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ACTION OF BACTERIOPHAGE ON MIXED STRAIN STARTER CULTURES. I. NATURE AND CHARACTERISTICS OF THE "NASCENT PHENOMENON"

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Evans (5) described the "nascent phenomenon" in connection with streptococcal bacteriophage action, whereby a small amount of susceptible culture in a mixture prevented the development of turbidity by a normally resistant strain of bacteria. Anderson and Meanwell (2) reported retarded acid development by a normally resistant mixed strain starter with the addition of a particular single strain culture and its homologous bacteriophage. Nichols and Wolf (7) tested 14 strains of starter bacteria with their corresponding strains of bacteriophage and found four strains of bacteriophage which caused this phenomenon.

The present study was initiated to determine the incidence of the "nascent phenomenon" amongst strains of bacteria and bacteriophage used in this laboratory, and to clarify its effect on acid production of mixed strain starters. Experiments revealed the nature of this phenomenon and indicated that the term "nascent" is not appropriate for describing this type of bacteriophage action.[1]

METHODS

The "nascent phenomenon" was determined by a method similar to that used by Nichols and Wolf (7). Three 100-ml. quantities of skimmilk in 6-oz. bottles were taken from a water bath at 32° C. and inoculated with 1 per cent of a strain of bacteria not sensitive to the bacteriophage being used. One bottle was retained as a control. About 2,500 or more particles per milliliter of a strain of bacteriophage were added to each of the other two bottles, and 1 per cent of the homologous strain of bacteria was added to one of the bottles containing bacteriophage. Determinations of titratable acidity were made after incubation at 32° C. for 6 and 7 hr. The nascent effect was considered to occur when the acidity of the control was considerably greater than that of skimmilk which received inocula of both strains of bacteria and of bacteriophage. The skimmilk inoculated with bacteriophage and the nonhomologous strain of bacteria served as a control and revealed any cross lysis.

The most probable numbers of bacteriophage in whey filtrates were determined by the limiting active dilution method outlined by Collins (3). The modified trypticase soy agar described by Mull (6) was used for determining bacterial populations by the standard plate count (1) at 32° C.

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[1] Although the term "nascent" is not appropriate, it is used in this report for purposes of convenience.

RESULTS

The following combinations of lactic streptococci and homologous strains of bacteriophage were tested: 712-F56; 565-F54; E8-F63; 318B/27-F74; W2-F24; H1,10-PF10; H1,2-PF2; HP-F59; ML1-F69; MM-P3, GS3-P2; NA1-P4; NA3-P5; and FV-P1. Only two strains of bacteriophage resulted in definite evidence of the nascent phenomenon (table 1). Low titratable acidity values at 6 and 7 hr.,

TABLE 1

Reduction in plate count and acid production due to the nascent phenomenon

Culture(s) used	Bacteriophage added	Plate count at:		Titratable acidity at:	
		0 hr.	6 hr.	6 hr.	7 hr.
				(%)	(%)
W2	None	2.9×10^7	2.2×10^9	0.50	0.62
W2	F59	3.1×10^7	2.5×10^9	0.52	0.58
W2 and HP	F59	3.6×10^7	4.2×10^4	0.25[a]	0.28[a]
W2	F56	2.6×10^7	2.0×10^9	0.50	0.62
W2 and 712	F56	4.8×10^7	2.3×10^4	0.29[a]	0.29[a]
HP	None	2.0×10^7	4.2×10^8	0.45	0.59
HP	F56	2.0×10^7	4.6×10^8	0.44	0.58
HP and 712	F56	4.4×10^7	6.2×10^6	0.34[a]	0.37[a]

[a] Low acidity due to nascent phenomenon.

as well as low plate counts at 6 hr. for several trials, indicated that bacteriophage F59 was consistently capable of causing this phenomenon in cases where cultures W2 and HP were both included. It was further indicated that bacteriophage F56 was capable of giving the nascent effect in cases where either W2 or HP was cultured with 712. However, the effect on titratable acidity and plate count usually was less in cases where HP was used with combination 712-F56. These strains of bacteriophage did not cause lower titratable acidity values when the homologous bacteria were omitted. Although plate counts were low at 6 hr. in cases of the nascent effect, they were higher than those usually encountered following lysis of bacteria in skimmilk by an homologous strain of bacteriophage.

Since little was known about this phenomenon, secondary growth or mutant cultures were substituted for the normally considered resistant strain and, in separate experiments, for the sensitive host strain of combinations of bacteria and bacteriophage with which the nascent phenomenon had occurred. To prepare a secondary growth culture, sterile skimmilk was inoculated with 1 per cent of a culture and with about 2,500 particles per milliliter of the homologous bacteriophage. Titratable acidity was determined after the mixture had been incubated for 6 hr. at 32° C. to verify the occurrence of mass lysis. Before the titratable acidity determination was made, 1 per cent of the mixture was inoculated into sterile skimmilk and incubated at 32° C. for 16 hr. or until coagulation had occurred. Mutant cultures prepared in this manner usually were propagated three or four times before experiments were run to determine whether their use would result in the nascent phenomenon.

Experiments first were run to determine whether use of secondary growth cultures of the normally considered resistant bacteria would alter occurrence of

the nascent phenomenon. Although secondary growth cultures of *Streptococcus lactis* W2 were resistant to the homologous strain of bacteriophage, F24, and to bacteriophage strains F56 and F59 (table 2), the addition of either combination

TABLE 2

Effect on occurrence of the nascent phenomenon of substituting secondary growth cultures for the normally considered resistant bacteria

Culture added concurrently with the secondary growth culture	Bacteriophage added	Titratable acidity at:	
		6 hr.	7 hr.
		(%)	(%)
Average results obtained using 5 secondary growth cultures which originated from combination W2-F24.			
None	None	0.50	0.67
None	F24	0.51	0.68
None	F56	0.50	0.67
712	F56	0.31[a]	0.32[a]
None	F59	0.52	0.69
HP	F59	0.26[a]	0.27[a]
Average results obtained using 3 secondary growth cultures which originated from combination HP-F59.			
None	None	0.32	0.40
None	F59	0.33	0.38
None	F56	0.30	0.37
712	F56	0.33	0.40

[a] Low acidity due to nascent phenomenon.

712-F56 or HP-F59 resulted in drastic reduction in the acid produced by these secondary growth cultures. Selection of cells resistant to an homologous bacteriophage in these cases had not resulted in cultures resistant to the nascent phenomenon. However, the production of acid was not retarded by addition of combination 712-F56 to secondary growth cultures of HP. In these cases, the selection of cells resistant to F59 also had resulted in cultures which were resistant to the nascent phenomenon.

In experiments in which the sensitive host bacteria were replaced by mutant cultures (table 3), titratable acidity values showed that growth of primary cul-

TABLE 3

Effect on occurrence of the nascent phenomenon of substituting secondary growth cultures for the sensitive host bacteria

Culture added concurrently with the secondary growth culture	Bacteriophage added	Titratable acidity at:	
		6 hr.	7 hr.
		(%)	(%)
Average results obtained using 5 secondary growth cultures which originated from combination 712-F56.			
None	None	0.47	0.62
None	F56	0.48	0.62
W2	F56	0.55	0.65
HP	F56	0.53	0.65
Average results obtained using 5 secondary growth cultures which originated from combination HP-F59.			
None	None	0.41	0.48
None	F59	0.39	0.48
W2	F59	0.57	0.67

tures of W2 or HP was not retarded. These results indicated that host bacteria of the proper sensitivity are of primary importance to the occurrence of this phenomenon.

Experiments were run to determine whether permanent changes were demonstrable in the sensitivity to bacteriophage of bacteria arising after the occurrence of this phenomenon, and to determine possible changes in the range of activity of bacteriophage arising during its occurrence. To obtain cultures of bacteria arising after occurrence of the nascent action of bacteriophage, mixtures of W2 and 712 and of HP and 712 were inoculated with bacteriophage F56; mixtures of W2 and HP were inoculated with bacteriophage F59. After an incubation period of 6 hr. at 32° C., titratable acidity determinations were made to verify

TABLE 4

The action of bacteriophage on secondary growth cultures obtained after occurrence of the nascent phenomenon

Culture added concurrently with the secondary growth culture	Bacteriophage added	Titratable acidity at:	
		6 hr.	7 hr.
		(%)	(%)
Average results obtained using 5 secondary growth cultures which originated from the nascent action of bacteriophage F56 on cultures of W2 and 712.			
None	None	0.50	0.62
None	F24	0.30	0.31
None	F56	0.48	0.61
712	F56	0.33[a]	0.32[a]
Average results obtained using 2 secondary growth cultures which originated from the nascent action of bacteriophage F56 on cultures HP and 712.			
None	None	0.52	0.59
None	F59	0.24	0.23
None	F56	0.51	0.57
712	F56	0.31[a]	0.32[a]
Average results obtained using 5 secondary growth cultures which originated from the nascent action of bacteriophage F59 on cultures W2 and HP.			
None	None	0.37	0.43
None	F24	0.39	0.43
None	F59	0.37	0.42
HP	F59	0.38	0.43

[a] Low acidity due to nascent phenomenon.

occurrence of the nascent phenomenon. Before the titratable acidity determinations were made, 1 per cent of each mixture was transferred to sterile skimmilk. These secondary growth cultures were incubated at 32° C. for 16 hr. or until coagulated and usually were propagated three or four times before determinations of sensitivity to bacteriophage were made. After the quantity used in the preparation of the secondary culture had been removed, the remainder of each mixture was coagulated with added lactic acid and bacteria-free filtrates were prepared. Bacteriophage present in such filtrates were considered to have arisen during occurrence of the nascent phenomenon.

The action of bacteriophage on secondary growth cultures obtained after occurrence of the nascent phenomenon are given in table 4. Secondary growth cultures following the action of bacteriophage F56 on cultures W2 and 712 were

in all cases similar to primary cultures of W2. They produced acid rapidly and were lysed by bacteriophage F24. Except in the presence of culture 712, these secondary growth cultures were not retarded by F56. Similarly, secondary growth cultures following the action of F56 on cultures HP and 712 resembled primary growth of culture HP. However, the action of F59 on W2 and HP gave rise to cultures which did not resemble W2 or HP. These secondary growth cultures produced acid poorly and were resistant to bacteriophage strains F24 and F59. That the filtrate of bacteriophage F59 used in preparing the secondary growth cultures was not contaminated with F24 was evident after several attempts failed to demonstrate its presence.

The titers of bacteria-free filtrates prepared following occurrence of the nascent phenomenon are given in table 5. Although titers representing the

TABLE 5

Specificity of bacteriophage isolated following the nascent phenomenon

Bacteria and bacteriophage used for preparing filtrates		Titratable acidity at 6 hr. (%)	Most probable no. of bacteriophage /ml. of bacteria-free filtrate using culture:		
Culture(s)	Bacteriophage		W2	HP	712
W2	F59	0.52	< 0.33	.....	.....
W2 and HP	F59	0.26[a]	2.5	2.5×10^{6}[b]	.....
W2	F56	0.50	< 0.33	.....	.....
W2 and 712	F56	0.25[a]	2.5	.....	9.5×10^{8}
HP	F56	0.45	.....	< 0.33	.....
HP and 712	F56	0.24[a]	.....	< 0.33	2.5×10^{8}

[a] Low acidity due to nascent phenomenon.
[b] Highest dilution made.

strains of bacteriophage used to cause the nascent effect were high, the filtrates inhibited nonhomologous strains of bacteria only slightly in case of W2 and not at all in case of HP. These results indicated that the bacteriophage isolated were similar in specificity to those strains used to cause the nascent phenomenon.

With several trials of the nascent phenomenon in which W2 was used as the nonhomologous strain, 1-ml. quantities of the resulting filtrate inhibited growth of W2. However, attempts to propagate the inhibitory principle failed, and inhibition of W2 by these filtrates appeared either not to result from the action of bacteriophage or to result from the action of a strain of bacteriophage which could not be propagated on this culture. These results suggested that a large amount of filtrate containing either F56 or F59 would inhibit growth of W2 even in the absence of specific host bacteria. To determine the effect of large amounts of filtrates containing F56 or F59, an experiment was run to compare growth of 1 per cent W2 or HP with and without addition of 10 per cent of the appropriate undiluted filtrate. A recently prepared filtrate of bacteriophage F56, labeled F56A, also was used in this experiment because it contained a larger number of bacteriophage particles than the filtrate labeled F56. For comparison of filtrates F56 and F56A, 1 per cent of each filtrate was added to cultures of W2, and 10 per cent of each filtrate was added to cultures of HP. Plate counts and titratable acidity determinations were made at 6 hr. Bacteria-free filtrates

were prepared from the cultures showing retarded acid development, and filtrates were titered to determine inhibition of the nonhomologous strain of bacteria.

Even in the absence of specific host bacteria, 10 per cent of filtrate containing either bacteriophage F56 or F59 drastically reduced the acid produced during 6 hr. by culture W2 (table 6). Titers of the bacteria-free filtrates again indi-

TABLE 6

Effect of adding large amounts of filtrate F56 or F59 to nonhomologous culture W2 or HP

Culture used	Bacteriophage added		Plate count at		Titratable acidity at 6 hr. (%)	Most probable no. of bacteriophage/ml. of filtrate using culture W2
	Strain	Calculated titer at 0 hr.	0 hr.	6 hr.		
W2	None	.....	2.0×10^7	2.4×10^9	0.51	
W2	10% of F56	9.5×10^6	1.8×10^7	1.5×10^8	0.26	< 0.33
W2	1% of F56	9.5×10^5	1.7×10^7	1.9×10^9	0.50	
W2	1% of F56A	2.0×10^7	1.8×10^7	1.5×10^9	0.46	< 0.33
W2	10% of F59	.....	1.6×10^7	1.7×10^8	0.27	< 0.33
HP	None	.....	1.3×10^7	1.4×10^9	0.49	.....
HP	10% of F56	9.5×10^6	1.1×10^7	8.0×10^8	0.47	.....
HP	10% of F56A	2.0×10^8	1.3×10^7	8.0×10^8	0.43	.....

cated that inhibition of W2 was not accompanied by multiplication of an homologous strain of bacteriophage. Even with these low titratable acidity values, plate counts at 6 hr. were very high. One per cent of filtrate F56A caused slightly greater inhibition of W2 than did 1 per cent filtrate F56, which contained fewer bacteriophage. Neither F56 nor F56A caused drastic reduction in acid produced by HP, but F56A caused slightly greater inhibition.

That the inhibition of W2 by either filtrate F56 or F59 was due to the large numbers of bacteriophage particles and not to some other inhibitory principle seemed evident from the following experiments. Quantities of 10 ml. of filtrates F56 and F59 were added to 100-ml. bottles of skimmilk and heated in flowing steam for periods of 3 and 4 min. Bacteriophage titers indicated greatly reduced numbers of bacteriophage after 3 min.; no bacteriophage titers were found after 4 min. Also, bacteriophage-free whey was obtained from skimmilk cultures of 712 and HP. Neither heated filtrate nor an equal amount of bacteriophage-free whey reduced the acid produced in skimmilk during 6 hr. by 1 per

TABLE 7

Effect on acid production of adding different amounts to Streptococcus lactis W2 to skimmilk containing 3 per cent of filtrate F56

Amount of culture W2	Filtrate added	Titratable acidity at:			
		2 hr.	4 hr.	6 hr.	8 hr.
(%)		(%)	(%)	(%)	(%)
0.1	−	.....	0.22	0.32	0.57
0.1	+	.....	0.20	0.20	0.21
1.0	−	0.25	0.36	0.50	.....
1.0	+	0.24	0.28	0.47	.....
10	−	0.42	0.54	0.70	.....
10	+	0.37	0.52	0.72	.....

cent of culture W2. In a different experiment, bottles of skimmilk with and without 3 per cent of filtrate F56 were inoculated with different amounts of culture. Titratable acidity values (table 7) showed that as the amount of W2 was increased, inhibition caused by the filtrate decreased. Practically no acid was produced by 0.1 per cent culture during 8 hr. in the bottle containing filtrate. With 1 per cent culture the acidity values were slightly less in the diluted filtrate at 4 and 6 hr. Ten per cent culture of W2 resulted in acidity values which were slightly different at 2 hr. However, values for the culture which contained filtrate were approximately equal to those for the control at 4 and 6 hr. Thus, inhibition by a constant amount of filtrate was changed considerably by differences in the ratio of bacteria to bacteriophage.

In view of the importance of the ratio of bacteria to bacteriophage, plate counts were made at hourly intervals for cultures of 1 per cent W2 with and without addition of different amounts of filtrate F56A. The results (table 8)

TABLE 8

Effect of different numbers of bacteriophage F56 on 1 per cent culture of Streptococcus lactis W2

Amount of filtrate F56A added (ml./100 ml. skimmilk)	Calculated no. of bacteriophage particles /ml. at 0 hr.	Plate count at:					Titratable acidity at 6 hr. (%)
		0 hr.	1 hr.	2 hr.	4 hr.	6 hr.	
0	.....	2.0×10^7	4.0×10^7	1.2×10^8	1.0×10^9	2.1×10^9	0.54
1	2.5×10^6	1.7×10^7	2.0×10^7	7.1×10^7	5.4×10^8	2.2×10^9	0.52
3	8.0×10^6	1.8×10^7	1.2×10^7	4.7×10^7	2.6×10^8	1.5×10^9	0.48
5	1.2×10^7	1.7×10^7	5.0×10^6	1.2×10^7	1.4×10^8	9.7×10^8	0.38
10	2.5×10^7	1.8×10^7	1.5×10^6	2.0×10^6	1.9×10^7	1.5×10^8	0.27

confirmed those of table 7 in that inhibition of W2 was directly related to the number of particles of bacteriophage F56. As the number of bacteriophage particles was increased, the per cent titratable acidity at 6 hr. decreased, and plate counts indicated decreased numbers of viable bacteria. In cases of 5 and 10 ml. of filtrate, the numbers of bacteriophage particles were about equal to the plate counts at 0 hr., and at 1 hr. the plate counts were considerably lower than at 0 hr. Decreases in plate count possibly resulted from the absence of bacterial multiplication following that which appeared to be adsorption of particles of F56 to cells of W2. However, even with 10 ml. of filtrate, plate counts increased rapidly after 2 hr. Apparently, reproduction of the bacteriophage particles either progressed very slowly or stopped at some point before completion. Cells which had escaped attack then could have accounted for the increases in plate count and the considerable amounts of lactic acid.

In view of the failure of F56 to multiply on culture W2, plate counts and direct microscopic observations were compared at 4, 6 and 8 hr. during the action of F56 on 712 and on a mixture of 712 and W2. Microscopic observations revealed very large numbers of bacteria in the mixture and few bacteria in the culture of 712. The large numbers of bacteria present in the mixture and the cell debris made it difficult to obtain accurate counts of the bacteria, which

were found to occur as single cells rather than in pairs. Estimates of microscopic counts at 6 hr. were 6.5×10^7 for the mixture and 4.5×10^5 for the culture of 712, as compared to plate counts of 2.5×10^4 and 3.4×10^3, respectively. The presence of much larger numbers of bacteria not detected by the plate count in the mixture was considered to verify a difference in the action of F56 on W2 as compared to 712.

DISCUSSION

Inhibition of certain nonhomologous strains of bacteria by bacteriophage, usually referred to as the nascent phenomenon, was found to occur even in the absence of host bacteria in cases where very large numbers of bacteriophage particles were supplied. Large numbers of either F56 or F59 were found very effective in retarding the acid production of W2, and large numbers of F56 caused some reduction in the acid produced by HP. Data suggested that bacteriophage F56 retarded the acid production of W2 by attacking and preventing bacterial multiplication. That W2 cells were only inhibited and not lysed seemed evident both from the failure of F56 to multiply on W2, and from microscopic observation of large numbers of bacteria not detected by plate counts. Multiplication of the bacteriophage apparently stopped at some point prior to completion or progressed at an undetected, extremely low rate. Hence, the homologous host which previously has been found essential to occurrence of the nascent phenomenon seems necessary only as a means of increasing the number of bacteriophage to a level which insures that most of the nonhomologous bacteria will be attacked. Thus, the nonhomologous bacteria are prevented from multiplying and producing appreciable amounts of acid. Secondary resistant cultures of the host failed to replace the homologous host strain of bacteria in cases where very large numbers of bacteriophage were not supplied.

Since homologous host bacteria apparently are important only as a means of supplying large numbers of bacteriophage, the term nascent seems no longer applicable to this phenomenon. Rather, the explanation suggested by the data draws attention to the basic method by which bacteriophage multiply. Generally it has been considered that bacteriophage are quite specific in their action and that specificity is largely a matter of adsorption. The data of this paper suggest that in certain cases strain specificity may be more inconclusive and involve the metabolic processes within the bacterial cell. The relationship between bacterial cell and bacteriophage multiplication after adsorption was strikingly indicated by the fact that 712 supported complete multiplication of F56 and HP supported complete multiplication of F59 under conditions identical to those in which these strains of bacteriophage had merely inhibited the growth of W2. Evidently, differences between the metabolic processes of 712 and W2 and between those of HP and W2 influenced multiplication of the two strains of bacteriophage after adsorption. Utilization of W2 in comparison to the normal host bacteria may be useful as a means of separating adsorption from multiplication in studying these strains of bacteriophage. Furthermore, a difference is suggested between the requirements for multiplication of bacteriophage strains F56 and F59 and the requirements for F24, which multiplied readily on culture W2. Collins *et al.*

(4) found that F56 and F59 require calcium for multiplication (possibly not for adsorption), and that calcium is not required by F24. Although skimmilk contains adequate calcium, this difference may be associated with a more basic difference between the requirements of these different strains of bacteriophage. In experiments not yet reported, additions of yeast extract, casein hydrolysate, V8 juice, and $CaCl_2$ to the already very nutritive medium, skimmilk, failed to permit multiplication of F56 on W2.

Strains of bacteriophage which caused the nascent phenomenon required a definite specificity of the nonhomologous strain before multiplication and acid production could be retarded. This specificity was in one of two cases unchanged by the action of a different bacteriophage. The selection of cultures from W2 which were resistant to bacteriophage F24 did not result in cultures resistant to the nascent effects of bacteriophage strains F56 and F59. However, bacteriophage F56 failed to cause the nascent phenomenon when secondary growth cultures from HP were used. Thus, it appeared that bacteriophage F24 was more selective in its action than either F56 or F59, and that F59 was less selective than F56. These differences in selectivity of the different strains of bacteriophage may explain in part the observed differences in secondary growth cultures arising after the nascent action of bacteriophage strains F56 and F59. Secondary growth cultures arising after the action of F59 on W2 and HP were resistant to F24, and those arising after the action of F56 on HP and 712 were sensitive to F59. The previously mentioned differences in selectivity would indicate these results. However, they would not indicate the sensitivity to F24 of secondary growth cultures arising after the action of F56 on W2 and 712. It should be observed that in all cases where F56 was used to cause the nascent phenomenon, secondary growth cultures were similar to primary growth of the nonhomologous strain of bacteria; but in cases where F59 was used, secondary growth cultures did not resemble either strain used in the mixture. Furthermore, most probable numbers obtained by titering filtrates of mixtures against the homologous strain of bacteria used, indicated smaller numbers of F56 than of F59 (table 5). Thus, the number of bacteriophage present during occurrence of the nascent phenomenon and its influence on the ratio of bacteriophage to bacteria may be of considerable importance in determining the sensitivity of resulting cultures. A comparatively low number of bacteriophage particles might be insufficient to act upon all of the susceptible cells of the normally considered resistant strain. Consequently, the sensitivity of resulting cultures might not indicate accurately the comparative selectivity of the bacteriophage.

That secondary growth cultures of 712 and HP did not permit F56 and F59 to give the nascent effect and that these strains of bacteriophage could not multiply on the nonhomologous bacteria during occurrence of this phenomenon are important to an understanding of the action of bacteriophage on mixed strain starter cultures. For example, should strains of bacteria similar to W2 and 712 be present in a mixture, the entrance of bacteriophage similar in specificity to F56 would result in retardation of acid production by both strains. The total acid produced by the culture then would depend greatly on the remaining strains of

bacteria. However, since secondary cultures of 712 did not permit the nascent phenomenon, it seems possible that, following multiplication of uninfected cells, the strain similar to W2 might "recover" and produce significant amounts of lactic acid during subsequent propagations. In the absence of recovery the production of acid by such a mixture would be influenced to no greater extent than that which would result from mass lysis of the strains similar to 712 and W2.

SUMMARY

The nascent phenomenon was found to occur with two of 14 strains of bacteriophage. The acid-producing abilities of *Streptococcus lactis* W2 and *Streptococcus cremoris* HP were drastically reduced when either was cultured with bacteria-bacteriophage combination 712-F56; further, W2 produced very little acid when cultured with combination HP-F59. In cases where the homologous host bacteria were omitted and in cases where secondary growth cultures of the host bacteria were used, acid production was not reduced, unless very large numbers of bacteriophage were employed.

Large numbers of either F56 or F59 definitely retarded the acid production of W2, even in the absence of host bacteria. Particles of bacteriophage F56 were found capable of preventing normal multiplication of W2 cells. However, multiplication of the bacteriophage apparently stopped prior to completion, and bacteriophage capable of complete multiplication on W2 were absent. The homologous host which previously has been found necessary to occurrence of this phenomenon seemed essential only as a means of supplying large numbers of bacteriophage.

A definite specificity of the nonhomologous culture was found necessary to occurrence of the nascent phenomenon, and differences were found in the selective abilities of different strains of bacteriophage.

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ACTION OF BACTERIOPHAGE ON MIXED STRAIN STARTER CULTURES. II. RELATION TO ACID PRODUCTION OF THE PROPORTION OF RESISTANT BACTERIA

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Although mixed strain starters, which may contain several strains of lactic streptococci, are used extensively in the manufacture of certain dairy products, retarded acid development caused by the action of bacteriophage has remained of primary importance. The use of mixtures has been considered to decrease the occurrence of starter failure. However, their use also has been found to complicate diagnosis of starter difficulty and to make the effect of bacteriophage action less predictable. Among those who have reported retarded development of mixed strain cultures due to the action of bacteriophage are Anderson and Meanwell (2), Johns (7) and Nelson *et al.* (10). In addition to retarded acid development, this laboratory has encountered starter failure caused by bacteriophage, similar to that reported by Johns and Katznelson (8) and Babel (3), in which the retardation of acid production was as drastic and abrupt as that usually encountered with single strain starters. In view of these different degrees of retarded acid development during the use of mixed strain starters, this study was undertaken to determine the proportion of susceptible bacteria necessary to permit the action of bacteriophage to appreciably change the amount of acid produced by a starter composed of different strains of *S. lactis*.

METHODS

Since a previous study (5) had shown that the "nascent phenomenon" would occur upon mixing certain bacteria-bacteriophage combinations, such combinations were carefully excluded from mixtures. Cultures of *S. lactis* were propagated in litmus milk at 32° C. Fresh undiluted culture was added as inoculum for those experiments in which the amount of a culture required was 1 per cent or greater. For smaller amounts, cultures were diluted 1:3 in sterile distilled water to facilitate measurement of the inoculum. Bacterial populations were determined at 32° C. by the standard plate count (1) on tryptone-glucose-beef extract-milk agar.

The limiting active dilution method (6) was used for estimating the most probable number of bacteriophage particles per milliliter of whey filtrate. Dilutions were made in sterile skimmilk, and bottles having the desired titer were used as inoculum.

Experiments were run using 300-ml. quantities of sterile skimmilk. Culture and diluted filtrate were added to bottles of skimmilk which had been permitted to reach 32° C. in a water bath. Titratable acidity values and bacterial populations were determined at hourly intervals following inoculation. Results of duplicate experiments were averaged.

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RESULTS

Experiments were run to determine the effects of adding about 2,500 particles of bacteriophage per milliliter to cultures inoculated with equal parts of one susceptible and one resistant strain of bacteria. For an experiment of this type, four quantities of skimmilk were used. Three were inoculated with 1 per cent of the susceptible strain; two of these three and the fourth bottle were inoculated with 1 per cent of the resistant strain. Diluted bacteriophage filtrate then was added to the first and second bottles. Thus, the first bottle served to indicate growth of the susceptible strain with bacteriophage added, the second and third bottles served to indicate growth of the two strains with and without the addition of bacteriophage, and the fourth bottle indicated growth of the resistant culture in the absence of bacteriophage.

Eight different mixtures gave results similar to those reported in figure 1

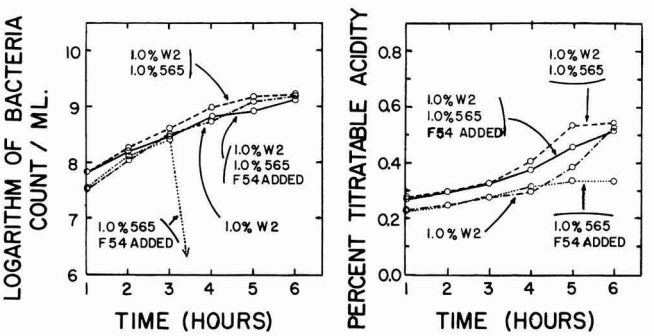

FIG. 1. Differences in bacterial population and titratable acidity caused by adding 2,500 particles per milliliter of bacteriophage F54 to a mixture of *Streptococcus lactis* strains 565 and W2.

for a mixture of *S. lactis* strains 565 and W2. That bacteriophage F54 caused mass lysis of 565 was indicated by a pronounced decrease in the plate count of surviving bacteria between 3 and 4 hr. Following mass lysis, increases in titratable acidity were very small, and the titratable acidity value at 6 hr. was only 0.34 per cent. The culture containing both strains of bacteria and bacteriophage F54 gave plate counts which were slightly lower at 3, 4 and 5 hr., immediately following the probable lysis of 565, than the same mixture without bacteriophage. At 5 hr. the plate count for this mixture was even lower than that for the culture containing only W2. The production of acid was slightly retarded after lysis of 565, but the acidity values for this mixture were greater until about 6 hr. than those for W2 growing alone. The low acidity value at 6 hr. for the mixture of W2 and 565 was attributed to experimental error.

Since the addition of bacteriophage to cultures inoculated with equal amounts of susceptible and resistant bacteria had caused only slightly retarded acid production, experiments were run to determine the effects of using smaller proportions of resistant bacteria. The resistant culture, 565, was used to make up 50,

25 and 5 per cent of the total bacterial inoculum for bottles which received both 565 and W2. Bacterial populations and titratable acidity values for mixtures with bacteriophage F24 were compared to those for identical mixtures without bacteriophage, and to those for cultures inoculated with amounts of resistant bacteria equal to those used in mixtures. Results of experiments in which the total bacterial inoculum for mixtures was 1 per cent and in which about 25 bacteriophage particles per milliliter were added are given in figure 2.

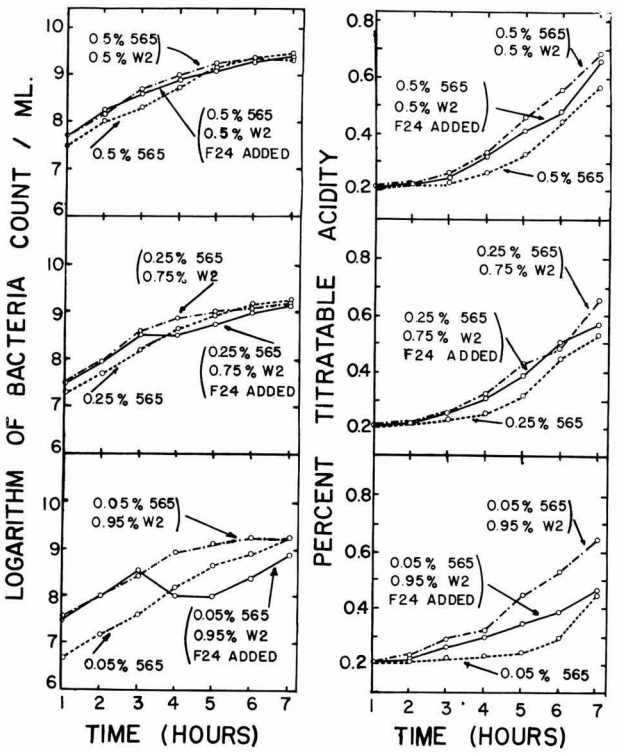

FIG. 2. Differences in bacterial population and titratable acidity caused by adding 25 particles per milliliter of bacteriophage F24 to mixtures of *Streptococcus lactis* strains W2 and 565.

Bacteriophages F24 caused slightly subnormal plate counts at 4 and 5 hr. where cultures 565 and W2 were used in equal proportions. Titratable acidity values also were slightly below normal at 5 and 6 hr., following the probable lysis of W2. However, the plate counts at 6 hr. were about equal for the two mixtures, and the titratable acidity values were only slightly different at 7 hr. Rapid recovery following the lysis of W2 was attributed to growth of strain 565. Although the plate count for 565 alone was about equal to that for the mixture with bacteriophage at 5 hr., the acidity values for 565 were somewhat lower

throughout 7 hr. The higher acidity values for the mixture were attributed to fermentation by W2 prior to mass lysis. These differences in titratable acidity were similar to those obtained in comparing a mixture of 1 per cent W2, 1 per cent 565 and bacteriophage F54 to a culture of 1 per cent W2 (figure 1).

The addition of bacteriophage caused greater differences in plate counts at 4 through 7 hr. for mixtures in which the amount of 565 was only 25 per cent of the total bacterial inoculum. That plate counts for the mixture which contained bacteriophage were lower than those for a culture of only 565 was surprising. It seemed that either the multiplication of 565 in the mixture had been retarded or that bacteriophage F24 had caused a slight reduction in the population of 565. However, care had been taken to select bacteria-bacteriophage combinations in order to exclude the nascent phenomenon. Titratable acidity values were slightly lower at 5 and 7 hr. for the mixture to which bacteriophage had been added. That the acidity values for these mixtures were approximately equal at 6 hr. probably was due to experimental error.

Use of 565 as only 5 per cent of the total bacterial inoculum resulted in exaggeration of effects which were less evident with larger amounts of resistant bacteria. Bacteriophage F24 caused a decrease in the number of surviving bacteria at about 4 hr., following the lysis of W2. The titratable acidity values increased at a much lower rate than those of the culture lacking bacteriophage. There was considerable lag in the production of acid by the culture of 565 which had received a comparatively small amount of bacterial inoculum. However, between 5 and 7 hr. the titratable acidity values increased rapidly, and at 7 hr. the titratable acidity for this culture was about equal to that in the mixture with added bacteriophage.

To simulate the cultural conditions used in the manufacture of cottage cheese, an experiment was run using a total bacterial inoculum of 5 per cent. Mixtures of cultures 565 and W2 were used with and without addition of about 2,500 particles of bacteriophage F24 per milliliter. The proportions of the resistant culture, 565, were 50, 25 and 10 per cent of the total inoculum. Titratable acidity values are plotted in figure 3.

Bacteriophage affected the amount of acid produced in cultures which received a total bacterial inoculum of 5 per cent culture in a manner similar to that which had been found using 1 per cent. The action of bacteriophage on a culture containing equal parts of 565 and W2 resulted in titratable acidity values which were slightly below normal at 3 through 7 hr., following the probable lysis of W2. At 3 and 4 hr., differences in titratable acidity for these mixtures were very small. In cases where 565 was used as 25 per cent of the inoculum, the action of bacteriophage caused slightly greater differences in acidity at 4 through 7 hr. Use of 565 as only 10 per cent of the total bacterial inoculum for mixtures permitted bacteriophage to cause greater differences in acidity values at 3 through 7 hr., but even with this comparatively small proportion of resistant bacteria, increases in titratable acidity for mixtures which contained bacteriophage were considerable. In each case the titratable acidity values were higher throughout 7 hr. for the mixture containing bacteriophage than for the

culture of resistant bacteria alone. However, increases in titratable acidity were about equal. Thus, the growth of susceptible and resistant bacteria appeared to establish the level of acidity at the time of mass lysis. Increases in titratable acidity above that level were similar to increases caused by the resistant bacteria growing alone, and the acid contributed by the susceptible culture following lysis appeared negligible. That the susceptible culture produced very little acid following mass lysis also was shown by the case in which bacteriophage F24 was added to 5 per cent of strain W2.

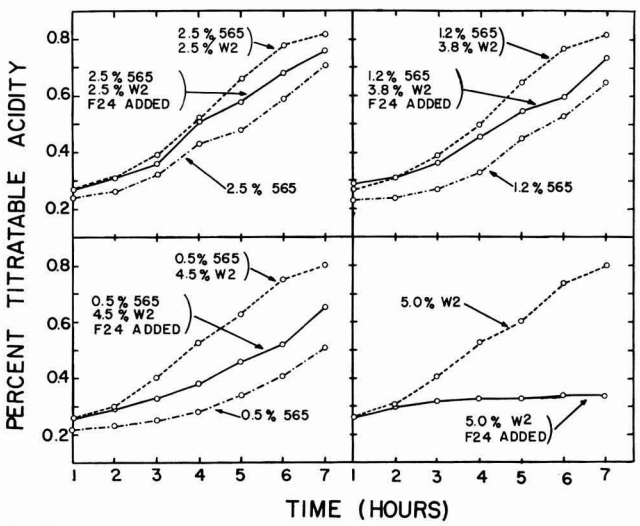

FIG. 3. Differences in titratable acidity caused by adding 2,500 particles per milliliter of bacteriophage F24 to mixtures of *Streptococcus lactis* strains W2 and 565.

DISCUSSION

Although the addition of bacteriophage to mixtures of susceptible and resistant bacteria resulted in subnormal acid production after mass lysis of the susceptible strain, the acidity values were higher than those for cultures of equal portions of resistant bacteria growing separately. However, subsequent increases in titratable acidity for such mixtures were similar to increases for cultures of only the resistant strain. Apparently, the total bacteria present prior to mass lysis determines the acidity at lysis for a mixture of susceptible and resistant bacteria. Following lysis of the susceptible strains, only the resistant strains of bacteria are important in the continued production of acid. Thus, the degree of subnormality in acid production which may be caused by the action of bacteriophage on a mixed strain starter is influenced by the time at which the susceptible bacteria are mass-lysed, by the proportion of bacteria which are not

susceptible to the strain or strains of bacteriophage present, and by the acid-production rate of the resistant bacteria.

With each of the mixtures used the rate of acid production was only decreased by the addition of bacteriophage. Even with mixtures which contained comparatively large proportions of susceptible bacteria, cases of suddenly interrupted acid production were not encountered. Titratable acidity values very closely resembled those obtained by Katznelson and Hood (9) following the addition of different amounts of penicillin to milk, and those obtained by Bradfield *et al.* (4) with milk which contained aureomycin. It is apparent that in cases of retarded production of acid by mixed strain starters one can not use titratable acidity values as a means of safely distinguishing between cases caused by antibiotics and those resulting from the action of bacteriophage.

Commercially prepared mixed strain cultures may contain several strains of lactic streptococci; in some cases 8 or 10 strains are used. Although the sudden appearance of several strains of bacteriophage in the cheese vat seems unlikely, continued use of a mixed strain starter may result in the build-up of a sufficient number of bacteriophage strains to cause subnormal development of acid following lysis of the major portion of bacteria. Such a gradual development of difficulty probably would become evident and would seem within limits of control. The employment of adequate sanitation practices and constant vigilance to discontinue for some time the use of starters upon evidence of retarded acid development would seem adequate. Gradual build-up of bacteriophage strains active against the bacteria of a mixed strain culture may be cited as an explanation for certain cases of retarded acid development. However, this interpretation does not appear to adequately explain sudden failure.

In view of the fact that the major portion of fast acid-producing bacteria in a mixture must be susceptible to permit the action of bacteriophage to cause appreciable subnormality of acid development, the use of equal amounts of two unrelated starters each day for cheesemaking with planned daily rotation of starters appears to have considerable merit as a precaution against starter difficulties due to the action of bacteriophage. Although this practice is seldom followed in the United States, the rotational use of two single strain starters each day has been practiced extensively in New Zealand. In the present study the effect on acid production caused by the action of bacteriophage was very slight in cases where susceptible bacteria were used as 50 per cent of the total bacterial inoculum. Even in cases where a susceptible strain was used as 75 per cent and 90 or 95 per cent of a mixture, the addition of bacteriophage did not cause the acid production to lag more than about 1 hr. and 2 hr., respectively, behind that for control cultures which lacked bacteriophage. Thus, the rotational use of equal amounts of two unrelated starters would seem to give considerable protection against starter difficulties due to the action of bacteriophage. With certain dairy products which require lactic starter, single strain cultures are sufficient; with others, multiple type cultures may be required. The use of equal amounts of unrelated less complex starters and knowledge of the exact

strain or strains of bacteria used in their preparation would greatly simplify diagnosis of starter difficulties.

SUMMARY

Plate counts and titratable acidity values were subnormal following the action of bacteriophage on a mixture of one susceptible and one resistant strain of bacteria. When the susceptible strain was used to make up 50 per cent of the total bacterial inoculum, plate counts and titratable acidity values were only slightly below normal. Differences in plate counts were negligible 2 to 3 hr. after the bacteriophage had caused lysis of the susceptible strain. Use of the susceptible strain as 75 per cent of the mixture permitted bacteriophage to cause greater deviations in plate counts and in titratable acidity, but even in mixtures which contained susceptible bacteria as 90 and 95 per cent of the total bacterial inoculum, the production of acid was appreciable and greater than that in cultures which had been inoculated with only resistant bacteria in amounts equal to those used in mixtures.

ACKNOWLEDGMENT

This study was supported in part by funds from the California Dairy Industry Advisory Board.

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THE HEAT STABILITY OF EVAPORATED MILK AS A FUNCTION OF THE STEAM AND HYDROSTATIC PRESSURE APPLIED IN THE FOREWARMING OF MILK

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The usual pretreatment process used in the preparation of raw milk for the manufacture of evaporated milk consists of heating the milk, prior to evaporation, to 190 to 210° F., either by the direct injection of steam into the milk or indirectly by circulating the milk through tubular heat exchangers. Occasionally, the milk is heated to temperatures above 212° F. in an attempt to improve the heat stability.

It has been known for many years that if raw milk is heated to temperatures above 212° F., the heat stability of the subsequently produced evaporated milk is improved generally. Grindrod (2) found that evaporated milk made from raw milk which had been heated rapidly to elevated temperatures, such as 260° F., was improved in heat stability. He attributed the effect to disruption of the protein by the high velocity of the steam used. Later, Webb *et al.* (7) found that the heat stability of milk could be increased greatly by heating it indirectly in a heat exchanger to relatively high temperatures, for example, in the range 230 to 290° F. The time required for the cold milk to reach these temperatures was approximately 3 sec. and the time of holding at these temperatures was 25 sec.

The milk, when treated by either of the two preceding processes, was found to be unsatisfactory for use in the preparation of commercial sterilized evaporated milk because of the low viscosity and poor storage quality of the sterilized product. In order to reduce this difficulty, Webb *et al.* (7) suggested subjecting only part of the milk to the high-temperature process. It was presumed that the sterilization process could be controlled either by mixing the proper amount of high-temperature heated milk with milk processed in the usual manner, or by reducing the temperature of the heat treatment.

It was noted by Grindrod (3) and later by Bell *et al.* (1) that the very heat-stable evaporated milks produced by the high-temperature treatment were improved in storage stability if the sterilization process were severe enough to produce an increase in color of the sterilized milk. All attempts to produce sterilized evaporated milk light in color were accompanied by rapid fat separation on storage, followed often by gelation. Bell *et al.* (1) remarked that, "Long continuing fluidity and high heat stability are not correlated. Long continuing fluidity is rather due to changes caused by heat which are associated with a darker color and more cooked flavor."

While it appears that highly heat-stable milk produced by the processes reported so far is not satisfactory commercially, it is the purpose of this paper to

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report on a process for the production of an evaporated milk of above-normal heat stability but without the defects associated with highly heat-stable milk. However, this process appears to be limited to milk of good natural heat stability, that is, milk low in salt content, primarily calcium, in relation to protein.

EXPERIMENTAL PROCEDURE

The steam jet assembly was formed by passing a small steam line through the 1-in. cap of a tee which was located between the pump and hot well. The steam line projected through the cap approximately 1 in. This section, or nozzle, was detachable so that nozzles containing orifices of various sizes could be used. A thermometer was located in the line approximately 5 in. from the nozzle. A spring-loaded valve for applying back pressure was located in the line near the outlet to the hot well.

The general procedure followed in the preparation of above-normal, heat-stable evaporated milk was as follows: A 50-lb. lot of cold raw milk without previous heat treatment was heated almost instantaneously from 40 to approximately 210° F. by the injection of steam into the milk while it was being pumped by a positive displacement pump at the rate of 8 lb. per minute, and under 20 to 40 lb. gauge pressure, from the supply tank to the hot well. Variation in the operation of the steam injection process was obtained by changing the nozzle to one containing the proper number of orifices to give the desired manifold pressure and by constricting the milk outlet by means of the spring-loaded valve to give the desired back pressure on the milk.

The milk was held in the hot well less than 5 min. at 190 to 200° F. This was followed by evaporation, homogenization at 2,500 lb. per in.^{2} at 130 to 140° F., cooling to 40° F. and standardization to 7.93 per cent fat and 26.5 per cent total solids. The milk was filled into 14.5-oz. standard cans.

The sterilization process used was described by Nelson (5), but briefly, it consisted of a preheating period of 9 min. at 227° F., followed by a cooker process at the times and temperatures given in tables 1 and 2. Heat stability was determined by the number of minutes required in the cooker at the given temperature to produce a slight film grain in the sterilized evaporated milk. Color was estimated by the spectrophotometric method as used by Nelson (4).

EXPERIMENTAL RESULTS

The data in table 1 are typical of the effects produced by variations in steam and hydrostatic pressure used during the forewarming of raw milk on the time required in the cooker to produce equivalent grain formation in the evaporated milk. One week elapsed between the preparation of lots 1 and 5; therefore, these lots of milk are not identical. In San Jose, however, seasonal changes in heat stability are gradual and not so marked as in the colder regions.

The normal heat stability of evaporated milk prepared in the usual commercial manner in San Jose varies from 6.5 to 7.5 min. at 250° F. It is apparent that lot 1 is average in heat stability. The increased manifold pressure used in lots 2, 3 and 4 resulted in an increase in the heat stability of the evaporated

TABLE 1

The effect of variation in steam and hydrostatic pressure on the heat stability of evaporated milk

Lot	Steam manifold		Steam pressure at manifold	Hydrostatic pressure	Cooker	
	No. of holes	Dia. of each hole			Time	Temp.
		(*in.*)	(*psi*)	(*psi*)	(*min.*)	(° *F.*)
1	4	0.125	35	20	7.5	250
2	8	0.0625	60	20	11.5	250
3	2	0.125	70	20	13.0	250
4	6	0.0625	95	20	17.0	250
5	4	0.125	55	40	18.0	250

milk. The manifold pressure in lot 5 is reduced, but this is compensated by the increase in back pressure. Since the increase in manifold and back pressure is equivalent to an increase in temperature of the steam at the time of its condensation in the milk, it is probable that a fraction of the milk is heated to a relatively high temperature, although for a very short period of time. The evidence is without direct proof; however, the marked lowering of the volatile sulfide content (unpublished data) is in agreement with the data found by Townley and Gould (6) for milk heated at high temperatures.

In table 2, lots 1, 2, 3 and 4 represent milk in which the effects of long and

TABLE 2

A comparison of cooker processes with respect to the physical properties of evaporated milk prepared from jet-treated raw milk and stored 90 d. at 100° F.

Lot	Cooker process		Visc. at 75° F.	% reflectance at 520 M	Grain	Destabilizer added	Fat separation
	Time	Temp.					
	(*min.*)	(° *F.*)	(*M. U.*)			(*ml.*)	
1	13	250	30	64.5	sl. film	0	1+
1	5	253	68	77.5	hy. film	1.5	1+
2	14	250	55	60.8	sl. film	0	1−
2	5	253	64	76.2	sl. film	1.4	1−
3	13	250	44	62.0	sl. film	0	1+
3	5	253	87	75.5	film	2.4	1+
4	12	250	50	62.8	sl. film	0	1−
4	5	253	50	75.2	sl. film	2.0	1
5	3	260	68	75.2	sl. film	1.2	1
5	3	260	25	75.2	none	0	2
6	4	260	70	75.3	film	0	2+
6	5.2	255	58	76.2	sl. film	0	2+
6	8.6	248	84	70.1	film	0	1−

short cooker processes are compared. Since an effort was made to have an equivalent grain condition at the end of the process, it was necessary to destabilize that portion of the milk used for the short process by the addition of 10 per cent $CaCl_2$ or N phosphoric acid. The short-process milk is lighter in color than normal (normal is 66–68) and the long-process milk is darker in color than normal. The processes used on lot 5 are identical. The object is to note the effect of viscosity and destabilizer on the fat separation of the two lots. Lot 6 is commercially forewarmed milk and is added for comparison.

After 90 days' storage at 100° F., samples were inspected for gelatin and fat separation. No gelation or increase in viscosity was found. Fat separation was evident and was estimated by visual inspection. This is a commercial procedure and is based on the amount and toughness of the fat layer. Index 1 represents a thin layer of fat, incorporated easily into the milk; index 2 represents an objectionable amount of fat, incorporated with more difficulty into the milk; index 3 represents an objectionable amount of fat which cannot be stirred into the milk. The significant feature of these data is that no appreciable difference exists in fat separation between milk light and milk dark in color when a destabilizer is used in the milk light in color to develop a comparable viscosity. This is not true of milk forewarmed in the usual manner, such as lot 6. Lot 5, which does not have added destabilizer and is low in viscosity does show an increase in fat separation, but it is less than expected.

The use of the steam-jet process on milk low in natural heat stability was not successful. Pilot experiments performed in Wisconsin during the late winter and early spring were not successful in increasing the heat stability of raw milk above the average attained by commercial processes. This was not unexpected in view of the relatively poor heat stability of the milk at this season of the year. Later, however, when the heat stability of the milk had improved to some extent pilot experiments indicated no net gain in heat stability from the steam jet process.

DISCUSSION

The forewarming process described furnishes a convenient method for increasing the fat stability of milk used in the preparation of sterilized milk lighter than normal in color. It permits the development of a satisfactory viscosity which differentiates this process from similar processes in commercial use. Commercial processes use back pressure sufficient only to heat the milk to the desired temperature. This results in a moderate increase in heat stability, but a decided loss in viscosity occurs. Both processes require milk of good natural heat stability.

It is not known why milk low in natural heat stability fails to respond to this process. In general, these milks are low in protein in relation to the salt content, especially to calcium and magnesium. It is assumed generally that casein, which constitutes the greater portion of the protein, is affected directly by the salt balance in the heat coagulation of milk. This may be true, but the possibility exists that the salt balance operates to some extent indirectly on the casein through its effect on the denaturation and coagulation of the whey proteins.

SUMMARY

A process has been devised for the heat stabilization of milk which is based upon the regulation of the manifold steam pressure and the hydrostatic back pressure applied to the milk.

The process differs from those reported previously in that part of the milk is heated apparently to a high temperature but cooled with extreme rapidity by

the remainder of the milk to the desired temperature, preferably 200 to 210° F.

It appears that milk which can be stabilized by this process is suitable for manufacture of evaporated milk light in color.

ACKNOWLEDGMENT

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ALTERATIONS IN SPECIFIC GRAVITY DURING THE RIPENING OF BULL SPERMATOZOA

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Experiments aiming at a separation of bull spermatozoa, according to size, by the aid of a counter-current centrifuge, the principle of which was sketched by Lindahl (8), suggested that changes in specific density or volume occurred during the ripening[1] of the sperm cells. A review of literature failed to reveal any data concerning such changes. For our purpose it was necessary to define a series of ripening stages delimited by visible changes. As such we have made use of the loss of the residual protoplasm and the cessation of motility and distinguished three stages: Spermatozoa with residual drop, motile[2] ones without residual drop, and non-motile ones without residual drop. These three types have been denominated unripe, ripe, and over-ripe. To be able to follow alterations in the specific density of spermatozoa, we have carried out determinations of the mean density of the sperm cells in a number of ejaculates taken from normally fertile bulls, and related the obtained data statistically to the frequencies of unripe, ripe and over-ripe spermatozoa in the ejaculates.

Preliminary experiments were carried out during July and August, 1949, in the laboratory of the Bull Breeding Association at Falkenberg, and then continued in this Institute, where the present experiments were performed during September–October, 1950.

MATERIAL AND METHODS

All the eight bulls (Swedish red and white cattle), from which sperm samples were collected for the present work belong to "Enköpingsortens seminförening" and were used in regular service every 4th day. The average percentage of fertility[3] of these bulls varied from 54 to 58. Sperm samples were taken during the period from Aug. 21 to Sept. 22, 1951. Sperm was collected by the method of the artificial vagina. Each time three ejaculates were taken from the same bull in immediate succession to provide a desirable variation as to the frequencies of unripe, ripe and over-ripe spermatozoa. The ejaculates are numbered 1, 2 and 3 in the order in which they were collected. In all cases the sperm samples were diluted three times with a slightly modified phosphate-buffer solution of Phillips

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[1] Dealing with non-morphological changes in the spermatozoa, which seem to occur prior to as well as subsequent to the loss of residual protoplasm, i.e. the completion of morphological maturation, we prefer to use the term ripening instead of maturation.

[2] As we are not interested in any relation to fertility of these spermatozoa, but only want to fix an arbitrary limit between different stages of ripeness, we have not differentiated our spermatozoa in "progressively advancing" ones and others, but in "actively moving" and "non-motile" ones.

[3] Calculated as the number of pregnancies per 100 first-inseminations. Pregnancies were determined 2 mo. after insemination by palpation of uterus via rectum.

and Spitzer (11). When used in our experiments, the spermatozoa had been stored in this solution for about 4 hr. at 3–4° C. To remove the seminal plasma, the suspension was centrifuged for 10 min. at 2,300 g., a treatment that did not appear to cause any mechanical injury to the cells.

The specific gravity of the spermatozoa was determined by suspending equal amounts of the sperm cell sediment obtained above in a series of solutions of different specific gravities, the lightest having a lower specific density than any of the spermatozoa, the heaviest being of about the same density as the densest spermatozoa. The sperm cell suspensions in the appropriate media were centrifuged at 2,300 g. This was done in hematocrit tubes with the wide part gradually and smoothly running into the capillary part, thus permitting all sperm cells of greater density than that of the medium to sediment down into the graded capillary. The centrifugation lasted 10 min. Prolongation did not reduce the height of the sediment column any more. Provided that the packing coefficient of unripe, ripe and over-ripe spermatozoa differs little, the described procedure permits a calculation of the fraction of spermatozoa having a lower specific density than that of a certain test fluid. The total volume of all spermatozoa in the sample was obtained by centrifuging a corresponding sample of sperm cells in the lightest medium. The test solutions to be used must have a rather high specific gravity, being at the same time physiologically inert. Thus at concentrations sufficiently high to give the desired specific gravity, the dissolved compounds must neither exert an osmotic pressure strong enough to diminish the volume of the spermatozoa by reducing their water content nor increase the viscosity of the solution too much. For this purpose we have used water solutions of methylglucamine salt of umbradil (2,5-diiodine-4-pyridon-N-acetic acid).[4] By balancing the concentrations of the two components in a suitable way, pH was regulated to 7.1. It appeared appropriate to use five test solutions, the physical properties of which are given in table 1. The specific densities of these

TABLE 1

Umbradil-methylglucamine-salt solutions used as test liquids. pH = 7.1

Indication no.	Umbradil	Methylglucamine	Specific gravity	Osmotic pressure (atm.)
	(g./100 ml.)	*(g./100 ml.)*		
I	1.396	0.705	1.0918	18
II	2.990	1.510	1.2193	71
III	3.522	1.779	1.2596	105
IV	4.253	2.148	1.3167	186
V	4.651	2.349	1.3519	220

solutions were determined by weighing the solutions in pycnometers and their osmotic pressures were taken from a curve relating osmotic pressure to specific gravity for the actual concentration range. The osmotic pressures of this curve were calculated from vapour-lowering determinations according to Wright (15).

[4] We thank Professor E. Bárány, Uppsala, for having turned our attention to this group of compounds in our search for a suitable substance. We are very much indebted to Astra Ltd., Södertälje, for having generously placed umbradil and methylglucamine to our disposition.

As seen from this table, the osmotic pressures are high as compared with that of seminal plasma (c. 8 atm., calculated from data compiled by Anderson (1)). However, as is well known, mammal spermatozoa are rather resistant to hypertonic solutions, and the spermatozoa showed an intense motility even after 30 min. in the highest concentration of umbradil-salt solution. In this connection it must be emphasized that the umbradil and the methylglucamine ions are practically the only ones in the solution.

However, the accuracy of these results is in the fact dependent on whether the described procedure and the rather high osmotic pressures of the test solutions do not change the volume (and specific density) of sperm cells, a supposition which is *per se* improbable. Nevertheless, we were not able to detect any change in volume of sperm cells when they were transferrd to umbradil-salt solutions with osmotic pressure of up to 186 atm. These control experiments were performed in the following way. To reveal possibly occurring changes of volume in the least concentrated umbradil-salt solution, in which all spermatozoa sediment, equal amounts of sperm cells sedimented from the original sperm samples were suspended in pairs of hematocrit tubes containing isotonic diluter and the lightest test solution. After 10 min. the spermatozoa were centrifuged at 2,300 g. until the height of the sediment columns did not decrease any more (table 2).

TABLE 2

Volumes of samples of spermatozoa in diluter (osmotic pressure 9 atm.) and test solution I (osmotic pressure 21 atm.)

Sample	Depth of sediment column		Difference
	In diluter	In test solution I	
	(*mm.*)	(*mm.*)	(*mm.*)
1	6.0	6.0	< 0.2
2	12.0	12.0	< 0.2
3	26.0	27.0	$+1.0$
4	32.5	32.5	< 0.2

In dealing with the more concentrated umbradil-salt solutions, the densities of which are high enough to keep a part of the spermatozoa suspended, we had to proceed in another way. Thus, an appropriate amount of spermatozoa was suspended in the relevant umbradil-salt solution, centrifuged in a hematocrit tube, and the constant height of the sedimented spermatozoa read. Then the supernatant containing floating spermatozoa was extracted with a fine capillary, another umbradil-salt solution having a considerably lower osmotic pressure was filled into the broad part of the hematocrit tube, and the spermatozoa in the capillary part of the tube again suspended. After a repeated centrifugation the sediment column height was read again. The results of such experiments are presented in table 3. The high osmotic pressures do not reduce the volume of the spermatozoa. Thus the water contained in the spermatozoa must be most firmly bound.

As will be seen below, there exists possibilities of the treatment with the test solutions causing other errors.

TABLE 3

Volumes of samples of spermatozoa in different test solutions. Sediment columns in mm.

Sample no.	Depth of sediment columns		Difference
	In test solution III (Osm. press. 104 atm.)	In test solution I (Osm. press. 21 atm.)	
	(*mm.*)	(*mm.*)	
1	11.5	11.5	< 0.2
2	12.0	12.0	< 0.2
3	12.5	12.5	$< .02$
	In test solution IV (Osm. press. 146 atm.)	In test solution I (Osm. press 21 atm.)	
4	18.5	18.5	< 0.2
5	20.0	20.0	< 0.2
6	7.0	7.0	< 0.2

Even though volume changes in the sperms cells did not occur in the test solutions, other experiments indicate that the sperm cells are injured in some way in the umbradil-salt solution, as they show an increase in volume when transferred from the test solutions to the diluter (cf. also (13)). This is the reason why, in the experiments of table 3, the second solution is not diluter. No determinations on the effects of osmotic pressures greater than 186 atm. were made. In view of the above results, it has been assumed that little if any change occurs at pressures above 186 atm.

From the figures obtained by measuring the sediment columns, the percentage of spermatozoa having a specific density lower than that of a certain test solution may be calculated. These percentage figures were used for the construction of curves showing the distribution of spermatozoa related to specific gravity (fig. 1).

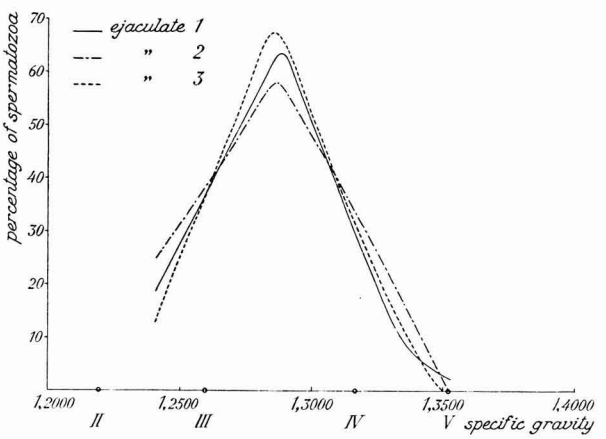

FIG. 1. Distribution of spermatozoa in a series of three ejaculates taken in immediate succession related to specific gravity. The specific gravity of test solutions II–V marked with circles.

The fraction of spermatozoa having specific gravities between those of two successive test solutions was considered to have a mean specific gravity equal to the mean of the specific gravities of the two solutions. Mean specific gravities (D) of the sperm cells of the ejaculates were calculated according to the formula

$$D = A_1 \cdot d_1 + A_2 \cdot d_2 \ . \ . \ . \ A_n \cdot d_n,$$

in which d_1, d_2 . . . d_n are the mean specific gravities of the fractions A_1, A_2 . . . A_n (expressed as decimal fractions) of spermatozoa sedimenting in the first but not in the second, in the second but not in the third test solution, and so on. In some cases, however, the spermatozoa were distributed only over a rather narrow range of specific gravity, our test solutions being too few to allow a sufficiently exact calculation in such cases. This is the reason why the figures for specific gravity are calculated from a smaller number of ejaculates in table 4 than the

TABLE 4

Means of percentage of unripe, ripe, and over-ripe spermatozoa, and of the specific gravities of the 1st, 2nd, and 3rd ejaculates

		1st ejaculate:	2nd ejaculate:	3rd ejaculate:
Unripe	%	3.7 ± 1.196	4.4 ± 0.687	5.4 ± 0.853
	no. of ejaculates	15	15	15
Ripe	%	89.1 ± 0.608	90.1 ± 1.274	90.8 ± 1.059
	no. of ejaculates	15	15	15
Over-ripe	%	6.6 ± 1.611	5.5 ± 0.960	3.9 ± 0.500
	no. of ejaculates	15	15	15
Specific gravity		1.2867 ± 0.0022	1.2897 ± 0.0040	1.2668 ± 0.0048
No. of ejaculates		12	14	13

other means. Obviously the determination of the mean specific density is subject to rather large errors.

After each determination of the sedimenting fraction, another estimation was made of the percentage of unripe, ripe and over-ripe spermatozoa in both the floating and the sedimenting fractions. This was done in a hemocytometer, which was kept at about 37° C. when counting the "non-motile" cells, whereas the total numbers of spermatozoa and of unripe sperm cells were counted at a rather low temperature. For each sample the total number of counted spermatozoa was 500 or more. Similar determinations were made as to ripe, unripe and over-ripe spermatozoa in the samples not treated.

RESULTS

The 15 series of ejaculates were taken during 32 days. Of the eight bulls, three were used once, three twice and two three times. During the experimental period there is a marked decrease in percentage of over-ripe spermatozoa in all the three ejaculates, this being especially marked after the end of August. There is no trend in the figures of unripe spermatozoa. Thus those of ripe ones show a rise corresponding to the decrease mentioned above. Nothing is known about the reason for these changes. However, it is evident that, in general, the percentage of unripe spermatozoa increases from the first to the third ejaculate

of each series, whereas that of over-ripe spermatozoa decreases. This is reflected by the mean values (table 4), which, however, do not differ significantly because of the great dispersion of the primary figures. The percentage of ripe spermatozoa is nearly equal in the three ejaculates constituting a series. The mean specific gravities of the spermatozoa of each of all the ejaculates range from 1.240 to 1.334. As seen from table 4, the mean specific gravity of the second ejaculates is higher than those of the first and the third. However, only the means of the second and third ejaculates differ significantly (degrees of freedom 25, $P < 0.005$).

The further treatment of our material revealed a negative correlation between the mean specific gravity of the spermatozoa (D) and the percentage of unripe spermatozoa, with the regression coefficient equal to 0.0022 and the correlation coefficient 0.4878 (degrees of freedom 37, $P < 0.005$). However, no significant correlation between ripe or over-ripe spermatozoa and the mean specific gravity could be ascertained. This implies that the unripe spermatozoa have a significantly lower specific density than the ripe and over-ripe ones, a conclusion which is further supported by the figures in table 5. Here the distri-

TABLE 5

Percentage of unripe, ripe, and over-ripe spermatozoa in the sedimenting and floating fractions of cells in test solutions II, III, and IV, and tests of significance for differences between fractions

Test sol.		Sedimenting	Floating	Degrees of freedom	P
		(%)	(%)		
II	Unripe	2.97	6.10	85	< 0.0005
	Ripe	90.81	90.88	85	> 0.5
	Over-ripe	6.45	3.03	85	< 0.0005
III	Unripe	2.91	6.26	87	< 0.0005
	Ripe	90.23	90.72	87	> 0.5
	Over-ripe	7.05	3.10	87	< 0.0005
IV	Unripe	2.30	4.84	41	< 0.01
	Ripe	90.06	91.63	41	< 0.5
	Over-ripe	7.72	3.52	41	< 0.025

butions of unripe, ripe and over-ripe spermatozoa in the sedimenting and the floating fractions in test solutions II, III and IV are compared. In all three cases the frequency of unripe spermatozoa is significantly higher in the floating fraction than in the sedimenting one, indicating a lower mean specific gravity of this kind of spermatozoa than that of the test solution in question. However, the opposite behaviour is shown by the over-ripe spermatozoa, whereas the ripe ones are equally frequent in the two fractions. It seems thus justified to conclude that the mean specific density of the over-ripe spermatozoa is higher than that of the test solutions used. Density of the ripe spermatozoa must, on the other hand, be evenly distributed within the range of specific density covered by the test solutions. The general conclusion suggested by these results is that the specific density of the spermatozoa increases during ripening. Probably the unripe and ripe spermatozoa differ more as to specific density than the ripe and

over-ripe ones, as no correlation between the frequencies of the two last-mentioned kinds of cells and the specific gravity of the ejaculates could be detected.

It is possible that the treatment with the test solutions exercised an influence upon the proportions between the three kinds of spermatozoa. If the mobility mechanism of ripe spermatozoa is sufficiently damaged by the test solutions, a number of spermatozoa will falsely be counted as over-ripe ones. Similarly an error would be introduced if the treatment with the test solutions should reduce the water content of the densest ripe spermatozoa, changing these into over-ripe ones. Finally a loss of the residual protoplasmic drop may be induced by the spermatozoa having been repeatedly manipulated. In order to rule out these objections, we have, by the aid of an analysis of variance, compared the frequencies of the three kinds of spermatozoa counted directly and after fractionation in test solutions II–IV. To calculate the percentage (x) of a certain kind of spermatozoa from the numbers available for the sedimenting and floating fractions, the following formula was used:

$$x = \frac{p_f(A\text{-}a) + p_s a}{A},$$

A = total volume of sperm cells in the sample (sedimenting in test solution I); a = sedimenting volume in the relevant test solution; p_f and p_s = percentage of the actual kind of spermatozoa in the floating and sedimenting fractions, respectively. The resulting figures are collected in table 6. As a control of the measurements and countings, the percentage of the three kinds of cells of each sample has been summarized, giving very nearly 100. The four samples do not differ significantly.

TABLE 6

Mean percentages of unripe, of ripe, and of over-ripe spermatozoa counted directly, and calculated from found frequencies in sedimenting and floating fractions in test solutions II, III, and IV

		Calculated from frequencies in test solution			Test of significance for differences	
	Counted directly	II	III	IV	Degrees of freedom	P
Unripe	4.5	3.7	4.1	3.9	141	> 0.5
Ripe	90.0	90.6	90.4	90.9	141	> 0.3
Over-ripe	5.3	4.7	5.0	5.3	141	> 0.5
Sum	99.8	99.0	99.5	100.1		

DISCUSSION

Sperm cells are highly specialized. Morphologically the nucleus is very much condensed and the reduced cell-body forms the very thin protoplasmic cover of the head (nucleons), the middle piece and the tail. The well known intense negative birefringence of the head depends *inter alia* upon the high concentration of cromatin in a highly dehydrated form. Obviously dehydration plays an important role in the development of the sperm head. Whether this is also the case, as to the middle piece and the tail, is difficult to decide from morphological data. However, the ability of the locomotion mechanism of the spermatozoa

to resist very high osmotic pressures speaks in favor of the view that the differentiations of the cell body are also associated with dehydration (cf. below). The dehydration processes will account for the very high specific gravity of the spermatozoa found by the present writers. Whereas the mean of all our determinations is about 1.280, a small fraction of the spermatozoa in some cases even sedimented in test solution V, indicating a specific gravity of about 1.350. Because of the difficulty of controlling the effect of the very high osmotic pressure of this test solution, the highest specific densities may be considered somewhat unreliable. These are, however, of very little importance for the calculation of the means. The specific density of other free mammalian cells may, for comparison, be represented by that of human blood cells of 1.0970 (12).

The high specific density of sperm cells may be accounted for, in the first place, by the high content of nucleic acids of the spermatozoan head. Specific volumes of desoxyribonucleic acid prepared according to Hammarsten (5) by different authors are collected by Jungner (6). The values range from 0.47 to 0.66, one of the lowest, corresponding to a specific gravity of 2.00, being considered most probable by Jungner. A corresponding collocation of values of specific volumes of proteins is made by Pedersen (10), the values ranging from 0.75 to 0.72, the corresponding specific densities being 1.34 to 1.39.

The resistance of the sperm cells to very high osmotic pressures applies to the cell volume as well as to the mobility mechanism. Thus the substances of both the nucleus and the cell body seem to be dehydrated. This means that the cells already are deprived of free water, the water present, furthermore, being very firmly bound. Smith and Polge (13), when discussing the ability of spermatozoa in surviving freezing under certain conditions, suggest that "spermatozoa may . . . have a low content of free water . . ." These writers also failed to detect any formation of crystals within the spermatozoa at freezing. However, nothing is known about the mechanism underlying the loss of free and loosely bound water.

Spermatozoa increase their volume in diluter after having been treated with test solutions. This probably depends upon changes in the surface layers regulating permeability caused either by the high osmotic pressures or by the absence of ions necessary for the maintenance of normal conditions in the outer cell surface. In both cases an exchange between the medium and the cell interior will be rendered possible, abolishing the conditions in the interior necessary for the preservation of the dehydrated state of the cellular constituents.

As dehydration constitutes a very important process in the morphogenesis of spermatozoa, it is highly probable that it also accounts for the rise in specific gravity, during ripening, found by the present writers. However, it must be expected that the structures upon which the movements of the spermatozoa depend will cease functioning when dehydrated too far. We therefore look upon the "non-motile" spermatozoa in ejaculates from normal bulls in regular service as aged, or over-ripe, and not as dead.[5] This view is supported by the fact that

[5] The consistency of this interpretation is supported by the fact that treatment with ions capable of increasing the water-binding capacity (*e.g.*, sulphocyanide) also will induce motility in non-motile spermatozoa (unpublished experiments).

disintegrating spermatozoa occur rather infrequently. If non-motile spermatozoa should be dead, disintegrating sperm cells would be expected to occur much more frequently. The detachment of the galea capitis is considered by Blom (2, 3, 4) to be an early morphological sign of regressive changes induced in spermatozoa at a prolonged stay in cauda epididymis, the "spermatic veil" described by Williams and Savage (14) being looked upon as an introductory stage. However, spermatozoa deprived of their galea are very sparse in ejaculates from bulls in regular service, and frequently are found only after a long period of abstinence. Now and then spermatozoa without galea were observed in our samples. Unfortunately their frequency in different fractions was not registered. A staining method for the differentiation of live and dead spermatozoa was devised by Lasley *et al.* (7). However, MacLeod (9) applying this technique to human spermatozoa found the staining properties of the cells to be reversible and not correlated with the viability in so far as motility was used as a criterion of viability.

The rise in specific gravity at the transition of unripe spermatozoa into ripe ones may partly be attributed to the loss of the residual protoplasmic drop, provided that the latter has a comparatively low density.

The great variation in specific gravity found in the three kinds of spermatozoa probably is due either to a corresponding variation in the proportion between the mass of nucleus and the mass of cell body, these two parts probably differing in density, or to a variation in dehydration. We hope to be able to settle these question in the near future.

SUMMARY

Determinations of the specific density of spermatozoa in ejaculates collected from normal dairy bulls in regular service were performed by centrifugation in umbradil-methylglucamine-salt solutions of different specific gravities. A negative correlation between the mean specific gravity of the spermatozoa and the frequencies of unripe spermatozoa (having a residual protoplasmic drop) was found, indicating a lower specific gravity in these than in ripe and over-ripe (non-motile) ones. The distribution of the three kinds of spermatozoa between the sedimenting and floating fractions of cells supports this conclusion, and suggests a higher specific density of over-ripe than of ripe spermatozoa. The process of ripening thus implies a continuous rise in density.

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THE BACTERIOLOGY OF SURFACE-TAINT BUTTER: A REVIEW[1]

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INTRODUCTION

Butter manufactured from properly pasteurized, high quality cream may develop a very objectionable flavor and odor in a few days when stored at temperatures as low as 5° C., if it has been contaminated during manufacture with certain types of bacteria. A defect in this category, which seems to develop only in butter made from pasteurized cream, has been most commonly described as "surface taint." Several different organisms have been suspected of causing surface taint, but now it is generally agreed that the typical defect is produced only by *Pseudomonas putrefaciens.*

This organism first was described and tentatively designated as *Achromobacter putrefaciens* in 1931 by Derby and Hammer (8). In a later publication, Long and Hammer (31) stated that additional work had indicated that this organism belonged to the genus *Pseudomonas.*

DEFINITIONS

The defect in butter caused by *Ps. putrefaciens* has been reported under a variety of names in different sections of the world. In general the terms applied to it attempt to convey the impression of a taint suggesting the decomposition of protein. In Australia, it is usually known as "decomposed odor" or "rabbito." The term "foetid" is used in New Zealand. In Denmark and the United States "putrid" is a common designation. Probably the most popular term describing the defect in Canada and the United States is surface taint. This latter designation was adopted because the defect seemed to manifest itself initially on the surface of the butter. In reality it is a misnomer because the characteristic flavor and odor very quickly involve the entire mass of the affected butter. Pont (40) pointed out that very frequently in older samples of butter the characteristic odor was not evident at the original surface and became apparent only when a block of butter was broken and fresh surfaces were exposed. Commercially the defect is often called proteolytic.

HISTORICAL

As early as 1899, Gilruth (16) demonstrated the production of a foetid odor in New Zealand butter. This defect was caused by the organism described as *Bacillus fluorescens liquefaciens,* which he had previously isolated from water. In 1900, Eckles (11) investigated an outbreak of putrid butter in Iowa. This

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butter had a strong disagreeable taste and a putrid smell, which rendered it unfit for table use. He isolated two organisms capable of causing this condition, the less objectionable being *B. fluorescens liquefaciens.*

It was pointed out by Derby and Hammer (8) that surface taint was first recognized as a definite butter defect in Canada in 1919. They further stated that the name surface taint was suggested by Marker, who in his position as Dairy Commissioner of Alberta, Canada had examined many churnings of butter possessing the characteristic odor associated with this defect. MacKay (36) presented data which showed that during 1928 and 1929 considerable surface taint butter was observed in the Canadian provinces of Alberta, Saskatchewan and Manitoba. During 1928, the percentages of the total pounds of butter examined that exhibited surface taint varied from 1.45 to 3.12 for the different provinces, while during 1929 the percentages ranged from 1.41 to 2.52. Sadler and Vollum (43) studied surface taint butter received in Vancouver, British Columbia. They noted that conditions at the creameries from which the butter samples were obtained permitted contamination following pasteurization.

The first report in Western Australia of a butter defect resembling surface taint was made by Hampshire (18) on his investigation into an outbreak of stinking butter in 1927. He emphasized inefficient grading, neutralization and pasteurization, and poor quality wash water as contributing factors. In his studies, the presence of many spore-forming bacteria in the butter was interpreted as indicating faulty grading, while a large number of coliform organisms in the vat after pasteurization indicated faulty pasteurization. In Southern Australia, Lock (26) stated that rabbito would develop during warm weather, in flush periods when insufficient time was allowed for cleaning, after heavy showers late in the season, when lumpy cream was not thoroughly mixed prior to pasteurizing, and in badly worked butter. He observed that the degree of rabbito varied with the amount of contamination, curd content, temperature and method of working. Loftus-Hills *et al.* (27) likewise investigated the rabbito defect in Australian butter. They concluded the defect was essentially a summer one and it did not seem to be more common in any particular district. They were able to reproduce the typical defect by working a culture of microorganisms isolated from rabbito butter into sterile butter.

Macy (37) stated that in butter obtained from perfectly pasteurized, sweet cream, conditions are quite ideal for the development of bacteria that can degrade the nitrogenous constituents of this product. Among the factors mentioned by this investigator as contributing to the ideal growth conditions were: (a) the pasteurization process rids the cream of its miscellaneous flora of microorganisms, which reduces competition for those appearing later; (b) the relatively low salt content of much sweet cream butter; and (c) if neutralization is overdone, the acidity is lowered to a point where conditions are much more favorable for contaminating organisms.

The relationship of overneutralization of the cream to the development of surface taint has been a point of much discussion during the years which have elapsed since the foregoing conditions were advanced as contributing causes of

the rapid growth of organisms on the nitrogenous components in butter. More recent observations, both in the laboratory and in commercial channels, have seemed to point toward the fact that surface taint is much more prevalent in butter made from high quality cream that requires little or no neutralization before churning than it is in butter churned from overneutralized sour cream.

SPORADIC OCCURRENCE OF SURFACE TAINT

Brown (1) reported that this defect has been noted frequently in butter at different times from widely separated districts. He also pointed out that in a number of instances some churnings from a single vat of cream have been affected, while others have shown no trace of the fault. It was cited further that there is no record of government grading officials ever having noted this particular odor in either pasteurized or unpasteurized cream or in butter made from unpasteurized cream. The irregular and sporadic occurrence of this defect, and the fleeting and transient nature of the characteristic odor also were noted by Pont (40).

From data collected on a large number of commercial samples, Derby and Hammer (8) concluded that there appeared to be rather distinct variations in the flavor and odor of samples of butter sent to the laboratory as exhibiting the surface taint defect. It also is conceivable that inexperienced graders who did not have sufficient opportunities to examine typical surface taint butter would tend to place in this category some samples of butter that would be more properly graded as cheesy. This variability in itself could be responsible materially for the apparently very sporadic occurrence of outbreaks of the defect.

Hood (19), however, has indicated that surface taint butter may be detected by the experienced grader by both taste and smell. Graders associate a distinct flavor with this type of butter, but the very characteristic odor is the most dependable criterion used in making the final decision on a questionable sample. He further pointed out that when the suspected flavor is present, and the characteristic odor is not sufficiently pronounced to pass judgment, graders now follow the practice of holding small samples of the butter in stoppered glass containers. Butter with the defect acquires, in 24 to 48 hr. at room temperature, a characteristic putrid odor which is easily recognized and is so distinct as to cause no confusion with other undesirable butter defects.

STAGES IN THE DEVELOPMENT OF SURFACE TAINT

Itzerott (22) stated that several stages in the development of the rabbito defect have been recognized. Usually the putrid odor is preceded by what has been termed the condensed stage, in which there is an odor resembling that of condensed milk. It is emphasized that the transition from the condensed to the putrid state usually is rapid, and frequently the butter becomes putrid without the condensed stage being observed.

It was pointed out by Hood (19) that surface taint begins to appear in butter about 8 to 10 days after manufacture, but that the degree of development depends somewhat on previous storage temperatures. Rapid development

takes place at temperatures within the range of 40 to 45° F. The rapid development of this defect was emphasized also by Brown (1). He stated that butter only 4 days old often has been known to be affected, and the trouble appeared to develop afterwards with extreme rapidity. This investigator also observed that usually butter which is only slightly affected tastes very flat, with sometimes a slightly cooked flavor.

Derby (7) found that butter made from pasteurized cream inoculated with a small amount of surface taint butter and held for a number of hours before churning commonly developed the defect. The development of a pronounced surface taint in the butter required from 2 to 3 days at 15.5° C. and from 7 to 10 days at 5.5° C. In later work, Claydon and Hammer (2) observed that when *Achr. putrefaciens* cultures were inoculated into pasteurized cream and the cream churned, the defect rapidly developed in the unsalted butter. In most instances the defect developed in 1 day at 21° C. and in 7 days or less at 5° C. They further noted that the defect passed through the same stages as those occurring in commercial butter showing the typical defect.

METHOD OF DEVELOPMENT OF TYPICAL SURFACE TAINT IN BUTTER

Derby and Hammer (8) found that surface taint could not be produced in butter by inoculating a normal product, either salted or unsalted, with surface taint butter, but could be developed by inoculating the defective butter into pasteurized cream and churning the cream. From 2 to 4 days were required for the surface taint to develop at 15.6° C. (60° F.) and from 7 to 10 days at 5° C. (41° F.). These workers concluded that the failure to develop surface taint by the inoculation of pure cultures of organisms or defective butter into normal butter, when it could be produced by inoculating these materials into cream and churning the cream, indicates that the organisms cannot spread widely through a mass of butter. It is also possible that in the cases where pure cultures of *Ps. putrefaciens* or defective butter were inoculated into cream enough cells were produced in this medium to cause the defect without any further growth in the butter. Furthermore, it should be noted that the foregoing data are somewhat at variance with the results of Loftus-Hills *et al.* (27), who reported that they were able to reproduce the typical defect by working a culture of microorganisms isolated from rabbito butter into sterile butter.

Experiments conducted by Wolochow (50) and Wolochow *et al.* (52) showed that in order to produce typical surface taint butter the cream must be given an adequate heat treatment prior to contamination with the causative organisms. In their studies, raw and pasteurized creams were inoculated from broth cultures of *Ps. putrefaciens* and incubated for 12 to 18 hr. prior to churning. The butter made from pasteurized cream developed surface taint in every case, while in no case did the defect appear in butter made from raw cream. Furthermore, surface taint butter was not obtained from the churning of inoculated whipping cream commercially pasteurized, presumably, at a lower temperature. Finally, it was observed that butter churned from inoculated cream that had been pasteurized by the vacreator process did not develop surface taint, but when some

of the vacreated cream was repasteurized in the laboratory at 81° C. for 10 min. before inoculation the typical defect appeared in the resulting butter.

The latter investigators also observed that when *Ps. putrefaciens* was inoculated into sterile skimmilk a typical "sweaty feet" odor was produced. Neutral or slightly acid conditions, and a high heat treatment of the skimmilk were necessary for the sweaty feet odor to develop. In alkaline skimmilk the odor was putrid. Later, Wolochow *et al.* (57) stated that surface taint of butter and the sweaty feet odor of heated milk were likely identical, because the odor produced by *Ps. putrefaciens* in high temperature pasteurized milk, cream, or butter serum is the sweaty feet odor, whereas the odor invariably produced by the same organism in high temperature pasteurized cream butter is the surface taint odor. Dunkley *et al.* (10) reported that the acid mixtures obtained by steam distillation of acidified skimmilk cultures of *Ps. putrefaciens* contained formic, acetic, butyric and isovaleric acids. From these studies they concluded that the substance causing the sweaty feet odor of skimmilk cultures of *Ps. putrefaciens* was closely related chemically to isovaleric acid.

EFFECT OF THE AMOUNT OF WORKING AND REWORKING ON THE APPEARANCE OF SURFACE TAINT

Long and Hammer (29, 30) studied the effect of moisture dispersion on the development of the putrid defect in unsalted butter churned from cream inoculated just prior to churning with *Achr. putrefaciens* by holding underworked, moderately worked, and thoroughly worked butter at 21° C. and also at approximately 5° C. They found that at 21° C. the under-worked and moderately worked samples showed the defect earlier than the thoroughly worked sample in both trials. At approximately 5° C. the defect developed much more slowly than at 21° C., but again the under-worked and moderately worked samples deteriorated sooner than the thoroughly worked butter. In all three trials the moderately worked butter developed the defect as rapidly as the under-worked sample. The later report described the effects of reworking unsalted butter made from pasteurized cream that had been inoculated with *Achr. putrefaciens* just prior to churning. Both the under-worked and moderately worked samples that were reworked after storage for 3 days at 10° C. developed the putrid defect more rapidly at this storage temperature than did comparable samples that were not reworked.

Claydon and Hammer (2) noted that although, in experimental butter, salt tended to prevent development of the putrid defect by *Achr. putrefaciens*, it was not entirely effective unless the butter was thoroughly worked. It was observed likewise by Wolochow *et al.* (52) in their studies with laboratory-churned salted butter that more complete working tended to prevent or delay the appearance of surface taint. Itzerott (22) indicated that the rabbito defect occurred most frequently and developed most rapidly in butter showing an open texture and free moisture. In commercial practice it has been observed that salted butter direct from the churn will never show the defect even on long incubation at

favorable temperatures. Only after the butter is printed does the characteristic odor develop.

EFFECT OF THE ADDITION OF BUTTER CULTURE

Derby and Hammer (8) studied the influence of butter culture in restraining the development of surface taint by adding 10 per cent of the culture just before churning. When a pure culture of *Achr. putrefaciens* was used to inoculate the pasteurized cream, both the salted and unsalted samples with added butter culture were still normal after storage for 20 days at 15.6° C. They concluded that it is improbable that the amount of butter culture in the cream which prevented the development of surface taint under the conditions of these experiments will always prevent the development of this defect. In a later investigation, Claydon and Hammer (2) found that the addition of 5 per cent of butter culture to pasteurized cream inoculated with *Achr. putrefaciens* prevented the development of surface taint in the resulting butter during 6 days storage at 21° C. In these trials the butter culture was added in the evening and the cream was held overnight before churning. These workers stated that the addition of butter culture is not effective under all conditions, since a number of the commercial putrid samples examined in their studies were made from cream with added butter culture. Elliker (13) presented data which indicated that the factor responsible for the inhibition of *Ps. putrefaciens* by butter starters is *Streptococcus lactis* or related bacteria. The specific inhibitory factor is presumably the acid produced by these organisms.

RELATION OF pH AND ACIDITY TO THE DEVELOPMENT OF SURFACE TAINT

Acidity determinations on representative samples of butter by Hood and White (21) showed that many of the surface taint samples came within the same acidity range noted for normal butter; however, Brown (1) encountered this defect in comparatively high-acid butter, as well as in butter having a less pronounced acid flavor.

Macy (37) pointed out that low acidity in the butter is unquestionably an influencing factor in the appearance of surface taint and many other putrefactive changes related to it. If neutralization of the cream is overdone, the acidity is lowered to a point where conditions are much more favorable for an attack by the causative organisms. The occurrence of the surface taint defect in butter has coincided with the development of a low-acid type of butter, as noted by Pont (40).

Cullity and Griffin (6) also have stressed that high acidity in the cream at churning apparently retards the development of this defect. Investigations conducted by Itzerott (22) indicated that cream acidities ranging from 0.06 to 0.15 per cent had little effect on the time required for this defect to develop in the butter made from such cream. Acidities above 0.15 per cent appeared to have a retarding effect, however. It was noted that high acidities in conjunction with low temperatures definitely inhibited the defect. He concluded that since the bulk of Australian butter was made from cream with an acidity below 0.15 per

cent, acidity alone could have exercised little control, but its effect in combination with salt may have been of some importance.

Claydon and Hammer (2) determined the pH values of the sera of several samples of commercial putrid butter. These values ranged from pH 5.8 to 6.8, which indicated that this defect was not necessarily confined to butter with a high pH value but developed over the same range as most bacterial defects. None of the pH values cited above indicated over-neutralization. In laboratory churning trials, *Achr. putrefaciens* was added to portions of cream adjusted to pH values from 5.2 to 7.8. The putrid defect developed in every sample of unsalted butter made in these churnings. On the other hand, when the pH of the cream was adjusted to 4.5, the defect did not develop in the resulting butter. They stated that these results indicated that *Achr. putrefaciens* was capable of producing the putrid defect in unsalted butter over a wide pH range. Wolochow (50) likewise found that the pH of surface taint butter fell within the range observed for normal commercial creamery butter.

RELATION OF PS. PUTREFACIENS TO THE DISAPPEARANCE OF DIACETYL

Studies conducted by Wolochow *et al.* (57) showed that diacetyl had a marked effect in delaying or reducing the development of surface taint in butter. The effect became considerably greater as the diacetyl content was increased. The supressing effect did not appear to be related to the inhibition of the growth of *Ps. putrefaciens,* because the growth of this organism was not inhibited in milk by 100 parts per million of diacetyl, a much higher concentration than is present in commercial butter. Elliker and Horrall (14) observed that the development of a putrid odor and flavor in commercial butter was accompanied, and in many cases was preceded, by the loss of typical butter aroma. Results on laboratory-churned butter contaminated with wash water containing a pure culture of *Ps. putrefaciens* showed that a definite decrease in the concentration of added diacetyl accompanied the growth of *Ps. putrefaciens* in the butter during storage. In some trials the diacetyl content was reduced to less than one-half the original amount after 4 days of storage at 70° F. In later work, Elliker and Horrall (15) prepared butter from autoclaved cream to which starter was added shortly before churning. The butter samples that were inoculated with *Ps. putrefaciens* showed some loss of diacetyl during a 7-day storage period at 21.1° C. These workers concluded from their observations that the ability of *Ps. putrefaciens* to produce a flat-flavored butter is perhaps more prevalent than is commonly realized.

Elliker (12) reported that almost all the diacetyl destroyed by *Ps. putrefaciens* during 10 days of growth in butter stored at 15.6° C. could be recovered as acetylmethylcarbinol and 2,3-butylene glycol. About three-fourths of that recovered was present as acetylmethylcarbinol and one-fourth as 2,3-butylene glycol. The fact that practically all the diacetyl originally present in these butter samples could be recovered in the form of the two reduction products of this compound brings up the question as to whether this conversion should be called destruction. It would seem more logical merely to state that the diacetyl was reduced to acetylmethylcarbinol and 2,3-butylene glycol because of an equili-

bration brought about by the lowered oxidation-reduction potential of the system as a result of the growth of *Ps. putrefaciens* in the butter.

SOURCES OF CONTAMINATION

Hood (19) listed poor sanitary conditions in the plant and dirty churns as probable causes of surface taint. Derby and Hammer (8) stated that surface taint is not caused by contamination of the surface of a piece of butter but rather by contamination of the cream or the unworked butter. They were of the opinion that the organisms causing this defect gained entrance in the plant following the pasteurization of the cream, and suggested that the churn was very likely to be involved in this defect. Loftus-Hill *et al.* (27) reported in their studies on rabbito that the origin of the causative organisms was not located, but the churns were considered the most likely source. They cautioned that other sources of infection, such as the water supply storage tanks or the cream vats, could be the cause of trouble.

It has been pointed out by Cullity and Griffin (6) that foci of contamination are built up in a factory by initial contamination from the water supply. The most likely sites of contamination are in the churn, or in any other piece of buttermaking equipment where cream or other food material may lodge. Long and Hammer (32) isolated *Ps. putrefaciens* from creamery water supplies and from the floors and sewers in dairy plants. This organism also was obtained from parts of a butter printer in a plant that was having difficulty with putrid butter. Other isolations were made from three of four churns that were examined by culturing material from around bolt heads, from between staves and from the junction of staves and ends. Another culture of this organism was obtained from the lining of a leaky milk vat. Pont (40) reported that the original source of infection with *Achr. putrefaciens* in the butter factory appears to be the water supply, but that secondary and, in many cases, more serious foci of infection may be set up in churns and other equipment. He stressed that wooden equipment was the type most likely to be involved.

In a recent study of a large number of samples of New Zealand butter, Thomson (47) has demonstrated a correlation between poor hygienic conditions in butter factories and the incidence of *Ps. putrefaciens* contamination of the butter. Out of 91 samples from which isolations were made, 69 had total bacterial counts over 50,000 per gram, 53 had yeast counts over 500 per gram, and 46 had coliform contamination classified as either "poor" or "bad." He stated that those districts having factories with a generally higher standard of factory sanitation have a relatively low incidence, whereas districts with lower hygienic standards have a much higher incidence. Most of the results suggested that the major source of contamination was unclean equipment.

In studies on *Ps. putrefaciens*, Long and Hammer (31) isolated this organism from raw milk, pasteurized milk, raw sweet cream, putrid salted butter and normal salted butter. Other materials yielding the organism were soil, water samples from a variety of sources, creamery floors, creamery sewers and creamery equipment. Data on the reported sources of *Ps. putrefaciens* are summarized

by Wolochow *et al.* (54). They listed the following materials as sources that have yielded this organism at least once: (1) soil, (2) farm well water, (3) creamery water, (4) stream, lake and roadside water, (5) creamery floors, sewers and equipment, (6) raw milk and cream, (7) pasteurized milk and cream, (8) abnormal butter, (9) normal butter and (10) sour hams.

RELATION OF WATER SUPPLY TO THE APPEARANCE OF SURFACE TAINT

Macy (37) pointed out that outbreaks, in Minnesota butter, of a defect resembling surface taint were in nearly every case traced to an infected water supply. The difficulty was overcome by seeking a new source of water or by resorting to the treatment of the water at hand. *Ps. putrefaciens* has been obtained by Hammer (17) from water used to wash butter. These isolations were made in connection with studies on creameries having difficulty with butter deterioration.

Linneboe (25) examined a number of creamery water supplies and farm waters for organisms of the *Achr. putrefaciens* type. Of 52 creamery water supplies, nine contained the organism as the water left the well; five other creamery waters were contaminated with the organisms after the water left the well. Also, it was found that six out of 55 farm waters contained the organism.

Corley and Hammer (4) found *Ps. putrefaciens* in a considerable number of water samples. These workers also noted that some water samples having high bacterial counts did not cause flavor deterioration when used to wash laboratory-churned unsalted butter, but the tendency was for samples showing high bacterial counts to be associated more generally with serious deterioration in butter than were samples having low counts. Long and Corley (28) frequently noted that water collected directly from a creamery well was satisfactory, while that collected at the churn was unsatisfactory. They concluded that in these instances the contamination evidently occurred either in the storage tank or in the piping. In a later publication, Corley *et al.* (5) reported on a detailed investigation of the water supplies of 70 Iowa creameries over a period of 18 mo. These investigators observed that some supplies regularly were satisfactory and some regularly were unsatisfactory, while others varied in quality from one examination to another. *Ps. putrefaciens* was isolated from approximately 5 per cent of the water samples examined. They concluded that various water supplies, acceptable from a public health standpoint, were not suitable for use in butter manufacture because of the presence of organisms causing spoilage in butter.

A recent paper by Hood (20) emphasized that the routine bacteriological analysis applied by public health laboratories to creamery water supplies is quite inadequate. A potable water according to present health laboratory standards may contain, in relatively large numbers, proteolytic and other types of bacteria capable of growing at low temperatures with detrimental effects if introduced into butter. He further stated that for the treatment of water supplies containing surface taint organisms, two to three p.p.m. of available chlorine have been found to be sufficient.

CONTROL MEASURES

Derby and Hammer (8) emphasized that creamery sanitation is extremely important when one is concerned with any microbiological defect of butter. They maintained that butter should be manufactured under such careful conditions that even if it were exposed for a short time to temperatures favorable for the growth of organisms there would be no danger of serious bacterial deterioration. It was pointed out by Macy (38) that fresh cream, thoroughly pasteurized, with cream and butter protected from recontamination from vat to churn to finished package of butter, pure water, uncontaminated starter and thorough working of the butter are among the most important precautions if one wishes to avoid this type of defect.

According to Pont (40) most investigators are in agreement on the following measures for the control of surface taint, roughly in the order of their importance: (1) purification of water supplies by chlorination or filtration; (2) elimination of secondary sources of infection, particularly in churns and woodwork generally (in many cases complete replacement of wooden parts of infected churns has been found necessary); (3) attention to factors concerned with satisfactory physical condition of butter (thorough working is stressed); (4) adequate pasteurization temperatures; (5) increased salt concentrations (up to 1.7 per cent); and (6) higher acidities at churning (up to 0.15 per cent). Other workers (6, 22, 50) have emphasized some or all of these points in their discussions of the control of this defect.

McCallum *et al.* (39) stated that since a satisfactory cure for surface taint butter is not known, control appears to be a matter of prevention through elimination of the causative agent at its source. They stressed that rigorous plant sanitation, efficient pasteurization, prevention of recontamination of cream, treatment of water supplies and sterilization of printing equipment and wrapping material are essential for the control of the defect.

EFFECT OF TEMPERATURE ON PS. PUTREFACIENS

Brown (1) emphasized the fact that the organisms capable of causing surface taint will develop the defect in butter stored at relatively low temperatures, while Macy (37) stated that high temperature of storage is an influencing factor in the appearance of surface taint. Pont (40) noted that the rate at which rabbito developed was dependent on the temperature. At comparatively high holding temperatures it may appear in 2 to 4 days, whereas at 5° C. and lower a week or more may elapse before the defect becomes evident in the butter.

According to Itzerott (22), temperature was definitely the greatest factor influencing the development of the rabbito defect. He noted that with temperatures between 60 and 90° F. the defect developed within 2 to 4 days, whereas temperatures below 55° F. had a definite retarding effect. Butter given a heavy inoculation of rabbito organisms and held at temperatures in the range of 12 to 15° F. for a period of 3 mo. showed no signs of the defect on removal from storage. After thawing, the inoculated butter rapidly developed the defect when exposed to temperatures between 65 and 95° F. The characteristic taint was usually

well developed after holding at these temperatures for 30 to 40 hr. These studies showed that the low temperatures prevailing during cold storage do not destroy the organisms but merely inactivate them for the duration of the storage period.

Derby and Hammer (8) found that none of their *Achr. putrefaciens* cultures survived 5 min. at 61.1° C., regardless of whether the cultures were young or old or whether they came from agar slants or from milk. In later work, Long and Hammer (31) subjected 1-day and 7-day milk cultures of several strains of *Ps. putrefaciens* to a temperature of 61.7° C. for varying periods of time. The cultures regularly survived a 0.5 min. exposure, while none except the 7-day culture of one strain and the 1-day and 7-day cultures of another strain survived 1 min., and these were inactivated in 2 min. From these data they concluded that it was unlikely that this organism could survive either the high temperature-short time or the holder method of pasteurization.

Wolochow *et al.* (53) reported on the heat resistance of one strain of *Ps. putrefaciens*. They found that, when suspended in milk, organisms of this strain survived 54.4° C. for 5 min., but did not survive exposure to 62.8° C. for 1 min.

CHLORINE RESISTANCE OF PS. PUTREFACIENS

Long and Hammer (34) observed that when *Ps. putrefaciens* was suspended in sterile distilled water or in pasteurized and filtered well water, it was rather easily destroyed by chlorine in the form of hypochlorite, provided excessive numbers of the organism were not present. With distilled water, destruction was especially active; 1 p.p.m. of chlorine usually was effective in 5 sec. The results with well water were more variable, but 5 p.p.m. of chlorine usually destroyed the organism in 5 sec. In a later paper, Long and Hammer (35) reported that when the numbers of *Ps. putrefaciens* organisms in relation to the amount of chlorine or its period of action were excessive, destruction was unsatisfactory. There was some evidence of variation in chlorine resistance among the strains of the organism that they tested.

RELATION OF SURFACE TAINT TO TOTAL POPULATION OF MICROORGANISMS

Samples of surface taint butter examined by Derby and Hammer (8) often contained large numbers of bacteria, as determined by the plate method. With some of these samples the counts were very high, but in a few instances the counts were comparatively low. Similarly, high yeast and mold counts were usually obtained on samples of butter exhibiting this defect. It was noted that the counts on these three types of organisms were, in a large percentage of the samples examined, higher on the surface portion than on the interior portion of the same sample. The total number of organisms found suggested to these investigators that considerable growth must have occurred because such high counts would not be expected in butter made from pasteurized cream unless growth had taken place, regardless of the quality of the raw material or the manufacturing methods.

Wolochow (49) found that the total and proteolytic counts on surface taint butter fell within the range of normal commercial creamery butter when the

plates were incubated at 50 to 70° F. for periods up to 5 days. In a later report, Wolochow, *et al.* (55) concluded that the numbers of bacteria in commercial surface taint butter were, in general higher than in normal commercial butter, but that the organisms producing surface taint constituted only small minority populations in the defective samples. They stated that the numbers of organisms observed did not appear to be sufficiently large to account for the surface taint defect on the basis of count.

Pont (40) has also noted that surface taint butter typically is associated with excessively high numbers of microorganisms. He emphasized that in the case of print butter taken from retail trade channels, high bacterial counts are not necessarily indicative of unsatisfactory manufacturing hygiene but may to some extent be a reflection of the conditions of temperature and handling to which the butter has been subjected. In his opinion the incidence of destructive organisms like *Achr. putrefaciens* is much more important in determining keeping quality than total or group counts.

TYPES OF ORGANISMS ASSOCIATED WITH PS. PUTREFACIENS

Derby and Hammer (8) noted that the general types of bacteria found in surface taint butter by picking colonies into litmus milk from beef infusion agar plates were essentially the same as those found in any lot of butter containing considerable numbers of organisms. Micrococci were especially conspicuous, and streptococci and non-spore-forming, gram-negative rods regularly were present. These workers also observed that the organisms which predominated on these plates did not produce surface taint in butter churned from pasteurized cream inoculated with these organisms.

In a study of the incidence of *Ps. putrefaciens* in dairy plant equipment, Long and Hammer (33) found that the flora on certain plates smeared with material from churns definitely resembled the flora on plates prepared from putrid butter. Regardless of whether or not *Ps. putrefaciens* was present, these plates usually contained micrococci, spore-forming bacteria, gram-negative rods, and often yeasts and molds. Cordes (3) also observed that surface taint butter invariably contained large numbers of bacteria and yeasts. White (48) isolated *Ps. putrefaciens* from print butter showing extensive surface discoloration caused by *Ps. nigrifaciens*. This suggested to him that there is a close association of these two organisms in regard to original sources of contamination.

OTHER ORGANISMS SUSPECTED OF CAUSING SURFACE TAINT

Shutt (45, 46) reported that surface flavor butter was almost unknown in city creameries, where the water supplies are known to be pure, but that this flavor has been quite prevalent in butter from creameries in the rural districts, especially after prolonged periods of wet weather. In his studies on creamery water, all suspected supplies were found to be contaminated with *Pseudomonas fluorescens*. Lock (26) stated that in Southern Australia *Ps. fluorescens* likewise was found frequently in water, and was considered a possible cause of rabbito butter.

In Canada, Wolochow *et al.* (51) made several hundred isolations of bacteria from creamery water supplies in a search for organisms having the ability to produce surface taint. *Ps. fluorescens* was very commonly present, and this organism was able to produce a pronounced rancidity in butter. In a later paper, these workers (56) reported that *Ps. fluorescens* was widely spread in Alberta creamery butter and water. They suggested the possibility of this organism causing a defect of pasteurized-cream butter chemically different from surface taint but for a temporary period organoleptically indistinguishable from it. All of their experimental surface taint butter samples containing inoculations from pure cultures of *Ps. fluorescens* became rancid upon further incubation. The latter report described a yellow-pigmented bacterium, believed to be a new species, which was isolated from the water supplies of one Alberta creamery. It produced a mild surface taint in butter churned from inoculated, high-temperature pasteurized cream. They believed this organism to be less important than *Ps. putrefaciens* in the production of commercial surface taint. The tentative name *Flavobacterium maloloris* was suggested for this species. Reynolds and Thornton (42) stated that workers in their laboratory had previously observed that *F. maloloris* was an infrequent cause of surface taint in butter. The data they presented were from an examination of 589 isolations of yellow bacteria from 140 samples of Alberta water; only two of the cultures in this collection proved to be *F. maloloris.*

MEDIA AND METHODS FOR ISOLATION OF PS. PUTREFACIENS

Derby and Hammer (8) isolated *Achr. putrefaciens* from several samples of surface taint butter by the use of an enrichment method which consisted of inoculating butter into litmus milk, holding this mixture at 5° C., then plating on beef infusion agar and picking colonies into litmus milk. These workers observed that organisms capable of producing surface taint could not be isolated from a considerable number of samples of surface taint butter, although with some of the samples the defect could be carried through a series of experimental churnings by using defective butter to inoculate the pasteurized cream.

Claydon and Hammer (2) noted that *Achr. putrefaciens* was most easily isolated from commercial putrid butter by inoculating the butter into thoroughly pasteurized cream, churning the cream and smearing portions of the resulting butter on beef infusion agar plates after the defect had developed. It appeared that when the organisms were in clumps, as was the case with the smearing procedure, the colonies developed more readily than from isolated cells. Inoculation of the defective experimental butter into pasteurized cream for production of second or third generation samples occasionally resulted in isolation of the organism when it had not been obtained previously. They also observed that incubation of the inoculated cream overnight at 10° C. facilitated isolation of this organism, as did incubation of the experimental butter and the smeared plates at 5 to 10° C., rather than at 21° C. It was further noted that the age of the butter seemed to be related to the ease with which *Achr. putrefaciens* was isolated, since fresh samples yielded the organism more readily than older samples.

In later work, Long and Hammer (31) reported that a special gelatin-agar containing an iron salt was very useful in the isolation of *Ps. putrefaciens* because of the luxuriant growth and high color production with it. On this medium *Ps. putrefaciens* developed readily, and after several days colonies were fairly large, raised and brown to reddish brown or pink. It was found with certain materials that enrichment in litmus milk at 3° C., followed by smearing on the special medium, aided in isolating the organism, whereas with other materials direct smears were more successful. These workers stated that in attempting isolations from butter, the serum was more satisfactory than the butter itself. It was emphasized that even with this improved medium growth may not occur when the numbers of organisms in the inoculating material are small. It was also pointed out that better results were obtained when plates were smeared than when poured in the usual way.

Recently an investigation of the nutrition of *Ps. putrefaciens* was conducted by Reinbold (41). As a result, this investigator developed a plating medium containing casitone (Difco) and l-malic acid which was suitable for the cultivation of this organism. Ten different cultures of *Ps. putrefaciens* were used in a comparison of this medium with the medium developed by Long and Hammer (31). Plates containing equal portions of the same dilution of a test culture were poured with each medium. The number of colonies on the new medium was significantly higher than on the medium of Long and Hammer in six of ten cultures. No difference in colony counts was obtained from three of the other cultures, while the number of colonies from the tenth culture was significantly higher on the medium of Long and Hammer. It was also noted that there was no difference in colony size, although pigmentation was enhanced on the new medium.

RELATION OF THE AGE OF BUTTER TO THE RECOVERY OF PS. PUTREFACIENS

The difficulty in isolating *Achr. putrefaciens* from most samples of putrid butter and the failure to obtain it from other samples suggested to Claydon and Hammer (2) that the organism died rather rapidly after causing the defect. Results of their experiments indicated that, with laboratory-churned butter containing *Achr. putrefaciens*, the organisms became more difficult to isolate as the butter aged. These workers found that occasionally, even with a large initial inoculation, and particularly with salt present, *Achr. putrefaciens* was not obtained from defective butter after 20 days; in one instance with a smaller inoculation, it was not obtained after 3 days. They stated that under similar conditions difficulty would be expected in attempts to isolate the organism from commercial putrid butter which had been held for some time, particularly at 21° C., or above.

In a later study, Long and Hammer (31) investigated further the viability of *Ps. putrefaciens* in butter. Each of the two lots of laboratory-churned butter included a salted and unsalted portion, held at 21 and 3° C. These investigators examined these samples for *Ps. putrefaciens* at the time of manufacture and at intervals thereafter by smearing serum on the special gelatin agar and also by adding serum to litmus milk for enrichment at 3° C. In the unsalted butter

held at 21° C. the number of *Ps. putrefaciens* after 24 hr. were much larger than originally and no reduction could be detected after 18 days; at 3° C. the numbers had greatly increased after 1 wk., and there was no decrease after 16 wk. In the butter containing 2.5 per cent salt and stored at 21° C., there was an increase in numbers of *Ps. putrefaciens* after 24 hr.; later there was a definite decrease. In the salted samples held at 3° C., there was a slight increase in numbers up to 8 wk. and then a decrease.

Wolochow *et al.* (55) determined the numbers of organisms in five laboratory-churned unsalted butter samples made from cream inoculated with *Ps. putrefaciens*. The samples were stored at 10 to 15° C. Counts on tryptone-glucose-beef extract-two per cent skimmilk agar were determined at intervals up to 23 days. In three of these samples the counts were still increasing at the end of this storage period, the maximum numbers ranging from 10 to 17 million organisms per gram of butter.

RELATION OF SALT TO THE GROWTH AND SURVIVAL OF PS. PUTREFACIENS

Hood and White (21) observed surface taint in butter having a salt content as high as 2.67 per cent. Derby and Hammer (8) inoculated pasteurized cream with *Achr. putrefaciens*, churned the cream and added varying amounts of salt to portions of the resulting butter. All samples were held at 15.6° C. The unsalted and slightly salted (0.75 per cent) portions developed surface taint in 4 days, whereas the medium-salted (1.5 per cent) portion was still normal after 20 days storage. Based on the above results, these workers stated that salt had a great influence on restraining the development of surface taint, but that it was improbable that the medium salt concentration used in this experiment would always prevent the development of this defect.

Claydon and Hammer (2) determined the salt content of a number of samples of commercial putrid butter. They found a range of salt content in these samples from 1.08 to 2.41 per cent. These investigators also noted that, in laboratory-churned butter, salt was not effective in preventing the development of the defect unless the butter was thoroughly worked. It was noted by Wolochow (49) that the salt content of surface taint butter fell within the range for normal commercial butter. Macy (37) emphasized that low salt content was definitely an influencing factor in the appearance of surface taint.

Itzerott (22) studied the effect of salt in conjunction with acidity on the development of the rabbito defect. He concluded from his work that a salt concentration of 1.7 per cent in butter made from cream with an acidity between 0.06 and 0.15 per cent will give a reasonable amount of protection against the development of the defect. His experiments indicated that the salt had a greater effect than acidity in checking the growth of organisms.

Long and Hammer (31) tested the salt resistance of 15 representative cultures of *Ps. putrefaciens* in litmus milk. They found that in milk containing 4 per cent of salt all the cultures grew; with 6 per cent of salt, only six of the cultures grew; with 8 per cent of salt, one culture showed slight growth after 3 wk.; and with 10 per cent of salt none of the cultures grew. In experiments

conducted by Wolochow *et al.* (57), it was observed that *Ps. putrefaciens* grew in nutrient broth containing up to 6 per cent of salt and in skimmilk containing up to 4 per cent. Growth also occurred in butter serum containing 1.2 per cent of salt but not in serum containing 6.8 per cent.

RELATION OF CURD TO THE GROWTH AND SURVIVAL OF PS. PUTREFACIENS

Hood and White (21) analyzed a number of samples of butter for curd content and found that the values for surface taint butter corresponded closely to the values secured for normal butter. Later work by Wolochow (49) also showed that the curd content of surface taint butter fell within the range noted for normal commercial creamery butter.

RELATION OF PH AND ACIDITY TO THE GROWTH AND SURVIVAL OF PS. PUTREFACIENS

Derby and Hammer (8) found that *Achr. putrefaciens* failed to grow in skimmilk acidified with lactic acid to 0.30 or 0.31 per cent, but did develop when the milk was acidified to 0.27, 0.28 or 0.29 per cent. In a later study, Long and Hammer (29) investigated the acid resistance of *Ps. putrefaciens* by adding varying amounts of lactic acid to sterile skimmilk. At approximately pH 5.3, *Ps. putrefaciens* survived only a relatively short time. With values appreciably above pH 5.3, the organism multiplied in the acidified milk; with values below pH 5.3, it was inactivated in less than 48 hr. In milk acidified to pH 4.9, the organism was rendered inactive in 8 hr. Wolochow *et al.* (54) presented data on the growth of *Ps. putrefaciens* at varying pH levels in several buffered liquid media. The pH range permitting growth varied with the medium, but that in some instances growth was not prevented until the pH was below 5.5 or above 9.5. They concluded that *Ps. putrefaciens* was able to initiate growth and to grow over a pH range considerably wider than is commonly found in the serum of normal or surface taint butter.

NUTRITIONAL REQUIREMENTS OF PS. PUTREFACIENS

Long and Hammer (31) reported that the growth of *Ps. putrefaciens* on beef infusion agar was not improved by the addition of various amounts of the amino acids alanine, asparagine, cysteine, cystine, glycine and tryptophane. Addition of the oxidizing materials, potassium permanaganate, hydrogen peroxide and potassium persulfate did not aid the growth of *Ps. putrefaciens* when used in small amounts and retarded growth when used in relatively large amounts. The addition of certain reducing compounds was of some value, sodium thiosulfate apparently being best suited to the organism. Certain other compounds previously reported as growth factors for some other organisms were also investigated. Among those tested were α-naphthaleneacetic acid, benzoic acid, γ-(indole-3)-*n*-butyric acid, hydrocinnamic acid, indole-3-acetic acid, nicotinic acid, phenylacetic acid, riboflavin, thiamin and 1,2,5,6-dibenzanthracene. Benzoic acid had a questionable beneficial effect, and none of the others in this group aided growth.

Scheunemann and Behrens (44) used a synthetic medium to investigate the

nutritional requirements of *Ps. putrefaciens*. Experiments employing various concentrations of growth factors in this medium showed that thiamin hydrochloride, nicotinic acid, betain hydrochloride and vitamin B_6 stimulated growth. A markedly lesser growth stimulation was exhibited by choline hydrochloride and pantothenic acid.

Reinbold (41) found that the amino acids used by most of his cultures were glutamic acid, aspartic acid, isoleucine, leucine and tyrosine. Of other nitrogenous compounds tested, the most readily utilized ones were protolysate (Mead Johnson), casamino acids (Difco), and casitone (Difco). The organic acids most readily used were propionic, butyric, pelargonic, lauric, lactic, succinic, sebacic, fumaric, l-malic, α-bromopropionic, glyceric and gluconic. Of these latter acids, l-malic, fumaric, and lactic were foremost in promoting growth and pigmentation of the organisms. The action on carbohydrates was variable. Under the conditions of his experiments, the only carbohydrate used by all of the test cultures was glucose. Other carbohydrates utilized by at least one of his test organisms were maltose, sucrose, dextrin, soluble starch, cellobiose, l-arabinose, glycogen, fructose and inulin. Within the test conditions used, the nine B-complex vitamins either collectively or individually did not stimulate growth.

Doudoroff *et al.* (9) showed that *Ps. putrefaciens* could be adapted to utilize sucrose at a much greater rate than the constituent hexoses. Demonstration of a sucrose phosphorylase, phosphoglucomutase and phosphohexoisomerase in dry cell preparations of *Ps. putrefaciens* helped to elucidate the metabolic pathway by which the glucose portion of sucrose is utilized by this organism. The finding of a sucrose phosphorylase in the enzyme system of *Ps. putrefaciens* also points to the extremely important part that phosphate plays in its metabolic processes.

In a more recent paper, Klein and Doudoroff (24) reported on studies with a mutant strain of *Ps. putrefaciens* which was capable of utilizing glucose. The experiments on the wild type of the organism, which could not grow with glucose as a sole carbon source, and on the mutant were conducted with washed-cell suspensions, using the Warburg technique. This mutant strain of *Ps. putrefaciens* oxidized glucose rapidly and utilized it for growth. They stated that the utilization of glucose by this mutant was strictly adaptive in nature, and that, apart from the enzyme initiating oxidation, the wild type, like the mutant, had all the enzymes necessary for the subsequent metabolism of glucose.

The results of these experiments are at variance with the work of Reinbold (41), who presented data showing that under the conditions of his experiments all of the ten cultures of *Ps. putrefaciens* tested utilized glucose. It is conceivable in the experimental method used by Reinbold that some carryover of essential intermediates was accomplished when the test medium which contained glucose was inoculated with the test culture, thereby furnishing enough of a critical substance so that the culture could initiate growth. On the other hand, in the work of Klein and Doudoroff, the cells collected by centrifugation of liquid cultures were washed with phosphate buffer before being used in the Warburg flasks; consequently, these test organisms had to initiate growth with only the components present within the cells themselves.

In the paper by Klein and Doudoroff (24), it was stated that because neither wild-type nor glucose-utilizing mutant cells were capable of oxidizing fructose and repeated attempts to isolate a mutant utilizing fructose had proved unsuccessful; the conclusion was made that free fructose was completely unavailable to *Ps. putrefaciens*. Subsequent experiments, reported in a very recent note by Klein (23), however, have revealed that fructose can be metabolized by this organism if this sugar is present in high concentrations. Fructose oxidation differed from the oxidation of other sugars studied in that it was nonadaptive in nature.

SUMMARY

Surface taint became a problem to the dairy industry with the introduction of pasteurized cream for buttermaking. This heat treatment seems to transform the cream to a satisfactory medium for the growth of the causative organism. Butter churned from pasteurized cream that has been contaminated with *Ps. putrefaciens* usually develops a putrid odor and flavor within a very few days, even when stored at temperatures as low as 5° C.

Ps. putrefaciens has been isolated most frequently from the water used in washing the butter and from various pieces of equipment in dairy plants encountering difficulty with surface taint. This organism is relatively easily inactivated by chlorine; therefore, adequate treatment of an infected water supply with this chemical will render it suitable for use in butter manufacture. In some instances, certain pieces of equipment such as wooden churns become so extensively infected with this organism that the replacement of a badly worn unit is the simplest method of stopping a bad outbreak of surface taint in the butter from a particular plant.

The incidence of surface taint can be lowered very markedly by following good sanitary practices in the dairy plant. Several groups of investigators have made suggestions for the prevention and control of surface taint. Although the practical methods suggested for the control of this defect are quite effective, there is still no really rapid and simple procedure for determining whether any particular churning of suspected butter actually contains *Ps. putrefaciens*, and, if so, just how extensive the contamination with these organisms is.

The quantitative estimation and isolation of *Ps. putrefaciens* from butter has been a problem worked on for many years by investigators in Australia, New Zealand, Canada and the United States. A large part of this research has been directed at the development of a more suitable medium for the growth of this organism. It has been apparent for quite some time that *Ps. putrefaciens* loses viability rather quickly when stored; hence, part of the difficulty encountered in recovering this organism from butter can be traced to the fact that the number of viable cells remaining in the infected sample may be very low. Also, there is very definite evidence that ordinary distilled water is highly toxic to *Ps. putrefaciens;* therefore, any technique employing unbuffered water as the dilution medium immediately lowers the possibility that any of the causative organisms will be recovered. Regardless of the technique used for the quantitative estimation or isolation of this organism from butter, it is imperative that as fresh samples

as possible be obtained for analysis because of the rapid rate at which the organism loses viability on storage.

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EFFECT OF FEEDING VARIOUS PERCENTAGES OF ARTIFICIALLY DEHYDRATED ALFALFA MEAL ON THE GROWTH AND CAROTENE INTAKE OF DAIRY CALVES[1]

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Poor or average quality hay has been found incapable of meeting the carotene and roughage requirements of the young dairy calf raised under a limited-whole milk and dry calf-starter system of feeding (4, 5). Since hay of required quality has not been generally produced in the northeastern states (2, 20), primarily because of weather conditions (19), the development of a system of feeding to insure adequate carotene and roughage intake is needed.

Inasmuch as the young calf, when fed starter and hay separately, generally prefers starter (4, 5), it would seem desirable to formulate a complete ration which would eliminate the preference factor and insure adequate carotene and roughage intake. The general desirability of self-feeding a complete ration to the young dairy calf has been reported previously (1, 9, 10, 14), as has the desirability of including roughage in the calf's ration (8, 17, 18).

High carotene potency, artificially dehydrated alfalfa meal is readily available on the market in the northeastern states and has been found to be an excellent source of carotene and roughage for young calves (4, 5). Therefore, a study to compare the effects of incorporating various percentages of artificially dehydrated alfalfa meal and starter into a complete ration fed *ad lib.* with a standard system of limited feeding of starter plus average quality hay *ad lib.* as a control was undertaken.

EXPERIMENTAL

Animals. Thirty-two 7-day-old calves, eight female and eight male Guernseys and eight female and eight male Holsteins, were placed on experiment from October, 1950, through March, 1951. Four female and two male Guernseys came from Lyman Farms, Middlefield, Conn., two female and four male Holsteins from Mansfield State Training School and Hospital, Mansfield, Conn. and the remaining calves from the University herd. All calves either nursed their dams for 48 hr. or were fed colostrum for the same period at the daily rate of 7 lb. for Guernseys and 8 lb. for Holsteins. Calves then were transported to the University research barn and placed in individual tie-stalls in a separate portion of the barn where the temperature was maintained at a minimum of 10° C.

Since it was impossible to predict in advance availability of calves from the three sources, calves were randomly assigned with restriction as to sex and breed

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to one of four ration groupings. This procedure was repeated so that two replications of the experimental design were achieved.

Rations. The four rations were: (a) Standard ration consisting of starter limited to 2.5 lb. daily for Guernsey calves and 3.0 lb. for Holsteins, and U. S. no. 2 field-cured and field-baled alfalfa hay fed *ad lib.;* (b) 25 per cent alfalfa ration consisting of a mixture of 25 per cent artificially dehydrated alfalfa meal and 75 per cent starter fed *ad lib.;* (c) 50 per cent alfalfa ration consisting of 50 per cent alfalfa and 50 per cent starter fed *ad lib.* and (d) 75 per cent alfalfa ration consisting of 74 per cent alfalfa and 26 per cent starter fed *ad lib.* These are described in table 1. Chemical analyses in table 1 represent average values of samples taken at successive 28-day intervals during the experimental period. The herd milk contained on the average 191 γ of carotene and 178 γ of vitamin A per pound.

TABLE 1

The ingredients and the mean chemical composition of the feed fed

	Rations			
	Standard			
	Field-cured and field-baled alfalfa hay starter	25% Alfalfa	50% Alfalfa	75% Alfalfa
		INGREDIENTS		
		(*lb./ton mixture*)		
Cracked yellow corn		469.50[b]	378.50[b]	252.50[b]	126.50[b]
Crimped oats		400.00	322.00	215.00	108.00
Wheat bran		300.00	250.50	160.50	94.50
Linseed oil meal, o.p.		140.00	113.00	75.00	37.00
Soybean oil meal, o.p.		279.25	225.25	150.25	74.25
Artificially dehydrated alfalfa meal		140.00	491.00	1000.00	1485.00
Dried skimmilk		100.00	81.00	54.00	27.00
Forta-Feed Lederle's 2-22C		40.00	32.00	21.00	11.00
Irradiated yeast[a]		1.25	1.25	1.25	1.25
Dicalcium phosphate		20.00	16.50	11.50	6.50
Iodized salt		10.00	8.00	5.00	2.00
Cane molasses		100.00	81.00	54.00	27.00
Total		2000.00	2000.00	2000.00	2000.00
		CHEMICAL COMPOSITION			
		(%)			
Dry matter	89.43	88.37	89.38	89.76	90.80
		(*% of dry matter*)			
Crude protein	18.63	21.95	22.67	22.39	21.95
Ether extract	1.75	4.08	3.92	4.10	4.16
Crude fiber	31.28	7.64	10.73	15.14	19.69
Nitrogen-free extract	37.77	59.97	55.70	50.72	45.67
Ash	10.56	6.35	6.97	7.65	8.52
Ca	1.19	0.62	0.74	1.04	1.42
P	0.18	0.66	0.57	0.44	0.37
Mg	0.24	0.31	0.29	0.29	0.31
		(*mg./lb.*)			
Carotene	3.88	1.88	5.11	10.20	17.60

[a] Expellar soybean meal plus Standard Brands type 142-F irradiated yeast equivalent to 1,600,000 I.U. of vitamin D/lb. mixture.

[b] All except corn, oats, bran and molasses pelleted.

TABLE 2

Effect of level of alfalfa meal included in the calf's ration upon feed consumption and its utilization

	Rations				Breeds		Sexes	
	Standard	25% alfalfa	50% alfalfa	75% alfalfa	Guernseys	Holsteins	Female	Male
	Total ration consumed (*lb.*)[a]							
Observed	374 ± 25	439 ± 34	322 ± 20	268 ± 32	302 ± 20	399 ± 24	348 ± 25	353 ± 26
Adjusted to live weight of individual calves at 7 d. of age	368	438	325	276	344	359	360	343
	Dry matter efficiency (Total gain wt./total dry matter intake)[b]							
Observed	0.366 ± 0.010	0.367 ± 0.012	0.390 ± 0.013	0.340 ± 0.017	0.357 ± 0.009	0.374 ± 0.011	0.352 ± 0.008	0.380 ± 0.011
	Digestible energy efficiency (Total gain wt./total T.D.N. intake)[c]							
Observed	0.502 ± 0.011	0.472 ± 0.016	0.533 ± 0.018	0.497 ± 0.024	0.487 ± 0.012	0.515 ± 0.014	0.482 ± 0.010	0.520 ± 0.015
	Carotene intake (γ)							
Mean daily intake/mean lb. live weight	72 ± 6	132 ± 12	235 ± 15	350 ± 38	199 ± 32	196 ± 29	198 ± 30	197 ± 32
	Weeks to deplete vitamin A stores							
Estimated	6.6 ± 0.4	7.5 ± 0.6	8.5 ± 0.6	5.7 ± 0.6	6.6 ± 0.4	7.6 ± 0.4	7.1 ± 0.5	7.0 ± 0.5
Adjusted to mean daily intake of carotene/mean lb. live weight	8.5	8.5	7.9	3.3	6.6	7.6	7.1	7.0

[a] Milk not included.
[b] Milk included.
[c] T.D.N. calculated from Morrison's (13) Appendix, Table 1.

The field-cured and field-baled alfalfa hay was identical in source to that described in a previous study (5). It graded on the average U. S. no. 2 alfalfa hay and contained 0.7 per cent timothy, 0.5 per cent foreign material, 38 per cent leafiness of legumes and 39 per cent color. The standard starter and the various mixtures of artificially dehydrated alfalfa meal and starter were prepared from one lot of ingredients. All but the corn, oats, bran and molasses were pelleted. A sample of the pellets from the standard starter measured 0.61 ± 0.21 cm. in length and 0.56 ± 0.03 cm. in diameter. Similar values for the 25, 50 and 75 per cent alfalfa rations were 0.57 ± 0.18 and 0.56 ± 0.03, 0.45 ± 0.10 and 0.55 ± 0.03 and 0.44 ± 0.11 and 0.54 ± 0.02, respectively.

After nursing their dams for 48 hr. or being fed colostrum from their dams, Guernsey calves received 7 lb. of Holstein herd milk daily the third through the seventh days and for successive 7-day periods thereafter 6, 5, 4 and 2 lb. daily. Corresponding amounts for Holstein calves were 8, 7, 5, 4 and 2 lb., respectively. Water was allowed *ad lib.* from the seventh day of age and, with the exception of calves on the control ration, all feeds were fed so as to allow a minimum weighback of 10 per cent.

To estimate the vitamin A storage, the calves were placed on a low-carotene ration from the 105th day of age through the 126th day of age. This ration, consisting of one-third beet pulp and two-thirds grain, was identical in composition and was fed at a level similar to that described previously (5, 6).

Observations and analyses. Feed intakes and refusals were weighed to the nearest 0.1 lb. Live weights were recorded and venous blood samples drawn for hemoglobin and plasma carotene and vitamin A determinations on the seventh day of age for each calf and at successive 7-day intervals thereafter. In addition, venous blood samples were obtained every second day from the 112th day through the 126th day for estimation of vitamin A stores. Height at withers, heart girth and girth of paunch were taken to the nearest 0.5 in. at 7 days of age and at successive 14-day intervals through the 105th day of age. Daily health observations were recorded.

Treatment of blood samples, analytical and statistical procedures were those employed previously (4). The data in tables 2, 3 and 4 are arithmetic means ± their standard errors.

In the analysis of variance, the variability among rations was considered first as standard *versus* complete rations. Secondly, among complete rations, there was a linear trend with increasing alfalfa and deviations from that trend. The total analysis besides embracing variability due to rations included that due to sex, breed and other effects. It was as follows:

Source of variation	*Degrees of freedom*		
Rations	3		
Standard *versus* complete rations		1	
Among complete rations		2	
Linear trend among complete rations			1
Deviations from linearity among complete rations			1
Breeds	1		
Sexes	1		
R × B	3		
R × S	3		
B × S	1		
R × B × S	3		
Error	16		
Total	31		

TABLE 3

Effect of level of alfalfa meal included in the calf's ration upon growth

Exptl. grouping	Observed values		105 d. value adjusted for 7 d. value
	At 7 d.	At 105 d.	
	Live weight (*lb.*)		
Standard	84 ± 8	213 ± 14	209
25% alfalfa	82 ± 6	232 ± 18	231
50% alfalfa	81 ± 5	200 ± 12	202
75% alfalfa	80 ± 6	167 ± 16	172
Guernseys	69 ± 2	170 ± 7	194
Holsteins	94 ± 3	236 ± 10	213
Females	78 ± 3	194 ± 10	200
Males	85 ± 5	212 ± 14	206
	Height (*in.*)		
Standard	28.5 ± 0.7	35.2 ± 0.6	35.1
25% alfalfa	28.8 ± 0.6	35.4 ± 0.6	35.1
50% alfalfa	28.4 ± 0.5	33.9 ± 0.7	33.8
75% alfalfa	27.8 ± 0.4	32.1 ± 0.5	32.6
Guernseys	27.4 ± 0.3	33.0 ± 0.4	33.7
Holsteins	29.3 ± 0.4	35.2 ± 0.4	34.6
Females	28.2 ± 0.3	34.1 ± 0.5	34.2
Males	28.6 ± 0.4	34.2 ± 0.6	34.1
	Heart girth (*in.*)		
Standard	29.2 ± 0.8	38.8 ± 0.7	38.7
25% alfalfa	29.3 ± 0.8	39.7 ± 1.0	39.6
50% alfalfa	29.8 ± 0.9	38.2 ± 0.9	37.8
75% alfalfa	28.6 ± 0.8	35.2 ± 0.9	35.6
Guernseys	27.7 ± 0.3	36.2 ± 0.5	37.2
Holsteins	30.8 ± 0.5	39.8 ± 0.6	38.7
Females	28.9 ± 0.5	37.8 ± 0.6	38.0
Males	29.6 ± 0.6	38.1 ± 0.8	37.9
	Girth of paunch (*in.*)		
Standard	29.2 ± 0.8	50.3 ± 1.4	50.2
25% alfalfa	28.8 ± 0.6	48.3 ± 1.4	48.5
50% alfalfa	29.8 ± 0.8	48.8 ± 1.0	48.2
75% alfalfa	28.1 ± 0.5	46.4 ± 1.5	47.1
Guernseys	28.1 ± 0.3	46.2 ± 0.2	46.9
Holsteins	29.8 ± 0.6	50.8 ± 0.3	50.1
Females	28.9 ± 0.5	47.2 ± 0.2	47.3
Males	29.0 ± 0.5	49.8 ± 1.0	49.7

The period that would have been required to deplete each calf of its vitamin A stores was calculated from the average level of the vitamin A in the blood from the 112th through the 126th day, as above. The equivalent depletion period for any of these given average levels was obtained by reference to previously estab-tablished data.[2]

[2] Calculation of time to deplete each calf of its vitamin A stores was accomplished by the following formula: $X = \bar{x} + b^1(y - \bar{y}) - 15$ in which X = expected week for blood plasma level of vitamin A to decrease to 4.0 γ per cent; $\bar{x}$ = mean week in which blood plasma level of vitamin A was observed, that is 17; b^1 = no. of week for blood plasma level of vitamin A to decrease 1.0 γ per cent (for Guernseys 0.3986 and for Holsteins 0.4021, both values derived from calves actually

TABLE 4

Effect of level of alfalfa meal included in the calf's ration upon hemoglobin and plasma carotene vitamin A

Exptl. grouping	Observed values		Mean 14 d. through 105 d. adjusted for 7 d. value
	At 7 d.	Mean 14 d. through 105 d.	
	Hemoglobin (*g.* %)		
Standard	11.15 ± 0.58	10.94 ± 0.28	10.56
25% alfalfa	10.59 ± 0.90	11.25 ± 0.42	11.08
50% alfalfa	9.90 ± 0.63	10.37 ± 0.25	10.46
75% alfalfa	8.91 ± 0.81	9.42 ± 0.32	9.88
Guernseys	10.04 ± 0.55	10.41 ± 0.18	10.44
Holsteins	10.23 ± 0.55	10.58 ± 0.36	10.54
Females	10.61 ± 0.36	10.70 ± 0.20	10.52
Males	9.66 ± 0.67	10.28 ± 0.34	10.47
	Plasma carotene (γ%)		
Standard	30 ± 5	125 ± 17	125
25% alfalfa	33 ± 7	125 ± 16	125
50% alfalfa	40 ± 9	208 ± 29	209
75% alfalfa	34 ± 11	191 ± 34	191
Guernseys	46 ± 7	215 ± 19	216
Holsteins	22 ± 2	110 ± 8	109
Females	43 ± 7	116 ± 20	159
Males	25 ± 3	158 ± 19	166
	Plasma vitamin A (γ%)		
Standard	13.9 ± 1.3	19.7 ± 0.7	19.8
25% alfalfa	17.1 ± 2.2	23.3 ± 1.1	23.3
50% alfalfa	17.7 ± 2.4	22.6 ± 0.7	22.5
75% alfalfa	16.6 ± 1.3	16.8 ± 1.2	16.8
Guernseys	15.8 ± 1.6	20.0 ± 1.0	20.1
Holsteins	16.8 ± 1.0	21.1 ± 0.8	21.1
Females	18.4 ± 1.4	20.4 ± 0.9	20.2
Males	14.2 ± 0.9	20.8 ± 1.0	20.9

RESULTS

Feed. A comparison of total feed consumed (table 2) and the trend of this consumption with increase in age (fig. 1) of those calves fed the complete rations, 25, 50 and 75 per cent alfalfa rations, showed an inverse linear relationship to the level of dehydrated alfalfa incorporated in the ration ($P < 0.01$). Those calves fed the standard ration, limited starter and U. S. no. 2 field-cured and field-baled hay *ad lib.*, were found to consume approximately the same amount of feed as the 50 per cent alfalfa ration calves, less than the 25 per cent alfalfa ration calves ($P < 0.05$) and more than the 75 per cent alfalfa ration calves ($P < 0.01$). Holstein calves consumed significantly greater amounts of feed

depleted to less than 4.0 γ per cent blood plasma vitamin A (6)); y = blood plasma level of vitamin A desired to predict X, that is 4.0 γ per cent; $\bar{y}$ = mean blood plasma level vitamin A observed during the 16th, 17th and 18th wk. and 15 = week of age started on depletion. The formula for Guernseys reduced to $X = 0.4 + 0.3986\ y$ and for Holstein $X = 0.4 + 0.4021\ y$

than Guernsey calves ($P < 0.01$); however, adjustment of the feed data to initial weight at 7 days of age demonstrated that breed difference was directly related to initial weight of the calves rather than a characteristic breed difference. No real difference between sexes in feed consumed was observed.

Utilization of dry matter or of calculated T.D.N. as evidenced by live weight increases (table 2) was more efficient in the 50 per cent alfalfa ration calves than in the 25 and 75 per cent alfalfa calves and the standard ration calves. Although Holsteins required less feed per unit of gain than Guernsey calves, this difference

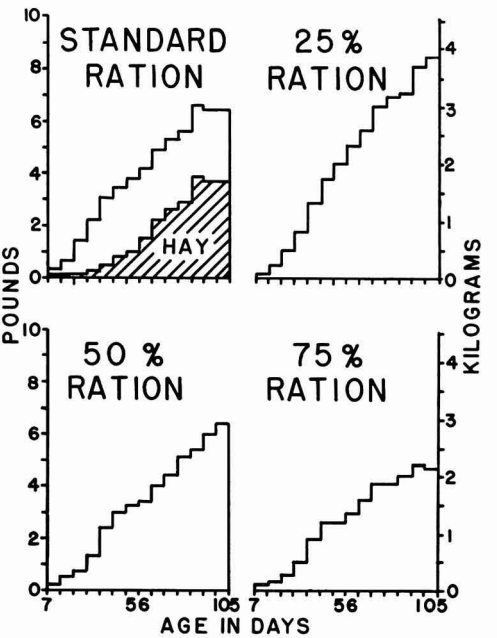

FIG. 1. The effect of level of alfalfa meal included in the young calf's ration upon feed intake with increase in age.

was found not to be statistically significant. Male calves utilized both dry matter and T.D.N. more effectively than females calves ($P < 0.05$). It should be pointed out here that some sorting of the 25, 50 and 75 per cent alfalfa rations occurred with calves exhibiting on the average neither preference for the non-pelleted nor pelleted portion of the rations. This might have affected the calculations of the efficiency of utilization of T.D.N.

Mean daily intake γ of carotene per pound of mean body weight (table 2) was directly related to the level of artificially dehydrated alfalfa included in the ration ($P < 0.01$). The complete rations, 25, 50 and 75 per cent alfalfa, provided a significantly greater intake of carotene than did the standard ration ($P < 0.01$).

During 29 per cent[3] of the total calf week for those calves receiving the standard ration, the daily intake of carotene was less than 60 γ[4] per mean pound of body weight. Similar data for the 25, 50 and 75 per cent alfalfa rations were 10, 3 and 0 per cent, respectively. The period during which the calves received daily less than 60 γ carotene per mean pound of body weight were for the standard ration between 7 and 42 days of age, for the 25 per cent alfalfa ration between 7 and 21 days of age and for the 50 per cent alfalfa ration between 7 and 14 days of age.

Utilization of the carotene ingested, as evidenced by predicted vitamin A stores (table 2), indicated maximum utilization by those calves receiving the 50 per cent alfalfa ration followed by the 25 per cent, standard and 75 per cent rations, respectively. Guernsey calves utilized less carotene than Holstein calves ($P < 0.10$). When the mean daily intake of carotene per mean pound of body-weight was equalized between groups by regression, the lower levels of carotene were utilized more effectively than the higher levels ($P < 0.01$ for the linear trend of the complete rations), and the Guernseys were less efficient than Holsteins ($P < 0.05$).

Growth. Live weight, height at withers and heart girth measurements at the termination of the experiment (table 3) were inversely related to the level of alfalfa included in the complete rations ($P < 0.01$). The standard ration provided greater increases in these measurements than the 75 per cent ration and a greater increase in height at withers than the 50 per cent ration. The 25 per cent ration calves made greater gains in live weight and heart girth measurements ($P < 0.02$) than did the standard ration calves, but no real differences were obtained in height at withers.

The level of alfalfa in the complete rations appeared to have a direct effect on the girth of paunch at 105 days of age (table 3); however, neither the differences between rations nor the linear trend were found to be statistically significant. The standard ration calves had greater girth of paunch than the 75 per cent ration calves ($P < 0.05$). Calculation of relative girth of paunch with size of calf (girth of paunch/height at withers) indicated that, with the exception of the 25 per cent ration calves which were slightly smaller, the girth of paunch in relation to size was essentially the same in all ration groups.

Guernsey as well as female calves were smaller in all growth measurements at the termination of the experiment on the 105th day of age than Holstein and male calves. Adjustment of the terminal measurements for initial measurements by regression, however, demonstrated that the differences in live weight for Guernseys and Holsteins and live weight, height at withers and heart girth for sexes were essentially attributable to differences in initial values.

Blood. Hemoglobin, as well as plasma carotene and vitamin A, (table 4)

[3] $\left[\dfrac{\text{Total individual calf week in which} < 60\ \gamma \text{ carotene per mean lb. of body weight was consumed.}}{(\text{Total expt. week})\ (\text{Total calves in expt. group})}\right] = \left[\begin{array}{c}\text{Per cent calf week in which} < 60\ \gamma \\ \text{carotene per mean lb.} \\ \text{of body weight was consumed.}\end{array}\right]$

[4] Based on the National Research Council's recommended carotene allowance for dairy calves (12).

were found to be related to the level of alfalfa incorporated in the complete rations. With an increase in the per cent alfalfa, there was a corresponding increase in the level of plasma carotene ($P < 0.01$), whereas the levels of hemoglobin and plasma vitamin A decreased ($P < 0.01$). Inspection of the data for plasma carotene and vitamin A indicate some departure from a linear relationship between these substances and per cent alfalfa ($P < 0.05$ for deviations from linearity).

Those calves fed the standard ration (table 4) had hemoglobin levels significantly greater than the 75 per cent ration calves ($P < 0.05$), plasma carotene levels smaller than 50 and 75 per cent alfalfa ration calves ($P < 0.01$) and plasma vitamin A levels greater than the 75 per cent alfalfa ration calves ($P < 0.05$) and smaller than the 25 and 50 per cent alfalfa ration calves ($P < 0.05$).

There were no statistically discernible differences between breeds or sexes in either hemoglobin or plasma vitamin A levels. Guernsey calves had significantly greater plasma carotene levels than Holstein calves ($P < 0.01$).

Health. Calculation of the per cent days free of scours for each calf and subsequent statistical analyses of these percentages indicated no relationship between incidence of scours and the various rations. Per cent days free of scours for Guernseys was 98.4, for Holsteins 99.6, for females 99.7 and for males 98.3. These differences between the breeds and between the sexes were found to be statistically significant ($P < 0.05$).

Besides the relative unthrifty general appearance of the calves receiving the 75 per cent alfalfa ration and the tendency for the male calves receiving this ration to be relatively full in the paunch, no other health observations were apparent.

DISCUSSION

The feasibility of feeding to the young dairy calf complete rations to insure adequate roughage and carotene intake has been demonstrated in this experiment. This would appear to be of real importance, especially when high quality roughage is not available.

The decrease in feed intake and growth with an increase in the level of alfalfa meal incorporated in the complete rations indicated that the calf is more sensitive to the level of roughage in the ration than are more mature dairy animals. Harshbarger and Salisbury (7) found subnormal growth in dairy heifers fed a complete ration containing 94 per cent timothy and optimum growth at a 70 per cent level. Corresponding values for the calf experiment reported herein were 75 and 25 per cent alfalfa.

Although not concerned with a comparison of roughage levels, Hibbs and Pounden have found mixtures of 54 parts roughage and 12 parts grain (10) and four parts roughage and one part grain (9) satisfactory for normal growth of young dairy calves inoculated with cud material. The latter has been found (3) to be of importance in the ability of the calf to digest roughage. Since no cud inoculations were employed in the present experiment, this might explain the subnormal growth obtained in those calves fed the 75 per cent alfalfa ration. Other factors such as associative digestibility (16), protein quality (11), mineral

imbalance, especially ratio of Ca to P, (12), other ration nutrient deficiencies or excesses or other factors such as the chick-growth depressant in alfalfa meal (15) also might have contributed to the poor response observed in those calves fed the 75 per cent alfalfa ration.

The poor utilization of the carotene by those calves fed the 75 per cent alfalfa ration can be explained on the basis of the relative inability of calves to utilize this high level of alfalfa. The decrease in efficiency of utilization of carotene with increasing levels of carotene contained in the rations would be expected in that, in general, increasing a particular nutrient in a ration results in a decrease in its efficiency of utilization.

No adequate explanation of the decrease in hemoglobin level with increase in the level of alfalfa included in the complete rations was apparent.

The breed difference in utilization of carotene and the per cent days free of scours confirmed previous observations from this station (5, 6).

SUMMARY

An attempt to formulate complete rations for the dairy calf insuring adequate roughage and carotene intake was undertaken. A comparison of mixtures of artificially dehydrated alfalfa meal and starter in the following percentages 25 and 75, 50 and 50 and 75 and 25 with a standard ration of U. S. no. 2 alfalfa hay fed *ad libitum* and restricted amounts of starter as a control ration was made, using a total of 32 Guernsey and Holstein female and male calves 7 days old.

With an increase in the percentage of alfalfa meal incorporated into the complete rations, there was a decrease in the amount of rations consumed. This decrease was accompanied by slower growth, as evidenced by live weight, height at withers and heart girth measurements. The growth of the calves on the standard ration was approximately equivalent to that on the 50 per cent alfalfa ration, greater than the 75 per cent alfalfa ration and less than the 25 per cent alfalfa ration. Efficiency of utilization of the feed consumed was greatest in those calves receiving the 50 per cent alfalfa ration.

Maximum utilization of carotene occurred in the 50 per cent alfalfa ration calves which had on the average predicated vitamin A stores of 8.5 wk. Corresponding values for the standard ration, 25 per cent and 75 per cent alfalfa rations were 6.6, 7.5 and 5.7 wk., respectively. Calculation of the daily intake of carotene per mean pound of body weight by week showed that during 29 per cent of the total calf week for those calves fed the standard ration, the carotene intake was less than 60 γ per mean pound of body weight. Similar values for the calves receiving the 25, 50 and 75 per cent alfalfa rations were 10, 3 and 0.

Guernsey calves made smaller increases in height at withers and heart girth, utilized less of the carotene ingested and had greater frequency of scours than Holstein calves. Females had fewer cases of scours than males.

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A STUDY OF OVULATIONS IN SIX FAMILIES OF HOLSTEIN–FRIESIANS[1]

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This report covers an analysis of variability in the incidence of multiple ovulations and quiet ovulations, and of the side on which ovulation occurred in an experimental herd of dairy cattle.

Numerous workers have reported an apparent differential function between the right and left ovary, greater in the right; none, however, has demonstrated associated variables or causes of variation.

Quiet ovulations are those for which there is no reported heat. Either the cow ovulates without being in heat or the outward expression of heat is so brief or so feeble as to be unnoticeable under the system of observation used. Sources of variability and the importance of such ovulations in detracting from satisfactory breeding efficiency appear not to have been given research consideration.

Multiple births are considered undesirable in cattle, as reported by Johansson (6) and Williams (10). Birth weights for twin calves are less than for singles and death rates are considerably higher; dystocia is more frequent in case of multiple births. Freemartins constitute approximately 92 per cent of all heifers born co-twin with bulls according to Swett *et al.* (9). The incidence of multiple ovulations and the embryonic death rate following single and multiple conceptions are antecedent characters that must be considered if the developmental physiology of twinning is to be understood.

EXPERIMENTAL PROCEDURE

The present study is a progress report and is based on records from the Emmons Blaine, Jr., Experimental herd of Holstein cattle located near Lake Mills, Wis. Detailed observations have been made routinely in this herd on various aspects of physiology of reproduction in connection with an experimental study of systems of mating.

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The collection of the data used in this study started October 15, 1947, and the study includes all the data available and satisfactory for use (some irregularities occurred in the timing of observations during the early months of the study) on the first reproductive period of all animals 15 mos. old or older by January 1, 1950, and on the second reproductive period of those animals whose second period had started on or before January 1, 1950. (Only outbred animals in the herd were old enough to be included in this report.) The first reproductive period designates the time between birth and first parturition in heifers, and the second reproductive period is the time between the first and second parturitions. Reproductive periods later than the second were not considered on any animals.

The operational procedures which were approximated during the earlier months of the study and which are currently used in gathering these reproductive data are as follows: Systematic heat checks are made twice daily at approximately 12-hr. intervals on all females over 6 mo. of age, the cattle being observed for 15 to 30 min. after they are turned out of confinement. Manual palpations of the genital organs, per rectum, are performed in the herd at 7-day intervals to determine the reproductive state of the animals scheduled for examination. Individual cows are scheduled for examinations 15 to 21 days after calving and weekly thereafter until involution of the uterus is complete. (Weekly examinations may be resumed in this connection if the uterine condition again becomes abnormal.) Heifers over 12 mo. of age, and cows over 14 days post partum, that have failed to show a heat period for 24 to 30 days are examined at the first regular examination day. If their heat periods are less than 18 days apart they are examined on the day of heat (this examination is not confined to the "herd examination day") and again 7 to 13 days later. Manual palpation of the ovaries to locate the corpus luteum 7 to 13 days after heat is performed on all animals 12 mo. of age and over.

An examination for satisfactory breeding conditions is made of all heifers during the last 7 days before 15 calendar mo. of age and of cows 67 to 74 days after calving. If approved for breeding, heifers are bred (by artificial insemination) at the first estrus on or after 15 mo. of age, and cows, on or after 75 days post partum. Cows and heifers are allowed a maximum of 233 "open" days to conceive after their breeding period begins. If open at the end of that time they are eliminated from the herd. All cows and heifers that have not returned in heat are examined 35 to 41 days after breeding to check for pregnancy.

The only reproductive abnormalities that receive therapeutic treatment are retained fetal membranes and immediate sequela, and infections of the genital organs which result in a generalized septicemia that may in turn endanger the life of the animal.

RESULTS AND DISCUSSION

Multiple ovulations and multiple births.

Johansson (6) gives 1.88 and 0.44 as the incidence of twin births in dairy and beef cattle, respectively. These percentages are based on the work of him-

self and numerous other workers. Lush (7) suggests there are possible differences between families within a breed in the incidence of multiple births. The relationship between multiple ovulations and multiple births, however, has not been explored.

The incidence of multiple ovulations in the present study was examined in the respective families or lines during the first and second reproductive periods (table 1). The *L* line, with 19.5 per cent multiple ovulations, was the highest in

TABLE 1

Single and multiple ovulations by families (first and second reproductive periods)

Line	Ovulations—1st period		Ovulations—2nd period	
	No. ovulations	% multiple	No. ovulations	% multiple
O	58	10.3	78	16.7
L	41	19.5	54	11.1
H	54	3.7	54	16.7
B	59	11.9	34	14.7
M	35	5.7	37	10.8
N	101	16.8	29	13.8
Totals	348	12.07	286	14.34

the first period and the *H* line with 3.7 per cent was the lowest. The Chi square test of significance showed a probability of chance occurrence of the observed differences between lines of approximately 0.08.

The over-all incidence of multiple ovulations was slightly higher in the second reproductive period than in the first (difference 2.27 per cent; P = 0.4–0.5). The range between the high lines (*O* and *H*, 16.7 per cent) and the low line (*M*, 10.8 per cent) was less than in the previous reproductive period and the Chi square test failed to approach significance for the differences between the families (P = 0.90–0.95). The over-all insignificant difference between the first and second reproductive periods fails to support (although it is in the right direction) Johansson's (6) conclusions that age is an important factor in twinning in cattle and also the work of others on various species which point to age as an important factor contributing to litter size. It may be that the first two reproductive periods are insufficient to demonstrate the age effect but it may also raise the question whether or not the increase in multiple births with age may not come about as a result of a decreased prenatal death rate in twin conceptions rather than from an increased incidence of multiple ovulations.

It has been suggested by Williams (10) that breeding too soon after parturition may lead to an abnormally high incidence of multiple births. This question was studied in an analysis of 286 ovulations, all from the second reproductive period. The post-partum interval was divided into two parts such that approximately 50 per cent of the ovulations occurred in the first period soon after parturition, and 50 per cent in the later period. The dividing point was between days 60 and 61 after parturition with 48.95 per cent of the ovulations occurring from day 1 to 60. The incidence of multiple ovulations during the first 60 days was 16.43 per cent on 140 ovulations, as compared to 14.33 per cent on 146 ovulations

for the period from day 61 to day 308 (difference 2.10; P = 0.3). This observation failed to offer definite evidence that breeding soon after parturition would lead to increased twinning, at least as far as the influence of ovulation rate is concerned.

Individual differences in the occurrence of multiple ovulations were studied in those cows for which there were records of at least two ovulations. The probability of a cow repeating a multiple ovulation in a given number of records on the basis of chance alone, was calculated by expanding the binomial $(p+q)^n$ where p equals the incidence of multiple ovulations in the whole sample, q, the incidence of single ovulations and n, the number of records on the cow. The last term of the expanded binomial q^n, gives the probability of the cow having only single ovulations; the next to the last term, npq^{n-1}, is the chance of her having only one multiple ovulation. The formula $1-(npq^{n-1}+q^n)$ gives the probability of a cow repeating multiple ovulations due to chance. The deviation of the actual proportion of these three kinds of cows (table 2) from the theoretical proportion was

TABLE 2

The distribution of multiple and single ovulations among 154 cows having at least two ovulations[a]

	Actually observed	Theoretical number
Cows with only single ovulations	100	90
Cows with only one multiple ovulation	32	49
Cows with more than one multiple ovulation	22	15

[a] Number of ovulations per cow ranged from 2 to 11, with 73 cows having either 2 or 3 ovulations.

highly significant ($P < 0.01$). It was therefore concluded that random occurrence of multiple ovulations within the herd could not account for the excessive number of two kinds of cows: (a) those animals that showed repeated multiple ovulations and (b) those which showed only single ovulations.

Whether or not a cow that repeats multiple ovulations is more likely to repeat them in sequence with one another than might be ascribed to chance was next investigated. A comparison was made between the proportion of multiple ovulations followed by multiples and the proportion of single ovulations that were followed by multiples. The percentages of the two kinds of sequences were 25.93 per cent and 12.67 per cent (difference 13.26 per cent; $P < 0.05$) based on 27 and 142 observations, respectively, in the first reproductive period, and 33.33 per cent and 16.34 per cent (difference 16.99 per cent; $P < 0.01$) based on 24 and 104 observations in the second reproductive period. Thus, there is a greater tendency for multiple ovulations to follow multiple and single to follow single than can be explained by chance alone. The incidence of repeatability is greater than can be accounted for by chance and in the individual that does repeat the tendency is to repeat in succession. Repeated multiple ovulations tend to distribute themselves during the observation period on a cow in sequences of two or more, rather than at random with respect to her different estrual periods. Multiple ovulations

therefore may result from an endocrine condition (disturbance?) more permanent than a single heat period.

Cole and Rodolfo (4) and Johansson (6) have reported a seasonal variation in the percentage of multiple births. Johansson reports one peak during the months of June and July which corresponds to conceptions in September and October and another in December and January, corresponding with conceptions in March and April. Cole and Rodolfo report in Hereford and Angus cattle the highest per cent of twin births in July, August and September, corresponding to conceptions in October, November and December.

The analysis of 348 ovulations during the first reproductive period in the present study demonstrated a significant monthly variation in the incidence of multiple ovulations (table 3). The peaks occurred during the months of March,

TABLE 3

Monthly variation in the incidence of multiple ovulation

Month	First period		Second period		First & second period	
	Total no. of ovulations	% multiple	Total no. of ovulations	% multiple	Total no. of ovulations	% multiple
1	32	6.25	37	10.81	69	8.69
2	44	13.64	32	21.88	76	17.11
3	26	15.38	25	12.00	51	13.73
4	30	13.33	21	23.81	51	17.65
5	29	27.59	21	28.57	50	28.00
6	33	6.06	24	16.67	57	10.53
7	33	3.03	18	27.77	51	11.76
8	37	8.11	15	13.33	52	9.62
9	14	0.00	16	0.00	30	0.00
10	25	28.00	30	6.67	55	16.36
11	18	11.11	19	10.53	37	10.81
12	27	11.11	28	3.57	55	7.27
	$X^2 = 20.23$ $P < 0.05$		$X^2 = 16.83$ $P = 0.2–0.1$		$X^2 = 20.76$ $P < 0.05$	

April, and May and again in October. This seems to be partly in agreement with each set of workers above because of the two peaks that were shown.

A significant monthly variation in the percentage of multiple ovulations was not noted during the second reproductive period ($P = 0.2–0.1$). The points of similarity between the first and second reproductive periods are the months of May and September. Both periods showed a high incidence in May and the lowest incidence in September. The points of difference were that during the second reproductive period a high frequency of multiple ovulations was maintained into the early summer, while in the first period a decided drop in frequency was noted after May. During the second period there was no peak noted in October, as there was during the first period in this report. The data from the two periods were pooled in further studies of the influence of month on multiple ovulations. A significant over-all monthly variation was then found ($P < 0.05$).

Data were available on 157 diagnosed pregnancies and subsequent calvings. Three sets of twins were born, giving a twinning incidence for the herd of 1.92

per cent, compared to the incidence of multiple ovulations of 13.10 per cent ($P < 0.01$). This suggests that the fertility at multiple ovulations may be markedly lower than the fertility at single ovulations. Consequently, a specific study was made of 294 ovulations at which breeding occurred. These included 252 single ovulations and 42 multiple ovulations. The incidence of multiple ovulations in these data is 14.29 per cent, which is very close to the average incidence of 13.10 per cent noted above in the herd as a whole. The conception rate at the single ovulations was 57.5 per cent and at the multiple ovulations was 28.6 per cent. This 28.6 per cent represents 12 conceptions of which only three resulted in twin births and nine in single births. It was concluded from this analysis that there is a highly significant difference ($P < 0.01$) in the conception rate of cows when bred at an estrus at which only a single ovum is shed, as compared to an estrus at which two or more ova are shed. It is suggested that either of two possible factors might be the cause of this difference in fertility: (a) that ova from multiple ovulations tend to be defective and either not capable of being fertilized or, if fertilized, of such low viability that they soon die, or (b) multiple ovulation is the result of a general endocrine dysfunction, with the result that there is an interference with sperm transport or that the uterus is not prepared properly to nourish the embryo or to allow placentation.

Quiet ovulations.

Quiet ovulations are defined as those ovulations that occurred without an observed heat period; 27.3 per cent of all ovulations reported were quiet. The time of occurrence of these quiet ovulations is important. If they occur soon after parturition, they are of minor importance, but if they occur with high frequency more than 60 days after parturition when an attempt is being made to breed the cows for a yearly freshening interval their importance will be increased.

Records of 286 corpora lutea from the second reproductive period were available, of which 208 resulted from ovulations associated with known estrual periods and 78 from quiet ovulations. The post-partum interval was divided into an early and a late part between days 60 and 61, the same division as in the study of multiple ovulations above. The incidence of quiet ovulations during the first 60 days was 44.3 per cent on 140 ovulations and 11.0 per cent of 146 for the period from day 61 to day 308 (difference 33.3 per cent; $P < 0.01$).

While there is a highly significant decrease in the incidence of quiet ovulations following the first 60-day portion of the post-partum interval, the incidence is still high enough to be of importance to the breeder who wishes to keep his cows at their maximum production by having them freshen yearly. It is suspected that an appreciable amount of time is lost due to quiet ovulations.

Side of ovulations.

It has been previously reported that the right ovary in the cow is more active than the left. Stalfors (cited by Reese and Turner (8)), reported, as a result of rectal examinations of 923 cows for pregnancy, that 62.5 per cent were pregnant in the right horn. The Idaho Experiment Station (5) concurred with these findings, as did Clark (3) and Casida (2). Casida *et al.* (1) found a significant dif-

ference in the total follicular volume between right and left ovaries, the right having the greater value, in an extensive study of the genitalia of heifer calves.

The data presented are in general agreement with these findings. The percentage of all single ovulations which occurred in the right ovary was 54.1 per cent and 59.6 per cent (difference 5.5 per cent; P = 0.2) for the first and second reproductive period, respectively, with an over-all average of 552 ovulations of 56.5 per cent in the right ovary.

The data were examined for possible differences between the families in the side of ovulation (table 4). An analysis of the data fails to show significant dif-

TABLE 4

Percentages of single ovulations in the right ovary and of subsequent pregnancies in the right uterine horn by families

Line	No. of ovulations	% of ovulations from right ovary	No. of pregnancies	% in right horn
O	117	60.7	33	75.8
L	81	59.3	24	54.2
H	98	57.1	28	50.0
B	81	56.8	25	48.0
M	66	51.5	15	46.7
N	109	52.3	20	40.0
Total	552	56.5	145	54.5

ference between families (P $\cong$ 0.8) in the frequency with which the right or left ovary functions.

The percentage of pregnancies that was on the right side (54.5 per cent) did not differ significantly from the percentage of ovulations that was on the right side (56.5 per cent). Further than this, the 138 ovulations from the right ovary and the 114 from the left at which breeding actually occurred gave conception rates of 57.2 per cent and 57.9 per cent for the ova from the two sides, respectively. Thus there was no evidence from these comparisons of differential fertility between the two sides of ovulation.

An analysis of the pregnancies by families according to the side of pregnancy brought out no significant differences (P $\cong$ 0.10). However, the small numbers, the relatively low probability and the wide range between lines warrant close attention being given this question as further data become available.

TABLE 5

Ovulation sequences from right and left ovaries

Sequences	Observed no. of sequences		Theoretical no. of sequences based[a] on the expansion of the binomial	
	1st period	2nd period	1st period	2nd period
Right followed by right	29	28	36.00	27.00
Right followed by left	38	18	30.54	18.30
Left followed by right	29	20	30.54	18.30
Left followed by left	27	10	25.92	12.40

[a] Based on an incidence of 54.1% and 59.6% of the ovulations in the right for the first and second reproductive periods, respectively.

If the functioning of each ovary was completely at random within the restrictions of the differential frequency of the ovulations between the right and left ovary, the sequences of two ovulations would then follow the expansion of the binomial, $(p + q)^2$, where p equals the incidence of ovulation from the right ovary and q the incidence from the left ovary (pp equals right followed by right; pq equals right followed by left; qp equals left followed by right, and qq equals left followed by left). A test of this hypothesis (table 5) that sequences of ovulations are at random showed no significant deviations from the theoretical ($P \cong 0.3$ for the first period and $P \cong 0.9$ for the second period). It was concluded from this analysis that there is no apparent tendency for cows to have a systematic sequence of ovulations from one ovary or the other.

SUMMARY

The incidence of multiple ovulations in the Emmons Blaine, Jr. herd of experimental Holstein cows was found to be 13.10 per cent, while the incidence of twinning was 1.92 per cent, a highly significant difference. The incidence of multiple ovulations was not significantly influenced by reproductive period, family, or length of time after parturition. There was a significant monthly variation in frequency of multiple ovulation in the first reproductive period and the monthly variation in the second period approached significance, being high in May and low in September in both cases. A highly significant tendency for multiple ovulations to follow in sequence was found. Significantly higher fertility was noted when cows were bred at an estrus from which only a single corpus luteum was formed as compared to an estrus from which two or more corpora lutea were formed.

The incidence of "quiet" ovulations (44.3 per cent) was significantly higher during the first 60 days after parturition than during the period 61 to 308 days post-partum. During this latter period their incidence was still sufficiently high (11.0 per cent) to constitute an important problem to the breeder.

Of the ovulations 56.5 per cent were from the right ovary. Studies of family and parity failed to show any significant deviations from this ratio. No apparent tendency was found for cows to have a systematic sequence of ovulations from one ovary or the other.

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EFFECT OF FEEDING METHOXYCHLOR-TREATED ALFALFA HAY TO DAIRY COWS[1]

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Following the consumption of DDT-dusted alfalfa hay, significant amounts of this insecticide appear in the milk and body fat of dairy cows, the amount present being proportional to the quantity of DDT residue on the hay (1). Because the methoxy analog of DDT (1,1,1-trichloro-2,2-bis (p-methoxyphenol) ethane) has a much lower order of toxicity than DDT in rats, rabbits and mice (6, 7), and because preliminary tests at this station showed it to be promising for the control of alfalfa weevil larvae, experiments were undertaken to determine the extent of accumulation of methoxychlor in the milk and tissues of dairy cows fed alfalfa hay that had been dusted with this insecticide.

MATERIALS AND METHODS

A 33-acre dry land field of alfalfa was divided into 16 1.5-acre plots with intervening buffer strips. These plots were assigned to four blocks of four plots each. One plot in each block then was dusted with the following levels of methoxychlor in pyrophyllite carrier: 1, 2, and 2.8 lb. per acre. One plot in each block was untreated. Thus, four plots received each treatment. Application was made with a power duster following the methods previously described (3) and at a time when insect counts showed large numbers of larvae of alfalfa weevil (*Hypera postica* Gyll.) on the alfalfa.

Insect counts were made the day of dusting (June 6, 1949) and at 4, 9 and 14 days following the application of methoxychlor. The alfalfa was cut 23 days after applying the methoxychlor. It originally was planned to cut the alfalfa on the day following the last insect count but rain delayed this for approximately 1 wk.[2] The alfalfa was raked into windrows and allowed to sun-cure, after which it was baled and stored in a barn until December, at which time the feeding trials began.

Eight Holstein dairy cows from the Station experimental herd were used for the feeding trials. Two cows received hay from the field plots receiving a given

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[2] This delay in harvesting the hay may have reduced the insecticide residue somewhat. However, results of other experiments conducted during two different years in northern Utah show that the residues reported in this paper are not out of line even though some rain did fall between dusting and cutting. For example, 0.9 lb. methoxychlor was applied per acre as a dust and residue of 2.6 ppm. was found 11 days later; 1.6 lb. were applied per acre and 10.6 ppm. were found 11 days later; and 1.8 lb. were applied per acre and 11.2 ppm. were found 27 days later.

treatment. In addition to the alfalfa hay, the cows received a grain ration which consisted of a mixture of 80 per cent barley and 20 per cent molasses-dried beet pulp, to which was added 2 per cent steamed bone meal and 1 per cent fine hay salt (NaCl). This ration was fed at the rate of 0.75 lb. of grain per day for each pound of butterfat produced during the previous week. If butterfat production dropped below 0.8 lb. per day, the grain ration was routinely discontinued.

Feeding began on December 18, 1949, and continued until April 10, 1950, a total of 113 days. Milk samples were obtained from each cow before feeding of the hay began, and at approximately weekly intervals throughout the period of feeding. Blood samples (100 ml.) were obtained before and at the end of the feeding period. The milk and blood samples were analyzed for methoxychlor, using the method of Prickett *et al.* (5), except that nitration was carried out according to the following schedule:

Time	Temp. (° *C.*)
Start	5
5 min. raise to	10
10 min. raise to	20
15 min. raise to	30
20 min. raise to	40
25 min. raise to	45
30 min. raise to	50
35 min. raise to	60
40 min. raise to	70
45 min. raise to	80
50 min. raise to	90
55 min. raise to	Boiling

Four of the cows, one from each treatment, were slaughtered at the end of the feeding period and selected tissues analyzed for their methoxychlor content, using the method of Prickett *et al.* (5) after the tissues were first ground in a food grinder. The methoxychlor residue on the hay was determined using the Fairing and Warrington method (2). The samples of hay used for analysis were obtained from every second bale throughout the period of feeding. All samples from a given field treatment then were combined and analysis made for methoxychlor. All readings were made on a Beckman quartz spectrophotometer.

RESULTS AND DISCUSSION

The control of alfalfa weevil larvae obtained following the application of methoxychlor to the alfalfa is given in table 1. All three dosages provided adequate protection, the two higher treatments giving excellent control for 14 days after application.

The methoxychlor residue and yield of the hays that were fed to the cows are given in table 2. The residue varied from 0 to 14 ppm. The yield was greater from the treated than from the untreated plots.

Analyses of milk from the eight cows, which were made at approximately weekly intervals throughout the period of feeding, showed that there was no methoxychlor in the milk at any time.

No methoxychlor was found in the blood before or at the end of the feeding period. Furthermore, there was no methoxychlor in kidney fat, kidney, liver or

TABLE 1

Control of alfalfa weevil larvae in alfalfa with methoxychlor, Cache Valley, Utah, June, 1949

Treatment			Weevil population[a]				Per cent control obtained		
Methoxychlor	Crop dust	Technical material	Day of dusting (June 6)	Days after treatment			4 d. after dusting	9 d. after dusting	14 d. after dusting
				4	9	14			
(%)	(*lb. per acre*)								
none	none	none	740	1,722	2,599	2,138			
5	22	1.0	503	131	287	304	88.8	83.8	79.1
10	21	2.0	547	91	169	153	92.9	91.2	90.3
15	18	2.8	764	68	223	180	96.2	91.7	91.8

[a] Weevil larvae/100 strokes of 15-in. net; based on 100 strokes in each of 4 replicates.

muscle of cows fed either treated or untreated hay. Kuntz *et al.* (3) have shown in rats that 100 ppm. methoxychlor must be present in the diet before this substance appears in the body fat. The highest residue on the hay ingested by the cows was 14 ppm. This level of methoxychlor is well below the level at which it would appear in the body fat of the cows, if there is a similarity in response of the two species of animals.

The daily feed consumption and milk and butterfat production of the cows during the period of feeding are given in table 3. The differences in production

TABLE 2

Average yield and residue on alfalfa hay treated with methoxychlor

Methoxychlor applied /acre	No. of plots	Yield	Methoxychlor residue
(*lb.*)		(*tons*)	(*ppm.*)
0	4	1.22	0.0
1.0	4	1.52	7.0
2.0	4	1.31	9.5
2.8	4	1.36	14.0

result from the various stages of lactation of the cows during the test. The reason for the low grain consumption of cow Hu 140 was that butterfat production dropped below 0.8 lb. on the 17th day of the test and grain feeding was discontinued.

The negative results reported for methoxychlor in milk, blood and tissues actually indicate that less than 0.1 ppm. methoxychlor was found, since this is the limit of sensitivity of the analytical method used. Color does not develop at the end point until this level of methoxychlor is exceeded. In no instance was any color characteristic of methoxychlor obtained in these samples.

TABLE 3

Average daily feed consumption and milk and butterfat production of dairy cows for 113-d. feeding period

Methoxychlor applied /acre	Cow no.	Hay consumed	Grain consumed	Milk produced	Butterfat	
					Produced	In milk
(*lb.*)		(*lb.*)	(*lb.*)	(*lb.*)	(*lb.*)	(%)
0	Hu 140	26.3	0.81	9.39	0.37	3.9
	E 186	32.4	3.23	23.0	0.75	3.3
1	Hu 63	34.8	5.86	28.3	1.00	3.5
	E 214	29.0	6.27	24.1	0.82	3.4
2	Hu 164	20.7	6.98	27.3	0.94	3.4
	A 143	26.3	6.13	28.8	0.90	3.1
2.8	E 180	34.2	8.92	46.8	1.47	3.1
	Hu 158	29.4	3.84	17.5	0.67	3.9

SUMMARY

Methoxychlor did not appear in measurable amounts in the blood, milk or tissues of six dairy cows consuming alfalfa hay that had been dusted in the field with methoxychlor. Two cows receiving untreated alfalfa hay likewise had no methoxychlor in their milk, blood or tissues.

There was no effect on milk or butterfat production or on feed consumption as a result of feeding methoxychlor-treated hay.

The methoxychlor residue on the hay was 0, 7, 9.5 and 14 ppm. for the alfalfa treated with 0, 1, 2 and 2.8 lb., respectively, of methoxychlor per acre.

There was no apparent effect of consuming the methoxychlor-treated hay upon the health of the cows.

Good economic control of alfalfa weevil larvae was obtained in the plots of alfalfa dusted with methoxychlor. The treated plots gave a somewhat greater yield of hay than the untreated plots.

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FACTORS INVOLVED IN CORROSION OF TINNED AND STAINLESS STEEL BY MILD ACIDIC SOLUTIONS USED IN CLEANING DAIRY EQUIPMENT

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Mild acidic solutions are used or suggested for use in the dairy industry for a variety of cleaning operations, such as removal of milkstone deposits, cleaning of high-temperature short-time pasteurizers (7), in some can cleaning operations (6) and alternating with alkaline compounds for cleaning of equipment (1). The metals used most widely at the present time in manufacture of dairy equipment are tin-plated steel, prepared either by hot dip or electroplating methods, and 18–8 stainless steel. The usual feeling (1) is that organic acids are "mild" in their corrosive action on dairy equipment metals. Data on corrosion of tin plate or stainless steel by some of the acids and acid dairy cleaners used on dairy equipment are available (2, 3, 5, 6, 8). However, comparative data on some acids presently used in dairy cleaning seem to be lacking. Therefore, it was considered desirable to study factors involved in corrosion of tin plate and stainless steel by acidic materials in present use as dairy cleaners, near the temperature and concentration limits of dairy cleaning, with the hope of obtaining comparative data of possible practical value.

MATERIALS AND METHODS

Hot-dip heavily plated tinned steel strips were prepared by cutting 3.5×1 in. strips from electroplated tinned steel sheets, tinning the edges by means of molten tin and a zinc flux and retinning the entire strip in molten tin, using a palm oil flux. Electroplated tinned steel strips were prepared by cutting 3×1 in. steel strips, cleaning with a foaming scouring powder, rinsing, pickling in diluted HCl and electroplating from a sodium stannate bath. The electroplated strips were flow brightened by dipping into hot vegetable oil. In this manner, electroplated strips with a tin coating between 0.2 and 0.3 mils (thousandths of an inch) were obtained. Stainless steel strips, 3×1 in., were cut from 18–8 sheet and buffed to approximately a medium polish. All strips were cleaned just before use with an alkaline detergent, rinsed, cleaned with a non-ionic synthetic detergent, rinsed with distilled water, shaken in carbon tetrachloride, dried at 100° C. for 0.5 hr., cooled in a desiccator for at least 0.5 hr. and weighed on an analytical balance.

All acidic solutions (see table 1 for grades of chemicals) were prepared in distilled water in a final concentration of 0.25 per cent by weight. Seventy ml. of each solution were placed in a 4-oz. wide-mouth jar. This solution volume covered the lower 2 in. of an immersed metal strip. A weighed strip was placed in each jar, the jar closed with a bakelite cap and held at various temperatures for 66 hr.

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Duplicate strips were treated with each solution at each temperature. Temperatures of 40 and 50° C. were maintained within 0.5° C. by means of thermostatically controlled water baths. Maintenance of a temperature of 60° C. within 1° C., was accomplished by means of a thermostatically controlled, gravity convection-type incubator. At the end of the exposure period, strips were removed from the jars, rinsed three times with distilled water, with light brushing during the second rinse, dried at 100° C. for 0.5 hr., cooled in a desiccator for at least 0.5 hr., weighed and duplicate weight changes averaged. Appearance of the strips was noted after weighing. All pH measurements were made at room temperature by means of a Beckman glass electrode pH meter.

RESULTS

Averages of losses in weights of treated tinned steel strips are listed in table 1.

TABLE 1

Weight losses of hot dip and electroplated tinned steel strips after treatment with acidic solutions at different temperatures

0.25% solution	Weight loss	Hot dip			Electroplated	
		40° C.	50° C.	60° C.	40° C.	50° C.
Phosphoric acid (C.P.)	mg.	2.05	1.85	3.55	2.30	3.40
	MDD[a]	3.60	3.20	6.20	4.00	5.90
Gluconic acid (tech.)	mg.	13.00	24.65	15.20	30.60	25.95
	MDD	22.70	42.90	26.40	53.20	45.10
Levulinic acid (tech.)	mg.	7.75	11.30	15.10	19.70	19.00
	MDD	13.50	19.60	26.50	34.30	33.00
Glycolic acid (tech.)	mg.	31.25	28.45	23.00	32.20	31.45
	MDD	54.30	49.50	40.00	56.00	54.30
Citric acid (C.P.)	mg.	21.10	21.65	36.60	43.30	43.90
	MDD	36.70	37.60	63.60	75.30	76.30
Acetic acid (C.P.)	mg.	10.30	14.10	17.40	14.65	19.40
	MDD	17.90	24.90	30.30	25.50	33.70
Sulfamic acid (C.P.)	mg.	29.50	23.70	22.90	32.10	40.20
	MDD	51.30	43.20	39.80	55.80	69.90
Sodium bisulfate (tech.)	mg.	38.50	41.10	35.50	28.15	30.30
	MDD	67.00	71.50	61.70	49.00	52.70

[a] MDD = Loss in mg./decimeter^2/day.

In general, losses in weight of hot dip strips were lower than those obtained with electroplated strips. Treatment with phosphoric acid resulted in very low weight losses at all temperatures. With gluconic acid and sodium bisulfate, hot dip strips lost more weight at 50° C. than at both 40 and 60° C. Electroplated strips lost more weight at 40 than 50° C. with gluconic acid, while the reverse occurred with sodium bisulfate. Levulinic, citric and acetic acids caused increasing losses in weight of hot dip strips with rise in temperature. With levulinic and citric acids, weight losses of electroplated strips were constant, while acetic acid showed an increase in weight loss with a 10° rise in temperature. Glycolic acid caused a slight decrease, sulfamic acid an increase in weight loss of electroplated strips with rise in temperature. Relating effects of the acidic solu-

tions by means of weight losses revealed that hot-dip tinned steel at 40 and 50° C. showed the greatest weight loss with sodium bisulfate, followed by glycolic, sulfamic, gluconic, citric, acetic, levulinic and phosphoric acids in that order. At 60° C., the descending order was citric, sodium bisulfate, glycolic, sulfamic, acetic, gluconic, levulinic and phosphoric. With electroplated tin strips at 40° C., the descending weight loss order was citric, glycolic, sulfamic, gluconic, sodium bisulfate, levulinic, acetic and phosphoric, while at 60° C. it was citric, sulfamic, glycolic, sodium bisulfate, gluconic, acetic, levulinic and phosphoric. It is pertinent to note that all of the MDD (milligrams per square decimeter per day) figures were below the "serious" weight losses represented by 100 MDD (2, 5), an indication of the essentially mild nature of the tested acidic solutions.

Observations on changes in appearance of tinned strips indicated fairly well defined relationships (table 2). In all cases, changes in appearance were most

TABLE 2

Appearance[a] *of hot dip and electroplated tinned steel strips after treatment with acidic solutions at different temperatures*

0.25% solution of:	pH	Hot dip			Electroplated	
	25–30° C.	40° C.	50° C.	60° C.	40° C.	50° C.
Sulfamic acid	1.67	slR / GSp	NC / SWSp	NC / SWSp	RP / SWRPSp	RP / SWslRPSp
Phosphoric acid	1.96	NC / SWSp	NC / SWSp	NC / SWSp	NC / SWSp	NC / SWSp
Sodium bisulfate	2.06	NC / slBrBSp	NC / GSp	NC / WSp	RP / SWRPSp	RP / SBRPslSp
Citric acid	2.55	NC / BBrSp	NC / BBrSp	NC / BBrSp	NC / GBSp	NC / GBSp
Glycolic acid	2.59	NC / BBrSp	NC / BBrSp	BSp / BSp	slR / BGSp	slR / BGSp
Gluconic acid	2.94	NC / BrBSp	NC / BrBSp	NC / BrBSp	slR / GBSp	slR / GBSp
Acetic acid	3.10	NC / IslSp	RP / IslSp	RP / IRP	RP / I	RP / IRP
Levulinic acid	3.14	NC / IslSp	NC / IslSp	NC / IRP	NC / Br	slR / I

[a] B—black, Br—brown, G—gray, I—iridescent, NC—no change, P—pits, R—rust, S—silver, sl—slight(ly), Sp—spangled, W—white. Appearance of portions of treated strips above and below air-liquid interfaces is indicated by the position of key letters above or below dashes (3).

intense at the air-liquid interfaces; liquid phases were next in intensity, while vapor-phase exposed strip portions showed the least change. A relationship between color of treated strips, particularly in the liquid phases, and pH of the solutions seemed to exist. Liquid phase portions of strips treated with solutions between pH 1.67 and 1.96 were colored silver, white or gray, those between pH 2.06 and 2.94 were gray, black and brown, while at pH 3.10 and 3.14 an iridescent rainbow-like film predominated. "Spangling," a term used here to describe seemingly etched patterns on tin surfaces, was noted with all solutions

except acetic and levulinic acids, the only organic acids used which were not hydroxy acids and which gave the highest pH readings. Of the organic acids, volatile acetic and levulinic acids caused pitting of the tinned steel strips. Inorganic sulfur-containing acidic materials; *i.e.*, sulfamic acid and sodium bisulfate, also caused pitting, while phosphoric acid did not. In general, appearance changes were more intense with the electroplated rather than hot dip strips.

Treatment of stainless steel strips by the acidic solutions resulted in extremely small weight changes (table 3). Only sulfamic acid caused weight losses at all

TABLE 3

Weight changes of stainless steel strips after treatment with acidic solutions at different temperatures

0.25% solution	pH	Weight change[a] at:		
	25–26° C.	40° C.	50° C.	60° C.
		(*mg.*)	(*mg.*)	(*mg.*)
Phosphoric acid	1.93	+0.20	+0.10	+0.35
Gluconic acid	2.92	+0.15	0.00	0.00
Levulinic acid	3.11	+0.20	+0.15	+0.30
Glycolic acid	2.53	+0.40	+0.05	+0.05
Citric acid	2.52	+0.20	+0.40	−0.60[b]
Acetic acid	3.12	+0.25	+0.30	−0.10
Sulfamic acid	1.66	−0.10	−0.20	−0.10
Sodium bisulfate	2.05	+0.10	−0.10	−0.05

[a] + weight gain, − weight loss.

[b] Strips treated with citric acid at this temperature had a light, white-gray film below the liquid level. All other strips were unchanged in appearance.

temperatures, while citric and acetic acids and sodium bisulfate caused weight losses only at 60° C., the highest temperature used. With all other solutions, small weight increases occurred. Strips treated with citric acid at 60° C. developed a light white-gray film and also lost the greatest amount of weight. All other strips were unchanged in appearance.

DISCUSSION

Acidic corrosion of tinned steel apparently is an extremely complex process involving many interrelated factors. No one effect, such as weight loss at a single temperature, for example, can be relied upon to accurately describe or predict the activity of an acid, for weight losses may vary in an unpredictable manner with temperature changes. However, certain trends which may be of practical value became apparent in this study.

Depending on the acidic compound and possibly related to chemical structure, weight losses of tinned steel remained constant, increased or decreased with changes in temperature. Further, the type and/or thickness of tin plate had a bearing on the intensity of the changes produced as greater weight losses, more pitting, more pronounced color changes, etc., were evident with electroplated as compared to hot-dip heavily plated strips. Probably in the case of electroplated strips a good deal of corrosion of steel, as well as tin, occurred. The pH of the solutions at room temperature had no apparent relation to weight losses of tinned

steel but did seem related to color changes and "spangling." Color changes possibly were due to formation of more or less soluble reaction products and, as the experimental method was essentially static, maximum macroscopic reactions probably were produced. In addition to pH of solutions, "spangling" seemed related to structure of hydroxy organic acids but not to structure of carboxylic or inorganic acids, weight losses, color production, type of tinplate and temperature. Pitting, particularly of thin electroplated tin strips, apparently was caused only by volatile organic acids and also related to the presence of sulfur within the molecules of inorganic acidic compounds. However, it is probable that the known and possibly variable porosity of tin plate also played a role in the pitting of test strips (8).

The low activity of phosphoric acid on tin has been recorded previously (8). The formation of insoluble tin phosphate as a protective corrosion product, resulting from interaction of the tin or tin oxide surface with phosphoric acid, seems entirely possible. Objections to excessive corrosion by phosphoric acid of iron and steel surfaces (2), such as those found in can washers, drains, etc., may possibly be removed by use of inhibitors (4). Preliminary experiments in this laboratory indicate that "spangling" of tin plate by phosphoric acid may be reduced by use of inhibitors.

Stainless steel was resistant to all of the acidic solutions at the temperatures employed. Citric acid was the only tested material which caused a visible change in appearance of stainless steel strips, and this occurred only at the highest test temperature.

CONCLUSIONS

Factors contributing to corrosion of tinned steel by mild acidic solutions were found to be temperature, pH, apparent volatility of organic acids, structure of acidic compounds and type of tinplate. Factors apparent as results of corrosion were weight loss, color changes, pitting and "spangling."

Weight loss of tinned steel changed or remained constant with change in temperature depending on the acidic compound and the type of tinplate. Under all test conditions, phosphoric acid caused only very slight weight losses as compared to various organic acids, sodium bisulfate and sulfamic acid.

A relationship between pH of acidic solutions and formation of colored deposits, as well as "spangling" of treated tinned steel strips, seemed to exist.

Pitting of tinned steel was caused only by volatile organic acids and also apparently was related to the presence of sulfur in molecules of inorganic acidic compounds.

Corrosion manifestations were more intense on thinly electroplated than on hot-dip heavily plated tinned steel.

Under the experimental conditions, stainless steel was not corroded appreciably by any of the acidic solutions tested.

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SOME CHARACTERISTICS OF THE LIPASE OF *GEOTRICHUM CANDIDUM*

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The conditions necessary for optimum lipase activity vary with the source of enzyme, the substrate and the methods used in obtaining the lipase preparation. Thus, it has been reported (4) that both *Aspergillus niger* and *Penicillium roqueforti* produce two types of lipase. The extracellular lipases were most active at pH 8.0, whereas the mycelial lipases exhibited maximum activity at pH 6.5. In addition, the optimum temperature for lipolysis varied with the substrate. Similarly, Tammisto (13) demonstrated that the heat-resistant intracellular lipase of *B. fluorescens liquefaciens* became heat sensitive when the cells were air-dried. Furthermore, the dried preparations exhibited a lower pH optimum than did the fresh cell preparation.

The lipase of *Candida lipolytica,* characterized by Peters and Nelson (7), was active over wide ranges of pH and temperature. Maximum cleavage of butterfat occurred at pH 6.2 to pH 6.5 and at 28 to 33° C. The activity of cell-free preparations was virtually unchanged by storage at 3 to 5° C. for 3 mo. However, considerable activity was lost during lyophilization and foam concentration. The same authors (8) also reported that favorable growth conditions resulted in low lipase production. The accumulation of lipase was enhanced by growing *C. lipolytica* without shaking and the addition of reducing substances to the cultures had little or no effect on the accumulation of lipase.

Although it has been reported that cysteine reverses the inactivation of milk lipase brought about by aeration (6) and that wheat germ lipase requires intact sulfhydryl groups for activity (12), the lipase of some microorganisms may differ in this regard. According to Fiore and Nord (2), the intracellular lipase of *Fusarium lini* contains no sulfhydryl or disulfide groups essential for activity. Similarly, microbial lipases also may vary in sensitivity to oxygen and other oxidizing agents. In this connection, it has been reported (3) that the lipase of *Clostridium perfringens* was unaffected by air or 10^{-2} *M* H_2O_2. Conversely, H_2O_2 or $KBrO_3$ have been added to cultures of *Geotrichum candidum* during fat synthesis studies (9) to prevent lipolysis of the accumulated fat.

Thus, it is apparent that growth and assay conditions directly influence lipase production and activity and, therefore, the behavior of an organism and its lipase in a given situation cannot be predicted. Although *G. candidum* rapidly hydrolyzes butterfat in cream (5), the characteristics of its lipolytic system are relatively unknown. It is the purpose of this paper to report some of the characteristics of the *G. candidum* lipase.

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METHODS AND MATERIALS

Bacteriological: G. candidum, strain 13, isolated from commercial raw cream in this laboratory, was selected as the test organism because of its known capacity to hydrolyze butterfat (11). Stock cultures were maintained on tryptone-glucose-beef extract-agar slants and were stored at 4° C. after initial incubation at 20° C.

Cell-free filtrates from broth cultures served as the source of the lipase. Active filtrates were obtained by growing the organism on a medium containing 10 g. Protolysate,[1] 1 g. Na_2HPO_4, and 0.1 g. KCl dissolved in 1 l. of distilled water. The medium was dispensed in 125-ml. quantities into cotton stoppered 1-l. Erlenmeyer flasks, adjusted to pH 7.0 and autoclaved at 121° C. for 15 min. The flasks were inoculated with 0.1 ml. of a water suspension prepared from a 24-hr. agar slant culture of *G. candidum,* and incubated without aeration for 4 days at 20° C. The cells were removed by filtration through asbestos-matted Gooch crucibles.

Lipase assay: The activity of filtrates was measured by titrating the fatty acids liberated from a buffered substrate. Except where indicated the emulsion used in the assay contained 1.0 ml. U.S.P. olive oil (or other substrates), 44 ml. of 0.3 per cent agar dissolved in buffer (pH 6.0), and 0.3 ml. toluene. The agar solution was prepared by dissolving 3 g. bacto-agar in 1 l. of buffer containing 250 ml. 0.2 *M* potassium biphthalate, 228 ml. 0.2 *M* NaOH and 522 ml. distilled water. The buffered substrate was sterilized at 121° C. for 15 min. and then stored at 4° C. until used. The system was brought to incubation temperature and 5 ml. of culture filtrate and 0.3 ml. toluene added. The reaction mixture was shaken until a stable emulsion was obtained and then incubated at 20° C. Aliquots of the emulsion were titrated daily during the 3-day incubation period. Duplicate or triplicate assays were performed in each trial. Controls containing either sterile growth medium or a heated culture filtrate were used. In no instance did the titration values of the controls change during the test period.

A 10-ml. aliquot of the reaction emulsion was diluted with 25 ml. of 1:1 neutral 95 per cent ethyl alcohol-diethyl ether, and the free fatty acids titrated with 0.1 *N* alcoholic KOH to a phenolphthalein end point. Initial titration values, obtained prior to incubation, were subtracted from the values obtained during incubation. The net titration values obtained in this manner were thus a measure of lipase activity. Experimental data are expressed as the net titration value (milliliters 0.1 *N* KOH) of the 10-ml. aliquot.

RESULTS

The data in figure 1 show the influence of pH and temperature on the activity of the extracellular lipase of *G. candidum.* Lipolysis was studied in emulsions buffered at pH 4.0, 5.0 and 6.0 with potassium biphthalate-NaOH mixtures, and in emulsions buffered at pH 6.0, 7.0 and 8.0 with KH_2PO_4-NaOH mixtures (10). Since there were no significant differences between the lipolytic activity obtained

[1] Mead Johnson & Company, dry enzymic digest of casein.

in a phosphate buffer and in a biphthalate buffer at pH 6.0, the data were combined into a single graph.

As can be seen from the graph, rapid lipolysis occurred in substrates buffered between pH 5.0 and 8.0, and at 20, 30 and 37° C. Maximum activity was observed at pH 6.0 and 30° C. The enzyme was completely inactive at pH 4.0. In this connection, additional experiments demonstrated that the lipase was destroyed when active filtrates were acidified to pH 3.0 and immediately reneutralized.

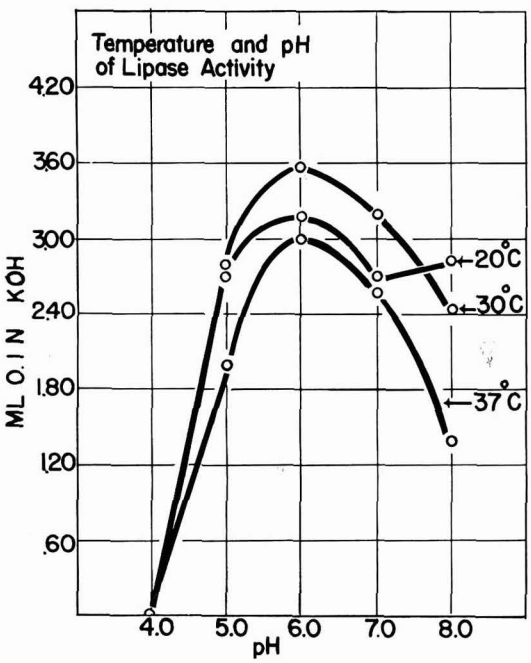

FIG. 1. *Effect of temperature and pH on activity of G. candidum lipase.* Assay flasks contained 45 ml. buffered 0.25 % agar, 2.5 ml. butter oil, 2.5 ml. filtrate from a 4-d. broth culture and 0.3 ml. toluene. The assay emulsion was incubated for 3 d. at 20, 30 and 37° C. Data are expressed as mean net titration value of 20-ml. aliquots from triplicate emulsions.

The enzyme hydrolyzed numerous substrates; however, natural fats such as butterfat and olive oil were attacked more readily than synthetic triglycerides, monoglycerides and fatty acid esters of the glycols. Tripropionin, tributyrin and tricaprylin at pH 6.0 were hydrolyzed to a moderate degree, whereas triacetin, tristearin, trilaurin, tripalmitin and trimyristin did not undergo lipolysis. Greater activity was obtained with glyceryl mono-oleate than with glyceryl monostearate, laurate or ricinoleate. Diglycol oleate, diglycol laurate and propylene glycol monolaurate yielded low titration values. The fatty acid esters of polyethylene glycol were not hydrolyzed.

The data represented by figure 2 show the influence of relative substrate con-

centration on the reaction rate when butterfat, olive oil and tributyrin were employed as substrates. Lipolysis increased when the concentration of olive oil was increased, and did not appear to reach a maximum with the concentrations studied. A similar response occurred when butterfat was tested; however, it was noted that with 5 ml. of culture filtrate maximum lipolysis occurred in the presence of 3 ml. of substrate. The influence of increased concentrations of tributyrin was different than that observed with the other substrates. In this in-

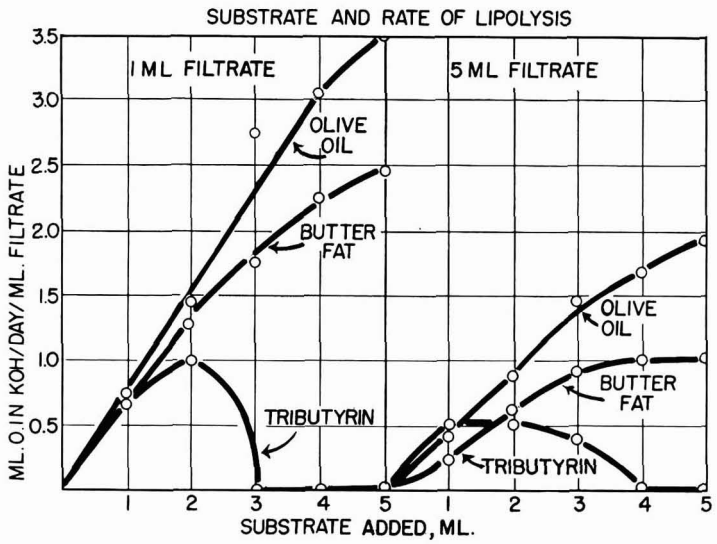

FIG. 2. Influence of relative substrate concentration on rate of lipolysis. Assay emulsion contained 40 ml. 0.3 % buffered agar (pH 6.0), the type of substrate and volume of culture filtrate as shown, and 0.3 ml. toluene. Duplicate emulsions were incubated at 20° C. for 3 d. Data are calculated from mean net titration values for 10-ml. aliquots.

stance the enzyme activity reached a maximum and decreased rapidly to zero. Complete inhibition was observed when high concentrations of tributyrin were present. This inhibition may have been caused by the presence of impurities in the substrate. The differences in rates obtained by comparing the substrates indicate relative substrate specificity. Variation in absolute substrate concentration and emulsion-forming properties of the substrates undoubtedly contribute to a small degree to these differences.

A study of the distribution of lipase in broth cultures demonstrated that filtrates were more active than the corresponding mycelial preparations. For this comparison the mycelia were macerated at 4° C. in a glass tissue-homogenizer in the presence of no. 600 carborundum and 4 ml. of biphthalate buffer at pH 6.0. When thoroughly ground, the homogenate was diluted to a volume equivalent to the culture filtrate by the addition of buffer. The lipolytic activity of such preparations compared to that of the culture filtrates is illustrated in figure 3. Al-

though the filtrates varied in lipolytic activity, in all instances the filtrate activity was greater than that of the corresponding mycelial preparation. Thus, the data suggest that the lipase of *G. candidum* is primarily an extracellular enzyme.

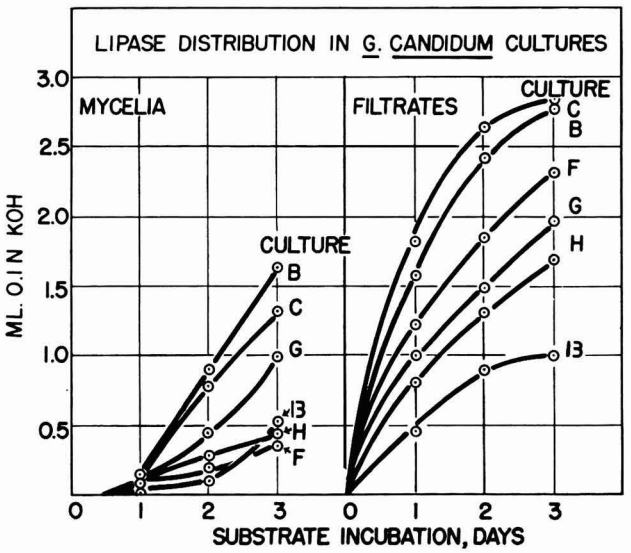

FIG. 3. The distribution of lipase in broth cultures of *G. candidum*. The lipase was obtained from mycelia and filtrate of a 4-day broth culture grown at 20° C. Activity was determined in an emulsion containing 44 ml. 0.3 % buffered agar (pH 6.0), 1 ml. USP olive oil, 5 ml. of enzyme preparation, and 0.3 ml. toluene. Other conditions are as in figure 2.

These data also emphasize the differences in lipolytic potential normally encountered in cultures classified as *G. candidum*.

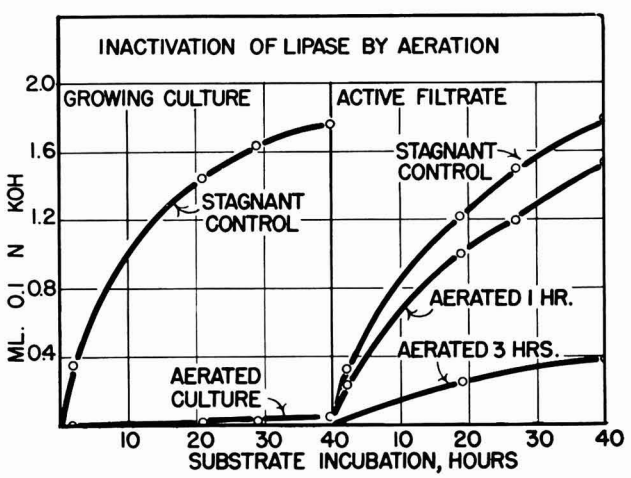

FIG. 4. Inactivation of lipase by aeration. Assay conditions as in figure 3.

The lipase was readily inactivated by mechanical aeration. Broth cultures grown at 26 to 30° C. and aerated by shaking [120 strokes 1.378 in. per min.] during growth were completely devoid of lipolytic activity. A progressive inactivation of lipase occurred when active filtrates obtained from stagnant cultures were aerated by mechanical shaking. These results, shown in figure 4, suggest that intact sulfhydryl groups may be necessary for lipolytic activity. To investigate this possibility, additional tests for the presence of active sulfhydryl groups were performed by the use of sulfhydryl group inhibitors. It was found that the lipase was unaffected by 1-hr. exposure to 10^{-4} *M* $AgNO_3$, $CuCl_2$ or 10^{-3} *M* H_2O_2. Conversely, the presence of oxygen or 10^{-4} *M* $HgCl_2$ caused a 50 per cent reduction in lipase activity. These results are illustrated in figure 5.

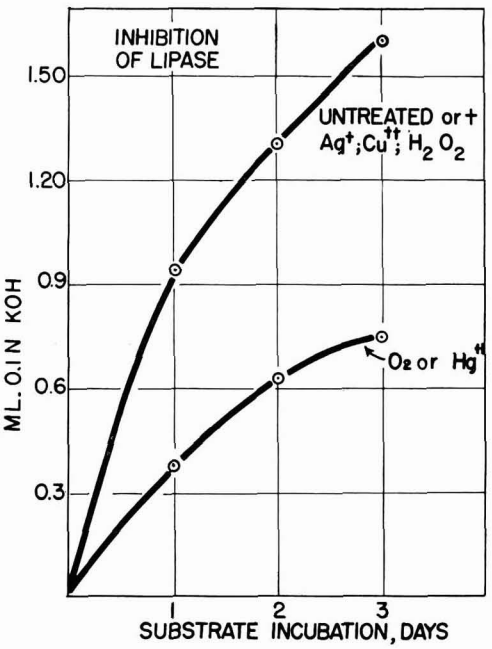

FIG. 5. Inhibition of lipase by metals and oxidizing agents. Assay conditions as in figure 3.

The lipase preparations inactivated with oxygen or mercury responded differently to the addition of reducing agents. After 1-hr. incubation with the inhibitor, the reducing agents were added and the mixture allowed to stand an additional 30 min. before being assayed. In this manner it was found that 5×10^{-3} *M* glutathione, cysteine or Na_2S at pH 6.0 partially reversed the mercury inactivation, whereas ascorbic acid was ineffective. Conversely, oxygen inactivation was irreversible with respect to these reducing agents under the same conditions. The data shown in figure 6 illustrate the effect of glutathione on both $HgCl_2$ and oxygen inactivated lipase.

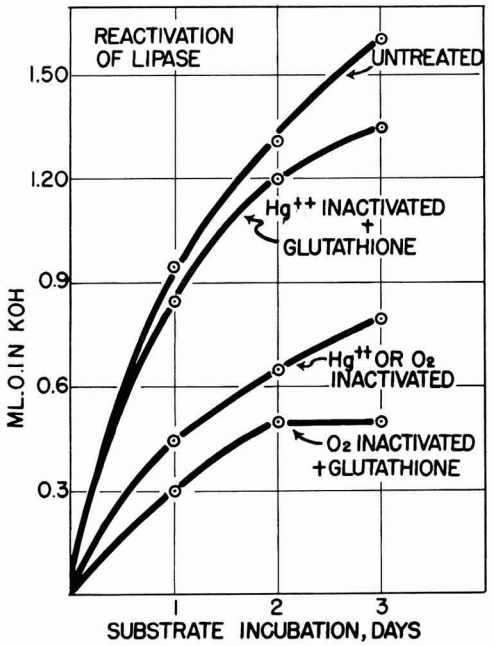

FIG. 6. Reactivation of inhibited lipase. Assay conditions as in figure 3.

DISCUSSION

Some of the characteristics of the lipolytic system elaborated by *G. candidum* closely parallel those which have been described for the lipase of *C. lipolytica* (7). Points of similarity are the optimum pH and temperature, oxygen sensitivity and extracellular occurrence of the enzyme.

A major point of difference between the above two lipases is the acid sensitivity exhibited by the *G. candidum* lipase. This enzyme was inactive at pH 4.0 and was destroyed when it was acidified to pH 3.0 and immediately reneutralized. These data are consistent with other investigations which have shown that (a) lipolysis is retarded when raw cream undergoes a rapid natural lactic acid fermentation (1) and (b) the rate of lipolysis decreases in direct proportion to the increase in acidity of cream supporting the growth of *G. candidum* (11).

A study of the substrate specificity shows that the extracellular lipolytic system of *G. candidum* contains a true lipase which is specific for triglycerides and which exhibits maximum activity on naturally occurring mixed triglycerides. Thus, the high levels of free water-insoluble fatty acids encountered in cream inoculated with *G. candidum* (11) may be due to the action of this enzyme. Data obtained by the use of inhibitors and reducing agents suggest the necessity for intact sulfhydryl groups for lipase activity.

SUMMARY AND CONCLUSIONS

Filtrates from cultures of *G. candidum* contain a true lipase which was active

at pH 5.0 to 8.0 over a temperature range from 20 to 37° C. Maximum activity occurred in substrate buffered at pH 6.0 and incubated at 30° C.

Naturally occurring mixed triglycerides were more readily hydrolyzed than were synthetic triglycerides and other esters. Lipolytic activity was dependent upon the type of substrate, substrate concentration, enzyme concentration and reaction time.

The lipase was inactive at pH 4.0 and was destroyed at pH 3.0. The enzyme was partially and irreversibly inactivated by oxygen. $HgCl_2$ inhibition was partially reversed by glutathione and other reducing agents.

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PROGRAM

FORTY-SEVENTH ANNUAL MEETING

OF THE

AMERICAN DAIRY SCIENCE ASSOCIATION

UNIVERSITY OF CALIFORNIA
DAVIS, CALIFORNIA

JUNE 24–26, 1952

GENERAL PROGRAM COMMITTEE

R. B. BECKER, Florida, *Chairman*

N. N. ALLEN, Wisconsin
RAMER LEIGHTON, Minnesota
E. L. JACK, California
S. W. MEAD, California

GENERAL PROGRAM

Monday, June 23, 1952

10:00 a.m. on — REGISTRATION, *Administration Building*
8:00 p.m. — INFORMAL GET-TOGETHER, *Recreation Hall*

Tuesday, June 24, 1952

8:00 a.m. — REGISTRATION, *Administration Building*
10:00 a.m. — OPENING SESSION, *Varsity Theater, Davis*

DR. PAUL F. SHARP, *Director, Agricultural Experiment Station, University of California*, Presiding

National Anthem

Leader—DR. L. E. ROSENBERG, *Chairman Division of Zoology, University of California*

Invocation

DR. HERBERT BOOTH SMITH, JR., *Pastor, Community Church, Davis*

Address

DR. ROBERT GORDON SPROUL, *President, University of California*

Presidential Address

DR. H. A. BENDIXEN, *State College of Washington*

Address. The Cow in the Atomic Age.

DR. STAFFORD L. WARREN, *Dean of the School of Medicine, University of California at Los Angeles*

1:30– 4:30 p.m. — SECTION MEETINGS

Manufacturing Section

Symposium: Some Aspects of the Effects of Heat on Milk.

Room 1, Chemistry Building

Production Section A

Artificial Breeding

Room 2205, Veterinary Science Building

Production Section B

Calf Nutrition

Room 101, Horticulture Building

Extension Section

Opening Business Session and Teaching Methods Exhibits

Room 100, Agricultural Engineering Building

8 : 00 p.m. FORMAL RECEPTION AND DANCE, *Recreation Hall*

Wednesday, June 25, 1952

9 : 00–11 : 00 a.m. SECTION MEETINGS

Manufacturing Section A

Cheese

Room 1, Chemistry Building

Manufacturing Section B

Milk Flavors, Frozen Milk, Dry Milk

Room 100, Hunt Hall

Production Section A

Breeding and Management

Room 2205, Veterinary Science Building

Production Section B

Milk Secretion

Room 101, Horticulture Building

Extension Section

4-H Club Work, Artificial Breeding

Room 100, Agricultural Engineering Building

11 : 00 a.m. **Production Section Business Meeting**

Room 2205, Veterinary Science Building

Manufacturing Section Business Meeting

Room 1, Chemistry Building

Extension Section Business Meeting and Committee Reports

Room 100, Agricultural Engineering Building

1 : 30– 4 : 30 p.m. **Manufacturing Section**

Cheese, Bacteriology, Cream and Butter

Room 100, Hunt Hall

Joint Session of Extension and Production Sections

Joint Committee Reports

Symposium on Bloat

Room 1, Chemistry Building

4 : 30 p.m. **Extension Section**
Business and Committee Meetings
Room 100, Agricultural Engineering Building

7 : 00 p.m. ANNUAL BANQUET
Installation of Officers
Announcements and Honor Awards
Recreation Hall

Thursday, June 26, 1952

9 : 00–12 : 00 a.m. SECTION MEETINGS
Manufacturing Section
Chemistry, Milk Heating, Evaporated Milk
Room 1, Chemistry Building
Production Section A
Reproduction
Room 2205, Veterinary Science Building
Production Section B
Feeding
Room 101, Horticulture Building
Extension Section
Dairy Herd Improvement Associations
Room 100, Agricultural Engineering Building

1 : 30– 3 : 00 p.m. **Manufacturing Section**
Symposium : The Economic Status of the Dairy Industry
Room 1, Chemistry Building
Production Section A
Milk Secretion
Room 2205, Veterinary Science Building
Production Section B
Rumen Function
Room 101, Horticulture Building
Extension Section
Publicity, Pipeline Milkers
Other Business
Room 100, Agricultural Engineering Building

3 : 00– 4 : 30 p.m. ASSOCIATION BUSINESS MEETING
Room 2205, Veterinary Science Building

ENTERTAINMENT FOR ALL

Monday, June 23 INFORMAL GET-TOGETHER, 8 : 00 p.m.
Recreation Hall

Tuesday, June 24 RECEPTION AND DANCE, 8 : 00 p.m.
Recreation Hall

Wednesday, June 25 ANNUAL BANQUET, 7 : 00 p.m.
Recreation Hall

ENTERTAINMENT FOR LADIES

Wednesday, June 25 Ladies' Tour, 9:00 a.m.

Thursday, June 26 Ladies' Luncheon and Fashion Show, 12:00 noon

CHILDREN'S PROGRAM

Age Group: 3–6 NURSERY SCHOOL
Tuesday, 1:30 p.m.–4:30 p.m.
Wednesday and Thursday, 9 a.m.–4:30 p.m.

Age Group: 6–12 ORGANIZED ATHLETICS AND SWIMMING
Tuesday, Wednesday and Thursday
9 a.m.–12:00 noon and 1:30 p.m.–4:30 p.m.

MANUFACTURING SECTION

Tuesday, June 24, 1952

1:30– 4:30 p.m. **SYMPOSIUM: SOME ASPECTS OF THE EFFECTS OF HEAT ON MILK.** E. L. JACK, *Presiding*
Room 1, Chemistry Building

Systems of Heat Transfer and their Relation to the Fluid Properties and Thermal Treatment of Milk.
R. L. PERRY, *University of California*
Discussion Leader: P. H. TRACY, *University of Illinois*

Some Physical Effects on Milk of HTST Sterilization.
A. P. STEWART, *Golden State Co. Ltd., San Francisco, Calif.*
Discussion Leader: J. H. HETRICK, *Dean Milk Co., Rockford, Ill.*

The Effect of Heat on Ionic Equilibria with Particular Reference to Calcium and Phosphorus. R. JENNESS, *University of Minnesota*
Discussion Leader: C. W. GEHRKE, *University of Missouri*

The Effect of Heat on Bacteria in Milk with particular Reference to Thermal Death Rates as Influenced by Various Factors. C. C. PROUTY, *Washington State College*
Discussion Leader: J. FRANK CONE, *Pennsylvania State College*

Wednesday, June 25, 1952

9:00–11:00 a.m. Section A. **CHEESE.** A. J. MORRIS, *Presiding*
Room 1, Chemistry Building

M1 The Occurrence and Survival of *Brucella abortus* in Italian Cheese Curd Made from Raw and Pasteurized Milk. HERBERT L. GILMAN, *New York State Veterinary College*, and J. C. MARQUARDT, *Department of Agriculture and Markets, Albany, N. Y.*

M2 Paper Partition Chromatography of the Free Amino

Acids of Foreign-type Cheeses. HAROLD J. FAGEN and RALPH V. HUSSONG, *Kraft Foods Co., Research Lab., Glenview, Ill.*

M3 A Direct Chromatographic Method for the Determination of the Lower Fatty Acids in Cheese. W. J. HARPER, *Ohio State University.*

M4 A Preliminary Report on the Relationship of the Lower Fatty Acids to the Ripening of Provolone Cheese. W. J. HARPER, *Ohio State University.*

M5 The Development of the Lower Fatty Acids in Swiss Cheese. O. J. KRETT, A. LEBOYER, H. E. DAUME and J. B. STINE, *Kraft Foods Co., Research Lab., Glenview, Ill.*

M6 The Use of White Mutants of *Penicillium roqueforti* in Cheese Making. H. A. MORRIS, J. J. JEZESKI and W. B. COMBS, *University of Minnesota.*

M7 A Measurement of Cheese Body. D. M. IRVINE and W. V. PRICE, *University of Wisconsin.*

9:00–11:00 a.m. Section B. **MILK FLAVORS, FROZEN MILK, DRY MILK.** O. F. GARRETT, *Presiding*
Room 100, Hunt Hall

M8 The Influence of Feeding Corn, Pea Vine, or Alfalfa Silage to Dairy Cows on pH and Titratable Acidity, Per Cent Butterfat and Total Solids and Milk Flavor and Production. H. C. HANSEN, E. D. MCGLASSON, R. H. ROSS, W. R. HARVEY and D. L. FOURT, *Idaho Agricultural Experiment Station.*

M9 Antioxidant Properties of Milk as Influenced by the Type of Pasture Fed to the Cow. V. N. KRUKOVSKY, *Cornell University.*

M10 Two Phenomena Related to Casein Precipitation in Frozen Storage of Milk. G. CHRISTIANSON, S. T. COULTER and ROBERT JENNESS, *University of Minnesota.*

M11 A Method for Measuring the Wettability of Milk Powders. U. S. ASHWORTH, *State College of Washington.*

M12 Some Observation of the Use of Surface-active Agents as a Means of Improving the Dispersability of Whole Milk Powder. H. A. HOLLENDER, *Purdue University.*

M13 A Study of the Particle Size of Nonfat Dry Milk Solids. J. J. JANZEN and A. M. SWANSON, *University of Wisconsin.*

M14 The Effect of Various Milk Serum Proteins and Sulfhydryl Groups on Bread Quality. B. L. LARSON,

University of Illinois and R. JENNESS and W. F. GEDDES, *University of Minnesota.*

11:00 a.m. **SECTION BUSINESS MEETING**
Room 1, Chemistry Building

1:30– 4:30 p.m. **CHEESE, BACTERIOLOGY, CREAM, BUTTER.** A. J. MORRIS, *Presiding*
Room 100, Hunt Hall

M15 Effect of Spoilage Bacteria on Flavor Component of Cottage Cheese. R. B. PARKER and P. R. ELLIKER, *Oregon Agricultural Experiment Station*

M16 Isolation of *Streptococcus thermophilus* Bacteriophage from Swiss Cheese Whey. D. D. DEANE, F. E. NELSON and F. C. RYSER, *Iowa Agricultural Experiment Station.*

M17 Some Factors Affecting the Action of Benzoyl Peroxide in the Bleaching of Milk for Cheese Manufacture. S. KURAMOTO and J. J. JEZESKI, *University of Minnesota.*

M18 Acetaldehyde Production by *Streptococcus lactis* and *Streptococcus lactis* var. *maltigenes.* E. ZURAW and M. E. MORGAN, *University of Connecticut.*

M19 Starter Activity Tests Using Non-fat Dry Milk Solids. N. S. GOLDING and L. MCCORKLE, *State College of Washington.*

M20 Preliminary Studies on the Thermal Resistance of Micrococci in Milk. A. N. MYHR and J. C. OLSON, JR., *University of Minnesota.*

M21 A Study of Methods for Evaluating the Keeping Quality of Sweet Cream Butter. B. M. ZAKARIASEN, L. ECKBERG and R. W. MYKLEBY, *Land O'Lakes Creameries, Inc., Minneapolis, Minnesota.*

M22 The Behavior of the Lipase from *Pseudomonas fragi* in Cream and Butter. S. A. NASHIF and F. E. NELSON, *Iowa Agricultural Experiment Station.*

M23 The Effect of Aging of Cream upon the Distribution Pattern of Free C_4, C_5 and C_6 Fatty Acids. I. I. PETERS, *Texas Agricultural Experiment Station.*

M24 The Role of Ascorbic Acid and Tocopherol in the Development of Oxidized Flavor in Cream and Butter. A. C. SMITH, M. LOEWENSTEIN, R. E. ANDERSON and H. C. OLSON, *Oklahoma A & M College*

M25 "Skunkweed" Flavor in Cream and Butter. H. C. OLSON, R. F. BEACHBOARD, R. E. ANDERSON, *Oklahoma A & M College;* L. F. EDMONDSON and C. LACEY, *Oklahoma State Dept. of Agr.*

Thursday, June 26, 1952

9:00–12:00 a.m. **CHEMISTRY, MILK HEATING, EVAPORATED MILK.** O. F. GARRETT, *Presiding*

Room 1, Chemistry Building

M26 Observations on the Keeping Quality of Pasteurized Milk, with and without Added Aureomycin, which Was Obtained from Various Sources and Stored at 45° F. J. C. OLSON, JR., D. S. WILLOUGHBY, E. L. THOMAS and H. A. MORRIS, *University of Minnesota.*

M27 The Glyceride Structure and Polymorphism of Butterfat. G. R. GREENBANK, *Bureau of Dairy Industry, U. S. D. A.*

M28 Isolation and Compositional Characterization of the Fat-membrane Proteins of Nonhomogenized and Homogenized Milk. J. R. BRUNNER, C. W. DUNCAN and G. M. TROUT, *Michigan State College.*

M29 Electrophoretic Characterization of the Fat-membrane Proteins of Nonhomogenized and Homogenized Milk. J. R. BRUNNER, H. A. LILLEVIK, G. M. TROUT and C. W. DUNCAN, *Michigan State College.*

M30 Preparation of Samples of Skimmilk for Electrophoretic Studies. J. TOBIAS, R. MCL. WHITNEY and P. H. TRACY, *University of Illinois.*

M31 Effect of Heating to 300° F. by Means of the Mallory Small-tube Heat Exchanger on the Electrophoretic Properties of Skimmilk. J. TOBIAS, R. MCL. WHITNEY and P. H. TRACY, *University of Illinois.*

M32 Limitations of the Use of Serum Protein Determinations in Evaluating Heat of Milk. H. A. HARLAND, S. T. COULTER and R. JENNESS, *University of Minnesota.*

M33 The Phosphatase Inactivation Curve in the HTST Pasteurization Range. S. A. HANSEN, F. W. WOOD and H. R. THORNTON, *University of Alberta, Edmonton, Canada.*

M34 Estimation of Calcium Ion Activity in Milk and Other Biological Fluids. G. CHRISTIANSON, R. JENNESS and S. T. COULTER, *University of Minnesota.*

M35 Observations on the Color of Evaporated Milk. S. PATTON, *Pennsylvania State College.*

M36 A Spectrographic Method for the Determination of Tin, Copper, Iron and Lead in Evaporated Milk and the Effects of Storage on Evaporated Milk. C. W. GEHRKE and C. VAN RUNYON, *University of Missouri.*

1:30– 3:00 p.m. **SYMPOSIUM: THE ECONOMIC STATUS OF THE DAIRY INDUSTRY.** E. L. JACK, *Presiding*

Room 1, Chemistry Building

Changing Economic Conditions in the Dairy Industry. H. C. TRELOGAN and L. HERRMANN, *Agricultural Research Administration, U. S. Department of Agriculture.*

The Impact of Technological Developments upon Economic Conditions in the Dairy Industry. G. W. SPRAGUE, *Western Condensing Co., Appleton, Wisconsin*

Discussion Leader: R. G. BRESSLER, JR., *University of California.*

3:00– 4:30 p.m. Association Business Meeting

Room 2205, Veterinary Science Building

PRODUCTION SECTION

Tuesday, June 24, 1952

1:30– 4:30 p.m. Section A. **ARTIFICIAL BREEDING.** G. HYATT, JR., *Chairman*

Room 2205, Veterinary Science Building

P1 Relationship of Maximum Daily Air Temperature, Mean Daily Air Temperature and Humidity to Physiological Reactions of Dairy Bulls. J. E. JOHNSTON and C. BRANTON, *Louisiana State University, Baton Rouge*

P2 Uniformity and Nutritional Studies with Monozygotic Bulls. H. H. OLSON, *University of Minnesota, St. Paul*

P3 The Effect of Frequency of Ejaculation on the Semen Characteristics and Libido of Young Bulls. F. N. BAKER and N. L. VANDEMARK, *University of Illinois, Urbana*

P4 The Influence of Gonadotropic Hormones on Semen Quality. J. H. BYERS, G. MCCURLEY and M. VONKROSIGK, *Oregon State College, Corvallis*

P5 The Use of Bacitracin and Terramycin in Semen Diluters and the Storage of Semen at –15° C., in a Glycerin-citrate-yolk Diluter. O. T. STALLCUP and H. K. MCCARTNEY, *University of Arkansas, Fayetteville*

P6 The Relationship between Dilution Rate of Bull Semen or the Number of Motile Spermatozoa and Fertility. C. BRANTON, H. C. KELLGREN and T. E. PATRICK, *Louisiana State University, Baton Rouge*

P7 The Reducing Components of Bull Semen as Determined by an Iodimetric Titration. B. L. LARSON and G. W. SALISBURY, *University of Illinois, Urbana*

P8 The Effect of Spermatozoa Concentration and Dilution

on the Respiratory Activity of Bull Semen. M. W. H. Bishop and G. W. Salisbury, *University of Illinois, Urbana*

P9 Oxygen Consumption of Semen from Dairy Bulls on Three Levels of Carotene and Vitamin A. M. von Krosigk, *Oregon State College, Corvallis*

P10 Reconstituted Skimmilk as a Diluent for Bovine Semen. G. B. Marion and H. H. Olson, *University of Minnesota*

P11 Impedance Change Frequency in Bull Semen. M. W. H. Bishop, R. C. Campbell and J. L. Hancock, *Agricultural Research Council, Animal Research Station, Cambridge, England*

1:30– 4:30 p.m. Section B. **CALF NUTRITION.** N. N. Allen, *Chairman Room 101, Horticulture Building*

P12 Simplified Calf Starter Containing Corn, Oats and Expeller or Solvent Soybean Oil Meal. K. E. Gardner, *University of Illinois, Urbana*

P13 Slacked Lime as a Preventive of Scours for Whey-fed Calves. D. C. Brown, J. Read and H. S. Willard, *Wyoming Agricultural Experiment Station, Laramie*

P14 Effect of Dietary Lipids on the Polyunsaturated Fatty Acids in Blood Plasma of Young Dairy Calves. R. S. Allen and J. H. Zaletel, *Iowa Agr. Expt. Station, Ames*

P15 The Effect of Vitamin Supplements on Carotene Utilization from Hay by Dairy Heifers. S. R. Skaggs, *New Mexico Agricultural Experiment Station, State College*

P16 The Value of Arsonic Acid Derivatives as a Growth Stimulant when Fed to Calves. G. C. Graf and C. W. Holdaway, *Virginia Polytechnic Institute, Blacksburg*

P17 Effect of Ration upon Riboflavin Levels in Calf Tissues. E. G. Moody, S. M. Hauge and N. S. Lundquist, *Purdue University, Lafayette, Indiana*

P18 Effect of Type of Dietary Lipid upon the Blood Plasma Lipids of Young Dairy Calves. N. L. Jacobson, J. H. Zaletel and R. S. Allen, *Iowa Agricultural Experiment Station, Ames*

P19 The Value of Various Levels of Aureomycin in Milk Replacements for Dairy Calves. C. B. Knodt and Earl B. Ross, *Pennsylvania Agr. Expt. Station, State College*

P20 The Influence of Aureomycin and Cud Inoculation on

the Growth of Dairy Calves. A. D. McGilliard, M. Ronning, E. R. Berousek and C. L. Norton, *Oklahoma A & M College, Stillwater*

P21 Effect of Type of Protein on the Response of Young Dairy Calves to Aureomycin with Data on the Microflora of the Feces. L. L. Rusoff, J. A. Alford and C. E. Hyde, *Louisiana Agr. Expt. Station, Baton Rouge*

P22 Effect of Aureomycin Supplementation on Changes in Weight and Body Measurements of Dairy Calves. N. L. Jacobson, J. G. Kaffetzakis and P. G. Homeyer, *Iowa Agr. Expt. Station, Ames*

Wednesday, June 25, 1952

9:00–11:00 a.m. Section A. **BREEDING AND MANAGEMENT.** G. Hyatt, Jr., *Chairman*

Room 2205, Veterinary Science Building

P23 The Effect of Stress Conditions on Dairy Cattle. G. C. Graf, *University of Minnesota, St. Paul*

P24 Factors Influencing the Production of High-quality Milk. J. L. Covington, W. L. Griebeler, J. B. Rodgers, I. R. Jones, P. M. Brandt and L. W. Bonnicksen, *Oregon State College, Corvallis*

P25 Methods of Milking and Milk-handling as Factors Affecting the Quality and Economy of Milk Produced. II. A Further Study of the Effect of Permanent Pipelines in the Dairy Barn on Milk Quality. M. H. Alexander, W. O. Nelson and E. E. Ormiston, *University of Illinois, Urbana*

P26 Growth Uniformity Trials with Identical Twin Dairy Heifers—Estimates of Heritability and Twin Efficiency. H. W. Thoele and M. C. Hervey, *University of Minnesota, St. Paul*

P27 Progress Report on the Production Records of Crossbred Dairy Cattle. J. P. LeMaster, G. W. Brandt and C. C. Brannon, *South Carolina Agr. Expt. Station*, and M. H. Fohrman, *Bureau of Dairy Industry, U.S.D.A.*

P28 The Partition of Evaporative Cooling between the Respiratory and Outer Surfaces in European and Indian Cattle. H. H. Kibler and S. Brody, *Missouri Agr. Expt. Station, Columbia*

P29 Mammary Development and Heart Girth Relationships and Changes with Age. V. L. Baldwin and M. C. Hervey, *University of Minnesota, St. Paul*

P30 A Summary of a Ten-year Comparison between Loose

Run and Stanchion Housing for Dairy Cattle. E. E. HEIZER, C. E. ZEHNER and V. R. SMITH, *University of Wisconsin, Madison*

9:00–11:00 a.m. Section B. **MILK SECRETION.** N. N. ALLEN, *Chairman*
Room 101, Horticulture Building

P31 A Study of Some Causative Mechanisms in Bovine Streptococcal Mastitis. C. P. MERILAN and H. A. HERMAN, *University of Missouri, Columbia*

P32 The Role of Plasma Cells in the Production of Globulins within the Mammary Gland and Time Studies on Antibody Response from Experimentally Induced Inflammation of the Udder. R. M. PORTER, *University of Minnesota, St. Paul*

P33 Histological Evidence of a Hyalin-fibrin Complex in the Bovine Mammary Gland. J. R. KUIKEN, D. L. HILL and N. S. LUNDQUIST, *Purdue University, West Lafayette, Indiana*

P34 Histological and Chemical Studies of Fat Metabolism in the Mammary Glands of Cows and Goats. M. L. Y. SMITH, B. C. HATZIOLOS and S. KUMAR, *University of Maryland, College Park*

P35 Blood Levels and Urinary Excretion of Certain Constituents in Ketotic Cows. P. J. VAN SOEST, T. H. BLOSSER, G. M. WARD, J. B. CRILLY and M. F. ADAMS, *State College of Washington, Pullman*

P36 The Effects of Sodium Acetate Given Orally upon Cows with Ketosis. W. J. MILLER and N. N. ALLEN, *University of Wisconsin, Madison*

P37 Pituitary-adrenal Cortical Syndrome in Ketosis of Dairy Cows as Evidenced by the Adrenaline Test, Eosinophil Levels and Replacement Therapy. J. C. SHAW, B. C. HATZILOS, E. C. LEFFEL, W. M. GILL and A. C. CHUNG, *University of Maryland, College Park*

11:00–12:00 a. m. **SECTION BUSINESS MEETING**
Room 2205, Veterinary Science Building

1:30– 4:30 p.m. **JOINT SESSION OF EXTENSION AND PRODUCTION SECTIONS.** R. D. LEIGHTON and N. N. ALLEN, *Co-chairmen*
Room 1, Chemistry Building

Joint Committee Reports—

Breeds Relations, A. R. PORTER, *Chairman*
Dairy Cattle Health, G. M. WERNER, *Chairman*
Dairy Cattle Breeding, C. D. MCGREW, *Chairman*
Type, I. W. RUPEL, *Chairman*

Purebred Dairy Cattle Association, J. F. CAVANAUGH, *Secretary*

Antibiotics, W. A. KRIENKE, *Chairman*

Symposium on Bloat

The Place of Legumes in the Pasture Program.
R. E. HODGSON, *Bureau of Dairy Industry*

The Status of Our Fundamental Information on Bloat.
H. H. COLE, *University of California*

Practical Methods for Prevention of Bloat.
S. W. MEAD, *University of California*

Treatment of Bloat.
G. H. HART, *University of California*

Panel Discussion

Thursday, June 26, 1952

9:00–12:00 a.m. Section A. **REPRODUCTION.** G. HYATT, JR., *Chairman*
Room 2205, Veterinary Science Building

P38 The Incidence of a Sterility Syndrome in the Rabbit Fed Soybean Hay and the Failure of Certain Supplements to Alleviate the Symptoms. K. A. KENDALL and R. L. HAYS, *University of Illinois, Urbana*

P39 A Study of the Birth Weights of Purebred and Crossbred Calves. R. W. TOUCHBERRY, *University of Illinois, Urbana*, and K. A. TABLER, *Bureau of Dairy Industry, U.S.D.A.*

P40 Thiouracil-induced Hypothyroidism in Sexually Mature Dairy Bulls. E. W. SWANSON and J. P. BOATMAN, *University of Tennessee, Knoxville*

P41 The Intracrypt Space in the Placentome of the Cow. H. W. WEETH and H. A. HERMAN, *University of Missouri, Columbia*

P42 The Effects of Relaxin on the Cow's Cervix. E. F. GRAHAM and A. E. DRACY, *South Dakota Agr. Expt. Station, Brookings*

P43 The Sampling of the Endometrium of the Bovine Using a Biopsy Technique. J. D. DONKER, *University of Minnesota, St. Paul*

P44 A Dairy Cattle Pregnancy Test. J. H. BYERS, *Oregon State College, Corvallis*

P45 Effect of Hormones on Uterine Motility and Sperm Transport in the Perfused Genital Tract of the Cow. R. L. HAYS and N. L. VANDEMARK, *University of Illinois, Urbana*

P46 Chromatographic Separation of the Neutral Steroids of Cow's Urine. J. P. MIXNER and H. L. SAUNDERS,

JR., *New Jersey Agr. Expt. Station, Sussex*, in cooperation with Bureau of Dairy Industry, USDA

P47 Comparison of Neutral Steroids from Cows' Urine after Various Hydrolytic Procedures. J. P. MIXNER and H. L. SAUNDERS, JR., *New Jersey Agr. Expt. Station, Sussex*, in cooperation with Bureau of Dairy Industry, USDA

P48 Preliminary Studies of Irregular Breeding in Dairy Cattle. E. G. MOODY, W. A. SMITH, J. W. CRUMBAKER and G. B. MCLEROY, *Arizona State College, Tempe*

9:00–12:00 a.m. Section B. **FEEDING.** N. N. ALLEN, *Chairman*
Room 101, Horticulture Building

P49 Three Years' Studies Using Sulfur Dioxide as a Preservative for Forage Crops. C. B. KNODT, S. R. SKAGGS and P. S. WILLIAMS, *Pennsylvania Agr. Expt. Station, State College*

P50 Grass Silage *vs.* Hay as the Supplementary Roughage for Milking Cows on Good Pasture—(Preliminary Report). S. H. MORRISON and J. F. DEAL, *University of Georgia, Athens*

P51 Self-Feeding a Ground Hay and Grain Ration to Dairy Cows. K. E. HARSHBARGER, *University of Illinois, Urbana*

P52 Alfalfa Hay *vs.* Prairie Hay for Dairy Calves. J. B. WILLIAMS, S. D. MUSGRAVE, C. L. NORTON and W. D. GALLUP, *Oklahoma A & M College, Stillwater*

P53 Effect of Kind of Pasture and the Feeding of Supplements on Persistency of Milk Production in Summer. D. M. SEATH, *University of Kentucky, Frankfort*

P54 Different Grazing Intervals on Ladino Clover-Fescue Pasture as Affecting Milk Production and Flavor of Milk—(Preliminary Report). S. H. MORRISON, J. J. SHEURING, R. A. MARDEN and J. F. DEAL, *University of Georgia, Athens*

P55 Soil Fertilization and Plant Development as Factors Relating to Nitrogen Digestibility. K. A. KENDALL, R. W. TOUCHBERRY and W. B. NEVENS, *University of Illinois, Urbana*

P56 Relationship between TDN and Energy Values of Feeds. L. A. MOORE, *Bureau of Dairy Industry, USDA*, and H. M. IRWIN and J. C. SHAW, *University of Maryland, College Park*

P57 The Relation of Sulfur Compounds to Lactation in Ruminants. I. R. JONES, J. R. HAAG and P. H. WESWIG, *Oregon State College, Corvallis*

P58 The Effect of Thyroprotein Feeding on the Level of the Protein-bound and Inorganic Serum Iodine in the Bovine. J. F. LONG, L. O. GILMORE, J. W. HIBBS and F. ELY, *Ohio State University* and the *Ohio Agr. Expt. Station*

P59 The Utilization of Pear Cannery Waste as a Feed for Dairy Cows. F. R. MURDOCK, A. S. HODGSON, T. H. BLOSSER and A. O. SHAW, *State College of Washington*

1:30– 3:00 p.m. Section A. **MILK SECRETION.** G. HYATT, JR., *Chairman*

Room 2205, Veterinary Science Building

P60 Hormones in Lactation; Administration of Hormones in Declining Phases of Lactation. J. D. DONKER and W. E. PETERSEN, *University of Minnesota, St. Paul*

P61 Induction of Lactation in Dairy Cattle by Diethylstilbestrol-progesterone Implants. J. MEITES, E. P. REINEKE and C. F. CAIRY, *Michigan State College, East Lansing*

P62 Experimental Development of the Mammary Gland of the Bovine. D. L. HILL, *University of Minnesota, St. Paul*

P63 The Metabolism of Lactose. W. J. RUTTER, E. M. CRAINE and R. G. HANSEN, *University of Illinois, Urbana*

P64 The Utilization of Carboxyl Labelled C^{14} Acetate by the Perfused Bovine Mammary Gland. L. S. MIX, W. E. PETERSEN and H. E. STRUSS, *University of Minnesota, St. Paul*

1:30– 3:00 p.m. Section B. **RUMEN FUNCTION.** N. N. ALLEN, *Chairman*

Room 101, Horticulture Building

P65 Early Development and Function of the Bovine Stomach. R. B. BECKER, P. T. DIX ARNOLD, S. P. MARSHALL and J. WING, *Florida Agr. Expt. Station, Gainesville*

P66 Effects on Ruminating Calves of Changing to Omasal-Abomasal Feeding through a Rumen Fistula. H. J. LARSEN and G. E. STODDARD, *Iowa Agr. Expt. Station, Ames*

P67 Rumen Synthesis of Protein and Amino Acids in the Bovine on Natural and Purified Rations. C. W. DUNCAN, C. F. HUFFMAN and I. P. AGRAWALA, *Michigan Agr. Expt. Station, East Lansing*

P68 Some Chemical and Nutritional Properties of the Rumen Contents of Dairy Cows. B. M. PATEL and

N. N. ALLEN, *University of Wisconsin, Madison*

P69 The Effect of Milk Production, Body Weight and Feed Digestibility of Feeding Hay and Grain in Various Ratios. G. E. STODDARD and T. G. MARTIN, *Iowa Agr. Expt. Station, Ames*

3:00– 4:30 p.m. **ASSOCIATION BUSINESS MEETING**
Room 2205, Veterinary Science Building

EXTENSION SECTION

Tuesday, June 24, 1952

1:30– 4:30 p.m. **OPENING BUSINESS SESSION AND TEACHING METHODS AND EXHIBITS**

R. LEIGHTON, *Chairman*
Room 100, Agricultural Engineering Building

E1 Identical Twins in Research. H. THOELE, *University of Minnesota, St. Paul*

E2 Visual Aid in Teaching through Colored Slides. G. C. ANDERSON, *University of Idaho, Moscow*

E3 Technicolor Film, "The Right Semen to Produce the Right Calves." A. C. BALTZER, *Michigan State College, East Lansing*

Report of Teaching Methods Committee

Film—"Weight Control through Diet," National Dairy Council

States Exhibits—Explanation and Discussion
Delmar J. Young, *Chairman in Charge, University of Delaware*
(Exhibits adjoining Room 100, Agricultural Engineering Building)

Wednesday, June 25, 1952

9:00–12:00 a.m. **4-H CLUB WORK, ARTIFICIAL BREEDING COMMITTEE REPORTS, BUSINESS MEETING**

R. LEIGHTON, *Chairman*
Room 100, Agricultural Engineering Building

E4 The Group System of Judging and its Merits. G. E. GORDON, *University of California, Davis*

E5 A Proposed Uniform Score Card for 4-H Judging, Fitting and Showmanship Contests. R. D. STEWART, *The American Guernsey Cattle Club*

E6 Artificial Breeding, Western States. H. P. EWALT, *Oregon*, and Associates

COMMITTEE REPORTS
BUSINESS MEETING
COMMITTEE APPOINTMENTS

1:30– 4:30 p.m. **JOINT MEETING OF EXTENSION AND PRODUCTION SECTIONS AND COMMITTEE REPORTS** (See Production Section Program)

4:30 p.m. Extension Section, *Room 100, Agricultural Engineering Building*
COMMITTEE AND BUSINESS MEETINGS

Thursday, June 26, 1952

9:00–12:00 a.m. **DAIRY HERD IMPROVEMENT ASSOCIATIONS**
I. E. PARKIN, *Chairman*
Room 100, Agricultural Engineering Building

E7 Dairy Records Committee Report. L. H. STINNETT, *Chairman, Oklahoma Agricultural and Mechanical College*
- a. The standard DHIA program and its major goals and minimum requirements
- b. Training and supervision of personnel
- c. Continuation and expansion of various systems of testing
- d. Assembling, processing, and utilization of DHIA data
- e. Analysis and interpretation of DHIA results

Discussion of report

E8 DHIA Regulations. E. J. PERRY, *Rutgers University*

E9 New Uses of Dairy Herd Improvement Records. J. F. KENDRICK, *Bureau of Dairy Industry, USDA*

E10 How DHIA Operates in Western States, Representatives from
California, G. E. GORDON
Oregon, H. P. EWALT
North Dakota, C. C. OLSON
Utah, L. RICH

1:30– 3:00 p.m. **PUBLICITY, PIPELINE MILKERS**
R. LEIGHTON, *Chairman*
Room 100, Agricultural Engineering Building

E11 May the Interpreters Cooperate. G. HEEBINK, *West Virginia University, Morgantown*

E12 Pipeline Milkers and Operational Problems. G. E. GORDON, *University of California, Davis*

UNFINISHED BUSINESS

3:00– 4:30 p.m. ASSOCIATION BUSINESS MEETING
Room 2205, Veterinary Science Building

VOLUME XXXV MAY, 1952 NUMBER 5

JOURNAL OF DAIRY SCIENCE

ABSTRACTS OF LITERATURE

Prepared in cooperation with the
International Association of Ice Cream Manufacturers
and the Milk Industry Foundation

ANIMAL DISEASES

W. D. POUNDEN, SECTION EDITOR

276. The effect upon cattle of Arizona waters of high fluoride content. W. E. RAND and H. J. SCHMIDT, Stanford Univ., Palo Alto, Cal. Am. J. Vet. Research, **13,** 46: 50–61. Jan., 1952.

Results and illustrations present the fluorine effects on cattle of 3 ranches, 2 beef and 1 dairy, where the F intake varied because of varying levels of F in drinking and irrigation water. Many of the animals examined had been on ranches as much as 10 yr. Periodic analyses of water showed a relatively constant level of F. Calculations of F intake showed that daily levels in the diet of 1 mg./kg. of body weight were tolerated by the cattle with only slight effect upon the teeth. Levels of 2 mg. F/kg. of body weight resulted in marked teeth changes, stiffness, emaciation and general unthriftiness. Urine samples of more than 10 ppm. F indicated current F intake in damaging amounts. F in the metacarpus of less than 3,000 ppm. indicated no damaging effect, but 4,000 ppm. or more was associated with effects other than harmless teeth changes. These field observations are in very close agreement with reports of experimentally produced fluorosis.

E. W. Swanson

277. Effect of massive doses of penicillin and dihydrostreptomycin, employed singly or in combination, on Staphylococcus pyogenes mammary infections. O. W. SCHALM and G. M. WOODS, Univ., of Cal., Davis. Am. J. Vet. Research, **13,** 46: 26–30. Jan., 1952.

A comparison has been made in a single herd of the effectiveness of 1.5 million-4 million units penicillin alone, 2-8 g. dihydrostreptomycin alone or combinations of these antibiotics in treating dry and lactating quarters infected with *Staph. pyogenes.* In lactating quarters only 33 and 20% cures were effected by the antibiotics alone, while 73% were cured by the massive combination. In dry quarters 65% were cured by penicillin alone and 73% by the combination. The importance of early treatment of infections is shown by the curing of only 18% lactating and 38% dry quarters which had been infected more than 12 mo. Many infections of several months duration were not yet cured following 6 courses of treatment (4 daily infusions with antibiotics in 50 ml. water). The use of massive doses is recommended for all cases of clinical mastitis of unknown etiology. E. W. Swanson

278. Susceptibility of cattle to Brucella suis following vaccination with Brucella abortus strain 19. F. V. WASHKO and L. M. HUTCHINGS, Purdue Univ. Agr. Expt. Sta., Lafayette, Ind. Am. J. Vet. Research, **13,** 46: 24–25. Jan., 1952.

Two heifers which had been vaccinated with *Br. abortus* strain 19 at 6 mo. of age were exposed while lactating to *Br. suis* by an intra-mammary inoculation. Inflammation of the inoculated quarter occurred in both cows and *Br. suis* was recovered in the milk intermittently after exposure, although all milk samples had been negative before. Blood serum-agglutination reactions indicated an active brucellosis. When slaughtered 2 mo. after exposure, *Br. suis* was recovered from the mammary gland and the supramammary lymph nodes. Thus, the vaccination was not effective against intramammary *Br. suis* infection.

E. W. Swanson

BUTTER

O. F. HUNZIKER, SECTION EDITOR

279. Försök med ett av vassle framställt antioxidationsmedel vid tillverkning av smör (Experiments using an antioxidant prepared from whey for butter manufacture). K. E. THOMÉ, T. OLSSON, L. O. LODIN and A. B. BUHRGARD, State Dairy Expt. Sta., Alnarp, Sweden. Meddelande no. **32.** 1951.

About 120 parallel churnings were made under commercial conditions using the antioxidant ABV prepared from whey (Swedish patent no. 129024, 1950). The butter was made from ripened cream and had a pH of approximately 4.8. Each churning with ABV was accompanied by a parallel churning of cream containing no ABV. For 1 series a "Special Salt" ($NaCl + Na_2CO_3 + NaH_2PO_4, 2H_2O$) was used. The butter was stored for 14 d. and 1 mo. at 13° C., and

for 3 mo. at – 20° C. Peroxide values were determined.

The addition of 3 kg. ABV to 1,000 l. cream at an early stage had a definite antioxidative effect and oily flavor did not develop. The buttermilk was discolored. When the preparation was added to both cream and butter, or to butter alone, it had a definite antioxidative effect, but the addition to cream only was most effective. Where the oxidative effect was pronounced, the product did not have a sufficient antioxidative effect to prevent oily flavor, but the "Special Salt" was fully effective. G. H. Wilster

280. The manufacture of aromatic butter. J. W. PETTE. Netherlands Milk & Dairy J., **2,** 1: 12–24. 1948.

In order to produce an aromatic butter, a weakly reducing starter must be used. The reducing power of a culture can be ascertained by applying the creatine test after 1, 2 and 3 d. of incubation. A positive test on the 3rd day indicates a weakly reducing starter. The aroma in butter also is increased by souring the cream in the presence of air and churning to rather large granules, followed by a minimum of washing. Avoid any contamination with foreign microorganisms, especially yeasts, which have the ability to reduce the flavor and aroma constituent.

W. W. Overcast

281. Butter spreader. E. J. VANI. U. S. Patent 2,589,000. 1 claim. March 11, 1952. Official Gaz. U. S. Pat. Office, **656,** 2: 512. 1952.

A 0.25-lb. stick of butter, inserted in this device, is advanced by a simple hand-operated mechanism, so that square pats of butter may be sliced off as desired. R. Whitaker

CHEESE

A. C. DAHLBERG, SECTION EDITOR

282. Forsøk med skimmelbekaemplse på ost (Experiments to control growth of mold on cheese). Danish Dairy Expt. Sta., Hillerod, Report no. **70.** 1951.

A total of 2,317 Edam, Gouda, Steppe and Swiss-type cheese containing 45% fat in moisture-free portion was used. The milk was pasteurized at 68–70° C. in a plate pasteurizer. The moisture content ranged from 44–47% before salting and fell to 39–43% in 10 wk. The salt content was 1.75–2.00% for Edam and 1.4–1.7% for the others. The following were tested: Nipagin T ($HO—C_6H_4—COO—CH_3$), sodium benzoate, Pandurol A, propionic acid, oils from A/S Ferrosan, mustard oil, Nipabutyl (C.M.D.) oil, Pentoxol. Water, alcohol (not denatured) or refined oil was used as carrier for these antimold products.

Application of chemicals to suppress the growth of mold is not to be recommended for non-paraffined cheese during ordinary ripening. Dipping the cheese in a bath of chemicals immediately before treatment with paraffin wax did not prevent mold growth during subsequent storage of the paraffined cheese. It was possible to keep the cheese free of mold for more than 3–4 wk. only in a few cases where the cheese had been treated with a mold preventive during the preceding storage.

Prevention of mold on paraffined cheese gave satisfactory results where the mold preventive had been worked into or painted on the paraffin, whereas treatment of the cheese itself immediately before paraffining proved ineffective.

G. H. Wilster

283. Forsøk med antimuggbehandling av ost (Experiments to control mold on cheese). A. T. BERGUM and K. WESTRE, Dairy Research Sta. of the Agr. College of Norway, Report no. **44.** Jan., 1951.

Gouda cheese containing 45% fat from 54 batches manufactured in 2 cheese factories were used in the experiments. The experimental and control cheese were identical until they were removed from the brine bath. Different treatment of the cheese to prevent mold growth on the surface consisted in one factory of: (a) dipping the cheese before waxing in 5 or 15% Nipagin solution, (b) placing cheese on shelves treated with Pandurol T, (c) dipping cheese before waxing in 25% Nipagin, benzoic acid solution (Nipagin = para-oxybenzoic acid methyl ester). In another factory treatment consisted of: (a) dipping cheese before ripening in 15% water solution of Nipagin M (Na salt of para-oxybenzoic acid methyl ester), (b) placing cheese on shelves treated with 2% water solution of Pandurol M, (c) cheese rubbed with paraffin oil containing 7% sodium benzoate.

Best results were obtained when the cheese were treated with a water solution of Nipagin. The oil treatment also was very satisfactory; the cheese thus treated had a good rind and the growth of mold was insignificant. The cost of treatment was 0.7 öre/kg. cheese for oil treatment, and 2.65 öre for Nipagin M Sodium treatment.

G. H. Wilster

284. Manufacture of cheese. L. L. RUSOFF and A. J. GELPI, JR. (assignors to Board of Supervisors of Louisiana State Univ. and Agricultural and Mechanical Coll.). U. S. Patent 2,585,501. 4 claims. Feb. 12, 1952. Official Gaz. U. S. Pat Office, **655,** 2: 486. 1952.

The surface of cheese is treated with an antibiotic prior to curing. R. Whitaker

285. Manufacture of bakers' cheese. M. M. KLOSER, T. L. KIMBALL and O. J. SCHRENK (assignors to Bowman Dairy Co.). U. S. Patent 2,586,830. 7 claims. Feb. 26, 1952. Official Gaz. U. S. Pat. Office, **655,** 4: 976. 1952.

Bakers' cheese is made by cooling coagulated skimmilk to below 65° F., then pumping it to a

filter press at not over 35 p.s.i. pressure. The curd in the press is cooled further with water and the moisture content reduced with cool air.

R. Whitaker

DAIRY BACTERIOLOGY

P. R. ELLIKER, SECTION EDITOR

286. Influence of refrigerated storage on dye reduction time of milk. C. K. JOHNS, Dept. of Agr., Ottawa, Can. J. Milk & Food Technol., **15:** 8–12. Jan.–Feb., 1952.

It appears that the 2-hr. icing of raw milk samples for methylene blue and resazurin tests are not justified. When resazurin was added to milk and stored overnight, the results were comparable to samples refrigerated for 2 hr. When milk was refrigerated overnight without methylene blue added, results agreed closely with the controls. The 2-hr. and overnight holding of milk with the dye slowed down the rate of the reduction of methylene blue. None of the differences were statistically significant.

If the tests are not run on the day of sampling the milk for the methylene blue test, milk should be stored overnight without the dye, while those samples used for the resazurin test should contain the dye.

H. H. Weiser

287. An improved procedure for microscopic grading of milk intended for pasteurization. M. E. MORGAN, P. MAC LEOD and E. O. ANDERSON, Storrs Agr. Expt. St., Storrs, Conn. J. Milk & Food Technol., **15:** 3–7. Jan.–Feb., 1952.

The authors have proposed a sequential analysis for microscopic grading of raw milk. The grading procedure suggests a bacterial standard of 200,000 clumps/ml., as well as additional standards of 100,000 and 400,000 clumps/ml.

Sequential tables provide for 3 grades of raw milk in which milk can be accepted or rejected as each microscopic field is counted. The main advantage of sequential grading provides for examination of fewer microscopic fields while achieving results comparable to the present standard procedure.

H. H. Weiser

288. Evaluation of food irradiation procedures. Quantitative chemical measurements utilizing high energy cathode rays. S. A. GOLDBLITH, B. E. PROCTOR and O. A. HAMMERLE, Dept. of Food Technol., M.I.T., Cambridge, Mass. Ind. Eng. Chem., **44,** 2: 310–314. Feb., 1952.

The sterilization of foods and drugs by ionizing radiations without heat may sometime be a reality. This study was made to develop a rapid routine technique to determine quantitatively the depth of penetration of the radiations and the area subjected to the radiations. Supervoltage cathode rays destroyed the test dye, methylene blue, in aqueous solutions, but in non-aqueous solutions reversible reduction occurred in part, as well as destruction. Applications of the findings to evaluations of beams of ionizing radiations have been made .

B. H. Webb

289. Preparation of diacetyl with butter cultures. M. W. MARCOUX (assignor to Armour and Co.). U. S. Patent 2,586,072. 5 claims. Feb. 19, 1952. Official Gaz. U. S. Pat. Office, **655,** 3: 696. 1952.

Diacetyl is produced by culturing milk with *Streptococcus diacetylactis* for not over 12 hr. at 75–80° F. until the acidity is about 0.55% as lactic acid.

R. Whitaker

DAIRY CHEMISTRY

H. H. SOMMER, SECTION EDITOR

290. Fractionation of the calcium in bovine blood by fluoride precipitation. A. H. CRAIGE, Univ. of Md., College Park. Am. J. Vet. Research, **13,** 46: 31–37. Jan., 1952.

Concentrations of NaF from 1–6 mg./ml. were found to precipitate increasing proportions of Ca from bovine blood plasma with fairly constant results from 3, 4 and 5 mg. NaF/ml. This method then was used to follow "free" and "bound" Ca under various conditions. 7 ml. of freshly drawn heparinized blood were blown into clean, dry centrifuge tubes containing 12 and 16 mg. NaF. After shaking twice at 15-min. intervals, the treated and untreated blood was centrifugalized and analyzed for Ca. By this method cows with ketosis had less free Ca than normal; cows with milk fever had less free and bound Ca than normal. Normal cows at parturition dropped more in bound than in free blood Ca, but seemed to recover normal bound levels ahead of normal free Ca. Injections of $CaCl_2$ which produced more acute symptoms of hypercalcemia than did Ca borogluconate also caused much larger increases in free blood Ca measured by this method. The free Ca level in normal bovine blood ranged from 5.2–6.4 mg./100 ml. plasma for stable-fed cows and 4.3–5.8 for pastured cows.

E. W. Swanson

291. Determination of 1,1,1-trichloro-2,2-bis (p-methoxyphenyl) ethane in milk and fatty materials. H. V. CLABORN and H. F. BECKMAN. Bureau of Entomology and Plant Quarantine, U.S.D.A., Kerrville, Tex. Anal. Chem., **24,** 1: 220–222. Jan., 1952.

The proposed use of methoxychlor for livestock pest control has indicated need for a more accurate method of determination than has been available. This method may be used to determine the methoxychlor deposited in fat tissue and excreted in milk. Fat from tissue or milk is dissolved in *n*-hexane. The methoxychlor is separated from the hexane solution of fat with nitromethane, which then is removed by evaporation. The sample is nitrated with fuming nitric acid at 100° C. for 30 min., the color measured by photoelectric colorimeter and methoxychlor determined by a standard curve.

B. H. Webb

292. Method of iodinating proteins. W. R. GRAHAM, JR., G. O. KOHLER and R. D. HOOVER (assignors to American Dairies, Inc., and The Quaker Oats Co.). U. S. Patent 2,586,425. 1 claim. Feb. 19, 1952. Official Gaz. U. S. Pat. Office, **655,** 3: 791. 1952.

Proteins, such as casein, are iodinated with free iodine with sodium paratoluene-sulphochloramine. R. Whitaker

DAIRY ENGINEERING

A. W. FARRALL, SECTION EDITOR

293. Frozen confection apparatus. C. ERICKSON and E. SPELLMAN. U. S. Patent 2,587,127. 16 claims. Feb. 26, 1952. Official Gaz. U. S. Pat. Office, **655,** 4: 1055. 1952.

An ice cream freezer of the horizontal continuous type, has a positive feed pump which introduces mix under pressure into the center of one end of the freezing cylinder. The frozen and whipped product is forced out of the other end through a dispensing outlet. R. Whitaker

294. Homogenizing apparatus. E. H. REEB (assignor to Star Metal Mfg. Co.). U. S. Patent 2,586,258. 10 claims. Feb. 19, 1952. Official Gaz. U. S. Pat. Office, **655,** 3: 747. 1952.

An emulsifier for oil-in-water type of emulsions such as milk, consists primarily of a rapidly rotating cylindrical bowl. The product is drawn into the bowl and discharged, by centrifugal force, between 2 concentrically grooved discs, into a collecting chamber. R. Whitaker

FEEDS AND FEEDING

W. A. KING, SECTION EDITOR

295. A study of normal bovine serum solids with vitamin K added as an oral prophylactic for calf scours. I. Use of healthy newborn calves. G. W. ANDERSON, W. M. DUPRE and J. P. LA MASTER, So. Carolina Agr. Expt. Sta., Clemson. Am. J. Vet. Research, **13,** 46: 5–9. Jan., 1952.

Serum solids prepared from slaughterhouse cattle blood, defibrinated, centrifuged, filtered and lyophilized was used in combination with vitamin K and enzymatic digested milk solids to feed to calves during the 1st day after birth. Part of the calves remained with their dams, the rest were separated at birth. Primary scours was not completely prevented by treatments, but there was evidence that treament was helpful in effecting rapid recovery. No deaths resulted from scours in 36 treated calves nor in 21 control calves.

E. W. Swanson

296. The effect of the ingestion of urea on the rate of wool production by Merino sheep. A. W. PEIRCE, Univ. of Adelaide, So. Australia. Australian J. Agr. Research, **2,** 4: 435–446. 1951.

Urea nitrogen was not as well utilized by Merino ewes as an equivalent amount of nitrogen from wheat gluten as measured by wool production and wool fiber diameter. When added to a low-protein high-fiber ration, urea had no significant effect, whereas there was a significant effect of added urea on a similar ration with added starch. It is suggested that urea is most useful when added to a diet low in protein and high in available carbohydrates but is not as useful to sheep as wheat gluten. The possible beneficial effects of sulfur on urea utilization are discussed. G. E. Stoddard

297. The influence of the amount of starch on the utilization of urea by sheep. W. A. PEIRCE, Univ. of Adelaide, So. Australia. Australian J. Agr. Research, **2,** 4: 435–446. 1951.

Urea was fed to Merino wethers on a low-protein ration with potatoes added as a source of starch at the rates of 50, 100, 150 and 200 g. daily. As measured by wool production, increases of 0, 7, 19 and 23%, respectively, for the 4 levels of potato feeding were obtained. A mixture of red palm oil and peanut oil added at the rate of 28 g. daily to each of the above rations to increase caloric intake gave similar increases of 0, 9, 15 and 15%, respectively, indicating little or no effect of added calories in the form of oil. Similar proportionate increases in wool fiber diameter were observed but with smaller percentage increases than total wool production.

G. E. Stoddard

298. Animal protein factor supplement produced by direct bacterial fermentation. Production and evaluation. H. M. HODGE, C. T. HANSON and R. J. ALLGEIER, U. S. Ind. Chemicals Co., Div. of Nat'l. Distillers Prod. Corp., Baltimore, Md. Ind. Eng. Chem., **44,** 1: 132–135. Jan., 1952.

This report describes production of B_{12} by anaerobic fermentation of plant proteins in various cereal grains and in yeast and bacterial cells. Some protein sources including dried skimmilk were unsatisfactory. The production of B_{12} with cultures of *Pseudomonas* sp. and *Proteus vulgaris* was 0.03 and 0.13 γ/ml., but a mixture of these cultures grown together on an anaerobic neutral medium produced 0.32 γ/ml. The product has been evaluated by experimental feeding on rats and chicks. B. H. Webb

HERD MANAGEMENT

H. A. HERMAN, SECTION EDITOR

299. Milking machine. D. F. AYRES (assignor to The DeLaval Separator Co.). U. S. Patent 2,587,680. 5 claims. March 4, 1952. Official Gaz. U. S. Pat. Office, **656,** 1: 87. 1952.

A portable milking machine built on a 2-wheeled cart is described. R. Whitaker

300. Test milking apparatus. J. W. L. ATTERLING, E. REDIN and H. RYDE (assignors to Aktiebolaget Manus). U. S. Patent 2,588,461. 16

claims. March 11, 1952. Official Gaz. U. S. Pat. Office, **656,** 2: 367. 1952.

A device is described, which attaches to a milking machine and which meters the volume of milk produced and delivers an aliquot portion into a test tube on the outside. R. Whitaker

301. Milk can cooler. H. W. Houseweart. U. S. Patent 2,588,927. 3 claims. March 11, 1952. Official Gaz. U. S. Pat. Office, **656,** 2: 494. 1952.

Milk in a can may be cooled easily by this device which consists of a lid for the can supporting a cooling coil capable of being rotated by the pressure of the coolant. R. Whitaker

ICE CREAM

C. D. DAHLE, SECTION EDITOR

302. Stabilizers for variegated ice cream. C. D. Dahle and W. F. Aull, Jr., Penn. State College, State College. Ice Cream Rev., **35,** 5: 52, 54–56, 58. Dec., 1951.

A stabilizing agent is necessary in preparation of variegating materials to prevent bleeding and iciness and to develop sufficient viscosity so that smeary streaks or pools in the ice cream will be avoided.

Six stabilizers, including pectin, Irish moss extract, cellulose gum, locust bean gum, gelatin and gum karaya were each used in preparation of strawberry and chocolate variegating syrups. Gum karaya was least efficient. Pectin proved satisfactory when used at the rate of 2% for chocolate or 1% for strawberry variegating syrups; Irish moss extract was effective when used at the rate of 0.3%; locust bean gum was satisfactory at 0.5%; cellulose gum was effective at a concentration of 0.75% in chocolate syrups or when used at slightly over 1% with strawberry syrups; gelatin (200 Bloom) was effective at 1% in chocolate syrup but this proved inadequate in the case of strawberry syrups.

The formula suggested for a suitable strawberry syrup included 45 lb. of 3:1 pack strawberries, 36.97 lb. of sugar, 18.45 lb. of water and stabilizer and 0.3 lb. of citric acid. The mixture is heated to 170° F. or higher depending upon the stabilizer used, cooled and held overnight at 40° F. or below prior to use.

The chocolate syrup formula included 13 lb. of cocoa, 40 lb. of sugar, 0.2 lb. of salt and 46.8 lb. of water and stabilizer. The syrup is processed in the same manner as the strawberry syrup. W. J. Caulfield

303. Method of adding emulsifying agents to prepared ice cream mixes. N. H. Nash (assignor to Lanco Prod. Corp.). U. S. Patent 2,587,369. 5 claims. Feb. 26, 1952. Official Gaz. U. S. Pat. Office, **655,** 4: 1119. 1952.

A cold solution of an ester of a higher fatty acid and a polyhydric alcohol in a suitable solvent is added at the rate of 0.1–0.2% by weight to ice cream mix after it has been pasteurized. R. Whitaker

304. H.T.S.T. pasteurization of ice cream mix. P. H. Tracy, J. Tobias and J. Ordal, Univ. of Illinois. Ice Cream Rev., **35,** 8: 145–147. Mar., 1952.

Pasteurization of ice cream mixes by the H.T.S.T. process at 175° F. for 30 sec. was found to be just as effective as use of 155° F. for 30 min. from the standpoint of bacterial destruction. To obtain bacterial destruction comparable with that resulting from pasteurization of mixes at 160° F. for 30 min., it was necessary to use a temperature of 177.5° F. for 30 sec. W. J. Caulfield

305. A study of some factors related to volume shrinkage of ice cream during storage. E. L. Thomas, W. B. Combs and S. T. Coulter, Univ. of Minnesota. Ice Cream Rev., **35,** 8: 134, 136, 138. Mar., 1952.

Heat treatment of mixes at 185° F. for 10 min. or 165° F. for 30 min. resulted in more shrinkage than when a longer or shorter holding period was used with either temperature. Heat treatments which yielded the maximum tendency towards shrinkage also coagulate significant amounts of the serum protein, but do not cause complete coagulation of these proteins. Increasing the globulin content of the mix by either 0.05 or 0.10% through addition of colostrum milk to the mix tended to increase shrinkage.

Dry ice-induced shrinkage is due primarily to internal pressure changes associated with alternate absorption and desorption of CO_2.

Jolting of qt. ice cream samples at the rate of 230 jolts/min. for 4 hr. during which the temperature of the ice cream increased from −18 to 6° F. did not accelerate shrinkage.

Shrinkage trends were found to be similar for spontaneous and vacuum-treatment methods. The vacuum treatment, however, failed to yield results which would predict either the rate or magnitude of shrinkage that can be expected to occur under spontaneous conditions. Spontaneous shrinkage could always be obtained provided the storage temperature is such that the ice cream lacks structural rigidity and the storage time is sufficiently prolonged. Time required for spontaneous shrinkage to occur in ice cream samples stored at 2° ± 3° F. ranged from 4–12 wk.

The authors conclude that there is need for much more research work on the shrinkage problem before it will be solved. W. J. Caulfield

306. Simplified laboratory equipment for testing mix, ice cream samples. J. Toth, Harry W. Dietect Co., Detroit, Mich. Ice Cream Rev., **33,** 6: 78–80. Jan., 1952.

Plans are presented for an efficient but relatively inexpensive ice cream plant laboratory de-

signed for checking composition of raw materials and ice cream mixes. Total cost of equipment in the laboratory was estimated to be under $1,100. Data show that the accuracy of fat and total solids determinations is comparable with that of the A.O.A.C. procedure.

W. J. Caulfield

307. Luick applies power take-off units for refrigeration of ice cream trucks. ANON. Ice Cream Rev., **35,** 8: 50, 157. Mar., 1952.

Complete automatic temperature control is provided on ice cream trucks operated by the Luick Ice Cream Co., Milwaukee, Wis., by equipping the trucks with refrigeration units operated as needed from the truck motor. During the period when the trucks are not in service, the refrigeration unit is operated by electricity.

Temperatures within the truck body are maintained at −5 to −14° F. Since temperature within the truck is at a suitable level for ice cream storage when trucks return to the plant, it has been possible to load the trucks in the afternoon for the following day's delivery. It is estimated that the compressor unit draws the equivalent of about 3.5 h.p. from the truck motor.

W. J. Caulfield

308. Credit in the ice cream industry. F. MURPHY, Hendries' Ice Cream, Milton, Mass. Ice Cream Rev., **35,** 6: 108–109. Jan., 1952.

The ice cream manufacturer should sell his product for cash. The lending of money is a function of the banks or other lending agencies and not a function of the ice cream industry. Since ice cream is sold for cash and it is a product with a rapid turnover, there should be little or no need for credit sales. W. J. Caulfield

309. Building ice cream gallonage through retail milk routes. ANON. Ice Cream Rev., **35,** 8: 48, 49, 92. Mar., 1952.

The retail milk route offers an important potential and profitable market for ice cream, in the opinion of several milk companies who have had experience in this field. Some of the essential rules for a successful and profitable operation are listed as follows: (a) Compensate the driver salesman with a liberal commission for his ice cream sales; (b) don't reduce prices except for specials; (c) try out the venture on 1 route to determine potential sales possibilities before equipping too many trucks.

The use of a well-insulated box on the milk trucks refrigerated with frozen brine slugs having eutectic points of from −8 to −12° F. has proved to be a practical and economical method of maintaining ice cream at a satisfactory temperature while on retail routes. This system of refrigeration has proved less expensive than use of dry ice and ice cream is maintained at a more satisfactory temperature. W. J. Caulfield

SANITATION AND CLEANSING

K. G. WECKEL, SECTION EDITOR

310. Sanitization of dairy farm utensils. A comparison of a cleaner-sanitizer containing Hyamine 1622 with an alkaline cleaner and hypochlorite sanitizer. W. E. BOTWRIGHT, Rohn and Haas Co., Phila., Pa. J. Milk & Food Technol., **15:** 29–33. Jan.–Feb., 1952.

The effect on thermoduric and total bacterial count was made on 31 farms using a cleaner-sanitizer composed of 10% Hyamine 1622, 5% Triton X-100, 30% sodium metasilicate pentahydrate and 55% tetrasodium pyrophosphate as compared to a standard cleaner and hypochlorite sanitizer. The detergent-sanitizer group was superior to the control group of compounds in reducing the bacterial count and also gave an excellent appearance to the washed utensils.

H. H. Weiser

311. Rengjøring og bakteriologisk kontroll av melksalgsmeierienes apparatur (Cleaning and bacteriological control of equipment in milk plants). ROLF HONORE, Dairy Research Sta. at the Agr. College of Norway, Report no. **42.** July, 1950.

The circulation method of cleaning equipment was studied in several commercial plants. In one, the equipment was not disassembled during 1.5 yr. Inspection then showed no visible deposit on milk pipes and the bacteriological condition of equipment was good. It was found that the bottle fillers could satisfactorily be a part of the equipment cleaned by the circulation method.

Examination of hands, boots and brooms indicated, that in cleaning the vats and tanks, serious infection of the metal surfaces might occur from these sources. If subsequent sterilization should fail, there is a risk of infecting the milk with pathogenic bacteria.

Tanks were flushed with hot water, or with an alkaline solution, with no scrubbing. They were sterilized with either steam or chlorine. After several months of cleaning by this method they appeared clean. The bacteriological condition was good. Sterilization of the pasteurizer, pipe lines and fittings after circulation-cleaning by heat proved more efficient than chlorine. For tanks, vigorous flushing of the surfaces with hypochloride was equal to heat sterilization.

G. H. Wilster

312. Better and more economical cleaning in ice cream plants. J. R. PERRY, Nat'l. Dairy Prod. Co., Inc., New York. Ice Cream Rev., **35,** 7: 48, 50, 60, 62, 65, 68, 71. Feb., 1952.

The use of new cleaning aids, developed for more efficient and more economical cleaning in ice cream plants, is discussed. Use of these efficient cleaning tools will save time, save materials, protect equipment from damage, lower processing

costs, improve product quality and boost the morale of personnel engaged in the clean-up operation. W. J. Caulfield

313. A single reagent field test for quaternary ammonium solutions. D. B. CONKLIN, Research Div., Wyandotte Chem. Corp., Wyandotte, Mich. J. Milk & Food Technol., **15:** 27–28. Jan.–Feb., 1952.

A qualitative method for testing the concentration of quaternary ammonium or detergent-quaternary compounds is based on the reaction between quaternary and a definite amount of brome phenol blue buffered on the acid side. When 200 ppm. or more of quaternary and dye solution are mixed, a blue color appears. If lesser amounts of the sanitizer are present, various shades of green appear. This is due to conversion of the dye to its blue complex plus the acidified yellow excess. The method is simple, low cost, quick and adaptable to many types of quaternary products.

H. H. Weiser

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