the iodine number of the milk fat.

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FURTHER STUDIES ON THE GROWTH-PROMOTING VALUE OF BUTTER FAT*

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In all of our work to date (4, 9, 10, 11) on the comparative nutritive value of butter fat and various vegetable oils we have used weanling rats 21 days old, and a basal ration of mineralized skimmed milk reinforced with vitamins A, D, and E. From this work we reached the conclusion that butter fat had nutritive value superior to the vegetable oils studied, and that this superiority probably lay in the existence in the butter fat of some saturated fatty acid or acids of high molecular weight.

We realized (9) that a young rat nursing its mother for 21 days would have all the advantages of the synthetic powers of the mammary gland for special fatty acids, which through storage could minimize any nutritional differences existing between butter fat and vegetable oils. To test this hypothesis, we set up experiments to show the response given to butter fat and corn oil by 14-, 21-, and 30-day-old rats on a basal ration of skim milk powder. The importance of young animals for studies of the comparative nutritive value of animal and vegetable oils and fats in the nutrition of the calf has been recognized by Gullickson, Fountaine and Fitch (6). Using animals 6 to 29 days of age at the start of the experiment, they were able to show remarkable growth differences between calves fed animal fats and those fed vegetable oils. All of the animal fats used by them (butter fat, lard, and tallow) showed distinct superiority for growth and general well being over the vegetable oils (coconut oil, corn oil, cottonseed oil, and soybean oil) when homogenized into skimmed milk. They reported as a matter of general observation that more marked differences were obtained the younger the calf when placed on experiment. In unpublished work we had used day-old pigs in similar studies, but with this species we did not find differences in the nutritive value of animal and vegetable oils. It is possible that on a milk diet the pig has special synthetic power enabling it to construct all the fatty acids needed for its optimum physiology.

The basal ration in the work published by the writers has consisted of raw skimmed milk, but certain disadvantages of this ration have become

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apparent. To obviate these objections, a basal ration of fat-free mineralized skim milk powder was substituted for the liquid skimmed milk. Growth responses of groups of rats fed butter fat and corn oil at levels of 25, 30, and 35 per cent of the skim milk powder ration were determined.

Recent papers have indicated the importance of the kind of carbohydrate in the diet in relation to the nutritive value of fats. Hoagland and Snider (7, 8) found lard to have growth-promoting value superior to hydrogenated cottonseed oil on a synthetic-type ration in which dextrose was the sole carbohydrate. This difference in nutritive value could not be accounted for by the linoleic acid content of the two fats nor on the basis of their digestibility coefficients. Freeman and Ivy (5), in a comparison of rats fed a "filled" milk in which the naturally occurring fat had been replaced by coconut oil with those fed an evaporated milk, found that over a 97-day period the growth of the animals fed the evaporated milk was greater than for a similar group maintained on the "filled" milk. Boer, *et al.* (1, 2, 3) reported considerably better growth of rats fed on a rice ration (polished rice 72, casein 5, brewers yeast 10, salts 3, butter fat 10) as compared with olive oil plus the non-saponifiable fraction of butter fat which was added as a source of vitamins A and D. Poor fur coats with loss of hair were noticed in the animals fed the olive oil diet.

Unpublished data using a synthetic type of dry ration constituted similar to milk have indicated that differences in growth response favorable to butterfat as compared to corn oil and coconut oil could be obtained when the carbohydrate fed was *lactose,* whereas when the sole carbohydrate was *sucrose* the growth responses resulting with these three fats were nearly alike. The above citations suggested the importance of studies to determine the relationship of the kind of carbohydrate in the diet to the relative growth promoting properties of fats.

EXPERIMENTAL

In these studies butter fat and corn oil (Mazola) were the only fats used. Fresh unsalted sweet-cream butter was obtained from the University of Wisconsin Creamery and the butter fat was prepared by decantation from the curd and water. All fats were reënforced with vitamins A and E such that each gram of fat contained 20 γ of β-carotene and 80 γ of α-tocopherol. Each rat received 12.5 γ of calciferol per week. Skim milk powder prepared by the spray-dried process was obtained in 100 pound lots to insure uniformity and was extracted for four separate 8-hour periods with diethyl ether with vigorous mechanical stirring. After thorough drying it was mineralized so that 10 grams of complete ration contained 1.5 mg. of elemental iron and 0.15 mg. each of elemental copper and manganese. In all of the following studies, unless otherwise indicated, groups consisted of six male rats, individually caged. All feeding was *ad libitum.*

Effect of age. This study was made with groups of rats of 14, 21, and 30 days of age when removed from their normal environment. The 14-day-old rats received an artificial milk free of butter fat and made up as follows:

10 grams skim milk powder (mineralized and extracted as above)
5 grams corn oil (20 γ of β-carotene per gram of fat)
4 cc. of 0.2 N lactic acid
12.5 γ of calciferol
0.3 mg. Thiamine
0.3 mg. Pyridoxine
0.3 mg. flavin
1.0 mg. calcium pantothenate
0.1 gram choline
Water to make 100 cc.

The skim milk powder was dissolved in water at 40° C. and the warm corn oil was homogenized into the mixture with a laboratory homogenizer. The vitamin supplements and the lactic acid were added and the mixture was brought to volume with water.

The 14-day-old rats were kept in an electrically heated cage and fed the corn oil milk at frequent intervals, using a dropper at first, until the rats were 21 days of age. At this time the rats were placed in individual cages and six were fed the butter fat ration while another six were fed the corn oil ration. At this same time "21-day-old" weanling rats were obtained from the dealer and placed on the butter fat and corn oil diets without a depletion period on the artificial corn oil milk. Similarly, at the end of one week two groups of "30-day-old" rats were obtained from the dealer and started on the butter fat and corn oil diets. Thus the experiment was set up with animals all having a similar history during the first 14 days of life.

The ration consisted of 70 parts of the extracted mineralized skim milk powder and 30 parts of the vitaminized fat. In each age group six animals were fed the butter fat ration and six others were fed the corn oil ration. The experiment was of six weeks' duration. The data are summarized in table 1 (Expt. 68).

TABLE 1

Effect of age. The figures show the average grams gain made in six weeks. Each figure represents six male rats except in Expt. 68 where in the "21-day-old" group each figure represents 12 male rats

	Age groups of animals								
	"14-day-old"			"21-day-old"			"30-day-old"		
	Butter fat	Corn oil	Difference	Butter fat	Corn oil	Difference	Butter fat	Corn oil	Difference
Expt. 68	221	198	23	219	200	19	215	210	5
Expt. 61	223	195	28	214	188	26	.....	.....	.....
Expt. 66	211	187	24	220	201	19	.....	.....	.....

In similar trials (Expts. 61 and 66), "21-day-old" rats were compared with litter-mate "14-day-old" rats, but in these experiments the diet was made up of 25 per cent of the vitamin supplemented fat and 75 per cent of ether extracted mineralized skim milk powder. These data are also given in table 1. The growth-promoting value of butter fat was superior to that of corn oil in all age groups. However the difference in growth-promoting value was greatest in the case of the "14-day-old" rats while in the case of the "30-day-old" animals, those fed the butter fat ration averaged only 5 grams greater gain in six weeks than those fed the corn oil ration.

Effect of fat level. The growth-promoting value of butter fat and corn oil at levels of 25, 30, and 35 per cent of the ether extracted skim milk powder basal ration was determined. The diets were supplemented in the usual manner and fed to weanling rats 21 days of age. The results are summarized in table 2. As the level of butter fat in the diet was raised, the gain in weight in six weeks ranged between 216 and 219 grams. As the corn oil level in the diet was raised from 25 to 30 to 35 per cent of the diet, the gain in weight decreased from 208 to 200 to 184 grams respectively. The greatest difference in the nutritive value of the two fats was at a level of 35 per cent. The gain in weight made in six weeks by the individual rats on the diets containing 35 per cent of fat was as follows:

Butter fat	*Corn oil*
226 grams	208 grams
225 "	194 "
219 "	193 "
213 "	183 "
208 "	180 "
206 "	149 "

There was a range of 20 grams in the gains made by the animals fed the butter fat ration and of 49 grams in those fed the corn oil ration. The lack of uniform growth in the corn oil group is typical. No explanation for the

TABLE 2

Effect of fat level on the comparative nutritive value of butter fat and corn oil with a basal ration of skim milk powder. Figures represent the average number of grams gained in six weeks

Diet	Number of rats	Grams gain in weight
Butter fat 25%, skim milk powder 75%	6	217
Corn oil 25%, " " " 75%	6	208
Difference		9
Butter fat 30%, skim milk powder 70%	12	219
Corn oil 30%, " " " 70%	12	200
Difference		19
Butter fat 35%, skim milk powder 65%	6	216
Corn oil 35%, " " " 65%	6	184
Difference		32

decreased rate of growth with an increasing percentage of corn oil in the ration is at present available.

Effect of various carbohydrates. A synthetic type ration similar to milk solids in composition, but with the carbohydrate and fat constituents varied, was made up according to the following formula:

Carbohydrate	32
Casein (fat free)	28
Salts IV[1]	6
Liver extract[2]	6
Fat	28
Thiamine	200 γ per 100 grams ration
Pyridoxine	300 γ per 100 grams ration
Choline	200 milligrams per 100 grams ration
β-carotene	20 γ per gram of fat
α-tocopherol	80 γ per gram of fat
calciferol	12.5 γ per rat per week

The casein was extracted twice with boiling 95 per cent ethanol for four hours, each alcohol extraction being followed by exhaustive ether extraction. Vigorous mechanical stirring assured good contact between the solvent and the casein during all extractions.

TABLE 3

The influence of carbohydrates on the growth promoting properties of fats. The figures are the average number of grams gained in six weeks

Group	Carbohydrate	Fat	Gain	Number of animals
			gms.	
1	Lactose	Butter fat	203	6
2	"	Corn oil	179	6
		Difference	24	
3	Dextrose	Butter fat	212	6
4	"	Corn oil	220	6
		Difference	8	
5	Sucrose	Butter fat	223	6
6	"	Corn oil	229	6
		Difference	6	
7	Dextrin	Butter fat	241	6
8	"	Corn oil	251	6
		Difference	10	
9	Starch	Butter fat	229	3
10	"	Corn oil	235	3
		Difference	6	

Ten groups of six male rats (3 rats each in groups IX and X) were fed this diet *ad libitum* for six weeks with the carbohydrate and fat combinations as shown in table 3. These rats were all placed on the synthetic milk (described above) when 14 days of age, and thus were on a ration free of

[1] Phillips, P. H., and Hart, E. B. Jour. Biol. Chem., 109: 657. 1935.

[2] A fat-free water extract. One part equals twenty parts of whole fresh liver.

butter fat from the fourteenth until the twenty-first day of age. The average gain in grams for each group of rats is also indicated in table 3, and the relationship of these differences is graphically portrayed in figure 1. With lactose as the sole source of carbohydrate, the six male rats fed the butter fat diet gained on the average 24 grams more in six weeks than those rats fed the corn-oil-lactose ration. However, when the carbohydrate portion of the ration consisted of dextrose, sucrose, dextrin, or starch, the rats in the corn oil groups averaged 6 to 10 grams greater gain in six weeks than the comparable animals on the butter fat ration.

In all, 30 rats were raised on the lactose ration of which 15 were fed the butter fat and 15 the corn oil diets. All 30 were fed the artificial-corn-oil

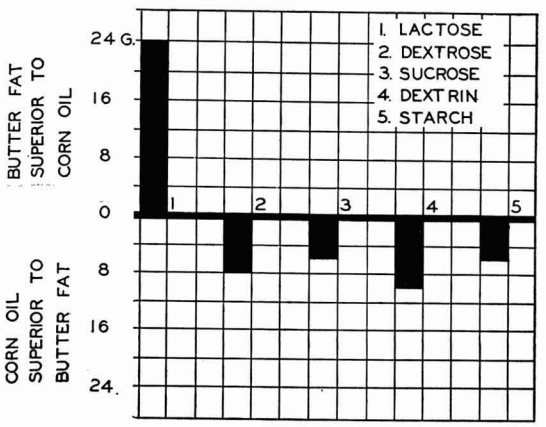

FIG. 1. The bars represent the difference in growth-promoting value of butter fat and corn oil on rations containing the carbohydrates as indicated.

milk from the fourteenth until the twenty-first day of age and then placed on experiment. The average total gain in grams at the end of six weeks was 197 for the animals fed the butter-fat-lactose ration and 168 for those fed the corn-oil-lactose ration, a difference of 29 grams.

DISCUSSION

The experiments reported here confirm our earlier work. The data indicate that growth in rats varies with the kind of fat fed when lactose is the sole carbohydrate in the ration. While digestibility determinations have not been made, this growth effect can not be related to any accepted digestibility values of the two fats involved. The linoleic acid content of the two fats tested is not responsible for the difference in nutritive value since corn oil contains a higher level of this essential nutrient than does butter fat. It is apparent that the explanation must lie in the existence in butter fat

of a specific compound or compounds which results in a more favorable physiology in the rats fed butter fat, to give a growth increment over those animals fed corn oil (and certain other vegetable oils (9)).

Restricting 14-day-old rats to an artificial milk, free of butter fat but containing 5 per cent of corn oil, until they were 21 days old was found to be an entirely feasible nutritional technique. Rats thus prepared had an average weight of 34 grams at 21 days, while their litter-mate controls which were allowed to suckle their mother through a normal 21-day period averaged 47 grams in weight. However, this difference was undoubtedly due to the abnormal environment and was largely overcome in the course of the six-week experimental period. In these studies with rats of various ages, the growth curves of the butter fat groups were quite similar no matter what the age (14, 21, or 30 days) at which the rat was removed from its normal environment. But in the case of the groups receiving corn oil, the slowest gain was made by the "14-day-old" animals. The growth of the "21-day-old" animals was only slightly better, while the growth of the "30-day-old" rats was markedly better, approaching that of the control group receiving butter fat. This indicates that stores of the growth promoting compounds are made by the nursing rat and by the rat fed the stock ration. Apparently it is difficult to so deplete a rat of these compounds that slowed growth results if the rat has been allowed a normal 21-day suckling period followed by about ten days' access to the stock ration. On an ordinary stock ration containing starch but not butter fat, synthesis of this factor by the rat undoubtedly occurs.

For the study of the nutritive value of fats, the ether-extracted-skim-milk powder is a more logical basal ration than the liquid-skimmed milk previously used. The fat is homogeneously mixed with the dry constituents in the case of the solid ration whereas it tended to separate out of the liquid diet so that the rats ate an abnormally high-fat diet soon after feeding time, leaving nearly skim milk to eat the rest of the day. The caloric intake is low per gram of ration ingested on the liquid diet as compared to the skim-milk-powder ration. This may in part account for the better growth obtained on the latter diet. Further, since the butter fat content of the skimmed milk was variable, usually between 0.04 and 0.08 per cent (0.5 per cent to 1.0 per cent of the total solids), one would expect the skim milk powder from which all the ether extractables had been removed to give a maximum differentiation between groups of rats fed butter fat and corn oil rations.

Certain similarities are apparent in the facts reported here using the rat as the experimental animal with the results found by Gullickson, *et al.* (6) using the calf. First, butter fat has been found to have a growth promoting value superior to certain vegetable oils. This difference is greater for both species at higher levels of fat in the diet. More marked differences in favor

of butter fat groups were found the younger the animal when placed on the experiment.

The peculiar relationship of lactose to the problem of the nutritional value of fats is well illustrated by the results of the experiments using synthetic-type rations. Only on the lactose ration did pronounced differences in weight occur between the groups fed butter fat and those fed corn oil, and in this case butter fat had superior growth promoting value. With all the other carbohydrates tested the difference in the growth promoting properties of the two fats was smaller and in favor of corn oil. From these facts it is evident that the variable nutritive value of the fats fed on the skim-milk-basal rations, either liquid or dry, is inherently related to the lactose content of the skimmed milk. It is to be noted that irrespective of the fat fed the lactose ration resulted in poorer growth than did rations containing any of the other carbohydrates, while the dextrin ration gave the greatest growth. However, when butter fat was fed in the lactose ration, the rate of growth did approach that obtained on rations containing the other carbohydrates. Thus it may be postulated that lactose depresses a certain beneficial intestinal flora which is present when dextrose, sucrose, dextrin, or starch is the carbohydrate of the ration. It is possible that the altered intestinal flora due to lactose feeding is unable to supply at an optimal rate certain fatty acids which are provided by the flora when other carbohydrates enter into the diet. Thus butter fat, or a fraction thereof (4, 11), may supply the deficiency due to its content of these acids, thereby effecting the metabolic processes of the animal directly. There is also the possibility that the ultimate compounds involved in these studies are entirely of floral origin, the butter fat merely complementing the lactose effect such that the beneficial flora may thrive, resulting in the intestinal syntheses of compounds, not necessarily fat soluble, comparable to those syntheses taking place on the other carbohydrates. In other words it seems that nature has put lactose and milk fat together as an optimum combination for the young animal.

SUMMARY AND CONCLUSIONS

1. Rats showed superior growth on butter fat as compared to corn oil when the sole carbohydrate in the diet was lactose. When the sole carbohydrate was dextrose, sucrose, dextrin, or starch the superiority of butter fat disappeared and corn oil gave rates of growth comparable or even slightly better than butter fat. The possible explanations of such results are discussed.

2. Ether extracted skim milk powder plus 35 per cent of fat as well as the synthetic type diet containing lactose as the sole carbohydrate were suitable rations for studying certain aspects of the nutritional value of fats.

3. Greater differences in the nutritive value of fats on a lactose containing diet were obtained the younger the rat at the start of the experiment.

However, from practical considerations, weanling rats 21 days of age may be used in these studies.

4. With the basal ration of ether-extracted-skim-milk powder, increasingly greater differences in the nutritive value of butter fat and corn oil resulted as the fat level was raised from 25 to 35 per cent of the diet.

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A MEASURE OF PERSISTENCY OF LACTATION IN DAIRY CATTLE[1]

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The variation in total milk production is influenced principally by three factors: 1) maximum initial production, 2) the persistency with which such yields are maintained, and 3) the length of the production period.

The importance of persistency in the economy of production has not been adequately investigated; however, sufficient evidence is available to justify the statement that, in general, cows having the highest degree of persistency are the most economical producers. Although several formulae for measuring persistency have been reported, none are entirely free from criticism. It is likely that no single formula can be so inclusive that it can apply equally well to all the different types or records that might be subjected to investigation. The formulae that have been reported may be classified into three groups. The first group includes the exponential curves which measure the rate of decline at any portion of the production period. The second group includes the regression coefficients which designate the slope of the lactation curve for only one portion of the period. The ratios of the production in one or more sections of the lactation to the production in different sections of the lactation constitutes the third type of formula.

REVIEW OF LITERATURE

Probably the first investigation dealing with persistency, although in an indirect manner, is that of Sturtevant (9). This work was designed primarily to measure the influence of time interval from calving on milk yields. Sturtevant found that the average decrease in production per month was approximately nine per cent. An average decline in production, however, does not explain many of the characteristics of the lactation curve itself since a large decline in production at the end of the period does not greatly influence the production for the entire lactation but contributes proportionately to the average decrease per month. It is therefore obvious that the mean decline in milk production determined by averaging the monthly percentage decline is not an accurate measure of persistency.

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Gaines (2, 3) has offered a rather complicated formula for measuring persistency. The exponential curve, $Y = Ae^{-kt}$ was fitted to the monthly yield of each lactation. The theoretical yield is represented by A, and the rate of change per month by k. The time factor is represented by t, and e is a constant. In addition to being too complex for average usage, it is also somewhat confusing in that a low k value designates high persistency and a high k value indicates low persistency. A similar formula has been derived by Gooch (4) which somewhat overcomes this criticism. He fitted the actual milk yields for the first 8 months of each lactation to the curve $y = Ae^{bt}$. In this formula, the factor e^b represents the degree of decline in production and a numerical increase in this factor designates improved persistency. Johansson and Hansson (5) have criticized this formula, as well as the one by Gaines, because they are not sensitive enough to measure sudden changes in the lactation curve.

Bonnier (1) and Kronacher (6) have used the linear regression coefficient of the first 13 weeks of lactation as a measure of persistency. Such a figure would be greatly influenced by the variable time required for different cows to reach peak production. In the event, that this factor is relatively constant in all of the lactations considered, the measure is still subject to criticism since it measures only the small portion of the lactation which usually shows comparatively little decline. Bonnier and Kronacher as well as others have suggested that a graphic presentation of the lactation curve is probably superior to any form of shape figure, numerical measure, index or ratio that can be devised.

More simple measures of persistency have been derived from the consideration of the ratios of the production in various portions of the lactation period. Sanders (7, 8) has used the ratio of the total production of the lactation to the maximum weekly production as a measure of decrease in yields and corrected for calving interval. If this measure can be properly corrected for some of the non-genetic influences, it is probably one of the simplest and most practical measures for persistency. According to Johansson and Hansson (5), Frederiksen and Ostergaard, and Terho have suggested measures very similar to the one proposed by Sanders.

The most extensive consideration has been given to persistency and causes of variations in milk and butterfat yields by Johansson and Hansson. In this detailed study, the authors have devised several practical measures of persistency. The lactations were divided into three 100-day periods. There are two measures which have been derived from these periods. The first is the ratio of the production in the second 100-day period to that in the first and is designated as $P_{2:1}$. This easily calculated ratio need not be corrected for calving interval, and it provides a practical means of expressing decline in production for the period considered. A similar measure is the ratio of the third period to that of the first. This measure of persistency

is designated by the symbol $P_{3:1}$. Although it is likewise easily calculated, it is necessary to correct this measure for calving interval before the results are comparable. Although neither of these measures express the continuous rate of change throughout the lactation period, they offer a practical means for measuring persistency in dairy cattle.

Turner (10) found that when all other conditions were uniform, the monthly milk or fat production during the lactation period, after maximum production has been reached, is a constant percentage of the preceding month. This observation tends to substantiate the assumption that the factors governing persistency are largely genetic.

DERIVATION OF THE FORMULA

The records used in this investigation were taken from the dairy herd at the University of Minnesota, St. Paul, Minnesota. Approximately 500 records of the Jersey, Holstein, and Guernsey breeds, made under standardized conditions, were considered. The actual milk yields were summarized from the barn records in 10-day intervals. Only the milk yields were considered in this study due to the fact that fat analyses were made irregularly and too infrequently to permit unbiased comparisons.

The lactation periods cover an interval of 368 days since most of the records were from cows on 365-day semi-official test. Various formulae, which have been suggested by other investigators, were applied to some of the records. It was necessary in all cases to rearrange the production records into intervals which suited the formula used. None of the formulae, however, seemed to utilize the available data to best advantage.

In order to get a visual concept of the nature of the entire production period, records of all cows made under standardized conditions were graphed. The graphs were arranged in the following order: 1) daughters of individual sires, 2) dams of daughters of individual sires, 3) consecutive lactations of all cows having more than one lactation, and 4) daughters of dams having 2 or more daughters. These graphs were very helpful in indicating the various characteristics and changes of the curve which must be considered in formulating any measure of persistency. It was observed from the graphs that the slope of the lactation curve during the first two months of production was the most variable portion of the curve.

The first consideration in the formulation of a persistency measure, therefore, was whether or not the portion of the lactation previous to peak production should be included. The association between the first two months' production and total production was found to be very low while any other two-month period gave comparatively higher correlations. These correlations, however, do not necessarily indicate the significance of the initial two-month production, but since no harmful effects could be anticipated if this period were eliminated, it was decided to disregard the first 48 days of production.

The remaining 320 days of the production period was first divided into 32 periods of 10 days each. In an attempt to evaluate the decline in yields, the production in the second 10-day period was divided by that in the first period, and in like manner the production in each subsequent interval was divided by the production in the preceding period. These fractions were summated and divided by 31, the number of ratios. After comparing the results of several calculations, it appeared that the fine division of the production period tended to overestimate the value of persistency. When these persistency values were compared to their respective graphs they did not seem to agree, so coarser divisions were tried. Eight divisions of the production period (320 days) gave values for persistency which seemed to agree fairly well with the graphs.

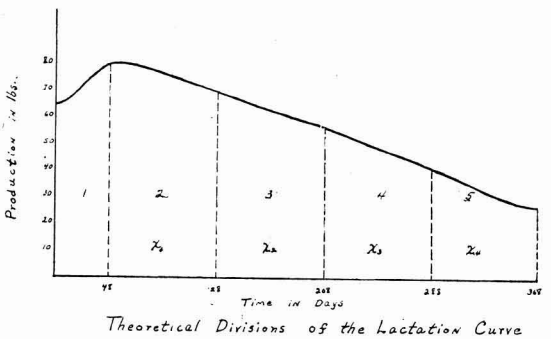

FIG. 1.

Another basis for division was also used. There appeared to be at least four physiological divisions for the lactation. Since the cows were not bred until four and one-half months following calving, the first 80-day period following peak production (48th–128th day) represents a portion of the lactation relatively free from individual depressing influences. A second period of 80 days would include the possible effects of conception and adjustment to pregnancy. A third 80-day period represents a division which is slightly influenced by pregnancy. The remaining 80 days include a period which is definitely influenced by advanced pregnancy and other complications and adjustments. The four divisions gave persistency values which compared favorably with estimations from the graphs of actual production. It was therefore decided to use four divisions of 80 days each in formulating a measure for the rate of decline in production.

Since the first 80-day period represents a portion of the curve which is comparatively free from individual physiological influences, it logically serves as a basis for comparison of yields in other periods. Thus the proportionate rate of decline in the first part of the lactation may be represented by the ratio in which the production in the second 80 days (128th to

208th) is divided by the production in the first 80 days (48th to 128th). Four divisions of the lactation permits three such ratios as the one just described. The relative rate of decline in the middle portion of the curve may be approximated by the ratio of the production in the third 80 days (208th to 288th) to that in the second 80 days (128th to 208th). The comparative decline in the last portion can likewise be represented by the ratio of the production in the fourth 80 days (288th to 368th) to that in the third period (208th to 288th). The three ratios that represent the comparative decrease in production for the first, middle and last portions of the lactation would be $\frac{X_2}{X_1}$, $\frac{X_3}{X_2}$ and $\frac{X_4}{X_3}$ respectively when X_1, X_2, and X_3 and X_4 represent the production in the four consecutive periods. Figure 1 illustrates the theoretical divisions of the lactation curve. If the values for each of the ratios are summated for a composite figure, the sum must necessarily be divided by three in order to keep the measures on a comparable basis.

It is questionable whether each ratio should contribute equally to the average persistency value. To determine the relative importance of each ratio presents a difficult problem. There are, however, some indications which favor the assumption that the three ratios contribute disproportionately to an average value. These indications are as follows: 1) the comparative levels of secretion are different during the three periods. The production considered in each of the three ratios was averaged and found to be in approximately the same ratio as 4:3:2, 2) correlation values between each of the three possible combinations of the ratios were markedly increased when they were multiplied by the factors 4, 3, and 2 respectively. Although these observations do not necessarily prove that the factors used are correct, they indicate that the ratios probably contribute in different proportions to the persistency value, and that the importance of each varies in approximately the ratio of 4:3:2.

After consideration of the above observations, it appeared advisable to multiply the ratios $\frac{(X_2}{X_1}$ $\frac{X_3}{X_2}$ $\frac{X_4)}{X_3}$ by the factors 4, 3, and 2 respectively. The denominator of the persistency figure would therefore become 9 instead of three.

The formula given below was derived from the previously discussed conditions concerning the lactation curve. The presentation of the formula in arithmetic terms should clarify any question which might arise concerning its function.

$$P = \frac{\frac{X_2\,(4)}{X_1} + \frac{X_3\,(3)}{X_2} + \frac{X_4\,(2)}{X_3}}{(4) + (3) + (2)}$$

or

$$P = 4/9\,\frac{X_2}{X_1} + 1/3\,\frac{X_3}{X_2} + 2/9\,\frac{X_4}{X_3}$$

Since the formula represents the summation of the ratios of the production in periods 2, 3, and 4 to that in periods 1, 2, and 3 respectively, multiplied by the constants, 4, 3, and 2 in like order and divided by the sum of the coefficients of the three ratios, the value of $\frac{X_2}{X_1}$ contributes 4/9, $\frac{X_3}{X_2}$ contributes 1/3 and $\frac{X_4}{X_3}$ contributes 2/9 of the total value of P.

While it is realized that this particular formula is most applicable to the type of records available for this study, the principles involved are basic for one method of evaluating decline in production during any lactation period. There are logical divisions for most lactations records, depending on the circumstances under which they are made. There may be records in which a larger or smaller number of divisions than 5 is warranted.

In order to make the general principles involved in the above formula more usable and applicable to different types of data, a general measure for persistency has been formulated in which any logical number of divisions of the lactation can be accommodated. Only two variables are involved: the number of divisions into which the curve is divided (n), and the production in any specific period (X_1 to X_n). The denominator of the fraction representing persistency included only one variable (n) and is merely another method of expressing partial factorials or the summation of consecutive figures within given limits. The formula is:

$$P = \frac{\frac{X_2 n}{X_1} + \frac{X_3(n-1)}{X_2} + \frac{X_4(n-2)}{X_3} + \cdots \frac{X_n(n-(n-2))}{X_{n-1}}}{n(n-1) - (n-1)\frac{(n-2)}{(2)}}$$

P – persistency.

X – (with the aid of subscripts) designates the production of any particular period.

n – the number of divisions into which the lactation is divided.

When compared to other measures the advantages of this formula are:

(1) The formula may be derived from lactation records of any given length. The number and length of the divisions of the lactation period is governed primarily by the calving interval.

(2) The latter stages of the lactation are included in the formula as well as those of the more productive portions. Some formulae include only the portions of the lactation directly following peak production. The decline in yields during this period is relatively small and nearly all cows show similar values for decrease during this period.

(3) The relative importance of the levels of production at various stages of the lactation period has also been taken into consideration. It is evident that some adjustment should be made for the decline in production toward

the latter part of the lactation period, because it does not have the same influence on total production as other portions of the lactation.

(4) The period of production prior to maximum yields (approximately 48 days) is excluded in order to eliminate the effects of variation in time to reach maximum production. Several factors, which are not genetic, tend to govern the production during the first few weeks of lactation, and the elimination of such a period is, therefore, indicated in a formula for persistency.

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SOME GENETIC ASPECTS OF PERSISTENCY IN DAIRY CATTLE[1]

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University of Minnesota

Only a limited number of investigations pertaining to the inheritance of persistency of milk and fat yields in dairy cattle have been reported, and there is considerable disagreement as to the causes of variance in persistency. All recognize non-genetic or environmental factors as playing an important role. Because of the heterogeneity of the data used in reported investigations, it is difficult to segregate the non-genetic from the genetic factors, a fact that has been emphasized by Johansson and Hansson (5).

On somewhat limited evidence Gaines (4) concluded that although environmental factors played an important part, persistency of milk yields is also affected by the genetic constitution of the cow. Also from similar evidence Becker and McGilliard (1) concluded that both the sire and the dam contribute to the persistency of their progeny. They also noted marked differences in the time required following calving to reach the maximum production—an important consideration in establishing measures of persistency. They found grades to reach maximum production sooner than registered Holstein and Jersey cattle. The possibility of some sex-linkage of factors for persistency is suggested in the work of Marchlewski (9) reporting evidence that some factors contributing to milk yield are sex-linked.

Johansson and Hansson (5) have shown that it is very difficult to determine a reasonably accurate estimation of the variance in persistency due to genetic factors, but they stated that if actual persistency could be measured with the same exactness as total yield it would, perhaps, show the same degree of hereditary determination. These workers believe that the present measures of persistency are not sensitive enough to detect or differentiate genetic and non-genetic variation, but that some of these indexes are suitable for practical use and serve a worth-while purpose. On the basis of the coefficient of intra-cow correlation and dam-daughter correlations, Johansson and Hansson estimate that 15 to 30 per cent of the total variance in persistency of yield as measured by their shape figures $P_{2:1}$ and $P_{3:1}$ or by the length of corrected dry period, is determined by heredity.

Csukas reports that the shape of the lactation curve is definitely inher-

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[1] Taken from data presented in a thesis submitted to the Graduate Faculty of the University of Minnesota by T. M. Ludwick in partial fulfillment of the requirements for the degree of Doctor of Philosophy. Prepared with the assistance of Work Projects Administration, official Project No. 166–1–71–124, Sub-project No. 447. Sponsor: University of Minnesota. Scientific Journal Series Paper No. 2037, Minnesota Agricultural Experiment Station.

ited but that three lactations are required to give a good estimation of the individual's true persistency curve. He also found that significant differences occur among consecutive lactations of the same cow; however, such differences were not as great as those occurring among different cows.

Kronacher (6) and Bonnier (2) have shown that the measure of persistency for consecutive lactations of the same cow are strikingly similar whereas the figures for different cows showed a wide range; the between-cow variations far exceeded the within-cow variations. Lortscher (7) and Nielsson (10) have shown that such non-genetic factors as age, season of calving, length of calving interval, level of nutrition, etc., have a definite effect on persistency of yields. The influence of these environmental factors on persistency and milk yields have been considered extensively by other workers and, for the most part, show good agreement.

EXPERIMENTAL PROCEDURE

In an effort to exclude as many non-genetic variables as possible, the data for this study include only records made under standard conditions, in the same herd. The data were taken from the dairy herd of the University of Minnesota, St. Paul, Minnesota. The records include one or more lactations from 130 registered Guernsey, Holstein and Jersey cows during the period 1919–1939. The standardized conditions which tended to eliminate many of the non-genetic factors influencing persistency are as follows:

(1) Heifers calving for the first time were not under one year and eleven months nor older than two years, three months.

(2) They were pregnant not sooner than four and one half months nor later than six months following the previous calving.

(3) All cows were milked the same number of times per day, and fed and managed in the same manner.

(4) All cows were fed hay and grain throughout the entire year as there was little or no pasture available.

It is obvious that these conditions should eliminate many of the environmental influences ordinarily affecting persistency; however, some non-genetic factors undoubtedly remain. Included in this group might be: 1) the differences in climatic conditions over a period of twenty years, 2) the differences in the quality of roughages and feed, 3) the variations in the qualifications of milkers from year to year, 4) the variations in conditions of the animal at time of first calving, 5) the differences between hand milking and machine milking. The last two conditions should have very little influence, however, since all the heifers were nearly the same size and age at calving, and only a very few records were made on machine milking. All lactations which met the standardized conditions were included in the investigations. The persistency index for each lactation was determined

according to method of Ludwick and Petersen (8). The data were analyzed from four different viewpoints in order to include as many hereditary influences as possible. The first observations include the various influences that the sire has on the persistency of his daughters. All sires having three or more daughters were included in the comparisons. The second analysis considers the importance of the dam on the persistency of her progeny. This influence was determined from several observations. The persistency values for the dams of daughters of individual sires were summarized. This division was made in order to determine the comparative influences of the dams and sires on the persistency of their progeny. Graphs and comparison of variability in persistency were also made of cows having two or more daughters by the same sire.

The third consideration includes some miscellaneous interacting factors which may be either genetic or environmental. In this group the influence of age and consecutive lactations are considered. The lactation curves of all cows having two or more lactations were superimposed on the same graphs for comparative purposes. The persistency values for all cows completing standard records were summarized by ages or lactations to determine the effects of development and age and its relation to persistency. Also included in this division is the correlation of persistency with maximum production and total production. The close association of persistency and total production is stressed. The influence of combining data from several different breeds is illustrated by the magnitude of such statistics as the coefficient of variability and the correlation coefficient.

The fourth phase of the study is devoted to the speculation or theorization as to the physiological basis for the inheritance of persistency.

DISCUSSION

Three facts have been summarized from these persistency values which indicate that persistency is definitely influenced by heredity. The first evidence is derived from the comparison of closely related and distantly related animals. The first group consists of comparisons of the persistency values of any two cows having the same dam and same sire. The variation in each group was estimated by the standard deviation of the differences between the persistency values. The second group includes cows having common dams but different sires, and the third group included cows having different dams but the same sire. The last group, which represents the least related animals, includes cows having neither parent in common. Table 1 should clarify the assumptions which may be drawn from the comparisons.

It is observed that the closely related animals show the least variations in persistency values and the most distantly related ones show the greatest variation. This would be expected if persistency were influenced largely

TABLE 1

The variation in persistency of closely related and distantly related animals

	Standard deviation of the difference between paired records
1. Cows havings the same dam and same sire	.052 ± .008
2. Cows having the same dam but different sires	.069 ± .007
3. Cows having different dams but same sires	.065 ± .005
4. Cows having different dams and different sires	.089 ± .002

by genetic factors. It is of interest to note that the variation in persistency of cows having either sire or dam, but not both, in common is very small. This fact would seem to indicate that both sire and dam contribute in about equal proportions to the persistency of their progeny. The method of comparing the performance of half-sisters, full-sisters, and non-related animals offers some promise in the study of inheritance in dairy cattle when sufficient data become available.

The second evidence for the inheritance of persistency is found in the similarity of consecutive lactations. Although there is a noticeable decline in persistency from the first to the second lactation, this decline is rather constant and can be predicted fairly well. Other investigators have also observed a close similarity among consecutive lactations.

A third evidence which indicates that persistency is influenced by heredity is found in the comparison of the average persistency values from the daughters of different sires when the influence of the daughter's dams is also considered. This evidence is summarized in table 2. It is apparent from this table that the sire has considerable influence on the persistency of his daughters. An average decrease of 20.1 per cent in persistency, as

TABLE 2

Comparison of persistency values between dams and daughters, arranged according to sires and breeds

Breed and No. of sire	Average persistency of dam	Average persistency of daughter	No. pairs	Increase in persistency	Decrease in persistency
				per cent	*per cent*
Guernsey					
3	0.885	0.836	9	.....	5.5
4	0.822	0.880	7	7.0	.....
Holstein					
5	0.873	0.798	5	.....	8.6
7	0.864	0.925	9	7.1	.....
8	0.910	0.927	6	1.8	.....
9	0.962	0.857	4	.....	10.9
10	0.923	0.808	4	.....	12.5
Jersey					
4	0.876	0.700	4	.....	20.1
5	0.740	0.842	4	13.8	.....
7	0.853	0.794	5	.....	6.9
8	0.852	0.830	4	.....	2.6

shown by Jersey sire No. 4, would not be expected unless the sire did contribute genetically to this character.

An important consideration in dairy production is the reliability of the first lactation in predicting future yields. Although there is good agreement among investigators concerning the decline in persistency following the first calving, table 3 is included in order to make comparisons with the other reports.

TABLE 3

Variations in persistency in different lactations

Lactation period	Mean persistency value	Standard error
1	0.876	0.008
2	0.781	0.013
3	0.773	0.016
4	0.735	Large
5	0.785	Very large
6	0.765	Very large

A second association which may add to the estimation of future performance is the relationship of persistency to total production. This association has been mentioned previously, but table 4 will serve to illustrate

TABLE 4

The average percentage of increase or decrease in persistency and total production of daughters above or below that of their dams for individual sires

No. of sire	Ave. increase in persistency of daughters over dams	Ave. decrease in persistency of daughters over dams	Ave. increase in production of daughters over dams	Ave. decrease in production of daughters over dams
	per cent	*per cent*	*per cent*	*per cent*
5	.....	6.6	.....	15.1
7	7.1	.....	11.4	.....
8	1.8	.....	.....	4.0
9	.....	10.9	.....	27.4
10	.....	12.5	.....	18.3

what might be expected in production when the persistency is known. Only the data for the Holstein breed has been included in this table due to the fact that only a limited number of Jersey and Guernsey records were available for this particular comparison. The Jersey and Guernsey records which are not listed did not, however, exhibit as close association between production and persistency as the Holstein records.

In the five Holstein sires considered, only one sire (No. 8) shows an inverse relationship between persistency and production. The remaining four show a high positive correlation between the two characters. Although the number of sires and comparisons considered in this portion of the investigation is relatively small the results would seem to indicate that the association of production and persistency is more likely a curvilinear than a linear function.

Although the daughters of Holstein sire No. 8 were more persistent than their dams, their average production was four per cent less. Sire No. 5, however, shows a 6.6 per cent decrease in persistency and a 15.1 per cent decrease in production of his daughters when compared to their dams. A possible range of four to five per cent might therefore be allowed for decrease or increase in persistency within the Holstein breed before any assumptions may even be considered for predicting production.

A third factor which affects the predictions of future performance is the variability among the different breeds. Many estimates of performance such as the mature equivalent factors, are based on data collected from several breeds. If these factors are to be applied to grade and "pure bred" cattle of all breeds, it seems likely that this might be a source of error. Data which include records from several breeds are subject to more variation than those including only one breed. This variation tends to limit the significance of statistics and results derived from such sources. Table 5 has been included to illustrate the variation among breeds relative to persistency and its association with maximum and total production.

TABLE 5

Differences among different breeds relative to persistency and production, and the influence of combining records from several breeds

	r_{xy}	r_{xz}	r_{yz}	cv_x	cv_y	cv_z
Guernsey	0.906 ± 0.04	0.00	0.372 ± 0.16	16.6	16.7	9.9
Holstein	0.902 ± 0.03	0.042	0.466 ± 0.09	18.8	19.9	8.6
Jersey	0.603 ± 0.08	0.052	0.509 ± 0.15	19.4	19.9	10.8
Combined Guernsey Holstein Jersey	0.969 ± 0.008	0.345 ± 0.06	0.471 ± 0.06	37.2	39.4	10.5

r = correlation coefficient.
x = maximum production.
y = total production.
z = persistency.
CV = coefficient of variation.

It is of interest to note that the percentage of variation is greatest for maximum and total production and least for persistency in all breeds. The percentage of variability for any of the free factors is relatively constant within any one breed, but when the breeds are combined, the per cent of variation is so great that little confidence can be placed in statistics derived from such data unless corrections are made to account for a portion of the variation or unless the statistics are of sufficient magnitude to offset such a variation. That the persistency values are the least affected by combining records of several breeds would seem to indicate that the formula used for persistency applies equally well to all breeds and is not greatly affected by level of production.

The analysis of numerous records eventually leads to the speculation of the primary action of the inherited factors which govern such complex

characters as milk production and persistency. It is likely that the genetic factors or genes which are basically responsible for these characters are those which govern the development and rate of function of various glands and organs of the body and also the extent of interdependence or interaction of such organs and glands. With the obvious complexity which may exist in the inheritance of milk production and persistency, it is easy to understand how the variation in these characters can be very large even when the non-genetic factors are kept as nearly uniform as possible.

It is well established that in most mammals the endocrine glands play an important role in mammary development and milk secretion, although the exact function of the endocrines for production in the bovine is not yet completely understood.

In consideration of the known function of the endocrine glands in milk secretion, there are several observations made from the data discussed in various portions of this investigation which might tend to support the supposition that a major part of the variation in persistency is a result of the inheritance of factors or genes, which govern the development and rate of function of various endocrine glands, and the interaction or interdependence of such glands.

The observations which tend to support such an assumption are rather indirect, however, three factors which may partially substantiate the suggested assumptions concerning persistency are:

(1) The age of the cow seems to have a definite influence on the maintenance of milk yields. It has been shown that the younger animals tend to have higher persistency values than older ones. Following the first calving there seems to be an adjustment to the different hormones which have probably functioned completely for the first time. Probably the most important of these is prolactin. This hormone is evidently necessary for continued lactation, and persistency seems to be highly correlated with prolactin secretion.

There appears to be no unquestioned explanation for the decrease in persistency with age. There is, however, some speculation which is entirely within reason. Assuming that prolactin secretion is one of the limiting factors in production, there may be a possible excess of this hormone in first lactation due to the limitation of production because of the size of the udder. With advanced age and pregnancies, the size of the udder is not the limiting factor but the secretion of prolactin may be. This theory would necessarily assume that secretion of prolactin is fairly constant throughout the life of the individual. At first calving the size of the udder is the limiting factor for production. During the subsequent lactations prolactin would be the limiting factor. The fact that age alone, regardless of lactation, causes a decrease in persistency does not seriously interfere with the theory because animals that calve at older ages have comparatively

larger udder development. The theory would, however, necessitate the abandonment of the idea that almost complete udder development, both alveolar and duct, takes place during the first five months of pregnancy.

(2) Increased frequency of milking also influences persistency. There is a possibility of extensive speculation as to the reasons for this action. The increased frequency of milking in heavy producing animals is usually thought to increase production by relieving excessive pressure within the gland and permitting further secretion. The production during advanced lactation, in which yields are comparatively low, has also been increased by increasing the frequency of milking. This observation might suggest that frequent stimulation of the cow to let down milk has a favorable effect on the production of prolactin. There might also be a close association of the oxytocic principle (pitocin) and prolactin.

(3) One of the most important non-genetic factors in persistency is the length of the calving interval. This factor is entirely dependent on the reproductive efficiency of the cow and the management of the herd. Very soon after conception there is usually a slight change in milk production. This may be the result of adjustment to the effect of the retention of the corpus luteum of pregnancy and other complicating conditions, that arise at such a time. The conditions associated with advanced stages of pregnancy, and particularly after the first 20 weeks following fertile service, are definitely inhibitory to production. The increased blood supply to the growing fetus may somewhat limit or inhibit the supply to the mammary gland and thereby reduce milk secretion. Placental hormones may also complicate the lactation yields. Although the endocrine glands play a very important role in most of the characters concerned in milk production, the interaction of these glandular products is extremely difficult to determine. When the endocrine influences are more specifically understood, it is very likely that the inheritance of production and persistency in dairy cattle will be much better comprehended.

SUMMARY

Three facts have been given which support the belief that persistency of lactation in dairy cattle is an inherited characteristic: 1) the variation in persistency values is much greater among non-related animals than among closely related ones, 2) most animals tend to keep their characteristic lactation curves throughout their productive period, 3) the influence of the sire or dam is easily detected in their progeny.

The first lactation usually gives a fair indication of future performance. The persistency values for the second lactation are usually about 10 per cent lower than the first (as measured by the formula

$$P = 4/9\,\frac{X_2}{X_1} + 1/3\,\frac{X_3}{X_2} + 2/9\,\frac{X_4}{X_3})$$

and subsequent lactations are very similar to the second.

Total production and persistency are closely correlated; however, some variation in production must be allowed before attempting to predict production from persistency values alone. The association of total production and persistency is probably of a curvilinear nature rather than linear.

The combining of data from several different breeds for the purpose of studying inherited factors in dairy cattle is usually an unreliable procedure. The variation in persistency, however, seems to be the least affected by combining data from several breeds.

It is postulated that a major portion of the variation in persistency is probably the result of the inheritance of factors or genes which govern the development and rate of function of various endocrine glands, and the interaction and interdependence of such glands, or the inherited or acquired ability of various tissues to respond to various glandular secretions. The mode of inheritance is undoubtedly complex.

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PROGRAM

THIRTY-EIGHTH ANNUAL MEETING

OF THE

AMERICAN DAIRY SCIENCE ASSOCIATION

UNIVERSITY OF MISSOURI
COLUMBIA, MISSOURI
JUNE 22–24, 1943

PROGRAM COMMITTEE

MANUFACTURING

P. F. SHARP, Golden State Company, Ltd. (*Chairman*)
O. F. GARRETT, New Jersey
G. C. NORTH, Beatrice Creamery Co.

EXTENSION

E. C. SCHEIDENHELM, Michigan (*Chairman*)
G. G. GIBSON, Texas
M. J. REGAN, Missouri

PRODUCTION

K. L. TURK, Maryland (*Chairman*)
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H. F. JUDKINS, Sealtest, Inc. (*Chairman*)
P. F. SHARP, Golden State Company, Ltd.
K. L. TURK, Maryland
E. C. SCHEIDENHELM, Michigan
A. C. RAGSDALE, Missouri

REGISTRATION

TIGER HOTEL AND ECKLES HALL

Monday, June 21 —2:00 P.M.– 9:00 P.M. —Tiger Hotel
Tuesday, June 22 —8:30 A.M.–12:00 noon—Tiger Hotel
1:00 P.M.– 5:00 P.M. —Eckles Hall
Wednesday, June 23—8:30 A.M.– 5:00 P.M. —Eckles Hall
Thursday, June 24—8:30 A.M.– 5:00 P.M. —Eckles Hall
Information Booths at Tiger and Daniel Boone Hotels daily.

COMMITTEE MEETINGS

ROOM ASSIGNMENTS

Suitable rooms will be available for all committees, and for other groups which may desire to meet, including special breakfasts, etc.

Reservations of committee rooms and posting of schedules for meetings should be made with H. A. Herman. Committee meetings and rooms will be posted at places of registration.

The following committee meetings have been scheduled. Others may be scheduled later.

Production Section

The following committees are requested to meet at 1:15 P.M. on Tuesday, June 22:

Breeds Relations. H. A. HERMAN, *Chairman*

Dairy Cattle Breeding. E. J. PERRY, *Chairman*

Measuring Results of Pasture Investigations. R. H. LUSH, *Chairman*

Feed Specifications. E. S. SAVAGE, *Chairman*

Silage Methods and Evaluations. C. B. BENDER, *Chairman*

If additional time for committee meetings is needed, these will be held 8:30–9:30 A.M., Wednesday, June 23.

SCHEDULE OF PROGRAM (Central War Time)

Time	General	Extension Section	Production Section	Manufacturing Section
Tues., June 22				
10:00–12:00	Opening Session			
12:15– 1:15	Lunch			
1:15– 2:30	Committees	Committees	Committees	Committees
2:30– 4:30		Joint Symposium		Symposium
4:30– 5:30	Tour—University Campus, Dairy Laboratories and Barn			
Evening 8:30	Reception			
Wed., June 23				
8:30– 9:30	Committees	Committees	Committees	Committees
9:30–11:45		Business and Reports	Papers (Div. A and B)	Symposium
11:45–12:00	Group Picture			
12:15– 1:15	Lunch			
1:30– 3:30		Committee Reports	Papers (Div. A and B)	Papers
3:30– 5:00		Business	Business	Business
Evening 6:30	Lakeside Supper			
8:30	Entertainment			
Thurs., June 24				
8:00– 9:00	Committees	Committees	Committees	Committees
9:00–11:00		Joint Symposium		Papers
11:00–12:00		Business	Business	Business
12:15– 1:15	Lunch			
1:30– 3:30	Joint Symposium—All Sections			
3:30– 5:00	Business			
Evening 6:30	Association Banquet			

PROGRAM FOR WOMEN

Tuesday, June 22

8:30 P.M.—Reception.

Wednesday, June 23

3:00 P.M.—Tea.
6:30 P.M.—Lakeside supper, Stephens College Lake.
8:30 P.M.—Entertainment.

Thursday, June 24

12:15 P.M.—Luncheon.
6:30 P.M.—Banquet.

Special features may be added to the women's program. Women are particularly invited to attend the opening session of the General Program. They also will be welcome at any of the Section Programs.

FOR THE CHILDREN

Supervised swimming, boating, picnics, playground and other entertainment will be provided.

GENERAL PROGRAM*

(Central War Time)

Tuesday, June 22, 1943

10:00–12:00 OPENING SESSION. J. H. NEFF HALL, *Room 204.*

Call to Order—ARTHUR C. RAGSDALE, *Chairman, Department of Dairy Husbandry, University of Missouri.*

Introductions—Officers of American Dairy Science Association and Guests. H. P. DAVIS, *President.*

Addresses of Welcome—FREDERICK A. MIDDLEBUSH, *President University of Missouri.*

M. F. MILLER, *Dean of the College of Agriculture and Director of the Agricultural Experiment Station, University of Missouri.*

Response and Address—H. P. DAVIS, *President, American Dairy Science Association.*

Guest Speaker—GEORGE E. HOLM, *Bureau of Dairy Industry, U.S.D.A.*

* All meeting rooms, entertainment, luncheon and banquet arrangements will be announced in special program mailed before meetings or at time of registration.

Announcements.

12:15– 1:15 **Lunch hour.**
1:15– 2:30 **Committees**—*Eckles Hall.*
4:30– 5:30 **Tour**—*University Campus, Dairy Laboratories and Barn.*
Evening 8:30 **Reception.**

Wednesday, June 23, 1943

8:30– 9:30 **Committees**—*Eckles Hall.*
11:45–12:00 **Group picture**—*Eckles Hall.*
12:15– 1:15 **Luncheon**—*University Farm Campus.*
Evening 6:30 **Lakeside Supper**—*Stephens College Lake.*
8:30 **Entertainment.**

Thursday, June 24, 1943

8:00– 9:00 **Committees**—*Eckles Hall.*
12:15– 1:15 **Luncheon**—*University Farm Compus.*
1:30– 3:30 **Symposium**—H. F. JUDKINS, *presiding.*

Utilization of the Milk Supply

1. Findings of Committee on Milk of the Food and Nutrition Board of the National Research Council. W. E. KRAUSS, Ohio Agricultural Experiment Station.
2. Will the Pigs, Chickens and Calves Miss the Skim Milk? L. A. WEAVER, *University of Missouri.*
3. The Past, Present and Future of Dry Milk. ROUD MCCANN, *Secretary, American Dry Milk Institute, Chicago.*
4. The Butter Industry's Part in a Milk Utilization Program. H. A. RUEHE, *Executive Secretary, American Butter Institute, Chicago.*
5. How It Has Been Done. W. T. CRIGHTON, *Producers Creamery, Springfield, Missouri.*

3:30– 5:00 **General Association Meeting.**
Evening 6:30 **Association Banquet—Presentation of Borden Awards.**

First Christian Church, Columbia, Mo.

SECTIONAL PROGRAMS

EXTENSION SECTION

June 22–24

Exhibits—Display of Extension Teaching Ideas.

Tuesday, June 22

1:15– 2:30 P.M.—Committee Meetings.

2:30– 4:30 P.M.—**Symposium.**

War-Time Problems in Dairy Cattle Feeding
(Joint Session of Extension and Production Sections)

See Production Section Program

4:30– 5:30 P.M.—**Tour—University Campus, Dairy Laboratories and Barn.**

Wednesday, June 23

8:30– 9:30 A.M.—Committee Meetings.

9:30–11:45 A.M.—**Business Session.** J. F. KENDRICK, *Chairman.*

Announcements and appointments of committees

Testing Committee Report

E. H. LOVELAND, *Chairman of Committee (In Charge)*
Panel Discussion "War-Time D.H.I.A. Adjustments"

A.	**Supervisors.**	
	1. Religious Objectors.	C. R. GEARHART, Pa. State.
	2. Women.	J. B. PARKER, Bureau of Dairying.
	3. Vocational Agricultural Boys.	RALPH F. EVANS, Univ. of Vermont.
B.	**Forms of Testing.**	
	1. Bi-Monthly.	E. C. SCHEIDENHELM, Mich. State.
	2. Combining Associations.	FLOYD JOHNSTON, Iowa State.
	3. Curtailed Calculations.	
C.	**Administering Large Testing Units.**	A. J. CRAMER, Univ. of Wisc.
D.	**Discussion.**	

1:30– 3:30 P.M.—Committee Reports.

Exhibit Committee Report

A. R. PORTER, *Chairman of Committee (In charge)*

A. Discussion of Exhibits by States.
B. Recommendations of Committee.

Type Rating Committee Report

E. C. SCHEIDENHELM, *Chairman of Committee (In charge)*

A. Report and Recommendations of Committee.

Sire Committee Report

E. J. PERRY, *Chairman of Committee (In charge)*

A. Some factors influencing success in field artificial insemination. I. F. ELLIOTT, G. W. SALISBURY AND S. J. BROWNELL, Cornell University.

B. Reciprocal use of D.H.I.A. and herd improvement registry records. J. F. KENDRICK, Bureau of Dairy Industry and C. A. HUTTON, University of Tennessee.

C. Use of incomplete records of sires and dams. G. A. BOWLING, Univ. of West Virginia and WARREN GIFFORD, Univ. of Arkansas.

Breeds Relations Committee Report

FLOYD JOHNSTON, *Iowa State College (In charge)*

A. Report of the Committee.

Dairy Cattle Health Committee Report

C. G. BRADT, *Chairman of Committee (In charge)*

A. Report of the Committee.

3:30– 5:00 P.M.—Business Session.

Thursday, June 24

8:00– 9:00 A.M.—Committee Meetings.

9:00–11:00 A.M.—**Symposium.**

Measuring Transmitting Ability in Dairy Sires and Cows

(Joint Session of Extension and Production Sections)

See Production Section Program

11:00–12:00 A.M.—Business Session. J. F. KENDRICK, *Chairman.*

1:30– 3:30 P.M.—**Symposium.**

Utilization of the Milk Supply

(Joint Session of Extension, Production and Manufacturing Sections)

See General Program

3:30– 5:00 P.M.—**Business Meeting of Association.**

PRODUCTION SECTION

Tuesday, June 22

1:15– 2:30 P.M.—Committee Meetings.

2:30– 4:30 P.M.—**Symposium.** E. S. SAVAGE, *Cornell University, Presiding.*

War-Time Problems in Dairy Cattle Feeding

(Joint Session of Production and Extension Sections)

A—Program of the feed industry and Government for conserving feed supplies. J. A. MCCONNELL, *Chairman of Feed Industry Council.*

B—Supply, needs and recommendations for meeting mineral and vitamin requirements of dairy cows under present conditions. C. F. HUFFMAN, *Michigan State College.*

C—General feed supplies with recommendations on how to meet protein shortages in dairy feeding. F. B. MORRISON, *Cornell University.*

D—What feeding problems are most acute? (Brief discussion by college representatives from various sections of country.)

E—Discussion.

4:30– 5:30 P.M.—Tour—University Campus, Dairy Laboratories and Barn.

Wednesday, June 23

8:30– 9:30 A.M.—Committee Meetings.

9:30–11:45 A.M.—Section Papers—See Divisions A and B.

1:30– 3:30 P.M.—Section Papers—See Divisions A and B.

3:30– 5:00 P.M.—Business Session.

Thursday, June 24

8:00– 9:00 A.M.—Committee Meetings.

9:00–11:00 A.M.—**Symposium.** WARREN GIFFORD, *University of Arkansas, Presiding.*

(Joint Session of Production and Extension Sections)

A—Methods available for measuring transmitting ability in sires and cows. J. L. LUSH AND R. H. NELSON, *Iowa State College.*

B—Program of the purebred dairy cattle association for uniform methods of proving sires and brood cows. H. W. NORTON, JR., *Secretary, Purebred Dairy Cattle Association.*

C—Proving sires and brood cows under field conditions and the part that artificial insemination can play in the program. S. J. BROWNELL, *Cornell University.*

D—International post-war cooperation in dairy cattle breeding. E. J. PERRY, *Rutgers University.*

E—Discussion.

11:00–12:00 A.M.—Business Session.

1:30– 3:30 P.M.—**Symposium.**

Utilization of the Milk Supply

(Joint Session of Extension, Production and Manufacturing Sections)

See General Program

3:30– 5:00 P.M.—Business Meeting of Association.

PRODUCTION SECTION—DIVISION A

Wednesday, June 23

9:30–11:45 A.M.

K. L. TURK, *Chairman*

Feeding

P1—Influence of quality of protein in the concentrate mixture on the milk production of dairy cows fed mixed hay and corn silage as roughages. R. W. BRATTON, G. W. SALISBURY AND F. B. MORRISON, *Cornell University.*

P2—Some factors affecting biological value of proteins for growing dairy heifers. E. W. SWANSON, H. A. HERMAN AND A. C. RAGSDALE, *University of Missouri.*

P3—Urea as a protein substitute in war time. I. W. RUPEL, G. BOHSTEDT AND E. B. HART, *University of Wisconsin.*

P4—Balance studies on milking cows fed molasses grass silage and phosphoric acid grass silage. W. A. KING, *New Jersey Agricultural Experiment Station.*

P5—Chemical analysis and silage quality. A. E. PERKINS, *Ohio Agricultural Experiment Station.*

P6—The fermentation in legume silage with wilting and with corn and cob meal as a preservative. R. W. STONE, F. R. MURDOCK AND S. I. BECHDEL, *Pennsylvania State College.*

P7—Citrus molasses—a new feed. R. B. BECKER, P. T. DIX ARNOLD, G. K. DAVIS AND E. L. FOUTS, *Florida Agricultural Experiment Station.*

P8—Value of prairie hay for milk production. A. H. KUHLMAN, *Oklahoma Agricultural Experiment Station.*

P9—Alyce clover hay vs. lespedeza hay for milking cows. D. M. SEATH, CECIL BRANTON AND L. L. RUSOFF, *Louisiana Agricultural Experiment Station.*

P10—Protein supplements for dairy calves. T. W. GULLICKSON AND RAYMOND HANSON, *University of Minnesota.*

1:30–3:30 P.M.

Minerals and Vitamins

P11—Mineral studies of Louisiana dairy cows. I. The hemoglobin, calcium and phosphorus analyses of blood. L. L. RUSOFF AND D. M. SEATH, *Louisiana Agricultural Experiment Station.*

P12—Study of poikilocytosis in dairy cattle (a preliminary report). J. T. REID, C. F. HUFFMAN AND C. W. DUNCAN, *Michigan State College.*

P13—Cobalt studies with dairy cattle in Michigan. C. W. DUNCAN, C. F. HUFFMAN, J. T. REID AND B. J. KILLHAM, *Michigan State College.*

P14—The effect of parturition and beginning lactation on the level of carotene and vitamin A in the blood plasma of dairy cows. T. S. SUTTON AND P. A. SOLDNER, *Ohio Agricultural Experiment Station and Ohio State University.*

P15—Carotene requirements for the maintenance of a normal spinal fluid pressure in dairy calves. L. A. MOORE AND M. H. BERRY, *University of Maryland,* AND J. F. SYKES, *Michigan State College.*

P16—The effect of additional vitamins A and D in a standard calf starter. E. A. KEYES, S. I. BECHDEL, AND W. J. S. THORPE, *Pennsylvania State College.*

P17—Changes in carotene content of prairie hay during storage. A. H. KUHLMAN AND W. D. GALLUP, *Oklahoma Agricultural Experiment Station.*

P18—Factors affecting the vitamin D and carotene content of alfalfa hay. G. C. WALLIS, *South Dakota Agricultural Experiment Station.*

P19—Urinary excretion of ascorbic acid in dairy cattle (a preliminary report). J. W. COTTER AND L. A. MOORE, *University of Maryland.*

PRODUCTION SECTION—DIVISION B

Wednesday, June 23

9:30–11:45 A.M.

DWIGHT ESPE, *Chairman*

Hormones

P20—The effect of thyroidectomy upon growth, appearance, general behavior, and reproduction in the bovine. A. A. SPIELMAN, *State College of Washington,* AND W. E. PETERSEN AND J. B. FITCH, *University of Minnesota.*

P21—The effect of thyroidectomy upon the lactation of the bovine. A. A. SPIELMAN, *State College of Washington,* W. E. PETERSEN AND J. B. FITCH, *University of Minnesota.*

P22—The hormonal preparation of dairy cows for lactation. R. P. REECE, *New Jersey Agricultural Experiment Station.*

P23—Stilbestrol as a stimulator and inhibitor of lactation. C. W. TURNER AND JOSEPH MEITES, *University of Missouri.*

P24—The influence of diethylstilbestrol dipropionate on the lactating mammary gland of the cow. R. P. REECE AND J. M. MURPHY, *New Jersey Agricultural Experiment Station.*

P25—The effect of pitocin on the lypolitic activity of mammary gland tissue and of milk. P. L. KELLY, *University of Arkansas.*

P26—The effect of thyroprotein on the growth rate of experimental animals. MARVIN KOGER AND C. W. TURNER, *University of Missouri.*

P27—Practical trials on the use of synthetic thyroprotein for increased production of milk and butterfat. E. P. REINEKE, *University of Missouri.*

P28—Experimental stimulation of mammary gland growth in female goats. JOHN P. MIXNER, *University of Missouri.*

1:30–3:30 P.M.

Health and Breeding

P29—The action of the milking machine in relation to milking and udder injury. W. E. PETERSEN, *University of Minnesota.*

P30—Further studies on the nutritional control of milk fever. W. E. KRAUSS, C. F. MONROE, J. W. HIBBS, AND T. S. SUTTON, *Ohio Agricultural Experiment Station.*

P31—The development of ketosis in dairy cattle. J. C. SHAW, *University of Connecticut.*

P32—The value of kelp meal in rations for dairy cattle. M. H. BERRY AND K. L. TURK, *University of Maryland.*

P33—Artificial breeding as a means of controlling genital infections in the dairy herd. H. A. HERMAN, E. R. BEROUSEK, AND E. W. SWANSON, *University of Missouri.*

P34—The effect of dilution rate on the liveability and the fertility of bull spermatozoa used for artificial insemination. G. W. SALISBURY, G. H. BECK, P. T. CUPPS, AND IRVINE ELLIOTT, *Cornell University.*

P35—Acid resistance in relation to longevity of bovine spermatozoa. SOLOMON MARGOLIN, O. L. LEPARD, AND J. W. BARTLETT, *New Jersey Agricultural Experiment Station.*

P36—Breeding efficiency in dairy cattle at various stages of estrus by artificial insemination. GEORGE W. TRIMBERGER AND H. P. DAVIS, *University of Nebraska.*

MANUFACTURING SECTION

Tuesday, June 22

1:15– 2:30 P.M.—Committee Meetings.

2:30– 4:30 P.M.—**Symposium,** H. F. JUDKINS, *Sealtest, Inc. Presiding.*

Dairy Technology Problems Resulting from War Restrictions

A—Significant sanitary regulations in relation to milk industry during wartime. H. J. BRUECKNER, *Cornell University.*

B—Whipping cream of low fat content. H. H. SOMMER, *University of Wisconsin.*

C—Butter stretchers and spreads. L. K. RIGGS, *Kraft Cheese Company, Chicago.*

D—Butter oil and its stability. G. E. HOLM, *U. S. Bureau of Dairy Industry.*

E—Evaporated milk of high solids content. B. H. WEBB, *U. S. Bureau of Dairy Industry.*

4:30– 5:30 P.M.—Tour—University Campus, Dairy Laboratories and Barn.

Wednesday, June 23

8:30– 9:30 A.M.—Committee Meetings.

9:30–11:45 A.M.—**Symposium,** R. WHITAKER, *Sealtest, Inc. Chairman.*

War Restrictions in Relation to Ice Cream

A—War restrictions applied to ice cream. C. A. IVERSON, *Iowa State College.*

B—Sweetening agents. B. E. HORRALL, *Purdue University.*

C—Body building on restricted milk solids. P. H. TRACY, *University of Illinois.*

D—Flavoring materials under war restrictions. E. C. SCOTT, *Swift and Company, Chicago.*

E—Ices and sherbets. J. H. ERB, *Ohio State University.*

1:30– 3:30 P.M.—Section Papers.

R. WHITAKER, *Chairman*

M1—Studies on the fat globule clustering phenomenon in milk. H. H. SOMMER, *University of Wisconsin.*

M2—Effect of pasteurization times and temperatures on certain properties of cream and milk. J. C. HENING, *New York State Agricultural Experiment Station.*

M3—Chlorine resistance of Pseudomonas putrefaciens. H. F. LONG AND B. W. HAMMER, *Iowa State College.*

M4—Isolation of Bacterium linens. OSCAR ALBERT, *Quebec Dairy School.*

M5—Significance of the coliform group of bacteria in American Cheddar cheese. M. W. YALE, *New York State Agricultural Experiment Station.*

M6—The quantitative determination of lactose-fermenting yeasts in sour cream. E. R. GARRISON, *University of Missouri.*

M7—Mold mycelia retained by butter, buttermilk and wash water during manufacture of butter. P. R. ELLIKER, *Purdue University Agricultural Experiment Station.*

M8—The preservation of sweet milk and cream on the farm. R. N. DAVIS, *University of Arizona.*

3:30– 5:00 P.M.—Business Session.

Thursday, June 24

8:00– 9:00 A.M.—Committee Meetings.

9:00–11:00 A.M.—Section Papers.

R. WHITAKER, *Chairman*

M9—Relation of the feed of the cow and various storage conditions to the types and amounts of carotenoid pigments in milk fat. O. F. GARRETT, *New Jersey State Agricultural Experiment Station.*

M10—The alkalinity of milk ash and its relation to the detection of neutralizer in dry milk. R. W. KUNKEL AND W. B. COMBS, *University of Minnesota.*

M11—Observations on the use of condensed and powdered sweet cream buttermilk in ice cream. E. L. THOMAS AND W. B. COMBS, *University of Minnesota.*

M12—Difficulties encountered in pasteurizing high acid milk for cheesemaking. S. T. COULTER AND W. B. COMBS, *University of Minnesota.*

M13—The influence of the composition of skim milk on the yield and quality of cottage cheese. H. C. OLSON, *Oklahoma Agricultural and Mechanical College.*

M14—Rate of salt migration in cheddar cheese curd. W. H. HOECKER AND B. W. HAMMER, *Iowa State College.*

M15—Fat acidities of cheddar cheese made with added lipase. F. J. BABEL AND B. W. HAMMER, *Iowa State College.*

M16—Preliminary studies of the fatty acids involved in lipase action in milk. I. A. GOULD, *Michigan State College.*

M17—Vitamin B complex preparations as an antioxidant in milk and milk products. N. P. TARASSIK, *University of California.*

11:00–12:00 A.M.—Business Session.

1:30– 3:30 P.M.—**Symposium.**

Utilization of the Milk Supply

(Joint Session of Extension, Production and Manufacturing Sections)

See General Program

3:30– 5:00 P.M.—Business Meeting of Association.

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CONTENTS

THE SCIENCE PRESS PRINTING COMPANY LANCASTER, PA.

PUBLICATIONS AND ABSTRACTORS

EDITORS

Dahle, C. D., Dahlberg, A. C., Elliker, P. R., Petersen, W. E., Tracy, P. H. and Weckel, K. G.

ABSTRACTORS

Archibald, J. G.
Babcock, C. J.
Bennett, F. W.
Berggren, Ruth E.
Brueckner, H. J.
Burgwald, L. H.
Bushnell, L. D.
Cole, W. C.
Cone, J. F.
Coulter, S. T.
Doan, F. J.
Dorsey, L. M.
Downs, P. A.
Ely, Fordyce
Erb, J. H.
Espe, D. L.
Frazier, W. C.
Fuller, S. A.
Garrett, O. F.
Glick, D. P.
Goss, E. F.
Horrall, B. E.
Huffman, C. F.
Irvine, O. R.
Josephson, D. V.
Lucas, P. S.
Lush, J. L.
Marquardt, J. C.
Martin, W. H.
Mueller, W. S.
Price, W. V.
Pyenson. Harry
Reece, Ralph P.
Stebnitz, V. C.
Thomsen, L. C.
Trout, G. M.
Webb, B. H.
Weckel, K. G.
Weiser, H. H.
White, G. C.
Yale, M. W.

JOURNALS

American Butter Review
American Milk Review
American Journal of Diseases of Children
American Journal of Physiology
American Journal of Public Health
Archives of Pediatrics
Australian Journal of the Council for Scientific and Industrial Research

Biochemical Journal

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Certified Milk
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Dairy Industries
Dairy World

Endocrinology

Food Industries
Food Manufacture
Food Research

Ice and Refrigeration
Ice Cream Field
Ice Cream Review
Ice Cream Trade Journal
Industrial and Engineering Chemistry

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Journal of Agricultural Science
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Journal of Animal Science
Journal of Bacteriology
Journal of Biological Chemistry
Journal of Dairy Research
Journal of Dairy Science
Journal of Endocrinology
Journal of Experimental Medicine
Journal of General Physiology
Journal of Genetics
Journal of Heredity
Journal of Industrial and Engineering Chemistry
Journal of Infectious Diseases
Journal of Milk Technology
Journal of Nutrition
Journal of Pathology and Bacteriology
Journal of Physical Chemistry
Journal of Physiology
Journal of Veterinary Research

Lancet
Le Lait

Milk Dealer
Milk Industry
Milk Plant Monthly

National Butter and Cheese Journal
New Zealand Journal of Science and Technology

Oil and Soap

Pacific Dairy Review
Proceedings of Society of Experimental Biology and Medicine

Refrigerating Engineering

Scientific Agriculture
Southern Dairy Products Journal

SPECIAL PUBLICATIONS

Federal Dairying and Bacteriological Establishment, Liebefeld, Berne, Switzerland

International Association of Ice Cream Manufacturers
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National Institute for Research in Dairying, Reading, England
New York Association of Dairy and Milk Inspectors

Prussian Dairy Research Institute, Kiel, Germany

State Agricultural Colleges and Experiment Stations

The Royal Technical College, Copenhagen, Denmark

United States Department of Agriculture

ABSTRACTS OF LITERATURE

BOOK REVIEWS

172. Fundamentals of Immunology. WILLIAM C. BOYD. 1943, Interscience Publishers, Inc., New York. 446 pages, XIV, 45 figs., 67 tables. Price, $5.50.

The whole field of immunology including blood groups is treated. The emphasis is on the fundamentals with enough of the application used to illustrate the principles involved except for the last chapter (86 pages) which is devoted to laboratory and clinical technique.

While it is intended primarily as a textbook for medical students, chemists, and biologists, it is an excellent treatise for reference for anyone interested in immunology. It presupposes no knowledge of the subject as all terms are clearly defined as they are met. The book is unique in that two kinds of type are used. In the larger type is a continuous coherent more elementary treatment of the subject for those whose interest in immunology is academic. In the smaller type is a more advance treatment containing the latest contributions in the field. In the latter controversial points are not evaded but evaluated. Extensive bibliographies are appended to each chapter. W.E.P.

173. The Biological Action of the Vitamins. A Symposium. E. A. EVANS, JR. Univ. of Chicago Press, Chicago, Ill. 1942. Price, $3.00.

This symposium covers the biochemical and physiological aspects of several vitamins of the B complex. The roles of vitamin K and phosphorous in body metabolism are also included. The topics covered, as indicated by the names of chapters in this volume, are: The Biological Action of the Vitamins by Elvehjem; cocarboxylase by Ochoa; Vitamin B_1: Clinical Aspects by Jolliffe; Riboflavin by Gyorgy; Human Riboflavin Deficiency by Sebrell; The Story of Pellagra and Its Treatment with Nicotinic Acid by Smith; Pyridoxine by Lepkovsky; Pantothenic Acid and the Microbiological Approach to the Study of Vitamins by Williams; Pantothenic Acid in Human Nutrition by Gordon; Biotin by du Vigneaud; Choline by Griffith; The Economy of Phosphorus in the Animal Organism by McLean; Vitamin K by MacCorquodale, and Vitamin K: Clinical Aspects by Smith and Warner.

Research workers and teachers in the field of nutrition and biochemistry should find this book of special value for its explanations of the relationships and functions of some of the B complex vitamins and biological enzymes.

A brief history of each topic is included and recent findings are discussed and interpreted on the basis of fundamental metabolic processes. The papers are well written and very complete, considering the space allowed. Good bibliographies accompany each chapter. Numerous tables, figures and illustrations enhance the effectiveness of the discussions. The author of each chapter is an authority on the subject he has discussed and the combining of these papers makes an interesting and informative volume.

Any teachers or research workers, in the dairy field, interested in this group of vitamins, will find the book highly useful because of its concise, up-to-date summaries with numerous references to sources of information.

R.K.W.

174. Food Poisoning. G. M. DACK. Univ. Chicago Press, Chicago, Ill., 1943. 138 pages. Price, $2.00.

The contents include discussions on the following subjects: Chemical poisoning in food, poisonous plants and animals, botulism, staphylococcus food poisoning, *Salmonella,* alpha type streptococci in relation to food poisoning, significance of other bacteria in food poisoning and infections to be differentiated from food poisoning.

In the introduction the author presents the derivation of the misnomer "ptomaine poisoning" and very briefly explains why the term is "unscientific and meaningless." Difficulties involved in and suggestions for investigating food-poisoning outbreaks are included.

The discussion on chemical poisoning is quite brief. Symptoms of the most common types are given. Antidotes and other treatment are not included. Botulism, staphylococcus food poisoning and *Salmonella* food-borne infections receive the most attention and consequently the descriptions of these three types are more complete. The chapter on botulism is quite timely in view of the increased interest in home canning. The great incidence of staphylococcus food poisoning is emphasized. Discussions on the latter subject include epidemiology, symptoms, treatment, laboratory diagnosis, and control. A suggested method for reheating pastries as a control procedure is included. Considerable emphasis is given to conditions governing production and to factors affecting and mode of action of staphylococcus enterotoxin.

This book would be considered somewhat elementary by investigators in this and related fields. Much specific information and some references regarding various phases of food poisoning have been omitted or very lightly passed over. For the benefit of the layman and the medical profession, the author might have explained and stressed more emphatically the need for discarding obsolete terms such as "ptomaine poisoning."

In spite of these shortcomings the book should prove quite valuable in bringing food sanitarians, public health workers and physicians up-to-date on the subject. It could also be recommended as a source of practical information for many a housewife and food manufacturer. P.R.E.

BREEDING

175. Studien über deutsche Rinderlandschläge. Das Mitteldeutsche Rotvieh. 1. Das Rotvieh in Hessen-Nassau. VOGEL, H. (U. GIESEN). Zeitschr. Zücht. Reihe B: Tierzücht. u. Zuchtungsbiol. 48: 164–187.

A review of the literature on weights, measurements, description, and production of the middle German red cattle in Hesse-Nassau. Included as "measures of the ideal" are data on prize winners at shows in different periods from 1887 to 1936. This breed is rather widely used for draft purposes also. Emphasis is laid also on their natural hardiness, economical utilization of feed, longevity, and general usefulness, even under unfavorable conditions. J.L.L.

CHEESE

176. The Problem of Bacteriophage in Cheese Making. Part I. Observations and Investigations on Slow Acid Production. E. B. ANDERSON AND L. J. MEANWELL, United Dairies Res. Lab., England. Jour. Dairy Res., *13*, No. 1: 58–72. 1942.

Failure of mixed culture starters as well as single strain starters as a result of bacteriophage infection was demonstrated. In a medium less acid than 0.5% a phage preparation was found to lose little strength during storage for 29 days at 22° C. (71.6° F.). It was ascertained that bulk starters could be protected from bacteriophage infection by cultivation of the bulk starters in airtight containers provided with a small opening, plugged with cotton-wool through which the milk can be inoculated.

The following phage detection test was found useful in a system of starter control:

Technique of phage detection test (P.T.T.)

Apparatus

Water bath, thermostatically controlled at 30° C. (86° F.)
1 ml. pipettes, sterile
Reductase tubes, sterile
Dippers, sterile
Bottles (sample) sterile

Materials

100 ml. quantities of milk autoclaved at 15 lb. for 20 min.
9 ml. tubes of Ringer solution, quarter strength.

Sampling

Examine a single pint and single bulk can for each starter and whey from one vat. Sample the bulk starter immediately the lid is removed and before plunging using a sterile dipper and a sterile bottle. Samples should be stored in the refrigerator until the series is complete, and then put on for test.

Method

The test must not be carried out in the starter preparation room. The test is applied to each starter in use at the following stages:

A. Mother culture
B. Pint culture for inoculation
C. Bulk starter
D. Whey at cutting corresponding to C.

To 100 ml. of autoclaved milk add 1 ml. of A, mix carefully and distribute in 10 ml. quantities in 8 sterile reductase tubes per starter to be tested.

To each of 2 add 1 ml. sterile Ringer solution = Control (a).
To each of 2 add 1 ml. of 1/100 dilution of B = (b).
To each of 2 add 1 ml. of 1/100 dilution of C = (c).
To each of 2 add 1 ml. of 1/100 dilution of D = (d).

Place the tubes in the water bath simultaneously. At the end of 6 hr. remove and determine the acidity, transferring the contents to a 150 ml. flask and rinsing out with 10 ml. distilled water.

Application

If the mean acidity of (b), (c) or (d) is more than 10% less than that of (a), phage may be presumed to be present in the culture under test.

S.T.C.

177. **The Effect of Varying Pitching Point and Rate of Scald on the Physical Properties and General Quality of Cheese Made From Milk of Varying Acidity and From Pasteurized Milk.** G. W. Scott Blair, F. M. V. Coppen and D. V. Dearden, Natl. Inst. Res. in Dairying, Univ. Reading, Eng. Jour. Dairy Res., *13,* No. 1: 73–84. 1942.

This is a continuation of work reported earlier (reviewed Jour. Dairy Sci., 25: A93, 1942). Cheese were made at varying controlled pitching points and rates of scald, the experiment being done both with sweet milk (0.145% acidity) and comparatively acid milk (0.17% acidity). In addition to physical tests made at regular intervals during the ripening period, the cheeses were judged for firmness and "spring" by panels of "experts," "non-experts" and "routine analysts."

The rheometer was considered to give the best measure of the physical properties of cheese. The "experts" individually and as a group were more self-consistent than the other panels in their judgment of firmness and

"spring." The pitching consistency was well correlated with the physical properties of the cheeses.

The curd from milk pasteurized at 74° C. (165° F.) was softer at any given temperature and time during the cooking process than curd from raw milk. The curd from milk pasteurized at 66° C. (150° F.) was slightly firmer than the curd from raw milk. S.T.C.

178. The Firmness of Rennet Curd; Its Measurement and Variations. S. J. ROWLAND AND D. SOULIDES, Natl. Inst. Res. in Dairying, Univ. Reading, Eng., Jour. Dairy Res. *13*, No. 1: 85–95. 1942.

The following method is described as suitable for measuring the firmness of rennet curd from milk coagulated under conditions simulating those of the Cheddar cheese making process: 0.02% of lactic acid is added to 100 ml. of milk contained in a glass jar 7.5 cm. in diameter and 8 cm. high. The milk is coagulated at 32° C. (89.6° F.) by a rennet concentration of 1 in 5000 using a time of 30 minutes. Curd firmness is measured as a function of the deformation produced during 15 seconds by a 6 gram weight loaded on a light disk 2.5 cm. in diameter placed on the middle of the curd surface.

Curd firmness was found to be increased by the addition of calcium chloride and by increases in acidity, amount of rennet and coagulation time. S.T.C.

CHEMISTRY

179. The Estimation of Solids in Milk. Part I. Determination of Solids-not-Fat by Various Methods of Hydrometry. S. M. BODEN, The West of Scotland Agr. Col., Glasgow, AND C. H. CAMPBELL, Scottish Cooperative Wholesale Soc., Glasgow. Jour. Dairy Res., *13*, No. 1: 45–57. 1942.

A comparison is made of the results secured in estimating the solids-not-fat content of milk by evaporation and by hydrometry. Tests were made in two laboratories on samples from 352 lots of milk. The method used in determining the solids by evaporation for most of the samples was as follows: approximately 1 gram of milk was syringed into an aluminum milk bottle cap. After 30 minutes in a water bath the caps were wiped individually and transferred to a steam oven operated at 98–99° C. (208.4–210.2° F.) for 2 hours followed by repetitions of 1 hour until the loss did not exceed 2 milligrams. Fat was determined by the Gerber method.

The authors stress the need for the standardization of evaporation techniques before attempting a permanent revision of the density formula. S.T.C.

180. Food Analysis. D. W. GROVER. Food Mfr., 18, 2: 41–45. 1943.

The author gives the English approved conversion factors in reporting proteins which are the same as those used in the United States of America. These factors are 5.7 for cereals, 6.38 for dairy products and 6.25 for meats, eggs, vegetables and fruits.

The Resazurin test has been adopted provisionally to determine quality in milk. It is claimed to be more effective than acidity values in measuring quality.

A polarographic method for determining dissolved oxygen in milk and its products has been developed. It is based upon a method used for water. This procedure is listed as a means of studying oxidative reactions which occur in dairy products.

Sampling cheese is recommended for uniformity by cutting a wedge and sampling it. When a trier must be employed to save the utility of the cheese the most representative sample is obtained by plugging diagonally and taking the outer half. This article will be continued in Vol. 18: 3; at that time the references supporting the above presentation will be given in detail. J.C.M.

181. Biennial Reviews of the Progress of Dairy Science. Section C, Dairy Chemistry. S. J. ROWLAND AND G. W. SCOTT BLAIR. Natl. Inst. Res. in Dairying, Univ. Reading, Eng., Jour. Dairy Res., *13,* No. 1: 93–113. 1942.

The progress of dairy chemistry from the middle of 1939 to the beginning of 1942 is reviewed under the general headings: composition of milk, chemistry of milk constituents, milk products, processing and manufacture, defects of milk and milk products, physical chemistry and physics, analysis of milk and milk products. There are 200 references. S.T.C.

CONCENTRATED AND DRIED MILK: BY-PRODUCTS

182. Bacteriological Aspects of the Manufacture of Spray-dried Milk and Whey Powders, Including Some Observations Concerning Moisture Content and Solubility. E. L. CROSSLEY AND W. A. JOHNSON, Aplin and Barrett Ltd., Yeovil, Somerset, Eng., Jour. Dairy Res., *13,* No. 1: 5–44. 1942.

This is an extensive bacteriological study of the operation over a period of 4½ years of two Kestner condensing and spray drying plants. Detailed investigations were made of the plant processes including handling of the raw milk, pasteurization, condensing in Kestner climbing film evaporators, handling of the condensed milk and spray drying. Milk, skim milk and whey were handled. Data were obtained showing the influence of processing variations on the moisture content and solubility of the powders.

The plate counts of 671 powder samples ranged from 200 to 19,500,000 per gram. The flora on standard milk agar at 37° C. (98.6° F.) comprised comparatively few species, thermoduric streptococci of the "*enterococcus*" and "*viridans*" groups predominating; *Str. durans* and *Str. thermophilus* were the commonest species. Five species of micrococci were commonly found. Spore-forming anaerobic bacilli were present in 14% of the samples.

Coliform organisms were rarely found in 1 ml. of reconstituted milk, but were isolated from 25% of the samples when 20 ml. of reconstituted milk was examined. False positives due to anaerobes were common, especially in stored samples and confirmatory tests of presumptive positives were considered essential. In fact, no true coliform organisms were found in 72 originally presumptive positive samples after 9 months storage at room temperature. It was shown that the coliform flora of dry milk was partly due to plant contamination by heat resisting strains. There was a variable decline in the bacterial count of the dry milk during storage. Some species died out more rapidly than others, the spore-forming species surviving longest.

The plate count of the fresh dry milk was not directly related to the raw milk counts, although probably affected by the numbers of thermoduric bacteria present in the raw milk and the storage conditions before processing.

High temperature short time pasteurization was used. Given a heating time of 20 seconds followed by a 3 to 5 minute holding period, a temperature below 159° F. (70° C.) yielded unsatisfactory bacteriological results while temperatures up to 167° F. (75° C.) could be employed without seriously decreasing dry milk solubility.

Considering the drying process itself inlet-air temperatures up to 170° C. (338° F.) produced no serious deteriorating of dry milk solubility. Inlet-air temperatures below 155° C. (311° F.) (commonest during winter operation) resulted in increased bacterial survival, especially of coliform organisms. Prolonged exposure of the dry milk to the outlet-air at 90° C. (194° F.) or less (as in the bag dust collectors), reduced the bacterial and moisture contents but significantly lowered the solubility.

Bacteriological cleanliness of the plants was considered a vital factor in producing low count dry milk. Conditions of operations which favored the multiplication of heat resistant streptococci were especially important. Continuous operation of the climbing film evaporator was found to result in increases in the plate count of the dry milk and in the incidence of coliform organisms. These increases were more pronounced after 10 hours continuous operation, so stoppage for plant cleaning every 7 to 9 hours was considered desirable. S.T.C.

DISEASE

183. Observations on Vibrio Foetus Infection in Cattle. W. N. PLASTRIDGE AND L. F. WILLIAMS, Storrs, Conn. Jour. Amer. Vet. Med. Assn., *102,* No. 791: 89. Feb., 1943.

"Conditions favorable for growth of 6 freshly isolated strains of *Vibrio foetus* of bovine origin were supplied by a soft agar medium consisting of liver infusion, 1% peptone, and 0.3% agar, and adjusted to pH 7.4; and by an atmosphere consisting of 10% CO_2 and 90% air. Initial growth appeared in a restricted zone from 0.5 to 1.0 cm. beneath the surface, and then extended upward to produce a grey zone of cells about 4 mm. in depth. The soft, liver-infusion agar was used in growing cells for antigen production.

"Findings on 9 aborted fetuses and blood samples from the aborting cows showed that *V. foetus* infection was detected in 5 by direct microscopic examination of stomach fluid, and in 6 by cultural methods, and in 8 by the agglutination test. All three procedures should be employed in diagnosing abortions caused by *V. foetus.*

". . . It appears that an agglutinin titer of 1:50 or less should be classed as *negative,* 1:100 as *suspicious,* and 1:200 or higher as *positive.*

"It is concluded that the agglutination test is of value in diagnosing abortions due to *V. foetus* and in determining the probable presence of *V. foetus* infection in a herd of cattle; that abortions due to *V. foetus* usually occur during the fifth, sixth and seventh months of pregnancy; and that *V. foetus* infection in cattle tends to be self-limiting, because of the transitory nature of infection and a tendency for initial infection to increase resistance to subsequent exposure." S.A.F.

FEEDS AND FEEDING

184. The Effect of a Vitamin A-rich Diet on the Vitamin A Content of the Colostrum of Dairy Cows. J. STEWART AND J. W. MCCALLUM, Moredun Inst., Gilmerton, Midlothian, Eng., Jour. Dairy Res., *13,* No. 1: 1–4, 1942.

In one experiment the rations of 69 cows were supplemented with 3 pounds of carrots per animal per day from the day the cows came off pasture in October until they calved during the months from November to April. Sixty-five control cows selected on the basis of calving date so that a control cow would calve on about the same date as a carrot-fed cow received the same rations except for the carrots. In another experiment 57 cows received $\frac{1}{7}$ pt. of cod liver oil per animal per day and 50 cows served as controls. The standard rations were known to be deficient in vitamin A. The vitamin A and carotene content of the colostrum from the

cows varied widely but there was no indication that the carotene and vitamin A content of the colostrum from the cows receiving the vitamin A rich concentrates was greater than that of the colostrum from the control cows. S.T.C.

185. Utilization of Urea by Young Calves. J. K. Loosli and C. M. McCay, School of Nutrition, Cornell Univ., Ithaca, N. Y. Jour. Nutr., *25*, No. 2: 197–202. Feb., 1943.

Two Holstein calves a few days of age were fed whole milk and a low protein basal ration containing 4.4% protein. Whole milk was discontinued at about two months of age. Four other calves were fed a similar ration in which 4 parts urea replaced an equivalent amount of starch. The calves on the low protein ration failed to grow, while the calves receiving urea to bring the protein equivalent to 16.2% gained 61 pounds in two months compared with 80 pounds gained by calves treated in a similar manner but fed a normal ration. Calves as young as two months of age were able to make fair gains in weight when urea constituted three-fourths of the dietary nitrogen.

The calves fed the basal ration were in negative nitrogen balance while those receiving the added urea were in a positive nitrogen balance, retaining 24% to 36% of the dietary nitrogen. The apparent digestibility of the dry matter and carbohydrates of the basal ration was 57% to 63%, while the dry matter and carbohydrates of the urea ration were 74% to 80% digested.

Feeding daily supplements of the B vitamins did not increase the rate of growth nor the efficiency of nitrogen utilization. C.F.H.

186. Seasonal Changes in the Lignin and Cellulose Content of Some Montana Grasses II. A. R. Patton, Mont. Agr. Expt. Sta. Jour. Anim. Sci., *2*, No. 1: 59–62. Feb., 1943.

The lignin and the cellulose contents of different grasses cut at different stages rather than at definite dates were investigated using a modification of the Crampton and Maynard method. In general, the increase in lignin and cellulose during growth appears to have been similar in the various species with a coefficient correlation between lignin and cellulose greater than 0.9 for 123 samples analyzed. Blue grama grass was an exception, however, since it had a very high lignin content in the early vegetative stage. C.F.H.

ICE CREAM

187. Frozen Desserts Need the Right Stabilizer. B. I. Masurovsky. Food Indus., *14*, No. 12. 1943.

Sherbets and ices should have a smooth texture. Lecithin as a means of attaining a desired texture is described.

Sherbets and ices due to their low fat content present a real problem, in texture control. The author cites 8 references in this field.

The author recommends using from 0.1 to 0.3% of lecithin in combination and partly replacing some of the common stabilizers used. The common stabilizers used were listed as algin, gelatin and avenex.

For sherbets the author suggests adding 1 gallon of a 12% ice cream mix to 8 gallons of the basic mixture, 1 gallon of cold pack fruit and 8 ounces of a 50% lactic acid solution. This sherbet mixture should yield a gain in volume of 25%.

Stabilizer combinations used were lecithin 0.3% and algin 0.2%. Another one was lecithin 0.3% and gelatin 0.2%. Lecithin 0.1, algin 0.2 and gelatin 0.2% was also used. Avenex 0.35% and 0.15 lecithin was tried. A control stabilizer for comparative purposes was made up of 0.2% algin, 0.2% gelatin and 0.1% avenex.

Texture, dipping qualities and general handling were improved by using purified lecithin. Orange and lemon flavors were improved in trials using lecithin as part of the stabilizer.

Freezing properties were helped with lecithin due to its wetting properties. J.C.M.

188. Research Discovers New Stabilizer. G. HADARY. Food Indus., *15*, No. 2: 76–77. 1943.

Extract of quince seed was found desirable as a substitute for gums and alginates as a stabilizer for dairy foods. Gums and alginates are difficult to obtain. Extract of quince seed has a real value in stabilizing milk drinks. Percentages of 0.03 to 0.04 stabilized cocoa solids in chocolate-flavored milk. No sediment was possible after 2 weeks at 40° F. with the extract of quince seed procedure.

Percentages of 0.03 to 0.04 of quince seed extract also gave good results as an ice cream stabilizer. The extract gave quicker whipping than the orthodox gelatin additions.

Procedures for preparing the extract are given. Where the seeds are attainable is given and 3 references relative to the subject are listed. The composition of quince seed is appended with other material essential for a better understanding of this problem. J.C.M.

189. Physically Handicapped Help Solve Labor Problem. GEORGE E. WILLIAMSON, Pres., Williamson Candy Co., Chicago, Ill. Ice Cream Field, *40*, No. 6: 16. 1942.

The author points out that for several years his company has been using disabled people as workers. To use them successfully he says it is necessary to consider both their disabilities and their abilities in selecting the work they are to do. When this is done it is claimed they offer no

more of a problem than the physically normal person, and in certain types of work they are just as efficient as the physically normal and are usually more punctual.

Specifications for many jobs do not call for strength, but do require accuracy, skill, and perhaps speed. The physically handicapped are doubly careful, because they cannot afford to take chances. This is responsible for the lack of accidents the Williamson Candy Co. has experienced with such employees. W.C.C.

190. **Are Sherbets and Ices the Industry's Salvation?** KEN FORREST, Merchandising Editor. Ice Cream Field, *41,* No. 2: 10. 1943.

There has been a sensational increase in sherbets and ices, the author states, since F. D. A. Order No. 8 became effective. He claims that 8,000,000 gallons were produced in 1940 whereas 30,000,000 gallons or more will be produced in 1943. The limiting factor will be the allotment of sugar.

It is predicted that it will not be possible for the industry to produce as much sherbet as ice cream under our present restrictions, although some manufacturers are attempting to do this. Examples are given in an attempt to establish this conclusion, and show that sugar will be the limiting factor. W.C.C.

191. **Sherbets and Ices.** C. D. DAHLE, Pa. State Col., State College, Pa. Ice Cream Field, *41,* No. 2: 20. 1943.

The restrictions imposed by Conservation Order M-271 issued Dec. 4, 1942, and Food Distribution Order No. 8, effective Feb. 1, 1943, have increased the interest of the ice cream industry in ices and sherbets. In the past, ices and sherbets have not been as palatable as they should have been, but by controlling overrun, composition and methods of freezing, satisfactory products can be made.

As a rule, ices and sherbets contain 30% to 35% sugar. Because of the restriction on sugar the author recommends using as much as 40% of the sweetening agents in the form of corn syrup solids. It is pointed out that an excess of sugar makes a soft product with more danger of "leakage," while a deficiency of sugar makes a harder product that is crumbly and less palatable. The author states that ices and sherbets should have about the same firmness as ice cream and that the amount of sugar used should depend upon (1) sugar content of an amount of fruit used, (2) sugar available and (3) overrun taken.

A brief discussion is given of the necessity of stabilizers of the proper type and concentration, the proper adjustment of acidity and in sherbets and advisability of using from 3% to 5% milk solids. Rapid freezing is recommended. A proposed formula for sherbet is also given. W.C.C.

192. Does Income Alone Govern Demand? ANONYMOUS. Ice Cream Field, *40*, No. 6: 26. 1942.

Reference is made to a report by the Bureau of Agricultural Economics which gives figures to show that as the family income increases the amount of ice cream consumed increases. According to these figures the amount consumed varies from 2.2 pounds for families with $500 annual incomes to 29.9 pounds for families with incomes of $5,000 or more.

It is contended that legislators should be made to realize that ice cream is the favorite "dairy food" of the middle income group. A chart is given to show the relation of income to per capita consumption of cheese, evaporated milk, and ice cream. W.C.C.

193. Canadians Adjust for War Production. T. H. BAULAND, Gen. Mgr., Borden Co., Ltd., Toronto, Canada. Ice Cream Field, *40*, No. 6: 12. 1942.

The limitations on sugar in Canada have been met by substituting other sweetening agents so that ice cream gallonage has been maintained at 100% of 1941 production. By the pooling of hauling facilities considerable saving has been accomplished in transportation. By the exchange of customers in various localities, between ice cream manufacturers, it has been possible for them to sell as much ice cream within a 35-mile radius as they formerly sold within a radius of from 150 to 200 miles. This has decreased delivery costs at least 50% and has reduced delivery mileage between 70% and 75%. W.C.C.

194. Ice Cream. ERIC HUMPHRISS. Food Mfr., *18*, 1: 17–19. 1943.

In England the first step was to limit the dairy products used in ice cream. Then the manufacture of ice cream was prohibited. To the prohibition order the industry reacted with disfavor since research had been completely designed to save materials essential to war. This work included adding 4% to 5% of rye or potato flour to the mix. It was explained that the food value of the product would be maintained. Palatability was secondary.

For the post war period new standards for ice cream should be established. These should assure the health qualities and food value of all ice creams. It is suggested that an 8% milk fat minimum be established. The author feels that yield should be legalized and limited at 100%. The ice cream should weigh about 5½ pounds per gallon thus with a yield limit the food-solids-per-gallon are assured.

The author feels that the temporary supervision of ice cream manufacture in England will stimulate better production in the future, as the industry is enjoying a period of rest which is stimulating plans for the future. J.C.M.

195. Examination of Raw Sugar. A. G. AREND. Food Mfr., *18*, 2: 55. 1943.

This article has an interest here as much sugar is used by the dairy industry.

A Cline-micro-camera is employed to study micro-organisms and sugar lice in the various samples.

The article gives details of using the camera and preparing the sugar. Solutions are required for this work. The water should be at a temperature below 15° C. Details of technique, interpretation, and supplies are given. J.C.M.

PHYSIOLOGY

196. The Ketogenic Activity of Extracts of the Anterior Pituitary. C. H. GRAY, King's College Hospital. Jour. Endocrinology, *3*, No. 2: 132. Aug., 1942.

The relationship between ketonuria and ketonaemia in the fasting rat injected with saline or pituitary anterior lobe extracts was investigated. That there is a renal threshold for ketone bodies in this species was confirmed although the actual level was not found to be so constant. It was considered possible that injections of anterior lobe extracts into fasting rats may lower the renal threshold for ketone bodies. The ketogenic activity was distributed fairly evenly among the albumen, globulin, and pseudoglobulin fractions of pH 5.5-soluble extracts of the anterior pituitary. The ketogenic activity of pH 5.5-soluble fractions of pituitary saline extracts was nearly as great as the crude saline extracts from which they were derived. The ketogenic activity of such fractions ran parallel with the glycotropic activity alone but the heat-stability of the latter indicated that the two factors may be separable. R.P.R.

197. Further Experiments on Lactation in Thyroidectomized Rats: The Role of the Parathyroids. S. J. FOLLEY, HELEN M. SCOTT WATSON, AND E. C. AMOROSO, Univ. of Reading and Royal Vet. Col. Jour. Endocrinology, *3*, No. 2: 178. Aug., 1942.

Thyroidectomy during lactation caused an almost complete cessation of lactation as judged by litter growth. Thyroidectomized rats mated and delivered their young but seldom began to rear their litters. Pregnancy was often prolonged. Autoplastic thyroid grafts containing parathyroid tissue made immediately after thyroid removal partially maintained lactation. Lactation was also partially maintained in thyroidectomized rats by injecting daily 10 or 20 Collip and Clark units of parathyroid extract. Rats thyroidectomized during lactation did not exhibit symptoms of tetany. It was concluded that failure of lactation after thyroidectomy is due at least partly to parathyroid deficiency. R.P.R.

198. **Effects of Oestrogen (Stilboestrol) on the Sperm Production of Adult Rams.** MIN-CHUEH CHANG, Cambridge Univ. Jour. Endocrinology, *3,* No. 2: 192. Aug., 1942.

Sperm production and sex drive of 2 Suffolk rams were studied before and after implantation of diethylstilbestrol tablets. Sperm production increased 7–9 days after implantation and the effect lasted for about 5 days. Sex drive, sperm morphology, and quality of spermatozoa were not influenced by the treatment. Spermatozoa obtained from the treated rams showed a high tendency to agglutinate. Five ewes inseminated with sperm collected from the rams during treatment were impregnated and gave birth to 8 normal lambs. The absorption rate of diethylstilbestrol was about 5 mg. per day. R.P.R.

199. **The Significance of Ketosis.** EATON M. MACKAY, The Scripps Metabolic Clinic. Jour. Clin. Endocrinology, *3,* No. 2: 101. Feb., 1943.

A review article in which the following phases are discussed: (1) antiketogenesis versus ketolysis; (2) origin of ketone bodies; (3) utilization of ketone bodies; (4) regulation of ketone body production; (5) fasting ketosis; and, (6) ketosis in diabetes mellitus. R.P.R.

200. **Thyroidal Action of Synthetic Thyroprotein.** E. P. REINEKE AND C. W. TURNER, Univ. Mo., Columbia. Jour. Clin. Endocrinology, *3,* No. 1: 1. Jan., 1943.

Experiments leading to the chemical formation of artificial thyroproteins with high thyroidal activity were described. Critical factors in the formation of such preparations were: (1) the degree of iodination of the protein; (2) the pH of the reaction medium; and (3) the temperature at which the iodination and incubation processes were carried out. Products formed under optimal conditions had several times the thyroidal activity of standard thyroid powder as judged by either biological assay or the yield of crystalline thyroxine which was obtained from them subsequent to hydrolysis. R.P.R.

201. **Administration of Non-Steroid Substances by the Implantation Technique.** A. S. PARKES, Natl. Inst. for Med. Res. Jour. Endocrinology, *3,* No. 2: 220. Aug., 1942.

Non-steroid substances were administered by the subcutaneous implantation of compressed tablets made with or without excipients. Cholesterol and benzyl sulphanilamide were found to be the most satisfactory excipients to use for improving the properties of the tablet or for delaying the absorption of highly soluble substances. The complete absorption of dextrose was

delayed for some weeks by incorporating it in 100 mg. tablets containing 90% cholesterol. Thyroxine was not absorbed in weighable amounts from tablets implanted for many months and no satisfactory excipient was found to expedite absorption. The implantation of 100-mg. tablets containing 15 and 25% adrenaline, using cholesterol as excipient, yielded about 5 mg. and 10 mg. respectively of the active substance in a month. Observations on female rats showed that 2–3 mg. of insulin were absorbed in a month from tablets containing 25% insulin. Chorionic gonadotropin was absorbed rapidly from tablets even when 90% cholesterol as excipient was added. Gonadotropic extract of sheep pituitary gland was much more efficient in immature female rats when administered over 5 days with cholesterol or sulphanilamide as excipient by the implantation technique than when administered by daily subcutaneous injection. R.P.R.

202. Ascorbic Acid-Gonadotropic Hormone Relationships in the Chick. FREDERICK N. ANDREWS AND RALPH E. ERB, Purdue Univ., Lafayette, Ind. Endocrinology, *32,* No. 2: 140. Feb., 1943.

The injection of ascorbic acid alone into one-day-old white Leghorn chicks had no influence on testicular weight. Ascorbic acid injected in combination with pregnant mare serum gonadotropin did not augment the action of the latter. There was no apparent relationship between blood plasma-ascorbic acid and the testis weight. R.P.R.

203. Anterior Pituitary-Stimulating Action of Yohimbine. NICHOLAS W. FUGO AND E. G. GROSS, State Univ. of Iowa, Iowa City. Endocrinology, *31,* No. 5: 529. Nov., 1942.

A study was made of the mode of action of Yohimbine in rats. Sexually immature female rats injected with one mg. doses of Yohimbine-HCl showed no precocious sexual development. Female rats injected with this drug for periods ranging from 40 to 101 days with doses varying from one to 4 mg. per day responded to lower doses with periods of prolonged estrus while with higher doses a condition resembling pseudopregnancy resulted. These animals exhibited atypical sexual behavior during estrus. Adult spayed rats showed no cornification of the vaginal epithelium following chronic administration of the drug, showing that the prolonged estrus observed in normal rats was not due to hyperemia. Hypophysectomized female rats showed no estrous condition when injected with yohimbine. Immature ovaries devoid of corpora lutea implanted into the anterior chamber of the eye of adult normal and castrated male rats showed the development of normal-appearing corpora lutea following injection of yohimbine into the rats. R.P.R.

204. Synthetic Thyroprotein, A New Drug Available in Veterinary Practice. E. P. REINEKE AND C. W. TURNER, Columbia, Mo. Jour. Amer. Vet. Med. Assn., *102,* No. 791: 105. Feb., 1943.

Through an iodination process, the authors have been able to synthesize a thyroprotein at low cost and thus make it available for experimental use on large animals. The preparations show thyroidal activity of about 4% of thyroxine by oral assay and approximately 11% when injected.

A few possibilities for the use of thyroid in large animals are pointed out. These fall into two groups, the first resulting from stimulated growth of young animals and the second from overcoming the normal sluggishness of body processes in older animals when normal thyroid secretions decrease. S.A.F.

205. The Temperature Phenomenon before Parturition and Its Clinical Importance. LEO WEISZ, Auburndale, Mass. Jour. Amer. Vet. Med. Assn., *102,* No. 791: 123. Feb., 1943.

The author has found that, in the cow and mare, the body temperature reaches the physiological maximum of 103.1° F. about one month antepartum. Twenty-four hours before parturition there is a drop of about 1.6° F. At first parturition and in very old dams the results are less reliable.

There is a decreased temperature in the sow two days before and in the doe a few hours before parturition. In the sheep and cat the drop in temperature is indistinct, while in the bitch the temperature drops below normal 24 hours before parturition. S.A.F.

MISCELLANEOUS

206. War Forces Progress in Food Processing. ERIC HARDY. Food Indus., *14,* No. 12. 1942.

Britain, Germany and Russia are developing new processed foods. Australia and South Africa are making new foods for troops.

Germany and Japan are making synthetic fats by modified procedures used in World War I.

At the Campden Experiment Station in England the relative sweetness of sugars is being studied.

Emulsions of milk, fat, and water are being used extensively as a spread for bread. A colloid mill is required for these mixtures. The material must pass through the mill at or below 41° F. The fat dispersion to successfully make these products must be between 45 to 55%. If milk and fat are used without other materials than a 40 to 60 fat to milk ratio is best. J.C.M.

207. British Food Technologists Improvise to Meet Shortages. H. B. CRONSHAW. Food Indus., *15*, No. 2: 57–58. 1943.

Frozen desserts are being made in England with very restricted materials. The writer describes in detail frozen desserts of English preferences that are unknown in the United States. These apparently are not affected by ice cream restrictions.

Sweeteners for frozen desserts are discussed. Corn sugar is as difficult to get in England as are other sugars. Saccharin is used to improve sweetening for frozen desserts and other foods. One part of soluble saccharin per 15 parts by weight of powdered dextrose is 30 times as sweet as sugar. Mild flavored honey to the limit of 25% is used to replace sugar.

Sugar mixtures are improved by adding 0.5% of an equal mixture of sodium citrate, sodium chloride and disodium hydrogen phosphate. Sodium alginate is regarded superior in frozen desserts to gelatin. Whole egg powder improves the yield and palatability of frozen desserts.

"Custard ice" is enjoying more use in England due to the restrictions on ice cream. It is indicated that both products are equally affected by stabilizers and sweeteners. J.C.M.

208. British Food Industry Geared to War Economy. ANONYMOUS. Food Indus., *15*, No. 2: 51–58. 1943.

Food Industries in England are subject to severe restrictions to fit into the war program. This involves materials, personnel and certain foods regarded as semi-luxury types.

Zoning and transportation restrictions that affect the dairy foods are in operation. Pooled transportation is practiced in some industries that are closely inter related. For the dairy industry limits of equipment and facilities are given in actual values. The article is presented as an aid to a better understanding if and when we are confronted with problems comparable to those in England. J.C.M.

209. Preservation of Foodstuffs. Indus. Eng. Chem., Indus. Ed., *35*, No. 1: 12–105. Jan., 1943.

A symposium consisting of 17 papers by different authors. Among those of interest to some members of the dairy industry are:

(a) Food requirements for overseas use. Captain Virgil O. Wodicka. Q. M. C. Subsistence Res. Lab., Chicago, Ill. p. 12.

(b) Food packing for overseas use. Captain Robert R. Melson. Q. M. C. Subsistence Res. Lab., Chicago, Ill. p. 16.

(c) Protection of foodstuffs against war gases. S. H. Katz, Edgewood Arsenal, Md. p. 20.

(d) Freezing foods. Domenic De Felice, N. Y. State Agr. Expt. Sta., Geneva, N. Y. p. 26.

(e) Cold storage of food. Hermann C. Lythgoe, Mass. Dept. of Public Health, Boston, Mass. p. 29.

(f) Stabilization of fats and fatty foods. H. S. Mitchell and H. C. Blaek, Swift & Co., Chicago, Ill. p. 50.

A review is presented of the attempts of various investigators to stabilize fats by means of anti-oxidants. Materials occurring naturally with food products have received most attention. The authors found that gum guaiac, an anti-oxidant not occurring with food materials, was an effective stabilizer for meat food fats. It is an excellent anti-oxidant for lard; it can be incorporated in packing materials which are to be used for fats and fatty foods; it gives promise of being useful in the stabilization of dehydrated meat and of poultry and dairy products.

(g) Short-time pasteurization of milk. C. Olin Ball, Owens-Illinois Can Co., Toledo, Ohio. p. 71.

The principles employed in the scientific advancement of heat sterilization of canned foods are applied in this paper to milk pasteurization processes. The slope values of thermal death time curves are important and they show a way to obtain lethality values of pasteurization processes referred to that of the standard process of 30 minutes at 143° F. A "phantom" thermal death time curve is described as one with direction or slope but without position with respect to destruction time. Calculations based on the bacteria-destroying value of the standard process as a reference show the comparative value of processes at other times and temperatures in destroying different types of bacteria. Using available data, 19.2 seconds at 161° F. has destructive power equivalent to 30 miuutes at 143° F. for *Br. suis;* but for *S. aertrycke* a process of 3 minutes 20.4 seconds at 161° F. is required to have destructive power equal to that of 143° F. for 30 minutes. These variations in equivalence with respect to different bacteria between processes at different temperatures are controlled by the slopes of phantom thermal death time curves for the bacteria. Applying this principle to the ability of the phosphatase test to prove the sufficiency of a pasteurization process, the author finds by making certain assumptions, that a process of 15 minutes at 161° F. should produce the same results in inactivating the enzyme as is produced by a process of 30 minutes at 143° F. Periods of time consumed in heating the milk to holding temperature and in cooling the milk must be considered. Such periods of rise and decline of temperature may contribute more lethal heat to the destruction of bacteria or to the inactivation of phosphatase than is contributed by the holding period of the process.

(h) Acid detergents in food sanitation. M. E. Parker, Beatrice Creamery Co., Chicago, Ill. p. 100.

Alkaline detergents have long been used for cleaning dairy and food equipment. They have been effective cleaners but in certain operations,

such as can washing, a concentration of milk solids is built up in the wash water which often leaves a film of these solids in the clean cans. This film supports bacterial growth and causes a bad odor in the closed can. Cans cleaned with alkaline detergents during the end of the day's run often receive an inoculation of proteolytic and oxidizing types of bacteria.

Acid compounds have not been acceptable as cleaners because of their inferior detergency and their corrosive action. The discovery of organic acids with low corrosive properties, the effective combination of such acids with surface-active agents and corrosion retarders, the inherent inhibiting effects upon quality-defective types of micro-organisms and the retarding effect upon calcareous formations have recently pointed the way toward the development of acceptable types of acid cleaners. Data are presented to show that cans alkali-washed but rinsed with steam charged with gluconic acid in sufficient concentration to give pH 6.0 to 6.5 or cans washed with a commercial acid cleaner were much cleaner than when only alkali was used. B.H.W.

210. Army Specifications for Dehydrated Foods. ANONYMOUS. Food Indus., *15*, No. 3: 52–53. 1943.

Specific specifications for Swiss Cheese Soup are given. This product prepared for the Army must contain 7% to 8% of dehydrated Swiss Cheese; and 3% to 4% of whole dry milk (spray process). These percentages are on the weight basis.

Tentative specifications for dehydrated foods for Army use have been issued by the Quartermaster Corps. Specific details for dried eggs are given with methods of inspection listed. These are of interest since dried eggs are frequently used in foods containing dairy foods as milk, cheese, and powdered milk. J.C.M.

211. Converts Food Trailer to Bus for Workers. ANONYMOUS. Food Indus., *15*, No. 3: 64–65. 1943.

The article explains how to convert a food trailer into a bus for workers so that it has a dual utility. A diagram of the converted trailer-bus is given. The capacity can be increased from 55 to 80 persons by making certain simple temporary alterations in the trailer.

The plan is well adapted to farm-factory operations where motor vehicles must be used for many purposes. J.C.M.

212. Choose Floors That Withstand Conditions of Processing. R. KANEGSBERG. Food Indus., *15*, No. 3: 58–61. 1943.

Chemical and physical properties of flooring are of first concern in processing rooms. Wood floors are suitable for areas where service is

light, and there is no exposure to acid, water, alkalies or other destructive materials. Concrete loading platform floors should have a top surface of emery aggregate.

Chemical resistance, resistance to surface wear, heat and cold are important. Slip proof is an essential; low asorption, cleanability, sound proof qualities and costs are also of importance.

Linoleum and rubber are not satisfactory for most processing rooms. Alkalies, moisture, oils and fat are determinative factors in their non-usability.

Concrete is the basis of all good dairy plant floors. It must be used properly; and special floor protective materials are almost essential.

J.C.M.

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