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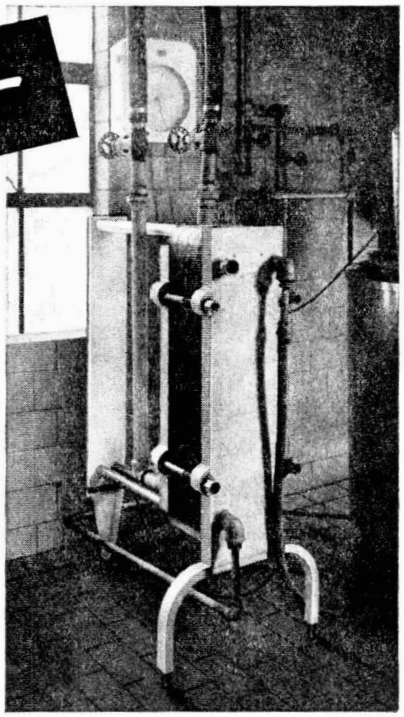

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VOLUME XXXVII September, 1954 NUMBER 9

BIOLOGICAL ACTIVITY OF PHOSPHATE ESTERS IN MAMMARY GLAND EXTRACTS[1,2]

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Mammary gland tissue contains enzymes capable of phosphorylating a variety of sugars including fructose and glucose *(8)*. Since a similarity exists between enzymatic reactions of various animal tissues, the products of the esterifications are probably the same as those resulting from other animal kinases. Purified brain hexokinase phosphorylates glucose in the six position *(5)*, while the product of purified rat liver fructokinase has been identified as fructose-1-phosphate *(6, 12, 14, 20)*. Galactose-1-phosphate is a product of galactokinase in yeast *(22)*, liver *(13)*, and bacteria *(18)*.

The presence of hexose phosphate esters in mammary tissue was established by Brenner *(3)* and Barrenscheen and Alders *(2)*. The latter noted that the lactating tissue contained a larger percentage of esters difficult to hydrolyze than nonlactating tissue. From lactating porpoise glands they were able to isolate the barium salt of a reducing hexose phosphate ester. Barkhash *(1)* was unable to find galactose phosphate esters in mammary tissue.

Caputto *et al. (4)* prepared a cell-free system from yeast which converted galactose-1-phosphate to glucose-1-phosphate. In this laboratory *(18)* a similar purified enzyme system specific for the α-esters has been obtained in preparations of a strain of *Lactobacillus bulgaricus*. This system, which was found to be reversible, required uridine-diphosphoglucose (UDPG) and manganese ions for activity *(11)*. The presence of the cofactor UDPG in rat mammary glands was established. Malpress and Morrison *(15)* observed that α-galactose-1-phosphate is a normal trace constituent of milk.

This information strengthened the possibility that galactose and glucose esters may be important in mammary gland metabolism. With preparations similar to those with which hexose phosphorylation was demonstrated *(8)* the reactions occurring in the presence of various hexose esters have been observed and are the subject of this report. Enzymes were found to be present in rat

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[1] Preliminary report: *Federation Proc.*, 12: 192. 1953.

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mammary gland homogenates which catalyze reactions with glucose, galactose, and fructose esters.

EXPERIMENTAL

Twenty per cent homogenates were prepared from a frozen powder of mammary tissue of lactating Holtzman rats, as described previously *(7)*. The homogenizing medium was either water or 0.015 *M* phosphate buffer (pH 7.4). The transfer of phosphate from adenosinetriphosphate (ATP) to an acceptor formed a new acid group which released a mole of carbon dioxide from the added bicarbonate. Manometric measure of the gas was an assay of the transphosphorylation. The following constituents (final concentration) were added in order: 0.027 *M* potassium bicarbonate, 0.0015 *M* potassium phosphate (pH 7.4), and water to make a total volume of 2.0 ml. in all flasks to 0.4 ml. of homogenate; 0.008 *M* magnesium sulfate and 0.05 *M* potassium fluoride were added in selected flasks. The concentration of ester substrate was 11 micromoles per flask. After the flasks were gassed with a mixture of 95% nitrogen–5% carbon dioxide, 10 micromoles of ATP were added from a side arm to start the reaction. Incubations were performed at 38° C. Control values were about half of the total carbon dioxide released in the presence of substrates and were discussed in a previous report *(7)*. Additional carbon dioxide resulting from the presence of a substrate was regarded as transphosphorylation, which is reported as micromoles of CO_2 released in excess of control values. All of the values obtained were corrected for the carbon dioxide retention of the incubation mixture. It was essential to determine relative retention since each type of ester with regard to position of the phosphate had a different effect on the retention.

The disodium salt of ATP (Pabst Laboratories) was neutralized with potassium hydroxide. Fructose-6-phosphate was a preparation of Nutritional Biochemicals Corporation; α-glucose-1-phosphate and glucose-6-phosphate were furnished by Dr. H. A. Lardy. The galactose esters were prepared in this laboratory *(18)*.

Since a hydrolytic cleavage of the hexose esters would produce new acid groups, the action of phosphatases would have given the same results as transphosphorylations. To determine specific phosphatase action, esters were incubated without addition of ATP. In addition, trichloroacetic acid (TCA) extracts were made before and after incubation with ATP; inorganic phosphate was determined on the clear centrifuged supernatant by the method of Fiske and Subbarow *(9)*.

Ketose was determined by the method of Roe *(17)* on the clear centrifuged TCA extracts before and after incubation. Fructose-6-phosphate and fructose-diphosphate have given 60.5 and 52.5%, respectively, of the color obtained with pure fructose in the Roe method *(23)*. Since these fructose esters presumably occurred in these experiments, a value of 56% was chosen arbitrarily for the calculation of ketose values which are reported in micromoles.

RESULTS

Low phosphatase activity. The changes of inorganic phosphate occurring during incubation of glucose esters (in the absence of ATP) with mammary gland homogenates are reported in Table 1. Because of the high phosphate levels where phosphate was used as buffer, measurement was possible only in homogenates prepared with water; the main buffering constituent therein was bicarbonate and thus the pH was about 8. Phosphatase activity was low; the six-ester of glucose was hydrolyzed more than the one-ester in the 1 hour incubation.

TABLE 1

The hydrolysis of phosphate esters[a] *by mammary homogenates*[b]

Substrate	No. of experiments	Micromoles inorganic phosphate released			
		Endogenous		From the substrate	
		Mean	Range	Mean	Range
Glucose-6-phosphate	5	1.0	0.7 to 1.3	1.5	0.5 to 2.3
α-glucose-1-phosphate	5	1.6	0.6 to 4.2	0.6	−0.6 to 1.6

[a] Concentration of ester substrate: 11 micromoles.
[b] Homogenates were prepared with water. Incubation time: 60 minutes.

As the conditions with regard to pH and ion concentration for assay of transphosphorylation were somewhat different than the above experiments, additional tests for phosphatase were made. If hydrolysis of the esters were occurring, the release of carbon dioxide in the absence of ATP would be expected. With conditions similar to those where transphosphorylation was obtained (with the exception that ATP was omitted) the presence of glucose or galactose esters caused no additional release of carbon dioxide. Thus at the lower pH and in the presence of phosphate ions there was even less phosphatase action than reported in Table 1.

Glycolytic esters. When ATP was present, the addition of glucose-6-phosphate, fructose-6-phosphate, and α-glucose-1-phosphate to mammary gland homogenates produced large amounts of carbon dioxide in excess of control values (Table 2). This indicated that each compound was readily esterified or converted to an intermediate which was esterified. During the transphosphorylation α-glucose-1-phosphate gave rise to rather large amounts of ketose material (Table 5), which was assumed to be fructose esters.

TABLE 2

Phosphorylation of glucose and fructose esters by mammary gland enzymes[a] *in the presence of ATP*

Substrate	No. of experiments	Micromoles of carbon dioxide in excess of controls in 60 minutes
Glucose-6-phosphate	3	5.5
Fructose-6-phosphate	5	5.6
α-glucose-1-phosphate	1	4.3

[a] Homogenates were prepared with phosphate buffer.

TABLE 3

Phosphorylation of galactose esters by mammary gland enzymes

Preparation	Homogenizing medium[a]	Experiment No.	Substrate		
			α-Gal-1-P	β-Gal-1-P	Gal-6-P
			(Micromoles of carbon dioxide in excess of controls due to substrate in 60 minutes)		
A	Water	36	4.4	1.9	2.0
		38[b]	2.5		
		39[c]	1.2		
B	Buffer	40	−0.4	0.0	4.3
C	Buffer	45	3.7	0.1	4.1
D	Buffer	54	5.1	2.4	3.8
		56[c]	4.5	0.6	4.3
E	Buffer	61	0.4	1.9	4.3

[a] Buffer refers to phosphate buffer at pH 7.4.
[b] Frozen and thawed twice.
[c] Frozen and thawed three times.

Galactose esters. The mammary gland homogenates were also capable of inducing esterification in the presence of added galactose-6-phosphate, α-galactose-1-phosphate and β-galactose-1-phosphate. Results obtained with several preparations showed that although activity was extensive it was not consistently present. Table 3 summarizes results of typical experiments; each of the effects noted was observed in more than one preparation and thus is a reproducible result. Preparation D was active in the presence of all three esters whereas B showed only activity for the six derivative. Preparation C had low activity for only the β-one-ester while E was low in activity only for the α-one-ester. The ability of preparation D to induce esterification in the presence of the β-ester was decreased by freezing and thawing three times, although the activity with the other esters was not changed appreciably. In preparation A, which was made with water rather than phosphate buffer, steps of freezing and thawing gradually decreased activity with the α-ester. The β-ester consistently was responsible for less extra carbon dioxide than the other two esters.

In some of the experiments the added galactose esters appeared to inhibit the apyrase which was responsible for most of the carbon dioxide of the control values. In Figure 1 the flask containing galactose-6-phosphate produced less carbon dioxide than the controls in the early phase of incubation. Since the rate of apyrase activity decreased with time *(7)*, the effect was not noticeable because of the esterification of galactose-6-phosphate. Table 4 summarizes the results of several experiments in which the inhibition was observed with galactose-6-phos-

TABLE 4

Apparent inhibition of apyrase by galactose esters

	Substrate	No. of experiments	Micromoles of carbon dioxide released (mean)	
			6 minutes	60 minutes
A.	Control	4	2.9	6.9
	α-galactose-1-phosphate		2.3	6.9
B.	Control	8	2.9	7.8
	galactose-6-phosphate		1.4	9.6

phate. Since the inhibition is noted at 6 minutes but not at 60 minutes, esterification is evident.

The increase of ketose during these incubations was estimated. Table 5 contains ketose values obtained with preparations which induced esterification with the specific substrate. None of the galactose esters was converted to a ketose material in large amounts. Since endogenous changes were not measured, the small values (0.1 to 0.3 micromoles) may have been due to glucose esters present in the preparations. This result was in contrast to the rapid conversion of glucose esters to ketose material.

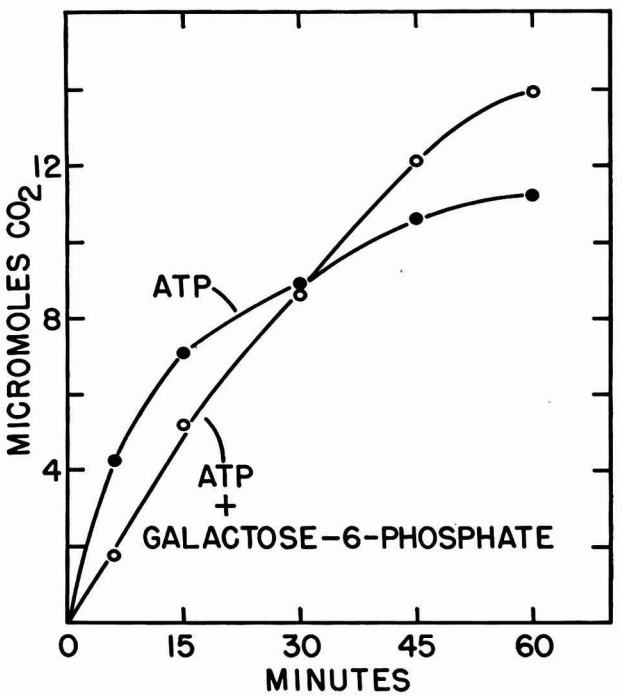

FIG. 1. Apparent inhibition of mammary gland apyrase by galactose-6-phosphate.

DISCUSSION

Knowledge of the phosphatase activity of mammary tissue under the conditions of these experiments is of importance since a phosphatase capable of splitting hexose esters would simulate esterification as measured here. Some hydrolysis of the esters occurs in bicarbonate buffer. This may be due to the alkaline phosphatase of mammary tissue reported by Folley *(10)*, which has a pH optimum of 9.3. When the incubation is buffered with large amounts of phosphate there is evidence for even less hydrolytic action. This may be due to the lower pH or may be the result of an inhibition by the phosphate ions as occurs in the case of intestinal phosphatase *(19)*. The low amount of enzymatic

TABLE 5

Increase of ketose[a] during incubation of phosphate esters with mammary preparations

Substrate	No. of experiments	Ketose		Increased ketose
		Zero time	60 minutes	
α-galactose-1-phosphate	4	0.7	0.8	0.1
β-galactose-1-phosphate	3	0.9	1.0	0.1
galactose-6-phosphate	3	2.6	2.9	0.3
α-glucose-1-phosphate	2	1.1	6.7	5.6

[a] Values are micromoles per flask.

splitting of hexose-esters by mammary gland is similar to the results obtained by Reithel *et al. (16)*.

The low order of phosphatase activity enhances the probability that the esters induce phosphorylation. The additional CO_2 released in the presence of an ester might have been interpreted as a stimulation of apyrase. The inhibitions noted with the same esters decrease the possibility of a stimulation. This indicates the presence of several glycolytic enzymes in the mammary gland. In the presence of ATP the fructose-6-phosphate is probably phosphorylated directly by phosphohexokinase to form hexosediphosphate. Since the glucose-1-phosphate is converted to ketose ester and since both the one and six ester of glucose gave rise to esterification, the glucose-1-phosphate evidently is converted to glucose-6-phosphate, whereon the latter is converted to fructose-6-phosphate. This provides evidence that in addition to the kinases and apyrase previously described *(7, 8)* mammary tissue contains the enzymes phosphoglucomutase (I), phosphohexoisomerase (II), and phosphohexokinase (III) which catalyze the following reactions:

(I) α-glucose-1-phosphate $\longleftrightarrow$ glucose-6-phosphate

(II) Glucose-6-phosphate $\longleftrightarrow$ fructose-6-phosphate

(III) Fructose-6-phosphate + ATP $\rightarrow$ fructose-diphosphate + ADP

Venkstern *(24)* has reported the presence of a phosphoglucomutase in mammary tissue.

In previous reports *(8)* on the phosphorylation of glucose by mammary homogenates two moles of phosphate were transferred from ATP for every mole of glucose esterified. It is probable that the product of the glucose phosphorylation is either the one or six ester of glucose which is esterified following the conversion to fructose-6-phosphate.

In the light of recent work on glucose-galactose interconversion, the enzymatic activity of α-glucose-1-phosphate may be of significance. At least it is possible for this intermediate to arise under proper conditions in the mammary tissue. Kosterlitz *(13)* has observed that α-galactose-1-phosphate is formed in liver tissue. If the glucose and galactose one-esters were in equilibrium in mammary homogenate one would expect glucose and fructose esters to arise during incubation of α-galactose-1-phosphate. This does not occur under the conditions of these experiments and does not acount for the enzymatic transphosphorylation which was observed.

Evidence was not obtained that galactose esters are metabolized by a scheme similar to glycolysis. Tagatose, a ketose, has been suggested *(21)* as a possible intermediate in galactose metabolism. Nonspecificity of the glycolytic enzymes or a series of similar enzymes could give rise to tagatose formation from galactose esters. Such possibilities do not account for the enzymatic activity observed here, since tagatose formation during the esterification would have been detected by the ketose assay. Thus the glycolytic enzymes observed in mammary tissue are specific in that they react with glucose esters but not those of galactose.

The ester actually participating and the product of the esterification mechanism resulting from the presence of galactose esters are unknown. The evidence is not sufficient to say whether the various galactose esters are interconverted.

SUMMARY

Glucose-6-phosphate, fructose-6-phosphate, and α-glucose-1-phosphate were phosphorylated by mammary gland homogenates. During the phosphorylation of α-glucose-1-phosphate fructose was formed. These findings present evidence for the presence of the enzymes phosphoglucomutase, phosphohexoisomerase, and phosphohexokinase in the tissue of lactating rat mammary glands. These enzymes are specific for glucose esters and have no activity with galactose esters.

Other enzymes are present which induce transphosphorylation in the presence of α-galactose-1-phosphate, β-galactose-1-phosphate, and galactose-6-phosphate. These esters were not converted to glycolytic intermediates or to ketose esters under the conditions studied.

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THE TECHNIQUE OF ELECTROEJACULATION AND ITS USE IN DAIRY BULLS[1]

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The widespread use of artificial insemination has increased the value of each ejaculate of semen from valuable sires. Since some males are either unwilling or unable to serve an artificial vagina, their usefulness is limited. Electrical stimulation is one of several methods that have been used in an attempt to collect semen from such animals.

One of the first to record the obtaining of semen by the use of electrical stimulus was Batelli *(1)*, who collected from guinea pigs. Moore and Gallagher *(15)* modified the technique and used it in an evaluation of the functioning of the accessory glands of the guinea pig. Gunn *(5)* was the first to make extensive use of electrical stimulus as a means of collecting semen from rams. Further work with this technique was done by Bonadonna *(2)*, Lambert and McKenzie *(6)*, Terrill *(17)*, and others. The stimulation was 60 cycle alternating current of about 30 volts applied by means of a rectal probe and a lumbar electrode. Laplaud and Cassou *(7)* constructed a bipolar rectal probe which permitted stimulation without an external lumbar electrode. Laurans and Clement *(10)*, Likar and Kamhi *(11)*, Mata and Cano *(14)*, and Ortavant *et al. (16)* made further use of the bipolar electrode in collecting semen from rams.

The bipolar probe was adapted by Thibault *et al. (18)* so that it could be used in the stimulation of bulls. His equipment consisted of a probe with 30 brass rings insulated from each other by ebony. He used 60 cycle alternating current which was varied from zero to thirty volts and back to zero every 3 to 5 seconds. Evidence is given on pH, volume, density, and motility of the semen to indicate that the semen samples collected were normal. Thibault *(19)* described in detail the electrical circuits, construction of the probe, and the technique used in restraining the bull and collecting the semen.

Laplaud *et al. (9)* further elaborated on the application of electrical stimulus as a method of obtaining semen from bulls. Laplaud and Cassou *(8)* cite an example of a young bull that had yielded 161 ejaculates in an artificial insemination center before an accident which affected his hindquarters prevented any attempt at service. A 6-ml. sample of semen was obtained by bipolar electrical stimulation and used to inseminate two cows. A pair of fraternal twins and a single calf were born from these matings. Lutwak-Mann and Rowson *(12)* made several chemical determinations of various fractions of bull semen collected by electrical stimulus. They found that the pre-sperm fraction was not from

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[1] University of Minnesota Scientific Journal Series Paper No. 3108, Minnesota Agricultural Experiment Station.

the accessory glands but probably from the urethral glands. Excesses of this fluid did not affect motility or fructolysis. Egron *(4)* found the semen collected by means of artificial vagina did not differ greatly from that obtained by electrical stimulus. The pH was slightly higher and the density of sperm was lower in semen resulting from electrical stimulation. Teasing the bull before collection increased the density. Briere *(3)* mentioned the applications of electroejaculation in his thesis.

Mascarenhas and Gomes *(13)* were the first to report large numbers of collections by electrical stimulation in the bull. They used the multi-ringed bipolar probe to apply both constant and impulse type of current. The impulse type of current is described in which the stimulation is passed from one group of rings to another in rapid succession. This type of stimulation gave less fluid and the side reactions were more marked than with the constant type. Initial experiments were performed by using a 15-v. current, but later work was done with a current of 5-10 v., which also proved satisfactory. A total of 48 separate collections were made from seven bulls. Generally, the semen was less dense but of higher volume so that the total numbers of sperm were essentially the same as in semen collections made with an artificial vagina.

EXPERIMENTAL PROCEDURE

Eighteen bulls were used as experimental animals in these trials. Holstein, Jersey, Guernsey, Brown Swiss, and Shorthorn breeds were represented and, except for a set of fraternal Holstein twins, all were purebred animals. Several different types of electrical stimuli were tried with varying degrees of success. The ordinary inductorium stimulus of high voltage peaks and low amperage was found to be highly stimulating to motor nerves, especially the sciatic, but was ineffective in causing either erection or ejaculation. The frequency of the stimulating current was varied from 15 to 900 cycles per second by means of a variable frequency oscillator. No advantage was found for either the higher or lower frequencies. Because of the expense and inconvenience of the equipment, standard 60-cycle current was used subsequently. A 30-v., 60-cycle alternating current which could be varied from zero to 30 v. by means of a rheostat also was tried. Because of undesirable fluctuations in the voltage applied to the bull as the current load increased, the rheostat was discarded in favor of a variable transformer as a means of varying the voltage level. An isolation step-down transformer was found to be necessary in isolating the circuit from the power source and to allow more gradual changes in voltage. Attempts were made to include resistors varying from 25 to 200 ohms in series with the bull in the final circuit. This was done to reduce marked changes in the current flow through the bull as the resistance changed because of feces accumulation and poor contact. Some mechanism, perhaps contraction of the rectum upon stimulation, causes the effective resistance of the bull to decrease as the amperage flow increases. Therefore, the added series resistor prevented a sufficiently high amperage level to achieve either an erection or an ejaculation.

Several rectal probes of various dimensions and specifications were used in these trials. The early attempts were made with only two electrodes. These were moved and placed by insertion of the gloved arm into the rectum. By localizing the stimulation over the glands and over the bulbo-cavernosus muscle, pronounced erection and a very little fluid could be obtained; however, in no case was an ejaculation obtained. One probe 1 in. in diameter with seven rings 1¾ in. apart was used with some success but did not give consistent results. A second probe, 1 in. in diameter with nine rings 1¼ in. apart, gave much more fluid, but ejaculation did not follow in all cases. A smaller probe, ¾ in. in diameter with six rings ¾ in. apart, gave very little stimulation even with the resulting high amperage. A probe 2 in. in diameter with three longitudinal electrodes was used but was found to be less desirable than the ringed probe of similar dimensions, because of more side reactions and more fluid secretion. The probe which seemed most satisfactory was 1½ in. in diameter with six rings 1¾ in. apart. This probe was made from a section of 1½ in. heavy steam hose 2 feet long. Each ring electrode was partially inlaid into the rubber. The electrodes were made from 2 turns of heavy solid core solder. A separate lead ran inside the probe and was soldered to each electrode. Most of the results reported were obtained with this type of probe and the electrical circuit shown in Figure 1.

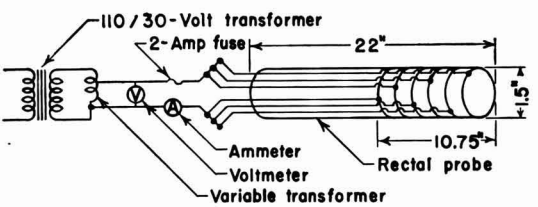

FIG. 1. Schematic diagram of stimulator.

Collection of the semen was made by means of a rubber cone which was held open by a section of radiator hose 1½ in. long, similar to that used for the artificial vagina. The opening was protected from dirt by a stiff rubber sheet with several intersecting slits cut in it. The semen was collected in a test tube which was surrounded by a larger test tube to serve as a warm water jacket to minimize cold shock. It is necessary to be able to see the semen at collection to aid in deciding on the number and intensity of subsequent stimulations.

The technique used became fairly well established as the trials progressed. The first step is administration of an enema of about two quarts of a warm 5% solution of sodium chloride. It is important to give an adequate enema to remove as much of the fecal material as possible in order to minimize its insulation of the electrodes. The preputial area should be clipped and brushed to reduce the amount of dirt and foreign material which otherwise may get in the semen. The probe is lightly lubricated with some noninsulating material and introduced into the rectum. The probe is inserted for varying distances,

depending on the size of the bull, but generally no more than 12 in. The best results, especially as far as erection is concerned, were generally obtained when the probe was maintained on the ventral side of the rectum. The voltage was gradually increased to about 3-5 v. and then back to zero every 5-10 seconds. The subsequent stimulations go progressively higher so that at about the fifth stimulation the maximum of 10-15 volts is reached. There is much variation between bulls, and from one time to another with the same bull, so that experience best governs the rapidity of increase. Erection and fluid secretion usually start at about the 5-v. level, but ejaculation usually occurs at the 10-15 v. maximum, at which time about 0.5 to 1.0 ampere of current is flowing. Too many stimulations at a sub-ejaculatory level yield much fluid and often make it difficult to obtain an ejaculation at any level. Too rapid an increase may lead to a pre-erection ejaculation and contamination of the semen with dirt, since the penis is not free of the sheath.

At the time of stimulation many motor nerves are also stimulated, and general tetany of the hind legs results. There is a general extension of the hind legs and a tendency to push forward. However, in no case has a bull been observed to establish unfavorable associations with either the collection stall or the method of collection. It was found that a minimum of restraint was necessary for best collection, the only requirement being a sturdy stanchion or stock to restrain the neck and to allow the bull to push forward. Good footing, such as earth or gravel, was needed, since the bulls slipped on concrete or wooden floors.

TABLE 1
Collection data

Bull No.	No. of successful electroejaculations	No. of attempts	Total volume of fractions containing sperm	Average volume
			(ml.)	*(ml.)*
1	29	30	327	11
2	2	3	33	16
3	3	4	30	10
4	0	3	0	0
5	1	1	10	10
6	6	6	74	12
7	9	10	93	10
8	13	13	189	14
9	9	10	216	24
10	12	12	95	8
11	12	12	77	6
12	2	2	32	16
13	3	3	36	12
14	1	1	9	9
15	2	2	33	16
16	2	2	39	19
17	3	3	63	21
18	1	1	8	8
19	1	1	11	11
Total	111	119	1,375	

RESULTS AND DISCUSSION

One hundred eleven separate collections were made from 18 different bulls. Semen was not obtained in eight attempts. Of the 19 bulls used, only one failed to yield any semen. Three separate attempts were made with this bull, but death due to pneumonia prevented further attempts. The volume of semen of the 111 successful collections averaged 12.3 ml. with a range of from 1 to 35 ml. (Table 1). The density ranged from very low to 1.27 billion per milliliter in almost an inverse relationship to volume. The extent of stimulation and degree of separation are the governing factors of the density-volume relationship. Usually, no attempt was made to fractionate the fluid portions from the more dense semen, since it was believed that the excess fluid was not so detrimental to the semen as was the necessary interruption in the sequence of collection when the fractions were separated. Of the unselected samples in which the total number of sperm was determined, there was no appreciable difference in total numbers of sperm between samples collected by means of artificial vagina and those collected by electrical stimulation. Although there were individual bull differences, the first few stimulations usually yielded some fluid, followed by the more concentrated semen. Further stimulation yielded more dilute semen and finally clear fluid. One normal bull was collected from by electrical stimulus about once weekly for a total of 30 collections (Table 2). He formed no un-

TABLE 2

Evaluation of unselected semen samples collected by electroejaculation from bull No. 1

Collection No.	Total volume	Total sperm No. in billions	Motility rate
	(ml.)		*(%)*
1	5	5.0	85
2	9	5.7	85
3	18	7.0	95
4	31	15.5	85
5	13	16.4	85
6	5	7.6	85
7	4	1.2	80
8	15	10.3	85
9	12	9.6	75
10[a]	10	10.5	95
11	7	6.4	80
12	20	21.0	85
13	5	4.0	50
14	11	13.4	80

[a] Collected by artificial vagina 5 minutes after collection No. 9.

favorable association with the treatment and was not affected in any other way. He did, on several occasions, serve an artificial vagina within a few minutes after collection by electroejaculation and produced a normal ejaculate.

Nine of the bulls in artificial insemination studs were impossible to collect from with the artificial vagina. Forty-seven collections were made from these bulls. Twenty-seven of these collections were diluted at usual rates and used in the field during the months of March, April, May, and November, 1953. Results

TABLE 3
Conception data on bulls which were electroejaculated

Bull	A	B	C	D
Number of first services	28	2,012	138	89
60-90-day nonreturns *(%)*	68.0	60.4	44.9	57.3
Previous year over-all *(%)* 60-90 day nonreturns	67.0	61.9	55.0	61.5

of this trial are shown in Table 3. The conception data for the remaining 10 collections is not available at the time of writing.

SUMMARY AND CONCLUSIONS

Evidence has been presented which indicates that it is possible by electrical stimulus to collect semen from some bulls which are either unable or unwilling to serve an artificial vagina. There have been no adverse effects or unfavorable associations in bulls collected from by electroejaculation. The semen so obtained was generally of greater volume but of lower density. However, the total numbers of spermatozoa were comparable to an ejaculate obtained by an artificial vagina.

Electroejaculation may serve as a valuable adjunct to the artificial vagina as a means of collecting semen in artificial insemination centers, especially for bulls which are difficult to collect semen from by means of the artificial vagina.

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CONCEPTION RATES IN DAIRY CATTLE FROM SERVICES AT VARIOUS INTERVALS AFTER PARTURITION[1]

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Previous reports agree in general on the advantages of a higher breeding efficiency for cows bred more than 60 days after parturition. All research previously published, however, was based on tabulations made from accurate records but without a carefully controlled experiment to obtain the data. The purpose of the research reported herein was to get additional information under carefully controlled conditions involving only experimental cows with normal reproductive organs, as determined by careful examinations, and with a history of good genital health after parturition.

VanDemark and Salisbury *(9)* analyzed the breeding records of 593 cows representing five dairy breeds in the University of Illinois herd. From a total of 1,674 pregnancies they concluded that fertility increased with the length of the postpartum interval to first service up to 80 days. The percentage of services that resulted in conceptions, for 212 services with a postpartum interval of 60 days or less to first service, was very low. They reported a range of 35 to 44% as the rate of conception for the three 20-day intervals to 60 days postpartum. Although highly significant, statistical analysis of the results indicated that a very large portion of the variance in services required for conception was accounted for by factors other than interval after calving. Shannon *et al. (8)* reported on the effect of the postpartum interval to first insemination on conception rate from the first service and from subsequent service in a cooperative artificial breeding organization in Illinois. The results of 7,071 cows inseminated at various intervals after calving indicated that a minimum of 50 days was required for satisfactory fertility.

Hofstad *(4)* presented data from 309 conceptions to show that breeding before the 60th day after parturition should be discouraged. In addition to a low conception rate, the cows bred before the 60th day after calving had a higher percentage of abortions, metritis, and retained placentae.

Jennings *(5)* reported on 191 foaling mares bred at different intervals after parturition. In his work, 110 mares were bred on the 9th day after foaling and only 48, or 43.7%, conceived. The abortion rate for this group was 12.8% — four times as great as that from 81 mares bred after a longer interval after parturition. In the latter group 54, or 67.3%, conceived; these had only 21% retained placentae, as compared with 29% in mares bred on the 9th day. Based

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on these results, Jennings recommended that 9th day breeding should be practiced as little as possible.

From work in Kentucky based on 19,703 services, Olds *(7)* concluded that the best time to breed is between 2 and 3 months after calving. Erb and Shaw *(3)*, in a summary of a survey of breeding failures in the state of Washington, stressed the advantage of waiting at least 50 days after calving before rebreeding dairy cows. Lasley and Bogart *(6)* reported that the percentage of cows settled increased as the time from parturition to insemination increased. Casida and Venzke *(1)* determined by rectal palpation that the uterus returns to normal size and tonus, on the average, approximately 26 days after calving.

The summary by Elting and LaMaster *(2)* showed that cows bred within 63 days after calving have less chance to conceive on the first service than those bred after a 63-day interval. Their data showed no difference in the number of additional services required for the cows that did not conceive on the first service among cows bred a short or a long interval after parturition. Conception usually followed on either the second, third, or fourth service, since 93.5% conceived from the first four services. Very little difference was indicated in the breeding efficiency between a 63-day interval and a longer period up to 6 months. To allow approximately 12 months between calvings, for a 305-day record with a 60-day dry period, they recommended that the first service be scheduled at 70 to 90 days after calving.

PROCEDURE

The Brown Swiss, Guernsey, Holstein, and Jersey cows used for this experiment had normal reproductive organs and a history of good genital health after a normal calving and were free from any disturbances or abnormalities in their genital tracts so far as could be determined by an examination before experimental breeding.

After the preliminary examination, the cows were assigned at random, for the time interval from parturition to first service, to one of the following three groups: 60 days or less, 61-90 days, and more than 90 days. These divisions were arbitrarily selected because they fit the general patterns of breeding on dairy farms. Fifty cows were bred in each group.

Cows with retained placentae, metritis, irregular estrus, abnormal genital organs or ovaries, or any other irregularity after parturition were not included in the experimental groups. It is generally known that such cows will have a much better reproductive performance if they receive veterinary treatment and are bred approximately 90 days after calving, provided that they appear to have normal genital health at that time. Among the cows that carried their calves to term, 91% were included in the experimental groups and 9% were eliminated because of one of the genital disturbances mentioned above.

Cows assigned to the experimental groups were bred either artificially or by natural service. The service sire was one that had a satisfactory breeding record, and the three cows (one in each group) used for comparison were all assigned

to the same bull. For a few cows it was necessary to deviate from this but the services were always to bulls with apparently the same fertility level. Conceptions were based on pregnancy examinations, but all females diagnosed as pregnant either dropped a calf or aborted.

RESULTS AND DISCUSSION

Fifty cows were bred in each of the three groups for different postpartum intervals to the first service. The results presented in Table 1 show that the cows bred the first time in 60 days or less after parturition had an average of

TABLE 1

Conception rate, number of previous estrus, and average number of days from parturition to first service and to conception for three experimental groups of cows in the Cornell dairy herd

Interval Parturition to 1st service	No. of cows	Previous estrus	Actual days—parturition		Conceived on 1st service	
			To 1st service	To conception		
(days)		*(av. No.)*	*(av. No.)*	*(av. No.)*	*(No.)*	*(%)*
60 or less	50	0.52	47.7	87.5	24	48[a]
(51-60)	24	0.70	55.2	74.5	16	67
(50 or less)	26	0.43	40.9	100.5[b]	8	31[a]
61-90	50	1.18	74.3	93.8	35	70
Over 90	50	3.12	118.8	130.3	38	76

[a] Statistically highly significant by chi-square test.

[b] Seven cows were "slow breeders" following the early service and required 101, 108, 111, 160, 206, 252, and 462 days from parturition to conception.

0.52 previous estrus and were fresh for an average of 47.7 days when bred the first time, and 24, or 48%, conceived. When this group was subdivided it was found that 24 cows were bred between 51 and 60 days postpartum and 16, or 66.7%, conceived. Among the 26 cows bred 50 days or less postpartum only 8, or 30.8%, conceived. Half of these, or 13, were bred 41-50 days postpartum and five conceived, compared to three conceptions for the 13 cows bred 40 days or less. These results indicated that good conception rates were obtained in cows bred more than 50 days after parturition and poor conception if the interval was less than this for normal cows with good genital health after parturition.

The group of cows bred 61 to 90 days after parturition had an average of 1.18 estrous periods previous to breeding and were bred for the first time an average of 74.3 days after calving, and 35, or 70%, conceived. Cows bred more than 90 days postpartum had an average of 3.12 previous estrous periods and were bred the first time an average of 118.8 days after parturition, and 38, or 76%, of them conceived. The low rate of conception among the cows bred for the first service within 50 days after parturition was statistically highly significant by chi-square test compared to the high rates of conception from first services more than 50 days after parturition.

An important part of the reproductive picture is the number of additional services required by the cows that did not conceive from the first service. This information is presented in Table 2. In the group bred 60 days or less after

TABLE 2

Conception rate at various services for cows bred at different intervals after parturition

Interval Parturition to 1st service	Cows bred		Service at which conception occurred						Sold before conception	Sold sterile	Services per conception to fertile cows
			1	2	3	4	5	Over 5			
(days)	*(No.)*										
60 or less	50	*(No.)*	24[a]	11	5	1	2	3(6) (9)	3[b]	1	2.09[e]
		(%)	48	22	10	2	4	6	6	2	—
(51-60)	24	*(No.)*	16	3	2	1	–	1(6)	1	–	1.65
		(%)	66.7	12.5	8.3	4.2	–	4.2	4.2	–	—
(50 or less)	26	*(No.)*	8	8	3	–	2	2(6) (9)	2	1	2.52[e]
		(%)	30.8[a]	30.8	11.5	–	7.7	7.7	7.7	3.8	—
61-90	50	*(No.)*	35	5	2	3	2	—	3[c]	–	1.55
		(%)	70	10	4	6	4	—	6	–	—
Over 90	50	*(No.)*	38	3	2	3	–	2(6) (6)	1[d]	1	1.54
		(%)	76	6	4	6	–	4	2	2	—

[a] Statistically highly significant by chi-square test.

[b] Three cows sold. One without additional service, one with 1 and another with 3 services, but sold before a return estrus was possible.

[c] Three cows sold. One each with 1, 2, and 3 services but sold before time had elapsed for a return estrus.

[d] One cow sold after 2 services but before time had expired for a return to estrus.

[e] Statistical test was made by analysis of variance for services per conception. When the cows first bred within 50 days after parturition are compared to those over 50 days, M. S. = 17.52 with 1 D. F., F = 9.56, which is highly significant since F.01 = 6.81.

parturition, 26 cows did not conceive from the first service. Three of these were sold before conception, one of which had no additional service, one had one, and the other had three before they were sold. The time interval between service and date of sale was too short to permit a return estrus. A similar situation prevailed in all three groups. These cows had to be sold for economic and herd management reasons. One cow was sterile after five services. The remaining 22 cows in this group conceived as follows: 11 at the second service, five at the third, one at the fourth, two at the fifth and sixth services, respectively, and one required nine services before conception. This group of cows had an average of 2.09 services per conception for the 46 cows that conceived. When this group was studied in more detail, it was found that the 24 cows bred from 51 to 60 days postpartum required an average of 1.65 services per conception to the fertile cows and those bred 50 days or less after parturition required 2.53 services. This low rate of conception was statistically highly significant when tested by analysis of variance.

For Group 2, in which the 50 cows were bred 61 to 90 days postpartum, 15 cows did not conceive from the first service. Three cows in this group were sold before conception; one each had one, two, and three services, but not enough

time for a return estrus. The 12 cows that required more than one service conceived as follows: five on the second, two on the third, three on the fourth, and two on the fifth service. The average number of services required per conception was 1.55 for the 47 cows in which conception occurred.

Group 3, in which 50 cows were bred after more than 90 days had elapsed since parturition, included only 12 cows that required more than one service. One of these was sold after the second service and before the time for a return estrus. One cow was sold sterile after five services. The remaining 10 conceived as follows: three at the second service, two at the third, three at the fourth, and two at the sixth. The services per conception averaged 1.54 for the 48 cows that conceived.

The above results on breeding efficiency in each group are reflected in the average number of days required from parturition to conception (Table 1). The averages in postpartum days to conception for each group were as follows: Group 1, bred 60 days postpartum or less, 87.5 days; Group 2, bred 61 to 90 days postpartum, 93.8 days; and Group 3, bred more than 90 days postpartum, 130.3 days. From the information presented in Table 1 it can be observed that the differences between Groups 1, 2, and 3 in postpartum days to first service, as an average, for the 50 cows in each group were 26.6 and 44.5 days, respectively, for the first service, but only 6.3 and 36.5 days for the average postpartum days to actual conception.

When the group bred 60 days or less after parturition was studied in more detail, it was found that the 24 cows bred from 51 to 60 days postpartum averaged 74.5 days from parturition to conception but the 26 cows bred 50 days or less after parturition averaged 100.5 days from parturition to conception. Seven of these cows required a considerable time and number of services before conception occurred. These results are so striking that the breeding of cows at an interval of 50 days or less after parturition cannot be justified. However, cows bred specifically for show purposes or for early fall freshening can be bred any time following 50 days postpartum if they possess excellent genital health, as did the cows in this experiment.

Information in Table 3 indicates that for cows bred within 60 days after parturition there is a distinct advantage to the cows with a previous estrus before service. The 17 cows with a previous estrus, when bred within 60 days after calving, had 70.6% conceptions on first service, as compared with 35.7% for 28 cows without a previous estrus. After cows have been fresh longer than

TABLE 3

Conception rates from first service at different post-partum intervals when cows are grouped according to number of estrous periods before service

Previous estrus	60 days or less			61-90 days		
	Cows	Conceptions		Cows	Conceptions	
(No.)	*(No.)*	*(No.)*	*(%)*	*(No.)*	*(No.)*	*(%)*
0	28	10	35.7	10	7	70.0
1	17	12	70.6	22	16	72.7
2	5	2	40.0	17	12	70.6
3	—	—	—	1	—	—

60 days there is no advantage in having a previous estrus. This can be expected on the basis of the longer time involved, resulting in a better genital condition, and the possibility of some cows with silent or unobserved periods of estrus.

After being assigned to the various groups, 82% of the cows in the group for service at an interval of 60 days or less had an estrus in time for service, and 94% had an estrus before 90 days, for the cows assigned to be bred between 61 and 90 days postpartum.

Another factor that should be considered to provide complete information is the number of cows with retained placentae and cases of metritis for the calvings resulting from these experimental services. Also included with this information, presented below, is the number of abortions in each group and the number of sterile cows. This information, with the first service given at the postpartum interval indicated, is as follows:

60 days or less	2 retained from 24 conceptions at first service 1 retained from 22 conceptions from additional services 1 aborted 1 sold sterile (5 services)
61 to 90 days	2 retained from 35 conceptions at first service 0 retained from 12 conceptions from additional services 1 aborted 0 sold sterile
More than 90 days	1 retained from 38 conceptions at first service 1 case of metritis from 38 conceptions at first service 2 retained from 10 conceptions from additional services 1 case of metritis from 10 conceptions from additional services 1 sold sterile (5 services)

The information presented above shows that when cows are in unquestionably good genital health the number of cases for retained placentae, metritis, or abortions is not increased with short or long postpartum intervals to first service. Since these cows were selected on the basis of good genital health, with only 9% eliminated for failure to meet these standards, the low number of 8, or 5.7%, of retained placentae from 139 normal parturitions and two abortions can be expected.

The results on frequency of retained placentae in this experiment can be compared with the incidence of retained placentae in the entire University dairy herd at Cornell from 1947 to 1953. For 1,083 calvings during this period there were 79, or 7.3%, retained placentae. These cows required considerable time and veterinary care before they were in good genital health. First service to these cows was at an average of 160 days after parturition. This abnormally long period was due to the time required to restore sufficiently good genital health to these cows to justify service. The results from service to these cows which were bred after the parturition at which they retained placentae, together with the conception rate at each service, were as follows: first, 47.1%; second, 19.6; third, 15.7; fourth, 3.9; fifth, 2.0; sixth, 2.0; ninth, 2.0; and 7.8% sterile cows. The average number of services per conception was 2.04 for the

fertile cows. Only six of the 79, or 7.6%, had retained placentae the following year. However, in checking on the time interval required from parturition to the conception time for previous and following conceptions, it was found that these cows had a relatively long postpartum interval to conception, and many of them had a history involving various reproductive disturbances for the previous two conceptions and for the parturition after the one at which the placenta was retained. This may indicate that the inability to discharge the placenta at the time of parturition is a reflection of reproductive inadequacies and disturbances for cows receiving a normal ration and kept under good management procedures.

There were no differences in the weights or sex ratios of the resulting calves in the three experimental groups.

Breeding 60 days or less after parturition showed no bad effects on the conception rate and genital health of the cows for the next pregnancy and calving after parturition from the experimental service. The average number of days to first service and the average time to conception for the next pregnancy were slightly shorter for the group bred 60 days or less after parturition in the previous year on experiment. The average of 1.69 services per conception for this group was also slightly better than that for either of the two other groups. Although these slight differences had no practical significance, it showed that there were no bad effects from these experimental services, for which only cows with good genital health were used. There were two sterile cows in each group; two, one, and one abortions; and two, one, and two cases of retained placentae for Groups 1, 2, and 3, respectively, for the next pregnancy after parturition from the experimental services.

SUMMARY

The results of this research with three groups of 50 cows each, bred 60 days or less, 61 to 90 days, and more than 90 days postpartum, showed a conception percentage from the first service of 48, 70, and 76%, respectively. Among the 50 cows bred 60 days or less, 24 were bred 51 to 60 days after parturition and 66.7% conceived, but only 30.8% conceived from the 26 bred 50 days or less postpartum.

The lower rate of conception for cows bred 50 days or less after parturition was highly significant. The above results are based on services only to cows with excellent genital health, since it is generally accepted that those with poor genital health will have unsatisfactory results from service with a short interval from parturition to first service. The results indicated that service at 50 days or less from the previous calving should be discouraged even for cows which are bred specifically for show or for changing to fall freshening. If the service is within 60 days after parturition, cows with a previous estrus have a much higher rate of conception than those that have not had an estrous period before the one at which service occurred.

There was only one sterile cow in each of the first and third groups, and none in the group bred 61 to 90 days postpartum.

Additional services required for the fertile cows which did not conceive from

the first service were considered. Including all services to fertile cows, the average number of services per conception were: first service 50 days or less after parturition, 2.52; 51 to 60 days, 1.65; 61 to 90 days, 1.55; and over 90 days, 1.54. The high rate for cows bred 50 days or less after parturition was statistically highly significant.

Although the average time from parturition to first service was only 40.9 days for the cows bred 50 days or less postpartum, the actual average days from parturition to conception was 100.5 days, which was decidedly longer than the 74.5 days for the cows first bred from 51 to 60 days. The group with first service from 61 to 90 days after calving averaged 93.8 days from parturition to conception.

The results for rate of conception from first service, average number of services per conception to fertile cows and the average days from parturition to conception indicated that for a good reproductive performance in dairy cows the first service should be over 50 days postpartum for normal cows with good genital health.

In this experiment, in which cows with unquestionably good genital health were used, there was no difference between the groups with short or long postpartum intervals to first service, in the number of cases of abortions, retained placentae, and metritis. Also, there was no difference in the above factors at the next calving and none in conception rate and average time required for the next pregnancy.

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LABORATORY TESTS, SINGLY AND IN COMBINATION, FOR EVALUATING FERTILITY OF SEMEN AND OF BULLS

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The evaluation of bull semen still remains one of the chief problems confronting the artificial breeding industry despite the fact that much research has been done. Branton *et al. (9)* have reviewed the literature covering this field. More recent work reports new approaches to the problem, including fructose content of semen and fructolysis *(1, 11, 16, 17, 24, 25, 26)*, objective methods of estimating spermatozoan motility *(4, 6, 8, 28)*, resazurin reduction time *(12, 13)*, metabolism of pyruvate *(27)*, and light-reflecting power of spermatozoa *(21, 22)*. The incubation of semen at body temperature has received considerable attention *(10, 23)*. Recently, studies of change in pH of semen were reported *(3)*. General studies of a number of different tests have been made by each of several workers *(2, 7, 14, 19, 20)*.

This paper reports studies carried out to evaluate some of the various tests that have been developed. Tests were studied that would be simple and rapid and thereby practical for routine use in a bull stud. Three trials were completed. The first two were carried out in the Foundation laboratory with semen from a nearby stud. The second included some of the most promising tests studied in the first trial plus additional tests. The third was carried out by an artificial breeding organization to study under field conditions one combination of tests.

EXPERIMENTAL PROCEDURE

In all trials, semen from bulls routinely breeding cows artificially was studied. Immediately after each collection of semen, a small sample sufficient for study was removed from the single or the combined ejaculates. The remainder of the semen was diluted and shipped from the stud for breeding cows. Semen samples were not discarded on the basis of tests made except a few with very low motility.

Motility of spermatozoa in aniline blue solution and in phosphate buffer was estimated with the samples at room temperature. For all other readings of spermatozoan motility the samples were held at 38° C. by means of a microscope stage incubator. Breeding efficiency was measured by 60- to 90-day nonreturns to first services on the day after collection of semen.

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Trial I.

The procedures for the tests run in this trial were as follows:

(a) Semen diluted at the rate of 1:50 in yolk-citrate-sulfanilamide (YCS) was incubated at 45° C. Motility of spermatozoa was estimated at intervals of 15 minutes. The diluter consisted of one part of 3.2% sodium citrate dihydrate in distilled water, one part egg yolk, and sulfanilamide at a final concentration of 0.3%.

(b) Methylene blue reduction time of spermatozoa at a temperature of 45° C. was determined. Five mg. of methylene blue chloride was dissolved in 100 ml. of a sodium citrate buffer prepared by adding 3.2 g. of the dihydrate salt to 100 ml. of distilled water. The procedure otherwise followed was that described by Beck and Salisbury *(5)*.

(c) pH of semen, diluted 1:10 in YCS or in 0.87% sodium chloride, was measured after incubation for 0, 1, 2, and 3 hours at 37° C. in a water bath. The samples were covered with mineral oil in small test tubes.

(d) Motility of spermatozoa diluted 1:50 in YCS was observed immediately after collection and at 2-day intervals during storage at 4° C. until no movement could be observed.

(e) Motility of spermatozoa, placed in a 3% aqueous solution of aniline blue and cooled slowly to 4° C., was estimated at 15-minute intervals. The semen was diluted at the rate of 1:40.

(f) Spermatozoa in undiluted semen were counted in an improved Neubauer haemacytometer. A Trenner automatic pipette facilitated dilution of the semen at the rate of 1:200 with a 4% solution of sodium chloride. Spermatozoa in all of the 400 small squares in the chamber were counted.

This trial was conducted during the months of August to November, inclusive, 1949. From two to ten samples of semen from each of five Holstein and seven Guernsey bulls were studied. There was a total of 65 or 72 samples, depending upon the tests. Each sample was represented by 33 to 309 first-service breedings, making a total number of 7,577.

Trial II.

Procedures for this trial were as follows:

(a) Methylene blue reduction time as in Trial I.

(b) Semen was diluted 1:50 in YCSPS (one part of yolk and one part of 2.9% sodium citrate dihydrate; the diluent also included 0.3% sulfanilamide and 500 units of penicillin and of dihydrostreptomycin per milliliter) and incubated at 38° C. and 4° C. Motility was observed after 0, 16, and 28 hours at 38° C. and after 28 and 52 hours at 4° C.

(c) Motility of spermatozoa was estimated after 0, 90, and 120 minutes in semen diluted 1:40 in a phosphate buffer consisting of 50 ml. of $M/15$ $Na_2HPO_4 \cdot 12H_2O$ and 6 ml. of $M/15$ KH_2PO_4 and held at 4° C. This study was initiated late in this trial because data which had been obtained after the beginning of the trial indicated that the brief survival of spermatozoa in aniline blue was due

largely to low pH. The phosphate buffer had a pH comparable to that of the semen-aniline blue mixture, which was approximately 5.5.

(d) Motility estimations were made at 0 and 2 hours after incubation at 38° C. in citrate-sulfa (CS). The ingredients were at the same concentrations as in YCSPS. The semen was diluted 1:50.

(e) Motility of spermatozoa in aniline blue was determined as in Trial I except that observations were made after 90 and 120 minutes.

(f) Spermatozoan motility was observed before and after temperature shock. A small test tube containing semen diluted 1:50 in CS at room temperature was plunged into water at 0° C., held there for 10 minutes, and then warmed for examination.

(g) Number of spermatozoa per milliliter of undiluted semen was measured by means of a Photelometer *(31)*.

This trial was initiated in December, 1950, and terminated in April, 1951. Each of the five Holstein and seven Guernsey bulls was represented by three to nine samples, and each sample by 77 to 472 first services. The number of samples per test varied from 26 to 61. There were 15,376 first services.

Trial III.

This trial was conducted in an artificial breeding organization by its personnel to study under field conditions one combination of tests. The stud was different from that in Trials I and II. The trial was run concurrently with Trial II. It included methylene blue reduction time, motility of spermatozoa in aniline blue solution after two hours at 40° C., and motility of spermatozoa immediately after collection and 1:40 dilution in YCSPS. Procedures were the same as for corresponding tests in Trial II. Seventy-one samples, with three to seven samples from each of the 14 bulls, were studied. Holstein, Guernsey, and Jersey breeds were represented. For each sample there were 55 to 239 first services performed on the day after collection, making an average of 146 services per collection and a total of 10,384 services.

RESULTS AND DISCUSSION

The characteristics of the semen from the bulls in the three trials are summarized in Table 1. The differences in breeding efficiencies among the trials may be due to variations among bulls or technicians or to other factors. Also, penicillin and streptomycin were included in the diluter in Trials II and III, but not in Trial I.

The simple correlation coefficients between nonreturn rates and some of the various methods of semen evaluation for the first two trials are presented in Tables 2 and 3. Correlations between various combinations of tests and nonreturn rates are in Table 4.

In Trials I and II the readings were made at various times or in various ways to determine the most satisfactory procedure. For example, the among-bull correlation between nonreturn rate and the estimation of progressive motility

TABLE 1
Semen characteristics of bulls (figures are averages)

	Trial I	*Trial II*	*Trial III*
Samples *(No.)*	65	65	71
Motile sperm in fresh semen *(%)*	68.5	68.7	75.5
Total sperm per ml. undiluted semen *(millions)*	1043	1210	1429
Total sperm per ml. diluted semen *(millions)*	9.9	8.5	10.5
Semen dilution rate *(1:)*	105.4	133.0	135.7
Methylene blue reduction time *(min.)*	15.6	21.4	15.7
60- to 90-day nonreturn rate *(%)*			
Averages	62.1	66.6	70.7
Ranges	49.6–70.6	55.6–72.2	64.6–76.0

of spermatozoa after 4 days in YCS was 0.71 (Table 2). The duration of total or progressive motility under these storage conditions is correlated to a much lesser degree with fertility (0.23 and 0.29, respectively). When evaluating semen in this manner one is justified, therefore, in making only one reading after 4 days of storage rather than following the much more laborious procedure of checking each sample at intervals of 2 days until motility ceases.

In Tables 2, 3, and 4 the among-bull correlations in almost all instances are higher than the total or within-bull figures. Stone *et al. (29)* and Erb *et al. (13)* made similar observations. This fact indicates that the possibilities of characterizing fertility of bulls by studying two or more samples of semen from each bull are much more promising than the characterization of individual semen samples in regard to fertility.

The main objective of these studies has been to find one test or a combination of tests which would enable a stud to predict, at the time semen is collected or shortly thereafter, the fertility of semen in the field and the fertility of bulls. In order to predict with accuracy, one must have a correlation coefficient close to 0.9 or higher. With a coefficient of 0.9 one is, by means of the test or tests, accounting for 81% (0.9^2) of the variation in fertility. If somewhat less than this percentage of the variation is accounted for, predictions will be less accurate. Furthermore, the fact that a correlation coefficient is statistically significant is no indication that it is of value for predicting fertility. For example, a correlation coefficient of 0.30 with 70 degrees of freedom is significant at the 1% level of probability. A test so correlated with fertility is, however, accounting for only 9% of the variation in fertility.

As can be seen in Tables 2 and 3, the only individual test in these studies which attained or approached a correlation coefficient of 0.9 with fertility was that on an among-bull basis for the incubation of spermatozoa in YCSPS at 38° C. Ludwick *et al. (23)* obtained a coefficient of 0.84 for a similar incubation test. The coefficient was calculated on an over-all or total basis. In preliminary results from additional trials with this test in this laboratory, the correlation coefficient has been considerably lower than that in Trial II. This test, however, merits additional investigation.

Since few individual tests listed in Tables 2 and 3 were sufficiently correlated with fertility to enable prediction, the correlations between nonreturn rate and

TABLE 2

Correlation between various tests and nonreturn rates, Trial I

Test	Storage time	Correlation coefficients		
		Total	Among bulls	Within bulls
	(days)			
Total motility during storage at 4° C. in YCS (72 samples)	0	0.20	0.13	0.25
	2	0.22	0.65	−0.01
	4	0.34	0.67	0.08
	6	0.34	0.59	0.18
	8	0.31	0.61	0.14
	10	0.33	0.53	0.19
	12	0.34	0.53	0.24
	18	0.21	0.26	0.18
	24	0.07	0.11	0.07
Drop in total motility during storage at 4° C. in YCS (72 samples)	2	−0.17	−0.71	−0.14
	4	−0.27	−0.69	0.02
	6	−0.26	−0.57	−0.07
	8	−0.24	−0.60	−0.04
	10	−0.25	−0.50	−0.10
	12	−0.29	−0.55	−0.15
	18	−0.13	−0.25	−0.06
	24	0.03	−0.07	0.07
Progressive motility during storage at 4° C. in YCS (72 samples)	0	0.20	0.62	0.04
	2	0.24	0.61	0.00
	4	0.32	0.71	0.09
	6	0.25	0.51	0.09
	8	0.33	0.67	0.14
	10	0.29	0.51	0.14
	12	0.38	0.34	0.19
	18	0.20	0.29	0.15
Drop in progressive motility during storage at 4° C. in YCS (72 samples)	2	−0.14	−0.45	−0.03
	4	−0.20	−0.64	−0.05
	6	−0.16	−0.25	−0.15
	8	−0.22	−0.37	−0.17
	10	−0.14	−0.15	−0.15
	12	−0.17	−0.30	−0.14
	18	−0.01	0.17	−0.06
Duration (days) of different degrees of motility at 4° C. in YCS (72 samples)				
Total motility		0.16	0.23	0.13
20% total motility		0.02	0.16	−0.08
50% total motility		0.27	0.36	0.20
60% total motility		0.31	0.48	0.23
Progressive motility		0.20	0.29	0.14
Duration of motility *(min.)* at 45° C. in YCS (73 samples)				
Total motility		0.12	0.37	−0.01
Progressive motility		0.14	0.44	0.00
Duration of motility *(min.)* in aniline blue at 4° C. (71 samples)				
Total motility		0.27	0.39	0.22
Progressive motility		0.13	0.25	0.08
Drop in total motility in aniline blue at 4° C. (71 samples)				
60 min.		−0.12	−0.33	0.00
120 min.		−0.03	−0.09	−0.01
Drop in progressive motility in aniline blue at 4° C. (71 samples)				
120 min.		0.17	0.77	−0.01

TABLE 2 (continued)

Test	Storage time	Correlation coefficients: Total	Among bulls	Within bulls
Total sperm per ml. diluted semen (65 samples)		0.26	0.57	0.01
Motile sperm per ml. diluted semen (65 samples)		0.30	0.53	0.10
Total sperm per ml. undiluted semen (65 samples)		0.27	0.54	0.01
pH drop in NaCl at 37° C. (72 samples)	1 hr.	0.26	0.53	0.02
	2 hr.	0.08	0.27	0.00
	3 hr.	0.21	0.42	0.08
pH drop in YCS at 37° C. (72 samples)	1 hr.	0.11	−0.16	0.19
	2 hr.	0.14	0.33	0.07
	3 hr.	0.08	0.26	0.02
Methylene blue reduction time *(min.)* (65 samples)		−0.37	−0.72	0.02

TABLE 3

Correlation between various tests and nonreturn rates, Trial II

Test	Samples	Correlation coefficients: Total	Among bulls	Within bulls
	(No.)			
Initial motility in YCSPS				
Total motility	61	0.38	0.61	0.29
Progressive motility	61	0.29	0.49	0.28
Storage in YCSPS at 4° C.				
After 28 hr. Total motility	59	0.28	0.52	0.15
Progressive motility	59	0.29	0.53	0.18
After 52 hr. Total motility	54	0.18	0.52	−0.03
Progressive motility	54	0.23	0.36	0.16
In aniline blue at 4° C. Drop in progressive motility				
After 90 min.	61	0.30	0.43	0.34
After 120 min.	61	0.31	0.50	0.31
In PO_4 buffer at pH 5.5				
At 4° C. Drop in progressive motility				
90 min.	26	0.50	0.76	0.47
120 min.	26	0.10	−0.14	0.09
Methylene blue reduction time	61	−0.33	−0.55	−0.14
Incubation in YCSPS at 38° C.				
After 16 hr. Total motility	59	0.54	0.86	0.39
Progressive motility	59	0.54	0.90	0.28
After 28 hr. Total motility	58	0.57	0.83	0.26
Progressive motility	58	0.42	0.70	0.14
After temperature shock in CS				
Total motility	61	0.01	0.08	−0.04
Progressive motility	61	0.05	−0.12	0.17
Drop in total motility	61	0.26	0.34	0.23
Drop in progressive motility	61	0.26	0.57	0.18
Incubation in CS at 38° C. for 2 hrs.				
Total motility	61	0.27	0.74	−0.01
Progressive motility	61	−0.12	0.12	−0.29
Drop in total motility	61	−0.06	−0.52	0.14
Drop in progressive motility	61	0.33	0.49	0.34
Total sperm per ml. undiluted semen	61	0.27	0.29	0.25
Total sperm per ml. diluted semen	59	0.22	0.24	0.21
Motile sperm per ml. diluted semen	59	0.32	0.41	0.26

TABLE 4

Multiple correlation coefficients of combinations of tests most highly correlated with nonreturn rates (Unless indicated otherwise, all are on among-bull basis)

Tests or combinations of tests	Correlation with nonreturn rate	
	Trial I	Trial II
Bulls *(No.)*	12	13
Samples *(No.)*	65	56
B[a]	−0.72	−0.53
F	0.72	0.54
M	0.08	0.40
BF	0.83	
BM	0.73	
FM	0.82	
BFM	0.90	0.73
BFMA	0.90	
BFMC	0.90	
BFMD	0.90	
BFME	0.90	
BFMG	0.91	
BFMH	0.90	
BFMW	0.92	
BFMEZ	0.90	
BFMGN	0.92	
BFMGW	0.92	
BFMGNE	0.94	
GNEW	0.79	
GNEWM	0.87	
BFMGNEW		
Total	0.45	
Among bulls	0.95	
Within bulls	0.39	
T		0.74
T_1		−0.72
T_2		0.86
T_3		0.90
T_6		−0.80
$BFMT_2$		0.87
$BFMT_3$		0.94
$BFMT_2T_3$		0.94
$T_1T_2T_3T_6$		0.93
BFT_3		0.94
BT_3M		0.91
T_3FM		0.93
T_2FM		0.87

[a] Key to symbols in table:

A pH of fresh semen in NaCl
B Methylene blue reduction time
C Sperm per ml. undiluted semen
D Initial progressive motility
E Total motility after 2 days in YCS at 4° C.
F Drop in progressive motility from initial progressive motility in YCS after 120 min. in aniline blue at 4° C.
G pH drop after 1 hr. in 0.87% NaCl at 37° C.
H Drop of total motility after 60 min. in YCS at 45° C.
M Initial total motility in YCS (trial I) or YCSPS (trial II)
N Drop in progressive motility after 120 min. in YCS at 45° C.
T Total motility after 2 hr. in CS at 38° C.
T_1 Drop in total motility in YCSPS after 16 hr. at 38° C.
T_2 Total motility in YCSPS after 16 hr. at 38° C.
T_3 Progressive motility in YCSPS after 16 hr. at 38° C.
T_6 Drop in total motility in YCSPS after 28 hr. at 38° C.
W (Initial total motility in YCS) ÷ (field dilution rate)

combinations of two or more tests were calculated. If a combination can be found in which the individual tests act in a supplementary or additive manner in accounting for or measuring sources of variation in fertility, enough of this variation may be accounted for to attain a correlation coefficient of 0.9 or higher and thereby to predict fertility with considerable accuracy. The relationship between such a combination of tests and of nonreturn rates may be determined by calculation of multiple correlation. Wallace and Snedecor *(30)* and Goulden *(18)* give detailed directions for computation. In the studies reported in this paper, multiple correlation coefficients on a total, among-bull and within-bull basis for various combinations of tests were calculated. The individual tests with the highest simple correlation coefficients or those quick and easy to perform, such as original total motility, were selected for this purpose. In Trial I, 59 multiple correlation coefficients for a combination of two tests, 45 for three tests, and 27 for four tests were computed. In Trial II, multiple correlation coefficients for 22 combinations of three tests, 13 for four tests, eight for five tests, two for six tests, and three for seven tests were calculated. Of these the most promising ones are presented in Table 4.

In Trial I the only combination of three tests that could be found with a correlation of 0.90 was methylene blue, drop in progressive motility after 120 minutes in aniline blue at 4° C., and initial total motility in YCS (BFM). As can be seen in the table, the addition of a number of individual tests to this combination did not increase the coefficient appreciably. Only by adding three to make a combination of six tests (BFMGNE) was the correlation coefficient increased considerably. With a combination of seven tests (BFMGNEW) a correlation coefficient of 0.95, accounting for 90% of the variation in fertility, was attained.

In Trial II the BFM combination, as well as some additional tests, was run. Here the correlation of BFM with fertility was only 0.73. This drop from the 0.90 value in Trial I could be expected, for in the first trial *(a)* these tests were selected from among many because individually they were those most highly correlated with fertility and *(b)* because the combination was the one most highly correlated with fertility of many combinations studied. With such selection one would expect that the correlations would be higher than the true or population values and that there would be regression toward the population figures when additional trials with these tests, selected a priori, are conducted.

Regression toward the population value probably also explains, at least in part, the comparatively low multiple correlation between the BFM combination and nonreturn rate for Trial III. The correlation coefficients were as follows: total, 0.31; among bulls, 0.55; and within bulls, 0.27. According to preliminary analyses of a fourth trial, the results of which will be reported later, still lower values have been obtained in this laboratory with this combination of tests. Factors, other than regression toward the population mean, causing these variations may have been: *(a)* Personnel. Men in Trials I and II, Trial III, and Trial IV were different. *(b)* Time. Erb and Waldo *(15)* compared semen tests with nonreturn rates over a 1-year period in a bull stud. The correlation coeffi-

cients varied greatly from month to month. *(c)* Range in nonreturn rates of individual bulls. These ranges decreased with successive trials, as can be seen in Table 1. *(d)* Cows.

In Trial II the combination of BFM with one of the readings of spermatozoan motility after incubation in YCSPS at 38° C. (T, T_1, T_2, T_3) gave coefficients above 0.90. One of the simplest and probably most practical combinations would be that of the three different readings of motility in the incubation test (T_2, T_3, T_4), which gave a coefficient of 0.93. A number of hours would elapse between collection of semen and completion of readings, but this would not be a serious disadvantage because, as stated above, all of these tests are probably of promising value only for establishing fertility of bulls and not of individual semen samples.

Although tests studied in combination with others were selected in most cases because individually they were comparatively highly correlated with fertility, such high correlation is not necessarily an indication that that test, when combined with one or more other tests, will significantly increase the multiple correlation coefficient. Conversely, a test with a low correlation may be of more value in this respect than one with a high coefficient. For a completely thorough study, therefore, one should use all individual tests, regardless of their correlation with fertility, in all combinations with all other tests. Such a study is extremely laborious. It is estimated that the time spent in all the calculations for the three trials reported in this paper was equivalent to that of a full-time statistical clerk working for one year.

In studies of this nature the attainment of correlation coefficients approaching 1.0 can not be expected. Laboratory tests can not measure variation in fertility contributed by processing methods in the laboratory, shipping or transportation conditions, technicians, and cows. Continual improvement of these sources of variation will, however, reduce the percentage of variation attributable to them and, therefore, increase the proportion measurable by laboratory tests. Furthermore, bulls in a stud have been selected for high fertility. If an unselected group of bulls representative of the population were available for study, one would expect a greater range in nonreturn rate and higher correlations between semen tests and fertility than with bulls commonly studied.

One criticism of most of the tests in these trials is that they lack objectivity and are subject to considerable error. This is especially true with estimations of motility. Electronic devices of the nature of those described by Rothschild *(28)* and Bosselaar and Spronk *(8)* may place estimations of spermatozoan motility or activity on an objective basis and would require little time.

Additional study of tests included in this paper and of others in various combinations and under varying environments and seasons is needed. In view of the work by Erb and Waldo *(15)*, the most promising ones, at least, should be studied over extended periods of time.

SUMMARY AND CONCLUSIONS

Three trials comparing correlations between nonreturn rates and single tests or combinations of tests for measuring quality of semen have been completed.

Trial I included 65 to 72 samples per test and 7,577 services; Trial II, 26 to 61 samples and 15,376 services; and Trial III, 71 samples and 10,384 services.

The only correlations with a magnitude of 0.9 or higher were those among bulls between nonreturn rate and *(a)* motility of spermatozoa after incubation at 38° C. in yolk-citrate plus antibacterial agents and *(b)* the combination of methylene blue reduction time, drop in progressive motility after 120 minutes in 3% aniline blue solution at 4° C., and initial total motility in YCS. With additional testing on an a priori basis in Trial II, the correlation for this combination was only 0.73, and in Trial III under field conditions, 0.55.

Throughout these studies the among-bull correlations were, in most instances, higher than the total or within-bull correlations. This fact indicates that the possibility of estimating differences in fertility among bulls is more promising than estimating differences among individual semen samples.

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FAT SEPARATION IN EVAPORATED MILK. III. GRAVITY SEPARATION AND HEAT STABILITY[1]

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Fat separation is a continuous problem in the production, handling, and storage of evaporated milk. This defect becomes most objectionable during prolonged quiescent storage at high temperatures. The butterfat rises to the upper surface forming a viscous, leathery layer, which may prevent pouring of the milk from a relatively small opening.

To retard fat separation, the manufacturer attempts to obtain effective homogenization and sufficient coagulation of the proteins during sterilization to give the product a high viscosity or "heavy body" *(1, 12, 14)*. These practical applications are aimed at three fundamental considerations: a reduction in the size of the fat globules, an increase in the viscosity of the suspending phase, and perhaps an alteration in the density difference between the fat globule mass and the suspending medium *(6)*.

A consideration of the fundamental factors controlling fat separation by necessity would include the phenomenon of heat stability, which might conceivably influence both the viscosity and the density difference between the fat particles and the suspending medium.

The effectiveness of homogenization is known to influence the heat stability *(2, 5)*. The interaction of these phenomena, therefore, made it necessary to consider heat stability simultaneously with the over-all problem of fat separation.

When milk is heated sufficiently, the protein undergoes a gradual but complete coagulation. During the process of sterilization of evaporated milk, this gradual coagulation of proteins, especially when carried out in a quiescent state, tends to form a gel structure, which accounts for the increased viscosity of sterilized evaporated milk as compared to the unsterilized product *(5)*. The manufacturing practice, therefore, has been designed to obtain a partial heat coagulation of the proteins. The individual factors that influence heat stability have been reviewed by Hunziker *(5)*. He considers the following factors to be of importance: *(a)* acidity, *(b)* albumin and globulin content, *(c)* concentration, *(d)* forewarming treatment, *(e)* homogenization, *(f)* possible actual differences in casein, *(g)* relative concentration of ions, *(h)* rennet forming organisms, and *(i)* total salt concentration.

Homogenization is less detrimental to heat stability in normal concentrated milk than in concentrated milk that is otherwise relatively unstable, e.g., milk

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with developed acidity. The destabilizing effect becomes greater as the pressure of homogenization is increased *(5)*. Webb and Bell *(11)* have shown that the temperature of homogenization (98.6-176° F.) of forewarmed concentrated milk has very little effect on the final heat stability.

A plausible mechanism for the destabilizing effect of homogenization was originally suggested by Tracy and Ruehe *(10)*. They attributed the destabilization to the adsorption of casein or phosphates on the newly created fat surfaces. More recently, Sommer *(8)* suggested that the destabilization might result from the adsorption of either cations or anions, resulting in a disruption of the ionic equilibria. The present commercial practice, therefore, must be adjusted to meet the two problems: maintenance of sufficient heat stability to permit sterilization and the prevention of excessive fat separation.

The primary purpose of this work was to determine the efficacy of the various fundamental factors in retarding fat separation in a commercial product. Thus, the size of the fat globules, viscosity, and density difference received direct consideration and heat stability received indirect consideration.

EXPERIMENTAL PROCEDURE

A general description of the method used for preparing the evaporated milk is given below. Where experiments involved departure from the normal process, the details are given with each such experiment.

Raw mixed milk from the University dairy plant was tested for butterfat and total solids by the Mojonnier technique and adjusted to a ratio of 0.44 fat to solids-not-fat by the use of cream containing 19% butterfat. The milk was forewarmed in steam-jacketed stainless steel open hot wells, which held approximately 10 gal. and which were equipped with small mechanical agitators. In all experiments, the forewarming temperature was 206° F.; only the holding time was varied to obtain relatively high heat stability. When the desired holding time had been completed, cold water was turned into the jackets to cool the milk to 180° F. within 2-3 minutes.

A nickel vacuum pan was used to concentrate the milk to slightly less than half its original volume. The temperature of evaporation was 130-135° F., and the water was removed at the rate of approximately 20 gal. per hour. Thus, the process of condensing lasted 30 minutes or less. The concentrated product was then homogenized in a Creamery Package homogenizer which had a rated capacity of 125 gal. per hour. A new, single service (Multi-Flo) valve was used each time the machine was assembled. The homogenized, concentrated milk was stored at 32-35° F.

A sample of the milk was tested for butterfat by the Mojonnier technique. Sufficient distilled water then was added to the milk to adjust the fat content to 7.95%, except that enough water was withheld for later addition so that it could serve as a carrier of "correction." The milk was filled into 14½ oz. cans, which had previously received an addition of correction, consisting of 0 to 4 oz. of calcium acetate, or 0 to 10 oz. of anhydrous disodium phosphate per 1,000 lb. of concentrated milk.

The sealed cans were placed into a sterilizer that had individual recesses 3 in. from the center of the reel. Thus, the cans were permitted to roll in a manner analogous to that of cans in an Anderson-Barngrover sterilizer. The speed of the reel was fixed at 11.5 r.p.m., and the reel was allowed to run throughout the coming-up process but was run only for the first 3 minutes of the holding period. The heating schedule during the coming-up process was as follows: 5 minutes to reach 212° F., 5 minutes holding at 212° F., and 5 minutes to reach 242° F. After holding at 242° F. for various times, cooling was accomplished by injecting a spray of cold water directly onto the cans with the reel revolving.

The cans of milk were opened immediately after cooling and examined for coagulation by viewing the milk in a thin film on the side of a glass beaker by transillumination. The appearance of visible grain particles was taken to be the most advanced degree of coagulation that would permit sterilization for subsequent observations. A minimum heat treatment of 14 minutes at 242° F. was considered to be essential for commercial sterility. Thus, from the series of samples it was possible to determine the quantity of correction, if any, that was necessary to prevent excessive coagulation in the milk that was to be stored for observations on fat separation. Where it was necessary to add correction to the milk, the details are given along with each experiment.

The heat stability of each batch of concentrated, homogenized milk was determined by varying only the time of holding in the sterilizer at 242° F. The entire process of sterilization was repeated a sufficient number of times to determine the heat stability in minutes, the end point being taken as the appearance of small grain particles.

Fat separation was determined by storing cans of the sterilized evaporated milk for 7 days at 100° F. and then removing a 25-ml. sample from the upper surface for determining the butterfat content *(6)*. The viscosity measurements were made at 100° F. with a modified Gardner mobilometer *(6)*. The method for calculating average viscosity was given in detail in a previous publication *(7)*.

EXPERIMENTAL PROCEDURE

Sterilizing time. The viscosity of different samples of unsterilized evaporated milk is low and uniform, but during the process of sterilization there is a gradual increase in the viscosity. It has been shown that a high viscosity in the finished product tends to retard fat separation *(1, 6, 12, 14)*. Observations by previous workers, however, were made on different samples of milk. Therefore, it was not possible to evaluate the total effect of sterilization on fat separation.

Evaporated milk was prepared by the general procedure up to the time of sterilization. Separate batches of the canned milk were then sterilized for varying lengths of time while the temperature was maintained at 242° F. A representative set of the results is given in Table 1. From these data, it is apparent that an increase in sterilizing time causes an increase in viscosity and a decrease in fat separation. This relation can be expressed simply by the equation $VN = k$, where V is velocity, N is viscosity, and k is a constant. If the

TABLE 1
The effect of sterilizing time on viscosity and fat separation

Forewarming time	Sterilizing time	Viscosity			Fat separation
		After 1 hr.	After 7 days	Calculated average	
(min.)	*(min.)*	*(cps.)*	*(cps.)*	*(cps.)*	*(% enrichment)*
15	14	7.3	6.0	6.2	39.6
15	18	8.8	7.0	7.3	27.0
15	22	11.0	9.3	9.6	12.4
15	26	14.3	10.4	11.1	8.3

velocity of rise of the fat globules is expressed by the test for fat separation *(6)*, and the k is taken as a constant in harmony with the conditions of the experiment, then fat separation multiplied by viscosity should give a constant value.

From the data in Table 1, it can be shown that viscosity multiplied by fat separation gives a progressively smaller value as the time of sterilization is increased. Thus, the indications are that fat separation is retarded more by an increase in time of sterilization than would be expected solely from the resulting increase in viscosity. It seems necessary, therefore, to consider other factors for an explanation of this phenomenon. One such factor might be the difference in density between the fat globule mass and the suspending medium.

Homogenization prior to forewarming. If there had been an increase in the density of the fat globule mass due to adsorption of proteins during sterilization, forewarming should produce a similar effect, but to a lesser extent. However, any material added to the fat globule during the normal process probably would be dislodged during homogenization, since homogenization is applied after forewarming. On the other hand, homogenization prior to forewarming would give an increased surface area of the butterfat, permitting additional adsorption.

Raw mixed milk from the University dairy plant was standardized and then heated to the desired temperature for homogenization. Immediately after homogenization at 2,000 lb. pressure, the milk was transferred to the hot wells and forewarmed. The forewarmed milk was concentrated and subsequently standardized to the normal composition of evaporated milk.

One major difficulty was encountered when the milk was homogenized prior to forewarming. Homogenization at low temperatures (93-135° F.) resulted in an exceedingly unstable concentrated product. To permit sterilization, it was necessary to homogenize the unconcentrated milk at 170° F., or above, or to rehomogenize the concentrated product at approximately 130° F. Therefore, it was not possible to carry out the experiment so that none of the albumin and globulin would be coagulated before homogenization.

The data in Table 2 show that the temperature of homogenization of unconcentrated milk has a marked influence on the heat stability of the forewarmed, concentrated product. As the temperature of homogenization was reduced from 175° F., there seemed to be a progressive reduction in the heat stability, even though forewarming at 206° F. was applied immediately following homogenization. When the unconcentrated milk was homogenized at a sufficiently high

TABLE 2

The heat stability of evaporated milk homogenized prior to forewarming

Milk sample	Temperature of homogenization	Forewarming time	Heat stability		
			Not rehomogenized	Rehomogenized at 1,000 lb.	Rehomogenized at 2,000 lb.
	(° F.)	(min.)	(min.)	(min.)	(min.)
A	170	25	20	—	—
B	164	0	11	—	13
B	164	10	13	—	17
B	164	20	13	—	17
C	130	20	2	2	2
C	175	20	15	21	20
D	130	20	2	2	2
D	175	20	15	22	22

temperature to produce a relatively stable concentrated product, the heat stability could be improved by rehomogenizing the concentrated milk at 130-135° F. This improvement in heat stability could be obtained by rehomogenizing the concentrated milk at either 1,000 or 2,000 lb. pressure.

As for fat separation observations, it was possible to utilize only a limited number of the samples that were homogenized prior to forewarming. The results indicated the possibility of producing evaporated milk with low fat separation tendencies when the temperature of homogenization was near 160° F.; on the other hand, when the homogenization temperature was 175° F., the fat separation was extensive.

Homogenization of cream. Since it was not possible to obtain direct information on the effect of coagulating albumin and globulin in the homogenized milk, an indirect approach seemed desirable. If the butterfat were not present during the forewarming process, there would be no adsorption of albumin and globulin onto the fat globules. Therefore, fat separation should be more rapid than under the normal process of manufacturing evaporated milk.

The above procedure has definite practical possibilities because the milk could be separated, allowing only the cream to pass through the homogenizer. The cream and skimmilk could be combined at any convenient stage in the process. Thus, the working capacity of the homogenizer would be increased threefold or more, and the separator would function also as a clarifier. However, this procedure might accentuate the problem of heat stability, since cream is extremely susceptible to the destabilizing effect of homogenization *(3, 10, 13)*.

With these considerations in mind, cream containing approximately 20% butterfat was heated to 136° F. and homogenized at 2,000 lb. pressure. Skimmilk was forewarmed at 206° F. for 25 minutes, then condensed to slightly less than half the original volume. These products were blended to give a mixture with the exact composition of evaporated milk. A part of the blended product was heated to 136° F. and rehomogenized at 2,000 lb. pressure. Each of the samples, with and without rehomogenization, was sterilized and stored for fat separation observations. A representative set of the results is given in Table 3.

Fat separation is apparently greater when the butterfat is withheld from

TABLE 3

Fat separation in evaporated milk made from homogenized cream and concentrated skimmilk

Rehomogenized	Viscosity			Fat separation
	After 1 hour	After 7 days	Calculated average	
	(cps.)	*(cps.)*	*(cps.)*	*(% enrichment)*
No	14.3	8.7	9.8	66.2
Yes	10.4	7.2	7.8	16.7

the normal forewarming process and homogenized in the form of cream. These results could be attributed to an adsorption of albumin and globulin during the normal forewarming process or to less effective homogenization of the high butterfat product. At any rate, fat separation was sufficiently pronounced to cast considerable doubt on the feasibility of separating the milk for fractional homogenization as a commercial process.

The heat stability of the above samples was sufficiently high to permit sterilization; it could be improved, however, by rehomogenizing the blended product at 136° F. This increase in the heat stability amounted to 4-5 minutes at 242° F. and was probably associated with an increased subdivision of the fat globules.

Repeated homogenization. To determine the effect of repeated homogenization on fat separation, evaporated milk was prepared according to the general procedure through the process of condensing. After standardization, the product was homogenized at 130-135° F. from one to seven times by repeatedly passing the milk through the homogenizer at 2,000 lb. pressure. The individual batches were sterilized and stored for fat separation observations. A representative set of the results is given in Table 4.

TABLE 4

The effect of repeated homogenization on fat separation

No. of times homogenized	Viscosity			Fat separation	Heat stability
	After 1 hour	After 7 days	Calculated average		
	(cps.)	*(cps.)*	*(cps.)*	*(% enrichment)*	*(min.)*
1	18.2	12.2	13.3	5.7	20
3	19.1	13.5	14.6	1.9	20
5	18.7	13.5	14.5	2.4	20
7	25.0	17.3	18.8	0.9	20

The data indicate that repeated homogenization under the conditions of this experiment has little effect on fat separation. This was especially true where homogenization was highly effective on the first passage of the milk through the machine. In addition, repeated homogenization did not alter the heat stability.

Temperature of homogenization of concentrated milk. Whole milk was standardized to the proper ratio of fat to solids-not-fat, forewarmed for 15 minutes, condensed, and adjusted to the desired temperature for homogenization. After homogenization, each batch of the milk was standardized to 7.95% butterfat.

TABLE 5

The effect of temperature of homogenization on fat separation

Temperature of homogenization	Viscosity			Fat separation	Heat stability
	After 1 hour	After 7 days	Calculated average		
(° F.)	*(cps.)*	*(cps.)*	*(cps.)*	*(% enrichment)*	*(min.)*
110	10.2	7.1	7.8	30.3	23
146	10.2	7.2	7.8	21.4	23
170	9.7	7.1	7.6	14.2	23

The remainder of the process was carried out according to the general procedure, which has been outlined previously. A representative set of the results is given in Table 5.

The data indicate that an increase in the temperature of homogenization results in less fat separation during subsequent storage. This could probably be attributed to an increase in the effectiveness of homogenization or to an increase in the extent of adsorption at the higher temperatures.

The heat stability was not altered by changes in the temperature of homogenization.

The effect of soybean lecithin on the efficiency of homogenization and fat separation. Holm *(4)* has pointed out that one of the forces to be overcome by homogenization is interfacial tension between the fat and the serum. Furthermore, Sommer *(9)* states that the size of fat globules attained in skimmilk with a given mechanical emulsifying action tends to vary inversely with the quantity of phospholipid that is added to butter oil. Thus, fat separation should be reduced through the addition of soybean lecithin to butter oil, since the emulsifying properties of soybean lecithin should alter the state of dispersion of the butterfat.

The previous experiments indicated that the dispersion of the fat globules exerted considerable influence on the final heat stability. Accordingly, the phospholipids of milk, through their emulsifying properties, might have an effect on heat stability.

The experiment was conducted as follows: butter from the University creamery was oiled off at 140° F. and filtered through cotton to obtain a clear butter oil. Sufficient soybean lecithin was dissolved in a part of the butter oil to represent 8% of the total weight. Skimmilk was forewarmed at 206° F. for 20 minutes, then condensed to slightly less than half its original volume. The condensed skimmilk and distilled water were blended with butter oil containing soybean lecithin and with butter oil alone to give products with the composition of evaporated milk. Each batch was mixed at 140° F. for 10 minutes in a motor driven emulsifier (Creamaid), and the resulting product was homogenized at 2,000 lb. pressure. The remainder of the process was carried out according to the general procedure.

The data, an example of which is given in Table 6, indicate fat separation was not materially altered by the addition of soybean lecithin. The results should be considered as quite significant, however, since the viscosity of the samples without added lecithin was much higher, thereby retarding fat separa-

TABLE 6

The effect of soybean lecithin on fat separation and heat stability

Per cent fat as soybean lecithin	Viscosity			Fat separation	Heat stability
	After 1 hour	After 7 days	Calculated average		
	(cps.)	*(cps.)*	*(cps.)*	*(% enrichment)*	*(min.)*
0.0	22.9	16.6	17.8	14.6	15
8.0	8.6	7.2	7.5	11.3	60

tion. Undoubtedly, the primary effect of the soybean lecithin was to increase the efficiency of homogenization. Microscopic observations showed a greater subdivision of the fat in the homogenized samples which contained soybean lecithin.

The soybean lecithin had a marked influence on the heat stability. Thus the results support the previous experiments where the state of dispersion of the butterfat had an influence on the heat stability. On the other hand, there is a possibility that the increase in heat stability resulted from a physico-chemical effect of the soybean lecithin.

DISCUSSION

In light of the present knowledge, there are three fundamental factors that control the rate of fat separation in evaporated milk. These factors are viscosity, size of the fat particles (effectiveness of homogenization), and density difference between the fat particles and the suspending medium.

The difference in density between the fat particles and the suspending medium is the factor which has the greatest appeal to potential investigators, because a reduction of this difference to zero is one way of completely eliminating fat separation. An increase in viscosity or effectiveness of homogenization can only prolong the time before objectionable fat separation would become apparent. Unfortunately, the means of increasing the relative density of the fat particles are extremely limited. Apparently this would depend on a closer association of materials like casein with the fat particles and in greater quantities than hitherto possible. With the present knowledge, this phenomenon can be accomplished only to a limited degree during the process of sterilization. Nevertheless, an advanced degree of coagulation gives a higher viscosity to retard fat separation. A high viscosity combined with effective homogenization is a relatively effective means of retarding fat separation. In commercial practice it seems advisable, therefore, to carry the coagulation process as far as possible without impairing the desirable properties of the finished product. This, of course, depends on the heat stability.

The method that was chosen for determining heat stability in this work had as its end point the appearance of small grain particles. It should be recalled, therefore, that the results are a measure of the resistance of the sample to coagulation by heat but do not indicate the maximum viscosity which could be produced in the evaporated milk. For example, one batch of evaporated milk showed

a heat stability of 20 minutes, and after sterilization for 19 minutes, had a viscosity of 18.2 cps.; another batch of evaporated milk showed a heat stability of 17 minutes and after sterilization for 15 minutes had a viscosity of 54.5 cps.

The increase in viscosity during sterilization is limited by the appearance of grain particles which, presumably, result from flocculation of the proteins. Since agitation of the cans of evaporated milk during sterilization hastens grain formation, it is logical to suspect that the hastening of grain formation comes from breaking of the gel structure, which permits flocculation of the proteins. It seems equally logical to expect that points of weakness within the gel structure would permit more rapid flocculation of the proteins and thereby enhance grain formation. Thus, the heat stability of evaporated milk, as determined in this work, would be reduced by the presence of weak points in the gel structure.

These weak points in the gel structure could conceivably arise either directly or indirectly from clumps of fat globules. If the weak points arise directly from clumps of fat globules, the result would be considered as physical, whereby there is a disruption in continuity of the protein phase. On the other hand, if the weak points arise indirectly from clumps of fat globules, the result would be considered as electro-chemical. The proteins that are enmeshed in the clumps and surrounding the clumps could be influenced by the electrical charge on the fat globules. Whether the proteins are either more heat stable or less heat stable than proteins that are free of the clumps, the effect would be the same. Consequently, there would not be a uniform rate of coagulation of the proteins throughout the milk, and weak points in the gel structure would result.

Thus, grain formation would be enhanced by the presence of clumps of fat globules. The concept that clumps formed during homogenization influence the heat stability of the finished product aids materially in explaining the results obtained in this work on heat stability. This is especially true where the heat stability of the milk could be improved by a second homogenization. In addition, the results extend the work reported by Doan *(3)*, who showed that increased fat clumping in cream is accompanied by a decreased stability of the cream toward coagulation by alcohol and by heat. The astonishing thing, however, is that heat stability should be influenced materially by the slight clumping which is visible in unsterilized evaporated milk.

The above comments on heat stability are intended merely as additions to the postulation presented by Tracy and Ruehe *(10)* and extended by Sommer *(8)*.

SUMMARY

The fundamental factors governing fat separation are considered in light of the normal commercial processing methods.

During sterilization there is an increase in viscosity; this is desirable for preventing fat separation during storage. Sterilization produces an additional reaction, which gives an increased retardation of fat separation. This phenomenon may be explained by assuming an increase in the density of the fat globule

mass. In harmony with the preceding supposition, there is considerable doubt about the feasibility of separation of milk for homogenizing the cream alone.

The most important factor governing fat separation is effective homogenization. In this respect the condition and temperature of the medium are extremely critical. At higher temperatures, homogenization is more effective. The maximum effectiveness of the homogenizer can be obtained if all known factors are adjusted to ensure complete dispersion of the butterfat.

The state of dispersion of the butterfat is of additional importance, since it affects the heat stability during sterilization. This work also shows that under certain conditions of homogenization, there is an increase in the heat stability.

ACKNOWLEDGMENT

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PRENATAL MORTALITY IN THE BOVINE BETWEEN PREGNANCY DIAGNOSIS AT 34-50 DAYS POST-INSEMINATION AND PARTURITION[1]

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Fertility of bulls used in artificial insemination is ordinarily measured by the percentage of cows that fail to return for service within a certain time interval after insemination. The interval on which the percentage is most commonly based is 60-90 days. The 60-90 day nonreturn rates are calculated at the end of the second month after the month of breeding. Obviously, there is a discrepancy between this method of calculating fertility and actual pregnancy, but at the same time this method serves a useful purpose.

Pregnancy diagnosis by rectal palpation early in gestation is a practical means of positively diagnosing pregnancy. The purpose of this study is to determine prenatal mortality between pregnancy diagnosis and normal parturition. The information obtained may be used to evaluate the accuracy of nonreturns as a measure of pregnancy.

Reynolds *(7)* reported that in domestic rabbits there seemed to be two periods in their gestation in which fetal mortality was increased. The first period was the 22d day of gestation, at which time the uterine tension is the greatest and the blood supply is lowered. The second period is the 2 days preceding parturition, at which time a large mass of fetal tissue is dependent upon a limited blood supply. Brambell *(3)* found that litter mortality occurred most often in wild rabbits between the 12th and 15th day of gestation. He also reported that individual fetal resorption and mortality was completely randomized throughout gestation.

Tanabe and Casida *(9)* found an embryonic mortality of 39.2% during the first 34 days of gestation in a select group of repeat breeder cows. Laing *(6)* reported 25% embryonic death in an experimental group of beef heifers that were slaughtered at 25 days post-insemination. Stewart *(8)* concluded that early embryonic death was the major contributor to the discrepancy between actual diagnosed pregnancies at 34-50 days and 30-60 day nonreturns. Burgess *(4)*, using nonreturn data, found that 16.2% returned to service at other than normal intervals. He estimated that 2 to 3% would return to service at a time greater than 120 days post-insemination. Hold *(5)* reported a 19.2% decrease in the status of pregnancy, based upon nonreturn data, between 3 weeks post-insemination and parturition, an 11.5% decrease in the period 3-12 weeks post-insemina-

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[2] Agent of the Bureau of Dairy Industry. These data were taken from a thesis presented by the senior author to the Graduate School of the University of Wisconsin in partial fulfillment of the requirement for a Master of Science Degree.

tion, and a 7.7% decrease from 12 weeks until parturition. Barrett *et al. (2)* observed a discrepancy of 14.9, 5.5, and 2.8% in favor of 30-60, 60-90, and 90-120 day nonreturns, respectively, as compared to pregnancy examinations at 35-49 days post-insemination. Adler *(1)* noted a difference of 5.5% between pregnancy diagnosis and 60-90 day nonreturns.

EXPERIMENTAL PROCEDURE

All data for this report were taken from records of the University dairy herds during the period from July, 1946, to January, 1952. All five major dairy breeds are represented in the data. Any datum was excluded if the animal was sold or died before the termination of pregnancy.

Routine pregnancy examinations were made upon all females 34-50 days post-insemination unless they had exhibited estrus prior to that time. Actual palpation of the amniotic vesicle was the sole criterion for a positive pregnancy diagnosis. The rectal palpation method described by Wisnicky and Casida *(10)* was used in all examinations. The return to estrus after a positive pregnancy diagnosis or observed abortions was used as evidence of embryonic and fetal mortality. Pregnancy was considered terminated the day the animal exhibited estrus or abortion occurred. All abortions attributed to disease were excluded from this study. A calf carried 240 days was considered a normal gestation. Except for two cases of stillbirth (one pair twins and one singleton), all calves born after 240 days were born alive.

This experiment covers 690 pregnancies in which complete data were recorded as to the actual termination of each pregnancy.

RESULTS AND DISCUSSION

The results are shown in Table 1. Forty-four of the 690 cows pregnant at 34-50 days post-insemination failed to maintain pregnancy to 239 days post-insemination. The mean length of gestation for the 646 cows that carried calves 240 days or longer was 278.5 days. The range in gestation for the same period

TABLE 1

A summary of the termination of pregnancy as evidenced by either the return to estrus or abortion in the interval from pregnancy diagnosis to 239 days post-insemination

Year	Total number pregnant 34-50	Days from insemination: From. preg. diag. to 60	Pregnancy terminations: 61-90	91-119	120-149	150-179	180-209	210-239	Total
1946-47	178	1	3	2	1	0	1	0	8
1948	128	2	2	2	1	1	3	0	11
1949	104	0	1	1	0	0	3	4	9
1950	137	0	1	2	2	0	1	0	6
1951	143	1	2	1	0	1	1	4	10
Total	690	4	9	8	4	2	9	8	44
Per cent loss of pregnancy		0.58	1.3	1.16	0.58	0.29	1.3	1.16	6.38

was 251 to 305 days. In each 30-day interval a small loss in pregnancy occurred. The interval with the least loss was the 150-179 days post-insemination interval. During this period only two animals returned to estrus. Statistical analyses of the variation of loss among intervals showed no significant difference ($P > 0.05$). The loss of pregnancy from pregnancy diagnosis to parturition had a range from a low of 4.38% in 1950 to a high of 8.65% in 1949. The mean was 6.38%, with a standard deviation of 1.87. This figure corresponds closely to the results obtained by Holt *(5)* using nonreturn data. The 60-day period 61-119 days post-insemination accounted for 2.46% of the total loss. The 60-day period 180-239 days post-insemination accounted for another 2.46%. The middle 60-day period accounted for only 0.87%. The remaining 0.58% was accounted for by the period from pregnancy diagnosis to 60 days post-insemination. This interval was variable in length, inasmuch as cows were examined for pregnancy over a 17-day span. The average length of this interval was only 18 days.

Since Barrett *et al. (2)* and Adler *(1)* have shown a difference of 5.5% between pregnancy diagnosis and 60-90 day nonreturns and this study indicates a loss in pregnancy of 6.38% from pregnancy diagnosis to normal parturition, about 12% should be subtracted from 60-90 day nonreturns to approximate the per cent resulting in a calf.

SUMMARY

Prenatal mortality was observed in 44 out of 690 females that were diagnosed pregnant at 34-50 days post-insemination. The fetal mortality between pregnancy diagnosis and parturition was 6.38% with a standard deviation of 1.87. During gestation the variation in loss of pregnancy between 30-day periods was not significant ($P > 0.05$).

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HISTOLOGY OF THE PITUITARY, TESTIS, AND ADRENAL IN RELATION TO REPRODUCTION IN THE BULL

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The relation between the histology of the endocrine organs and reproduction in bulls has not been studied extensively. Most of the reports dealing with normal and impaired fertility have been limited to the changes occurring in a single organ. In order to estimate the relative importance of the several possible causes of impaired fertility, the histology of the pituitary, adrenal, and testis in bulls is being studied at this station.

Smith and Smith *(11)* described the separation of the anterior lobe of the bovine pituitary into a central and peripheral zone. Bassett *(1)* described the central portion of the anterior lobe as a core running through the gland. She also stated that the peripheral portion of the lobe contains many alpha cells, whereas the central portion is devoid of alpha cells but rich in beta cells. Bassett *(1)* and Hall *(6)* described two kinds of beta cells in the anterior pituitary of cattle. Garm *(4)* described the following cell types in the anterior lobe of normal dairy cows: acidophils with small nucleus, acidophils with large nucleus, small basophils, large basophils, hyaline basophils, and heterotrophic amphophils. He believed that the last type of cell represents a late functional stage of hyaline basophils. He described the hyaline basophils as large basophils with pale pink homogenous areas in the cytoplasm. Cows with nymphomania showed no change in acidophils but an increase in frequency of large basophils, chromophobes with a large nucleus, hyaline basophils, and hypertrophic amphophils. Gilmore *et al. (5)* have studied the bovine hypophysis in relation to breed, age, milk production, and sex. They studied the gland by making statistical analyses of cell counts on comparable sections of the gland. They described an acidophil that seemed to be related to the sexual activity of the animal.

Friedman and Hall *(3)* and Smelser *(10)*, studying the pituitary of the steer, reported higher concentrations of gonadotrophin in the central core and of prolactin in the peripheral zone of the anterior lobe. These differences in the concentrations of hormones were not noted in the pituitaries of cows. Bassett *(1)* reported that the small basophil cells were virtually absent in steer glands but were increased and prominent in cows treated with stilbestrol and in pregnant cows.

Severinghaus *(9)* described a cycle of secretion for the chromophil cells of the anterior lobe of the pituitary in rats. He believed each type arises from chromophobes of the same type. He also called attention to the fact that different methods of fixation and staining may result in differences in classification of the acidophilic and basophilic cells.

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Weber *et al. (13)* described the glomerulosa of the bovine adrenal in cattle of different ages. They reported two types of cytoplasmic granules in the glomerulosa. One type appeared to be mitochondria and the second type, which was round and irregular in size, gave microchemical tests for phospholipid.

Wayman and Asdell *(12)* described the changes of the adrenals of nymphomaniac cows and pointed out the probable complex nature of adrenal activity in relation to this syndrome. Garm *(4)* reported a significant enlargement of the adrenals, confined primarily to the cortex, in all types of nymphomania. This enlargement was accompanied by a decreased amount of acetone-soluble birefringent crystals in the glomerulosa and a decrease in the urinary excretion of neutral ketosteroids. From cytological evidence, Garm concluded that the adrenals of nymphomaniac cows secrete abnormally large amounts of the salt-retaining hormone or hormones.

Phillips and Andrews *(8)* described the development of the spermatogenic tissue of bulls of different breeds. Growth and development of the tubules began at approximately 142 days of age, with the development of spermatozoa in many tubules at 224 and 261 days of age.

This report describes the histology of the pituitary, adrenals, and testis in normal dairy bulls and bulls with impaired breeding efficiency ranging down to total sterility.

MATERIALS AND METHODS

The bulls were slaughtered at a local slaughterhouse, and sections from the organs were placed as quickly as possible in freshly mixed formal-zenker, where they remained for 24 to 36 hours.

The pituitaries were split along the mid-sagittal plane to hasten fixation. At the time of collection, the adrenals were cut into many 5-7 mm. cross-sections, and two or three were placed in the fixative. In addition, if any portion of the gland appeared abnormal macroscopically, a section from that area also was saved for study. In both the pituitary and adrenal, it was found that better fixation occurred if the connective tissue capsule was left with the gland during fixation. One or two sections of testes approximately 1 cm. square and 5 mm. thick were also collected.

After fixation, the tissues were processed through a standard paraffin technique. The tissues were infiltrated with tissuemat (MP 52-55° C.) and embedded in tissuemat (MP 56-58° C.). Sections 6 and 8μ thick were cut in a refrigerated room after the blocks had been cooled.

The pituitaries were stained according to the Severinghaus technique. The adrenal glands and testes were stained with hematoxylin, eosin, and Azure II. During the early stages of the study several other fixatives and stains were used, but the method outlined above gave superior results.

The animals used in this study along with the available semen analyses and breeding histories are listed in Table 1. Since the animals have been collected from various sources, it has not been possible to obtain complete information on all of them.

TABLE 1

Animals used for collection of histological material

Bull No.	Breed	Age	Libido	Average fructose	Semen characteristics: Concen.	Percentage Live	Percentage Abnormal	Percentage motility	Fertility record
				(mg/100 ml.)	*(cu. mm.)*				
1	Holstein	3 yr. 4 mo.	normal	686	690,000	41	41	12	—
2	Holstein	2 yr. 8 mo.	normal	771	667,000	56	19	37	—
3	Holstein	3 yr. 2 mo.	normal	—	390,000	20	43.7	7	—
4	Holstein	3 yr. 4 mo.	normal	502	851,000	71.4	6.35	49	—
5	Holstein	mature	normal	525	1,692,000	—	—	—	71.8[a]
6	Jersey	mature	low	285	1,295,000	—	—	—	48[a]
7	Jersey	8 yr. 2 mo.	low	—	—	—	—	—	—
8	Jersey	7 yr. 1 mo.	low	—	—	—	—	—	24.1[b]
9	Guernsey	mature	low	—	low	—	—	low	—
10	Guernsey	mature	normal	—	normal	—	—	low	—
11	Guernsey	mature	low	—	low	—	—	low	49.6[a]
12	Hereford	2½ yr.	low	—	—	—	—	—	—
13[c]	Hereford	3 yr.	normal	—	1,000	20	80	1	0
14	Hereford steer	—	—	—	—	—	—	—	—
15	Holstein calf	150 days	—	—	—	—	—	—	—

[a] Fertility estimate based on 60-90 day nonreturn to first service while semen from the bulls was used for breeding.

[b] The last recorded conception from bull 8 in August, 1952, and he was slaughtered on October 21, 1953. This figure represents breeding per conception.

[c] Adrenals not studied in bull 13.

Bulls 1, 2, and 3 were inbred Holsteins previously described by Cupps *et al.* *(2)*. Treatment of these animals with follicle stimulating hormone increased the concentration of sperm without changing the other characteristics. Bull 3 was also injected intramuscularly with 50 I.U. of ACTH (Armour's achtar gel)[1] every other day for 8 weeks. During the injection period, the abnormal forms decreased from 43.7 to 29.2%. After cessation of treatment, the abnormal forms returned to the pretreatment levels. These bulls were slaughtered 4 to 6 months after the injections ceased. Bulls 4 and 5 were normal bulls. Bulls 6, 9, 10, and 11 were discarded by an artificial insemination stud because of lack of libido or because of poor quality semen. Bulls 7 and 8 had been returned from breeding herds for lack of libido and low fertility. Bull 12, a Hereford, had been removed from a breeding herd because he lacked sex drive. Bull 13, also a Hereford, had been used in natural service but had failed to settle any cows. A pituitary from animal 14, a steer, and the testis from number 15, an immature Holstein calf, were studied for comparative purposes.

RESULTS

The anterior lobes of the pituitaries of the normal bulls may be divided into two zones. The peripheral zone is composed largely of alpha cells. An occasional small beta cell may be seen in this zone of the gland. The central zone is deficient in alpha cells but is rich in beta cells. In mid-sagittal sections the central zone appears to be a core running through the lobe. Although the location and extent of this area is variable, it generally lies adjacent to the cleft in the anterior region of the lobe and centrally in the posterior region of the lobe. It usually does not extend the entire length of the lobe. The cells of the anterior lobe are arranged in cords and small acini which are surrounded by delicate strands of connective tissue. The entire lobe is very vascular, especially through the central zone.

The cell types described by other authors are seen in the normal glands. For descriptive purposes, the cell types in this article will be designated as follows: The alpha cell is one in which the cytoplasm is filled with bright red granules when fixed and stained as described above. The small beta cell is small and rounded, takes a dark blue stain, and contains a golgi apparatus lying close to the nucleus. The large beta cell is ovoid, takes a light blue stain, and contains a golgi apparatus peripherally in the cytoplasm. The chromophobe cell lacks a well-defined cell wall, and the cytoplasm takes very little stain. Cells with large nuclei similar to those described by Garm *(4)* are also present in the normal bull. Except for the size of the nucleus, they resemble the large beta cells and will be classified as such.

According to histological evidence, the chromophilic cells of the pituitary appear to undergo a cycle of secretion. The alpha cells may be seen in stages from many granules to cells with sparse granules. During the cycle of secretion,

[1] Courtesy of Armour Laboratories.

the small beta cells become rounded, the nucleus and golgi apparatus increase in size, the cell wall becomes well-defined, and the cytoplasm takes a deep blue color. Coarse blue granules then appear in the cytoplasm, giving the cell a granular appearance. At this stage an occasional red granule may be seen in the cytoplasm. These red-staining bodies may be mitochondria. Still later the nucleus begins to shrink and becomes pyknotic, and the blue granules become coarser. Then pale gray areas begin to appear in the cytoplasm. Later these areas become larger and begin to stain pink. Still later, the cell itself shrinks and takes a pink stain, and the cytoplasm becomes glassy in appearance. The large beta cells also undergo a cycle of secretion, and at certain stages it is difficult to distinguish between the two types of beta cells. However, at the stage of full secretory activity of large beta cells, the cytoplasm is filled with very fine purple granules that give the cell a purple hue in contrast to the bright blue of the small beta cells. During the stages of degeneration, the large beta cells form clear, pale gray areas in the cytoplasm, giving the cell a foamy appearance. The nucleus becomes pyknotic. At this stage the cell may become rectangular or very misshapen, the cytoplasm crowding around the other cells of the acinus and filling the entire central portion of the acinus. Figure 1 shows some of the various types of cells seen in the central zone of the pituitary of the normal bull.

The adrenal cortex of the normal bull may be divided into three zones, as has been described for the adrenals from other species. It differs from some species in that cords of cells from the glomerulosa seem to penetrate through the fasciculata and spread out to form part of the reticularis. The fasciculata is the widest of the three zones in the normal animal. The reticularis seems to be composed of a blending of the types of cells seen in the two other zones. In addition, strands of the connective tissue capsule of the adrenal may be seen growing through the cortex into the medulla. These ingrowths of connective tissue carry the glomerulosa with them so that glomerular tissue may be seen at the junction of the cortex and medulla. The zona glomerulosa of the normal bull consists of cords and cylinders of columnar cells with vesicular nuclei and vacuoles in the cytoplasm. As they approach the fasciculata, the cells tend to become compressed and lose their cord arrangement. After fixing and staining as described above, most of the glomerular cells contain one or more round, irregular-sized granules that stain bright red. Some of the cells contain large numbers of these granules making the glomerulosa appear red when viewed under the low powers of the microscope.

The fascicular cells are the largest in the adrenal cortex. They are rounded and contain an abundance of cytoplasm and large vesicular nuclei. The cytoplasm is granular and contains vacuoles that are smaller than those of the glomerular cells. The red granules are sparse, the cell being limited to two or three at most, and many cells contain none. Generally the granules are smaller and more regular than those seen in the glomerulosa.

The reticularis seems to be composed of cells of the other two layers merging together. Large, small, and degenerating cells are all seen in the reticularis. Some of the cells contain eosinophilic granules as described in the other two

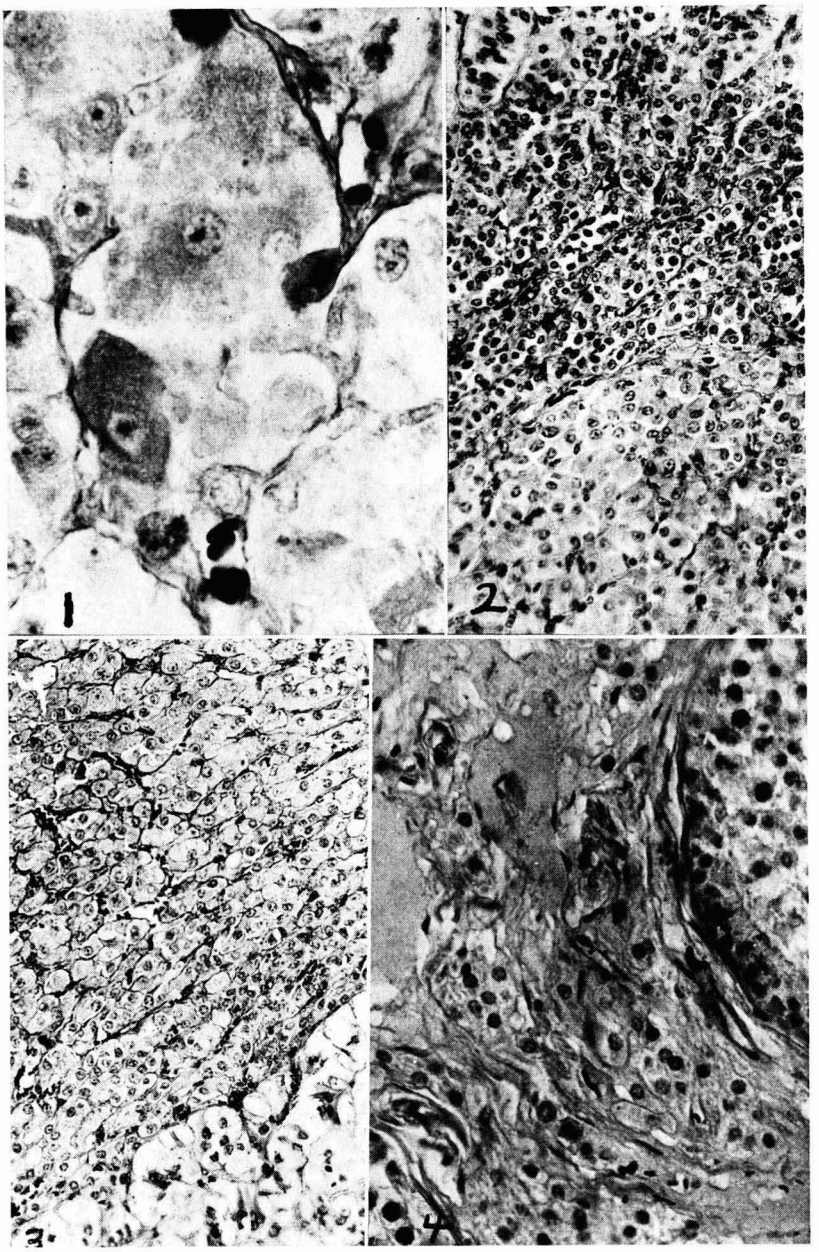

PLATE I

FIG. 1. Pituitary from a normal bull, No. 4. Large beta cells to left and below center and in the lower right-hand corner. Small active beta cells on left and top margin. Hyaline small beta cell in right center. × 862.5.

FIG. 2. Section of the glomerulosa and fasciculata of the adrenal, normal bull No. 4. × 187.5.

FIG. 3. Reticularis, normal bull No. 4. Medulla in lower right corner. × 187.5.

FIG. 4. Testis of normal bull No. 4. × 862.5.

layers. The growth pattern described above causes the boundary between the reticularis and fasciculata to be very irregular. Figures 2 and 3 are photomicrographs of the normal adrenal.

The testis of the normal bull is similar to that described in other species. The tubules are large, and all phases in the development and maturation of the spermatozoa may be seen. Different stages of development are seen in different tubules, suggesting a wave-like development of spermatogenesis in the individual tubule. There is a moderate amount of interstitial tissue, and the cells are characterized by well-defined cell walls, round vesicular nuclei, and vacuolated cytoplasm. The interstitial cells, sertoli cells, and some spermatogonia also contain small eosinophilic granules similar to those seen in the adrenal gland. Figure 4 shows the normal testis.

Changes observed in the pituitary glands of the low-fertility bulls are as follows: an increased hyaline degeneration of the small beta cells, a hypertrophy and hyperplasia of the large beta cells, a decrease in amount of the basophilic zone in the pituitary, and a failure of the beta cells to differentiate in a normal manner. Increased hyaline degeneration of the small beta cells was associated with a decreased concentration of spermatozoa in the semen. In bull 13 this degeneration was severe. The concentration of spermatozoa in his semen was 1,000 per cubic millimeter, and the seminiferous tubules resembled those seen in hypophysectomized animals of other species. Figure 5 is a photomicrograph of the pituitary and Figure 6 is a photomicrograph of the testis from this bull. In addition to number 13, bulls 1, 2, and 3 showed an increased degeneration of the small beta cells in the pituitary gland. In these bulls, the large beta cells appeared normal.

In comparison with normal and low-fertility bulls, the pituitary from the steer showed an interesting variation with respect to the small beta cells. Early stages of cells similar to the small beta cell were numerous. However, they stained green instead of blue, and granules were absent. An occasional degenerating cell similar to these small beta cells was seen. These cells contained a pyknotic nucleus and shrunken cytoplasm, but the cytoplasm did not become hyaline. The histological picture of the steer pituitary indicates a lack of differentiation and secretion by the small beta cell. There was an increased number of the small beta cells in the pituitary of bull 10. These cells appeared to be in full secretory activity (Figure 14).

The other major change that occurred in the pituitary of the low-fertility bull was a hyperplasia and hypertrophy of the large beta cells. This basophilism was accompanied by an increased degranulation of the alpha cells. Figure 7 shows the pituitary from a bull with this basophilism.

The histology of the other endocrine organs associated with the pituitary basophilism indicates a complex syndrome, and the limited number of bulls studied in this series may not have shown all the possible variations. The pituitary change described above is associated with two different conditions in the adrenal gland. The first type of change seen in the adrenal was hyperplasia of the adrenal cortex, especially of the glomerulosa and reticularis (Figures 8

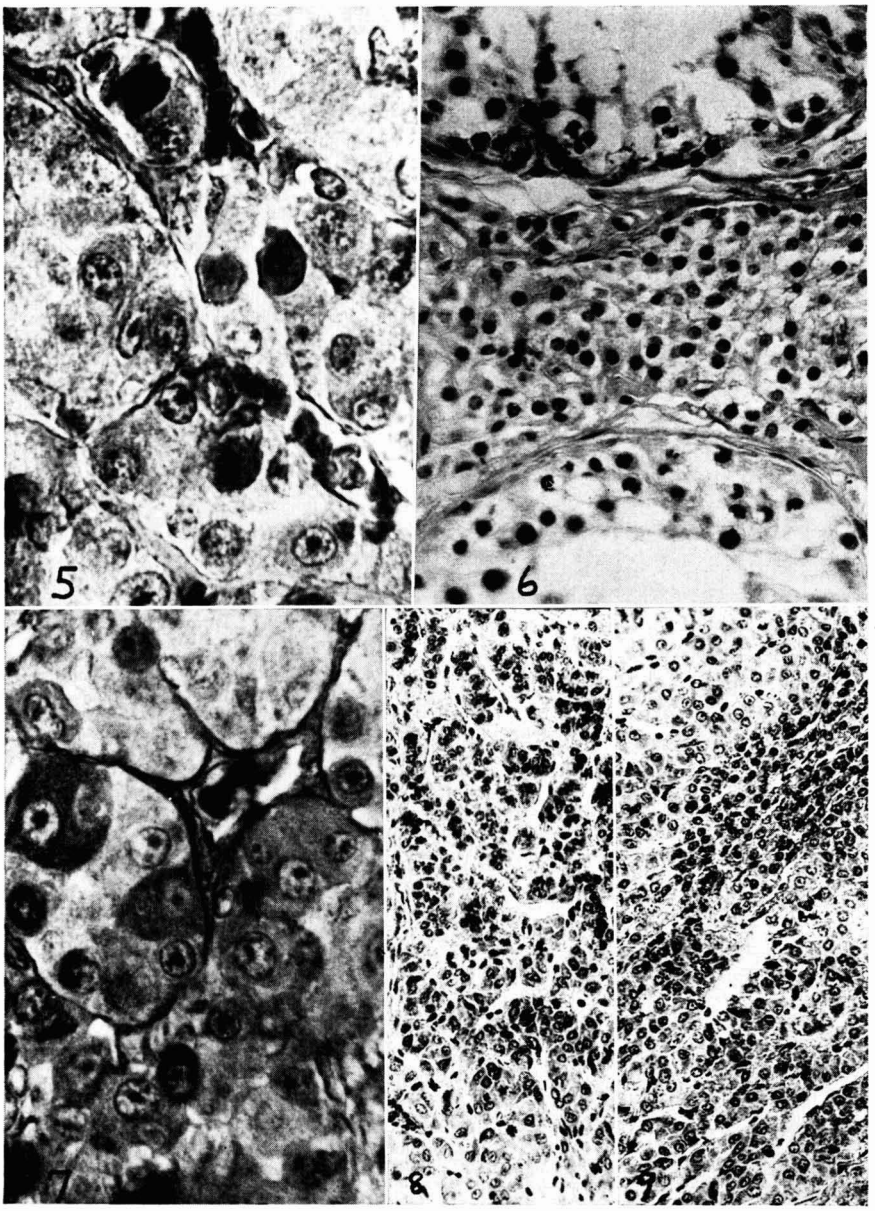

PLATE II

FIG. 5. Pituitary from bull No. 13. Note the extensive hyalizination of the small beta cells. × 862.5.

FIG. 6. Testis from bull No. 13. Note the degeneration of the seminiferous tubules. × 862.5

FIG. 7. Hypertrophy and hyperplasia of large beta cells of pituitary, bull No. 7. × 862.5

FIG. 8. Glomerulosa and fasciculata of adrenal gland. Fasciculata along the lower edge of the photograph. Adrenal hyperplasia, bull No. 9. × 187.5.

FIG. 9. Fasciculata and reticularis of adrenal gland. Adrenal hyperplasia, bull No. 9. × 187.5.

and 9). This hyperplasia was accompanied by increased degeneration of cortical cells and a decrease in the eosinophilic granules within the cells as compared with those of the normal gland. The fasciculata of these adrenals was narrow as compared with that in the normal bull.

Bulls 6, 7, and 9 showed the condition described above. The seminiferous tubules were essentially normal in these bulls and the concentration of spermatozoa was normal or low. When the concentration of sperm was low, the small beta cells showed an increased hyaline degeneration. The interstitial tissue appeared to be essentially normal histologically with the exception that the eosinophilic granules were sparse. The two other bulls (No. 10 and 11) that showed the basophilism contained tumors of the fasciculata of the adrenal gland (Figures 10, 11, and 12). The adrenals of these two bulls also showed a shrinking of the cells of the glomerulosa and reticularis with sparse granulation as compared with normal bull adrenals. The sparse granulation in these two zones of the adrenal was similar to that seen in bulls 6, 7, 8, and 9. The testes from bulls 10 and 11 were characterized by a hyperplasia of the interstitial tissue (Figure 13). However, the interstitial cells were shrunken and lacked vacuoles as compared with those of normal bulls.

The number of large beta cells in the pituitary of the steer increased, and many of them contained fine purple granules. Many of the acini contained accumulations of the fine granular material. Apparently during fixation and staining, shrinkage of this accumulated material caused the formation of a clear area around the cells. In some cases the material entirely surrounded the cell, giving the appearance of a cell within a cell. Beta cells with large nuclei were present and some of them contained the fine granular material described above. The golgi apparatus was prominent in many of the large beta cells. Many of the large beta cells contained pale gray areas in the cytoplasm.

Connective tissue was increased in the pituitary of bull 8. The beta cells were sparse and lacked differentiation, the interstitial cells resembled those seen in the immature bull, and the tubules had lost all the spermatogenic tissue and had become hyalinized. The adrenals lacked granulation. This may be the final stage of the syndrome described above.

The pituitary of bull 12 contained a very small amount of basophilic tissue. The histology of the other organs indicated a hypofunction of the basophilic tissue of the pituitary. Spermatogenesis was not complete, the interstitial tissue resembled that seen in the immature animal, and the adrenals were small as compared to the normal.

The adrenal cortices from bulls 1, 2, and 3 differed from those of the normal bulls and from those that showed hypertrophy of the large beta cells of the pituitary. The glomerulosa and fasciculata were relatively narrow. The glomerular cells were cuboidal to low columnar and contained a moderate number of granules. The cells of the fasciculata appeared normal. The major change in the adrenals of these bulls occurred in reticularis, which was thickened. Very few degenerating cells were present and there was an increased number of eosinophilic granules. The testes from these bulls showed deficient spermatogene-

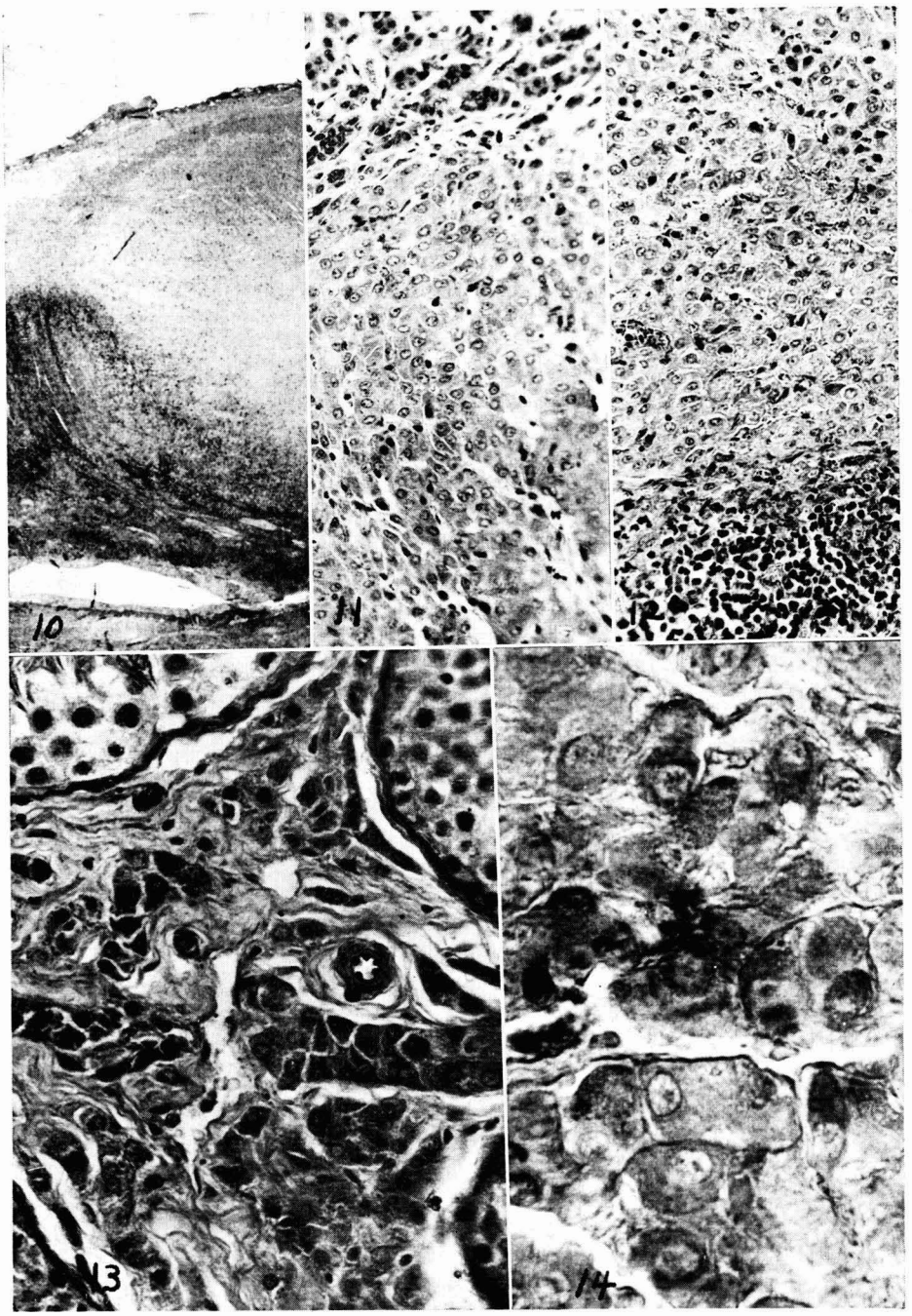

PLATE III

FIG. 10. Adrenal gland showing tumor in fasciculata, bull No. 10. × 15.

FIG. 11. Adrenal glomerulosa and fasciculata, bull No. 10. Fascicular tumor. × 187.5.

FIG. 12. Fasciculata and reticularis, bull No. 10. Fascicular tumor with leucocytes in the reticularis. × 187.5.

FIG. 13. Testis showing hyperplasia of interstitial tissue, and normal spermatogenesis, bull No. 10. × 862.5.

FIG. 14. Pituitary, bull No. 10. Basophilism with adrenal tumor. Hyperplasia of small basophils which are normal. × 862.5.

sis. Some of the tubules had degenerated and many contained no developing spermatozoa, nor were there any spermatozoa in the lumen. The interstitial tissue appeared normal although the cells were less vacuolated than those seen in the normal bull.

DISCUSSION

According to the histological changes in the endocrine organs studied, impaired fertility of these bulls may be divided into three broad groups. This grouping is somewhat arbitrary because certain individuals in the study showed some characteristics of more than one group.

The first group is characterized by bull 12. The primary reason for low fertility in this animal appeared to be a deficiency in the amount of basophilic tissue in the pituitary. Lack of differentiation of the beta cells, incomplete spermatogenesis, immature appearance of the interstitial tissue, lack of masculinity, and the animal's behavior also indicated a hypoactivity of the pituitary.

The syndrome shown by the second group of bulls (No. 1, 2, 3, and 13) is complex, with changes occurring in the pituitary, adrenal, and testes. The increased hyaline degeneration of the small beta cells, with the decreased rate of spermatogenesis, indicates that the small beta cells are associated with the growth and differentiation of the spermatogenic tissue. The semen contained many abnormal spermatozoa. Some were tailless, others showed other characteristic abnormalities, such as bent tails, coiled tails, abnormally large or small heads, and tails that appeared to be folded on the head in the form of a figure eight. The normal interstitial tissue, the normal libido, and the normal amount of fructose in the semen indicated that testosterone was being secreted in normal amounts. That the changes in the adrenal occurring in this syndrome are related to the production of poor-quality semen is indicated by the decrease in abnormal forms when treated with ACTH. The data collected at the present time indicate that changes in the secretion of more than one hormone are involved.

The third syndrome also is complex, involving the pituitary, adrenals, and testes. Bulls 6, 7, 8, 9, 10, and 11 showed features of this syndrome in its broad outlines. The features common to all these bulls were an increase in large beta cells of the pituitary and low motility of the sperm. On the basis of the adrenal histology, these bulls may be divided into two groups. Bulls 10 and 11 contained tumors of the fasciculata, and bulls 6, 7, 8, and 9 showed hyperplasia of the cortex.

Bull 10 produced semen that varied from normal to high in sperm concentration; his libido was normal. Bull 11 produced semen with a low sperm concentration; libido was poor and fertility was low. The 60-90 day nonreturn rate of the normal bull to 493 first services was 71.8%, and the 30-60 day nonreturn rate was 78.6%. The nonreturn rate on 129 first services for bull 11 was 49.6% for 60-90 days and 62.8% for 30-60 days. The quality of the semen at the time of slaughter was not as good as when the bull had been used in the stud. The decrease in nonreturn rates from 30-60 to 60-90 days in the normal bull and

bull 11 was 6.8 and 13.2%, respectively. The large percentage difference in the nonreturn rates from 30-60 to 60-90 days for bull 11 indicates that the vitality of his sperm was lowered to the point that, although they were capable of fertilization, the resulting embryos had a high mortality. Antibiotics were used in the egg yolk–citrate extender with which the semen used for breeding was diluted. Further work will be needed to test the possibility that the high embryonic mortality is the result of fertilization of ova by sperm of low vitality.

Semen from bulls 6, 7, and 9 also was low in motility. Bull 6 produced semen with a normal sperm concentration, 1,295,000 sperm per cubic millimeter. In bull 9 the concentration was low. Data on concentration for bulls 7 and 8 were not obtained. On the basis of the histological appearance of the testis, spermatogenesis was normal in bull 7 and had ceased in bull 8. The fructose content of the semen from bull 6 was low compared with that of the normal bulls 4 and 5. Conception rate for bull 6 was 48% 60-90 day nonreturn to 123 first services and 56.1% for 30-60 day nonreturn. The percentage drop in the 30-60 to 60-90 day nonreturn was 8.1%, intermediate between the normal and the other abnormal bull.

Changes in the large beta cells of the pituitary in this group consisted of hypertrophy and hyperplasia. In many respects the large beta cells resembled those seen in the steer but differed in that granules in the cells were not as abundant. Except for bulls 10 and 11, which contained tumors in the fasciculata, the adrenal changes resembled those described by Garm *(4)* in cows with nymphomania. Changes in the pituitaries of all these bulls resembled those described by Garm. According to him, the urinary secretion of 17-ketosteroids in cows with adrenal hyperplasia and basophilism similar to that seen in these bulls was low. The low fructose found in bull 6 and the low libido in bulls 6, 7, 8, 9, and 11 indicate that secretion of testosterone may be low. However, further work must be done to determine the excretion rate for ketosteroid substances.

On the basis of histological studies, it is not possible to determine which organ is primarily at fault. All the changes seen in the endocrine organs may be secondary to a more fundamental change in the metabolism of the animals. If the changes in the endocrine organs are secondary, treatment with hormone preparations probably will not be effective since they will correct only the secondary effects resulting from the malfunction of the organ secreting the hormone. Garm *(4)* has suggested that the primary change in cows with similar histological changes in the adrenals and pituitary is an over-secretion of the salt-retaining hormone or hormones from the adrenal. If this theory is correct, therapy to correct the condition will be very difficult. Since the ovary, testis, and adrenal may all secrete ketosteroids, chemical determination of total ketosteroids may not be a very accurate estimate of the primary changes occurring in such animals.

The lowered content of the eosinophilic granules, combined with other cell changes, indicates changes in the metabolism of the adrenals in this group of bulls. Since Weber *et al. (13)* have shown these granules in the adrenal to be phospholipid, it is possible that the metabolism or transport of fat and sterols is abnormal.

Mann and Parsons *(7)* injected 200 I.U. PMS daily into hypophysectomized male rabbits. After this treatment the fructose content of the seminal vesicle was low and the citric acid was high. This phase of the problem is being investigated to determine if the relation existing between the seminal fructose and citric acid in the semen of bulls with the third syndrome herein reported is similar to that found in rabbits by Mann and Parsons.

SUMMARY

On the basis of histological changes in the pituitary, adrenal, and testis, three syndromes related to impaired fertility in the bull have been described. The first syndrome was found in one bull (No. 12) and appeared to be a deficiency of the basophilic tissue in the anterior lobe of the pituitary.

The second type of syndrome was found in four bulls (No. 1, 2, 3, and 13). In these animals the large beta cells of the pituitary were normal, but many of the small beta cells showed hyaline degeneration. The glomerular and fascicular zones of the adrenal cortex were narrow but the reticularis showed hyperplasia. The testis showed faulty spermatogenesis with degeneration of some tubules and normal interstitial tissue. The concentration of spermatozoa in the semen was reduced, most of the sperm were dead, and many abnormal sperm were present. The libido and seminal fructose were normal.

Six bulls (No. 6, 7, 8, 9, 10, and 11) were grouped into a third syndrome because changes in the large beta cells and the alpha cells of the pituitary were similar. The large beta cells were increased in number and size; the alpha cells were sparse and many were lacking in granules. The small beta cells increased in number and appeared to be functional in bull 10. In bulls 6 and 7 the small beta cells appeared normal. In bulls 9 and 11 the small beta cells showed degenerative changes. In bull 8 these cells were not differentiated. Bulls 10 and 11 contained small tumors in the fasciculata of the adrenal. Bulls 6, 7, 8, and 9 showed hyperplasia of the adrenal cortex accompanied by degenerative changes in the individual cells. Libido was normal in bull 10 but low in bulls 6, 7, 8, 9, and 11. Seminal fructose was low in bull 6 of this group but was not measured in the other bulls. Motility of the spermatozoa was low, and fertility as measured by available data was impaired. The histology of the testis indicated that spermatogenesis had ceased in bull 8. Further study may reveal that the syndrome described for the third group of bulls is really more than one syndrome.

The pituitary, adrenal, and testis from two normal bulls (No. 4 and 5), the pituitary from a steer, and the testis from an immature calf were studied for standards of comparison with the low-fertility bulls.

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THE EAR AND LEAF-STALK CONTENTS OF CORN FORAGE AS FACTORS IN SILAGE EVALUATION[1]

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Estimates indicate an annual production of corn silage in the United States of about 40 million tons *(1)*. A need for a method of evaluating corn silage on a dollar basis frequently arises in tenant-landlord accountings, in computing costs of producing meat and milk, in farm management cost-accounting studies, and in placing economic interpretations upon the results of feeding experiments. Since corn silage is not an article of commerce, the dollar value of a ton of silage has in the past usually been calculated from *(a)* the value of the meat or milk produced from the feeding of silage; *(b)* the cost of production of the forage plus the cost of ensiling; *(c)* the market or farm value of hay, assigning to silage a fractional value of that of hay, such as one-third to two-fifths; or *(d)* formulas which specify a certain number of bushels of ears per ton and the hay-equivalent value of the leaf-stalk portion of the silage.

Since the feeding value of corn silage is influenced not only by its content of dry matter but also by the proportions of the dry matter which are formed by *(a)* ears and *(b)* the leaf-stalk portion, a method is needed for estimating the amounts of these two portions in a ton of corn forage or corn silage. This paper presents experimental data on the percentage dry matter content, together with the amounts of the ear and leaf-stalk fractions of corn forage from early-silage to advanced-silage stages of development. From these data a method has been derived for estimating the dollar value of corn forage or corn silage.

REVIEW

In a study of varieties of corn grown for silage, Burrill and McCluer *(2)* separated the forage into three portions, namely, ears, stalks, and leaves and husks, and made chemical analyses of each portion. Their investigation was one of the first in which evidence was presented to show the wide differences which exist among various fields of corn in the ear content of the forage at the silage-harvest stage. Numerous other investigations have confirmed these early findings. Whisenand *(7)* reported that the grain content of silage during a 5-year study ranged from 3.7 to 7.3 bu. per ton. His method of estimating the value of silage was to compute the market price of grain and stover, to determine the costs of harvesting and storage, and to increase the total of these costs by one-fifth to allow for an estimated one-sixth loss in weight caused by fermentation and drying. Pearson and Gaines *(6)* proposed two methods for evaluating

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corn silage. Their cost-accounting method was derived from records kept on 68 farms where silos were filled. The acre yield of the forage was 5.71 tons containing 28 bu. of grain, or 4.6 bu. per ton. Their second method, termed a nutritional method, was based upon an estimated digestible nutrient content of the grain and nongrain portions of the silage. The grain content was determined by harvesting the mature grain from a few representative rows of corn which were left standing until ripe.

Nevens *(3)* suggested a method of calculating the value of corn silage which is similar to the nutritional method of Pearson and Gaines but differs in that it is based upon determinations of the ear and leaf-stalk portions of corn forage at the silage stage as found at the Illinois Station *(4, 5)* during the years 1940 to 1946, inclusive.

EXPERIMENTAL

Five corn hybrids (Illinois Hybrids 206, 784, 972A-1, 2119W, and U. S. Hybrid 13), which had been shown by previous tests to be high yielding for grain and also suitable for silage production, were grown during 1947 to 1951, inclusive, in eight replicated blocks. Each block was 0.4 acre in size and contained four rows of each of the five hybrids. The corn was grown in 40-in. rows with plants spaced 12 to 14 in. apart in the row. There was practically no lodging and but little insect injury.

Acre yields of forage and proportions of the forage formed by ears and by the leaf-stalk portion were determined from samples of the standing forage. The first samples were taken when the kernels on the ears were in the early milk to milk stage of development. Subsequent samples were taken at approximately weekly intervals up to the time of complete harvest of the crop for silage. In sampling, every 35th plant from the four rows of each hybrid within a block was harvested by hand and brought together to form a sample containing from 20 to 30 plants. The samples were taken to the laboratory, where they were at once weighed and separated into ear and leaf-stalk portions. The ears were air-dried on racks for a period of several weeks and finally dried to complete dryness in an oven at 92° to 100° C. The remainder of the harvested plant was immediately chopped in a power cutter and subsampled. During the first two seasons, subsamples were reduced in moisture content in a forced-air drying chamber kept at a moderate temperature and finally dried to complete dryness at 92° to 100° C. In the last three seasons, the subsamples were brought to a dry basis by use of only one oven which was kept at a temperature of 92° to 100° C.

RESULTS

A summary of the sampling data is shown in Table 1. The values shown for each sampling date are means of eight samples of each of five hybrids, representing a total of 40 observations.

The yields of fresh forage differed considerably from year to year, since they were affected by season, fertility levels, and other environmental factors. The

TABLE 1

Yields of corn forage grown for silage with amounts of ears and leaf-stalk portion per ton of forage

Date of sampling	Forage				Leaves and stalks in 1 ton of fresh forage[a]		Part of forage formed by ears[a]	Ears		
	Fresh matter yield per A.	Dry matter		Dry matter yield per A.				Yield per acre[a]	In 1 ton of fresh forage[a]	
		Average content	Standard deviation		Average content	Standard deviation			Average content	Standard deviation
	(tons)	*(%)*	*(%)*	*(tons)*	*(lb.)*	*(lb.)*	*(%)*	*(lb.)*	*(lb.)*	*(lb.)*
August 28, 1947	10.43	17.4	0.74	1.81	308	9.9	11.5	417	40	9.1
September 4, 1947	10.25	19.6	0.55	2.01	290	9.8	25.9	1046	102	21.0
September 15, 1947	10.70	25.2	1.00	2.70	295	13.9	41.5	2236	209	37.8
September 22, 1947	10.52	27.7	0.90	2.91	286	15.9	48.4	2819	268	47.6
August 16, 1948	19.39	17.0	0.98	3.29	262	5.7	22.9	1512	78	25.9
August 24, 1948	18.78	22.2	0.85	4.17	279	14.5	37.1	3099	165	30.8
August 31, 1948	18.41	26.0	1.38	4.79	269	6.6	48.2	4621	251	36.4
September 7, 1948	17.04	31.4	1.73	5.36	282	10.9	55.1	5896	346	46.2
August 16, 1949	18.41	20.1	0.70	3.70	299	12.2	25.7	1896	103	25.9
August 23, 1949	17.30	23.2	1.00	4.02	305	10.8	34.3	2751	159	32.9
August 30, 1949	16.90	27.6	2.27	4.67	321	11.5	41.8	3904	231	52.5
August 22, 1950	13.14	19.6	0.69	2.58	323	14.4	17.7	907	69	25.2
August 29, 1950	13.46	21.5	1.39	2.89	310	10.6	28.0	1615	120	33.6
September 5, 1950	13.46	23.4	0.93	3.15	298	11.3	36.3	2288	170	24.5
September 12, 1950	13.46	26.7	1.11	3.60	286	6.9	46.4	3338	248	30.8
August 21, 1951	14.37	20.6	0.70	2.95	317	17.0	23.0	1365	95	26.6
August 28, 1951	15.83	20.5	0.81	3.25	282	15.8	31.2	2026	128	30.1
September 4, 1951	15.65	24.6	1.13	3.86	291	9.4	40.9	3146	201	32.9

[a] Dry matter basis.

dry-matter content of the forage rose markedly from one sampling date to the next, with a single exception which occurred in 1951. The dry-matter yields also increased at a marked rate. The increase in dry-matter yields during the comparatively short sampling intervals of 14 to 25 days ranged from 26 to 63%.

The most striking feature of the data is the marked increase in the ear portion of the forage expressed as a percentage of the total dry matter in fresh forage. At the first sampling date in 1947, for example, 11.4% of the dry-matter yield of the crop consisted of ears, whereas at the final sampling date, 25 days later, the dry matter of the ears comprised 48.4% of the total. During this period, the yield of dry matter in ears per acre rose from 416 lb. to 2,817 lb. At the first sampling in 1948, the quantity of ears (dry-matter basis) in a ton of fresh forage was only 78 lb. but at the last sampling, 22 days later, the quantity of ears had risen to 346 lb.

The leaf-stalk dry matter content of the forage remained within fairly narrow limits (262 to 323 lb. per ton of fresh forage) despite the changes in the percentage dry-matter content.

DISCUSSION

It is obvious from the data in Table 1 that the weight of the fresh matter alone is not a good index of the value of corn forage grown for silage. Harvesting corn forage at too early a stage means a great sacrifice in the potential yield. In 1948 the dry-matter yield of the crop increased to the extent of 2.07 tons per acre during a 22-day period. Harvesting at a far-advanced stage, however, does not solve the problem of satisfactory silage-making because corn forage ensiled at a stage when the dry-matter content is high is likely to mold. Much care is needed to ensile the crop at an optimum stage, which in experiments at this station has been found to be when the forage contains from 25 to 30% dry matter.

The within-year within-hybrid correlation between the pounds of ear dry matter per ton of forage and the percentage dry matter of the forage was 0.964. This correlation is based on 694 degrees of freedom and is significant at less than the 1% level of probability. This correlation indicates that the pounds of ear dry matter per ton of forage can be predicted with high precision from the percentage of dry matter of the forage. At this station numerous routine determinations of the dry-matter content of corn forage at the time of ensiling and of the resulting silage used in feeding trials have shown that there is little change in the dry-matter content of the corn forage from the time of ensiling to the time of removing the silage from the silo; consequently the above correlation indicates that one should be able to evaluate a ton of silage in terms of ear dry matter and leaf-stalk dry matter. The ear dry matter can be converted to an ear corn equivalent and the leaf-stalk dry matter to a hay equivalent. If water is added at the time of ensiling or if seepage occurs the dry matter content of the silage would automatically differ from that of the harvested forage.

The within-hybrid within-year regression of the pounds of ear dry matter per ton of forage on the percentage dry matter of the forage is shown in Figure 1.

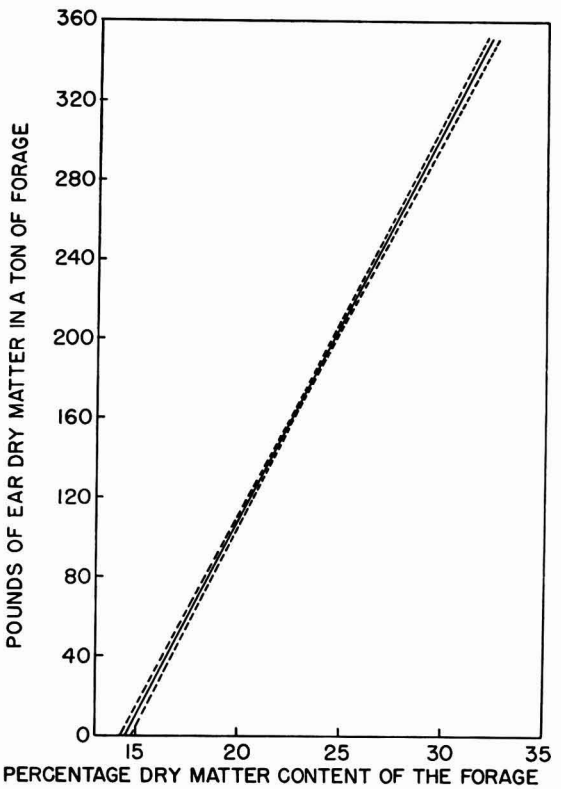

FIG. 1. The regression of the ear dry matter ($\hat{Y}$) on the percentage dry matter of the forage (X). The solid line is the regression equation $\hat{Y} = -285.0 + 19.6X$. The broken lines are the 0.01 fiducial limits. ($\hat{Y} \pm 2.58\sigma\hat{Y}$); $\sigma\hat{Y} = 21.66\sqrt{\frac{1}{720} + \frac{x^2}{11{,}205}}$

The 1% fiducial limits are plotted on each side of the regression line and the vertical distances between these lines indicate the precision of the estimates.

Suppose a dairy farmer is interested in selling part of his silage and by drying several samples he finds that his silage has an average dry matter content of 25%. A ton of silage containing 25% dry matter would contain 500 lb. of dry matter. By use of the regression equation of Figure 1, ($\hat{Y} = -285.0 + 19.6X$), it is found that a ton of silage containing 25% dry matter contains 205 lb. [$\hat{Y} = -285.0 + 19.6\ (25)$] of ear dry matter. If it is assumed that a bushel of ear corn containing 15% moisture weighs 70 lb., then the 205 lb. of ear dry matter is equivalent to [205 (0.0168)] = 3.44 bu. of corn. The 295 lb. of leaf-stalk dry matter is equivalent to [295 (1.1765)] = 347 lb. of stalks and leaves on a 15% moisture basis. Assuming that the stalks and leaves have a feeding value 90% as great as that of nonlegume hay, then the 347 lb. of stalks and leaves would be equivalent to 312 lb. of nonlegume hay. If prices of $1.50 per

bushel and $24 per ton are assumed for ear corn and nonlegume hay, respectively, the value of a ton of the farmer's silage is:

3.44 bu. ear corn @ $1.50 = $5.16
312 lb. hay @ $.012 = $3.74
Value of ton of silage = $8.90

The composition of a ton of forage is shown in Table 2 in terms of bushels of ears at a 15% moisture basis and pounds of stalks and leaves at a 15% moisture basis for forage containing from 15 to 32% dry matter.

TABLE 2

Ear and leaf-stalk content in one ton of corn silage at various stages of development

Dry matter of forage	Ears[a]	Leaves and stalks[b]	Dry matter of forage	Ears[a]	Leaves and stalks[b]
(%)	*(bu.)*	*(lb.)*	*(%)*	*(bu.)*	*(lb.)*
15	0.2	342	24	3.1	347
16	0.5	343	25	3.4	347
17	0.8	343	26	3.8	348
18	1.1	344	27	4.1	348
19	1.5	344	28	4.4	348
20	1.8	345	29	4.8	349
21	2.1	345	30	5.1	349
22	2.5	346	31	5.4	350
23	2.8	346	32	5.7	350

[a] 15% moisture basis; 70 lb. of ears per bushel.
[b] 15% moisture basis.

SUMMARY AND CONCLUSIONS

Determinations of the dry-matter content and yields of the ear and leaf-stalk portions of corn forage at stages ranging from early-silage to advanced-silage condition were carried out during five successive seasons. High correlations were found between the percentage dry-matter content of the forage and the ear content of the forage. The data make it possible to estimate with a high degree of precision the ear content of corn forage or corn silage having a known dry-matter content. A procedure is presented for making this estimate and converting it into terms of farm commodities, namely, bushels of ears and pounds of hay equivalent, to which market prices may be applied.

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INTERFERENCE BY GLYCEROL WITH DIFFERENTIAL STAINING OF BULL SPERMATOZOA AS USED WITH SEMEN THAWED FROM THE FROZEN STATE [1]

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The differential staining of live and dead spermatozoa has been proposed as a quality test for semen to be used in artificial insemination of dairy cattle *(2, 3, 4, 10, 12)*. Various factors, such as pH, osmotic concentration, stain concentration, ionizable salts, time allowed for staining and drying, and choice of buffer, have been found to affect the staining results *(1, 5)*. The staining procedure has been used with undiluted semen, with semen diluted with egg yolk–phosphate or citrate buffer mixtures, with boiled skimmilk, and with boiled homogenized milk.

More recently, glycerol has been found to be a necessary component of diluters used for low temperature storage of bull semen *(8, 9, 11)*. In the present studies of factors affecting the viability of frozen spermatozoa, both the per cent of motile spermatozoa and the per cent of live spermatozoa, as determined by differential staining, have been used as criteria. Discrepancies appeared in the comparative results obtained from these criteria, with glycerol diluters, and they are the subject of this report.

EXPERIMENTAL PROCEDURE

The differential stain used in these experiments was modified by Swanson and Bearden *(13)* from that proposed by Mayer *et al. (5)*. It consisted of 0.8 g. eosin Y, either 1.5 or 2.0 g. of fast green FCF, 3.0 g. of sodium citrate dihydrate, and 100 ml. of distilled water.

Three basic semen diluters used in these studies had the following compositions: *(a)* 20% of egg yolk plus 80% of a 3% aqueous solution of sodium citrate dihydrate; *(b)* boiled skimmilk; and *(c)* boiled homogenized milk. The boiled milks were heated to 92-95° C. for 10 minutes in a double boiler.

Eight semen samples from five dairy bulls were used in these experiments. Immediately after collection, the semen was diluted 1:10 with the basic semen diluters at 30° C., and the diluted semen was allowed to cool slowly to 5° C. over a period of approximately 4 hours. At this time the semen was diluted to a final rate of 1:20 by a five-step addition, during 48 minutes, of the basic diluter containing twice the volume of glycerol desired in the final diluter. One-ml. samples were then transferred to precooled 2-ml. molded Pyrex glass ampoules [2] which were sealed with an oxygen-gas fishtail burner.[3] These ampoules

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[1] Paper of the Journal Series, New Jersey Agricultural Experiment Station, Rutgers University — The State University of New Jersey, Department of Dairy Industry.

[2] Hughes-Murray, Inc., 472 Mulberry Street, Newark 5, N. J.

[3] No. 1012 Oxygen-Gas Fishtail Burner, American Gas Furnace Company, Elizabeth, N. J.

have a constriction diameter which will readily admit a breeding tube. Equilibration of the semen with glycerol continued for an 18-hour period at 5° C. Freezing of the diluted semen was accomplished by the procedure outlined by Polge and Lovelock *(8)*. The drop in temperature over the first 15 minutes approximated 2° C. per minute, after which the rate of drop in temperature averaged 0.7° C. per minute to –70° C. Total freezing time approximated 75 minutes.

The frozen semen samples were transferred to an alcohol bath in a chest cooled with dry ice to approximately –75° C. Examination of the semen was made after 24 to 36 hours of storage at –75° C. The frozen samples were thawed in tap water having a temperature of approximately 18° C.

The per cent of motile spermatozoa was estimated on the thawed semen, and the per cent of live spermatozoa was determined by differential staining, for which purpose approximately 300 spermatozoa were examined under the oil immersion objective of the microscope.

Two factorial design experiments were carried out in which the two measures of semen quality were compared as to their relative abilities to indicate spermatozoan viability. Each of these experiments included four semen samples and three basic semen diluters, each with four levels of glycerol. In the first experiment the final semen diluters contained 2.5, 3.75, 5.0, and 6.25% of glycerol by volume, respectively, and in Experiment 2 the glycerol levels were 5.0, 7.5, 10.0, and 12.5% by volume, respectively.

RESULTS AND DISCUSSION

Forty-six semen samples from dairy bulls were used to evaluate the normal expected relationship between per cent of motile spermatozoa and per cent of live spermatozoa as determined by differential staining. In this series the mean per cent motile spermatozoa was 60.6 and the mean per cent live spermatozoa was 69.2. The coefficient of correlation between the two measures was 0.59, which figure is highly significant statistically ($P < 0.01$). A regression equation to predict the per cent live spermatozoa, when the per cent of motile spermatozoa is known, is as follows:

Per cent live spermatozoa = 23.94 + 0.75 (per cent motile spermatozoa)

The evaluation of the three basic diluters containing various amounts of glycerol as measured by per cent of motile spermatozoa and by per cent of live spermatozoa is presented in Table 1. The columns of mean values at the right hand side of the table are of special interest and are graphed in Figure 1, together with predicted per cent of live spermatozoa based on the regression equation given above. It will be noted that these two measures of spermatozoan viability vary in a differential manner in relation to the glycerol content of the diluters. The per cent of live spermatozoa drops precipitously in relation to both the per cent of motile spermatozoa and the predicted per cent of live spermatozoa as the glycerol level exceeds 4%. This is true rather uniformly with all three diluters used.

TABLE 1

Effect of various diluters and glycerol levels on the percentage of motile sperm and percentage of live sperm after freezing to –75° C. (av. of 4 ejaculates)

Experiment	Glycerol	Diluter: Egg yolk–citrate Motile sperm	Egg yolk–citrate Live sperm	Skimmilk Motile sperm	Skimmilk Live sperm	Whole milk Motile sperm	Whole milk Live sperm	Mean Motile sperm	Mean Live sperm	Mean Predicted live sperm
	(%)	*(%)*	*(%)*	*(%)*	*(%)*	*(%)*	*(%)*	*(%)*	*(%)*	*(%)*
1	2.50	20.8	38.0	10.2	38.5	12.5	31.0	14.5	35.8	34.8
	3.75	40.5	50.5	18.2	46.5	27.5	42.5	28.8	46.5	45.4
	5.00	46.5	49.2	36.8	52.0	43.0	46.5	42.1	48.9	55.4
	6.25	41.2	45.8	40.2	43.8	42.0	43.0	40.9	44.2	54.5
	Mean	37.3	45.9	26.4	45.2	31.3	40.5	31.6	43.9	47.6
2	5.00	50.0	55.8	41.0	54.2	47.8	53.0	46.3	54.3	58.5
	7.50	53.0	52.5	50.0	52.0	49.5	47.2	50.8	50.6	61.9
	10.00	43.0	32.2	43.2	39.2	49.8	36.5	45.3	36.0	57.8
	12.50	34.5	20.0	44.5	22.0	40.8	19.2	39.9	20.4	53.8
	Mean	45.1	40.1	44.7	41.9	46.9	39.0	45.6	40.3	58.1

The data of the two experiments were subjected individually to an analysis of variance, and highly significant interactions were found in both instances between glycerol levels and the measures of semen viability used. It is evident from these comparisons that glycerol, in amounts above 4% in a diluter, interferes with the normal differential staining of spermatozoa, presumably by influencing the permeability of cell membranes of living and motile spermatozoa and allowing penetration of the stain into these cells. The analysis of Experiment 2 indicated that the diluters containing 7.5% glycerol gave a significantly greater per cent of motile spermatozoa post-freezing than did diluters containing 5, 10, or 12.5% of glycerol, agreeing well with Miller and VanDemark *(6, 7)* in this regard.

SUMMARY

The relative usefulness of two measures of spermatozoan viability (per cent of motile spermatozoa and per cent of live spermatozoa as determined by differential staining) was studied on frozen semen, using three semen diluters containing varying levels of glycerol. One semen diluter was composed of 20% of egg yolk and 80% of a 3% aqueous solution of sodium citrate dihydrate, the other two being boiled skimmilk and boiled homogenized milk. Glycerol levels, as used with the three diluters, varied from 2.5 to 12.5%. Results indicated that as the levels of glycerol in the diluters exceeded 4%, the per cent of live spermatozoa decreased in a very disproportionate manner to the per cent of motile spermatozoa and predicted per cent of live spermatozoa, becoming extreme at the high levels of glycerol. A plausible explanation of these observations is that the higher levels of glycerol increased the permeability of the living and motile sperm cells to the stain, allowing some of them to be counted as dead cells in the differential staining procedure. The per cent of motile spermatozoa is

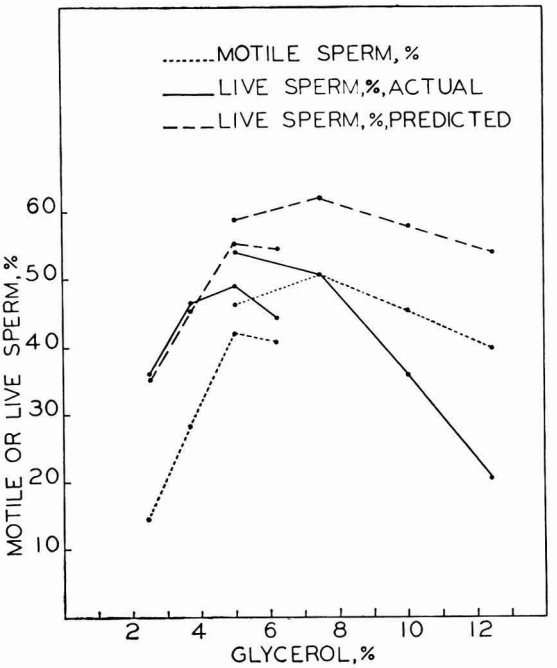

FIG. 1. The differential effect of diluter glycerol levels on the percentage of motile spermatozoa and the percentage of live spermatozoa as determined by differential staining after freezing to −75° C. (av. of 4 ejaculates).

judged to be the better measurement of spermatozoan viability in studies involving the use of glycerol as a constituent of semen diluters.

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LACTOSE CRYSTALLIZATION IN ICE CREAM. I. CONTROL OF CRYSTAL SIZE BY SEEDING

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It has been known for many years that when a supersaturated lactose solution is stored, relatively few crystal nuclei form initially. The supersaturated portion of the lactose precipitates on these centers, thereby producing large crystals. Lactose exists as a supersaturated solution in all ice cream, as Sommer *(9)* has pointed out, and, during storage, growth of crystals may cause sandiness, a texture defect. At present, however, no theory has been advanced which explains adequately why sandiness develops in some ice creams, but not in others.

It has long been a general practice to seed sweetened condensed milk as a means of controlling lactose crystal size. The seeding causes formation of abundant crystal centers on which the lactose present in a supersaturated condition is deposited, thus removing the danger of large crystal formation. Whitaker *(11)* mentioned a trial in which he induced copious crystallization by seeding ice cream with pulverized alpha lactose, but no data were given nor was any mention made about the development or prevention of sandiness in the sample. It was the purpose of this study to investigate the feasibility of seeding as a general means of preventing sandiness in ice cream. Since this work was completed, a patent has been granted *(2)* on this type of procedure.

EXPERIMENTAL PROCEDURE

The composition of the mixes used in this study varied from 11.5 to 18.3% milk solids-not-fat, and 39.8 to 42% total solids. Fresh cream was used as the source of fat and either nonfat dry milk solids or condensed skimmilk was used as the milk solids-not-fat concentrate. The mixes were pasturized at 160° F. for 30 minutes, homogenized at 2500 p.s.i., cooled and stored at 40° F. over night. They were frozen either in a 6-qt. counter freezer or a 60-gal. per hour Vogt continuous freezer. The samples were hardened quickly in a forced air hardening room at –10° F. or below.

Storage tests were conducted at 12° F., 2° F., and –10° F. After various intervals samples were removed from storage, judged for sandiness, and examined under the microscope, using glycerol as a diluent in which to disperse the sample. The microscope was equipped with a Bausch and Lomb micro light polarizer to facilitate detection and measurement of the lactose crystals.

RESULTS

At the start of this work, various types of seed lactose were added to the partially frozen ice cream in the freezer, or to the mix just prior to freezing, to

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supply nuclei for the development of lactose crystals. The size of the lactose crystals produced in ice cream was found to be directly related to the crystal size of the seeding materials. The data presented in Table 1 show the results of

TABLE 1

The effect of size and quantity of seed lactose upon the development of sandiness during storage at 12° F.

Sample No.	Seed lactose		Serum solids in mix	Occurrence of sandiness
	Type[a]	Quantity		
		(g/gal)	*(%)*	
7	—	none	12	Within 2 wk.
8	—	none	16	Sl. within 1 wk.
10	A	2	12	Sl. within 1 wk.
9	A	3	16	Sl. within 1 wk.
32	B	4	12	Sl. within 2 mo.
20	B	4	16	Sl. within 1 wk.
53	C	7	15	Within 6 wk.
54	C	13	15	Sl. within 6 wk.
55	C	20	15	Sl. within 6 wk.
76	D	0.7	12	None after 10 mo.
58	D	3	15	None after 10 mo.
56	D	5	15	None after 10 mo.
77	D	3	16	None after 10 mo.
110	D	4	18	None after 9 mo.
59	D	7	18	None after 10 mo.

[a] A—pulverized alpha lactose powders containing crystals as large as 70-100μ, few as small as 10μ.

B—pulverized alpha lactose powder with many 10-14μ, majority 35-50μ, some 100μ or more.

C—anhydrous alpha lactose rapidly rehydrated with majority of crystals 7-14μ, but many as large as 95μ.

D—anhydrous alpha lactose rapidly rehydrated and crushed with majority of crystals 3-7μ, a few 17-28μ, and occasionally one as large as 48μ.

varying the type and quantity of seed lactose in mixes with 12 to 18% serum solids. Initially, a pulverized alpha lactose powder was used, which contained crystals of wide size distribution; crystals as large as 70-100μ were common, with a few being as small as 10μ. This powder was effective in causing copious crystallization, but during storage at 12° F. the ice cream became sandy.

Microscopic examinations of the ice cream showed great masses of crystals. The data in Table 1, however, show that seed materials of the A, B, and C types were not supplying sufficient nuclei to prevent the growth of the lactose crystals to a perceptible size. Therefore, a powder, Type D, was prepared in which the majority of the crystals were 3-7μ in diameter, although it was not uncommon to find crystals 17-28μ and an occasional one as large as 48μ. This seed lactose was prepared by the method developed by Sharp *(8)*. Alpha lactose hydrate was heated under reduced pressure until the water of crystallization was removed. The water was then replaced rapidly by exposing the anhydrous lactose to steam. The original crystals were fractured and great numbers of crystal centers were formed as a result of the rapid absorption of moisture.

As the lactose concentration in the mix was increased, the necessity of having sufficient nuclei became more apparent. Since a greater quantity of lactose was

being crystallized, more centers were necessary to insure that only minute crystals developed. The data in Table 1 indicate that the number of crystal centers in the ice cream was dependent upon the quantity and type of seed lactose used. In general, if the lactose powder contained crystals of the size used in these experiments, 2 to 4 g. per gallon of ice cream mix was sufficient to provide adequate crystal centers. No improvement was noted by using greater quantities of seed material.

In the early experiments it was thought advisable not to seed the mix until it was saturated with respect to lactose. In a mix containing 12% serum solids (6.5% lactose) and 39% total solids there are 10.6 parts of lactose to 100 parts of water. Since lactose solubility at 32° F. is 11.9 parts in 100 parts of water, the mix becomes saturated when 11% of the water is frozen. The data of Cole *(1)* indicate this would be at approximately 27° F.

Further experimentation showed that the crystal nuclei were not lost quickly when added to a solution that was not saturated with lactose. The crysals remained undissolved for long periods. The data in Table 2 show the results of

TABLE 2

The effect of elapsed time after seeding[a] and before freezing upon lactose crystallization in ice cream

Sample No.	Time after seeding	Period of storage at 12° F.	
		6 days	2 weeks
	(min.)		
A	4	Great no., uniform size, $<7\mu$; not sandy	not sandy
B	7	Great no., fairly uniform, $<10\mu$; not sandy	not sandy
C	10	Great no., mostly $<10\mu$, some 20μ; not sandy	not sandy
D	12	Large no., 10-12μ; not sandy	not sandy
E	15	Large no., uniform 14μ; incipient sandiness	sandy
F	30	Much fewer, 24-38μ; sl. sandy	sandy
G	45	Only a few, 50μ or larger; incipient sandiness	sandy
H	60	Difficult to find crystals; not sandy	sandy

[a] 50 g. seed lactose dispersed in 10 gal. ice cream mix at 40° F.

an experiment in which 50 g. of the seed lactose was dispersed in 10 gal. of ice cream mix at 40° F. in the mix tank of a continuous freezer. Ice cream samples were taken after 4, 7, 10, 12, 15, 30, 45, and 60 minutes, hardened, and then stored at 12° F. Microscopic examinations showed that lactose crystals were still detectable in the ice cream coming from the freezer 30-45 minutes after seeding. The amount of seed lactose decreased progressively with each sample. The very small seed crystals particularly were lacking in the later samples.

After 6 days storage the samples were examined organoleptically and microscopically for crystallization. It may be seen from the data in Table 2 that the samples taken soon after seeding contained great masses of crystals under 10μ in size. In the samples taken 15 to 60 minutes after seeding, the crystals progressively decreased in numbers and increased in size. In the samples containing relatively few nuclei, the crystals were of sufficient size to be detectable organoleptically.

The results of many experiments conducted over the last 4 years testify to the deleterious effect of inadequate seeding. Samples containing too few nuclei became sandy more quickly than the unseeded control samples. This is also shown by the data in Table 2. The samples taken during the first 12 minutes had adequate nuclei, samples after 15 to 45 minutes were lacking in nuclei, and the 60-minute sample appeared to be normal unseeded ice cream. Within 6 days large crystals developed in the samples taken after 15, 30, and 45 minutes, whereas the samples taken before or after these had not become sandy. The presence of insufficient nuclei in these three samples accelerated the development of large crystals, whereas the normal samples showed a lack of lactose crystallization. After 2 weeks the normal sample became sandy, but the adequately seeded samples never did.

It was observed throughout this study that the size of the crystals alone did not determine whether a sample would be sandy. The data in Table 3 show there was a relationship between size and number of crystals necessary to produce the

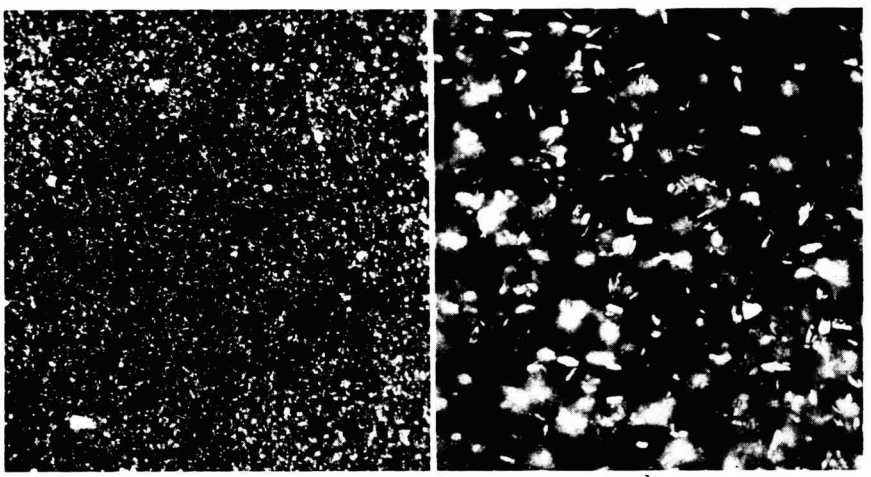
TABLE 3

Size and number of lactose crystals as related to sandiness

Sample No.	Serum solids in mix	Lactose crystals		Comments
		Size	Relative numbers	
	(%)	*(μ)*		
108a	11	14-21	Numerous	V. sl. sandy
b	11	28-42	Much fewer	Sl. sandy
c	11	42	Few	Sandy
10	12	15-24	Numerous	V. sl. sandy
7	12	60-70	Few	Sandy
56	15	17-21	Moderate	Not sandy
		< 7	Great numbers	.....
57	15	17-28	Few	Not sandy
		10-14	Numerous	.....
77	16	21-28	Very few	Not sandy
		< 7	Great numbers	.....
9	16	15-50	Few	V. sl. sandy
		< 7	Great numbers	.....
8	16	24-50	Moderate	Sl. sandy
59	18	14-35	Very few	Not sandy
		< 7	Great numbers	

defect. If relatively few crystals were present, they could be of the order of 30μ without causing sandiness. On the other hand, if large numbers were present, they had to be under 15μ in order to avoid sandiness. Homberger and Cole *(6)* also observed this relationship.

Figure 1, which shows the crystals in a seeded sample of ice cream after storage at 12° F., indicates that the typical tomahawk crystals of lactose are not characteristic of seeded ice cream. The thin plates or prisms found in such great numbers suggest that the crystallization took place under rather high precipitation pressure. Herrington *(5)* found such crystals formed when the supersaturation was high, whereas the tomahawk crystals appeared in more dilute solutions.



a. b.

FIG. 1. Lactose crystallization in seeded ice cream. Magnification a. 62x; b. 250x.

DISCUSSION

Whitaker *(11)* postulated that in mixes of high solids-not-fat the lactose may reach the labile zone of supersaturation at the temperatures encountered in the freezer. Agitation in this state induces crystallization, and as a result the lactose in high solids ice cream tends to crystallize, causing a sandy condition. In mixes of normal solids content, the lactose is said to be in the metastable zone in the freezer, spontaneous crystallization is not induced, and therefore the ice cream does not become sandy.

Herrington's *(4)* studies of spontaneous crystallization of supersaturated lactose solutions do not conflict with the view of Whitaker. Herrington found that the probability of nuclei formation was small with a slight degree of supercooling; it rose to a maximum and then fell again as supercooling was increased. He concluded that the chances of spontaneous crystallization did not seem great at temperatures only a few degrees below freezing, but as supercooling increased, the probability of crystallization increased until a maximum point must be reached. At still lower temperatures the solution became more stable again.

The addition to ice cream of crystal centers in the form of various insoluble substances has been shown by Whitaker *(11)* to cause lactose crystals to develop more rapidly. Certainly one of the explanations generally given for the tendency of fruit and nut ice cream to become sandy is the presence of nuclei or crystal centers in the flavoring material *(7, 9, 10)*. Sommer *(9)* found that the addition to ice cream of special skimmilk powders would not accomplish the intended purpose of creating numerous crystal nuclei so small that they were not perceptible as sandiness. He also suggested that clarification of mix to remove all gritty particles might be helpful in eliminating sandiness. In view of these findings and observations, it has come to be widely accepted that crystallization must be avoided if sandiness is to be prevented.

It seemed possible that sandiness developed in the above circumstances not only because of the presence of nuclei but primarily because of the lack of sufficient nuclei to cause removal of the supersaturated lactose as impalpable crystals. The results of this study proved this to be the case. Although high solids ice creams were stored under extremely adverse temperature conditions for long periods, sandiness never occurred when the mix was seeded adequately during freezing. Once the excess lactose crystallized, the danger of large crystals developing was eliminated.

Although the data presented here do not settle conclusively the question of whether lactose crystals form in all ice cream, they are additional evidence to support the belief that lactose crystals normally are not formed. The data show that when nuclei are present in small numbers sandiness is greatly accelerated. If crystal nuclei form during freezing or hardening of high solids ice cream, they are not formed in large numbers and therefore the product becomes sandy quickly. Seeding results in mass crystallization throughout the ice cream so that no crystals develop to a sensible size. There is no indication of a reduction in the number of crystal centers such as observed by Decker and Reid *(3)*.

SUMMARY

Adequate seeding of ice cream during freezing or of ice cream mix shortly before freezing has been found to prevent development of large lactose crystals. Removal of the supersaturated lactose in the form of minute crystals (not larger than 10μ) removes the danger of sandiness developing during storage. The presence of too few nuclei results in rapid growth of the crystals and acceleration of sandiness development. The results show large lactose crystals develop during storage of normal ice cream because of the inadequate number of nuclei to remove the supersaturated lactose as impalpable crystals.

ACKNOWLEDGMENT

The author is indebted to P. F. Sharp for his suggestions in initiating this work.

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RECENT PROGRESS IN THE MANUFACTURE AND USE OF LACTOSE: A REVIEW

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Whittier *(40)* presented a thorough review covering the literature up to 1944 and made available 327 references. In this review, the structure of lactose, its physicochemical properties, its manufacture, its general chemistry, and its fermentation products were ably described. Whittier and Webb *(41)* in their recent text on dairy by-products give an excellent discussion of modern manufacturing methods for lactose applicable to both casein and cheese whey and encompassing both the crude and refined grades of lactose. This discussion provides 28 references and presents excellent process diagrams. Fisher *(16)* provides a popular discussion of milk sugar which presents valuable statistics on the scope of the milk sugar industry and a good discussion of uses for lactose.

TRENDS IN MILK SUGAR PRODUCTION

We are indebted to Fisher *(16)* for valuable data on this topic. Until the important development of penicillin, milk sugar production was stabilized at some 7 million pounds per annum for the United States. Table 1 shows that the emergence of penicillin production approximately tripled the milk sugar requirement from 1943 to 1946 and that subsequently the requirement began to drop, though it was stabilized from 1949-1952 at a figure somewhat above 17 million pounds.

TABLE 1
Production of lactose

Million pounds	Year
7.3	1943
23	1946
21	1947
17	1948
>17	1949-1952

At present, no new direct use for lactose equal in scope to the penicillin requirement is at hand. It seems more reasonable to expect that the source material of lactose, namely whey, will find ever increasing use as a fermentation substrate, for it represents the least expensive form in which lactose occurs.

Some 10 billion pounds of whey results annually from cheese and casein making operations. About 9 billion pounds stems from cheese production. Approximately 0.6 billion pounds is a by-product of casein production. The milk sugar content of this whey is nearly 0.5 billion pounds. Only about 4% of the available milk sugar is being recovered as such, though much more could be recovered if the demand required it.

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MANUFACTURE OF MILK SUGAR

Modern milk sugar production is well described by Kastens and Baldauski *(22)*. Whey, after being limed to pH 6.8, is brought to a boil by steam injection. The albumin precipitates, and the clear liquor is decanted and filter-pressed. This filtrate is concentrated to 40% solids in a stainless steel triple-effect evaporator. The resulting crude sirup is held over night and reheated by steam injection and again filter-pressed. This filtrate is concentrated to 60% solids in a single-effect vacuum pan. It is then pumped to jacketed crystallizers and cooled to 50-55° F. More than 60% of the contained lactose crystallizes out. The slurry of sirup and crystals is fed by gravity into perforated basket centrifugals where the crystals are spun and washed with clear water. Washing is continued until the wash water is clear and colorless.

The mother liquor and wash water from the first crystallization are returned to the single-effect evaporator and reconcentrated to 65% solids and returned to the crystallizers. The steps thereafter follow a similar pattern to that for the first crystallization.

The crude crystals from the first crystallization can be dried directly as in a rotary drier. The resulting product is designated "crude lactose."

To obtain refined lactose, the crude crystals must be recrystallized. They are made into a solution of 30-40% concentration which is treated with activated charcoal and carefully filtered through filter paper. The clarified sirup is concentrated to 65-70% solids in a stainless steel single-effect evaporator and then transferred to a stainless steel crystallizer. The handling of the refined crystals follows the earlier pattern described.

The specifications for the established grades of lactose are shown in the following table taken from Kastens and Baldauski *(22)*.

TABLE 2
Lactose specifications

	H_2O	Ash	Protein	Lactose	Size
	(Max. %)	*(Max. %)*	*(Max. %)*	*(Min. %)*	
Crude	0.5	0.75	0.75	97.5	
USP	0.1	0.1	None	99.5	100% through 80 mesh
Edible	0.15	0.25	0.25	98.5	100% through 80 mesh

Peebles and Marquis *(30)* describe a patented process for making what may be roughly classified as an edible grade of lactose. Whey is evaporated to form a supersaturated solution of lactose and momentarily subjected to an elevated temperature (220-260° F.). This step causes the protein content to be irreversibly coagulated by heat so that the water-binding capacity of the proteins is reduced, which in turn lowers the viscosity of the whey concentrate. The material is then seeded with lactose crystals to induce crystallization. The slurry is then diluted with cold water and the lactose crystals are permitted to drop by hydraulic classification into a conical vessel. The crystals are removed, centrifuged, and dried. The overflow from the hydraulic classifier is concentrated and dried

to make a feed. About 35% of the total lactose content of the whey is recovered as lactose by this process.

Almy and Hull *(5)* outline a patented process for making a top grade of crude lactose. They begin with a sweet whey (pH no lower than 5.4) and neutralize to a pH of 7.0-7.5. To this whey is added 0.0025-0.06% of alkali metal tetraphosphate to reduce viscosity, stabilize the protein content of the whey against heat coagulation, and maintain calcium ions in soluble form. The treated whey is concentrated to 60% solids, and the lactose is crystallized, washed in a centrifuge, and dried. Large crystals are obtained which readily wash free from protein and salts. The crude lactose obtained is low in ash.

Almy and Garrett *(4)* describe in their patent a new process for crude lactose which makes use of ion exchange resins for adjusting pH and removing mineral components. They begin with sweet cheese whey and lower the pH to 4.6-4.8 with a cation exchange resin operating in its hydrogen cycle. This acidification is obtained by replacing metal ions with hydrogen ions. The acidulated whey is heated at 175-210° F. for 15 minutes to coagulate proteins. The whey is cooled and filtered. The deproteinated whey is then treated by passing it through first a cation removing resin bed and then through an anion removing resin. The effluent at this point contains 3-4% lactose, less than 0.1% total minerals, and less than 0.2% noncoagulable nitrogenous substances. It is rendered slightly acid (pH 6.5-6.8), concentrated to 30-40% solids, and spray dried. The resulting crude lactose product contains 95.2% lactose, 0.8% ash, 2.6% noncoagulable nitrogenous constituents as protein, and 1.4% moisture.

Wallace *(37)* describes further application of ion exchange processes to lactose production. He begins with sweet cheese whey (pH no lower than 5.8) and contacts the whey with a cationic exchange resin acting in the mixed alkali metal-hydrogen cycle to effect replacement of calcium and magnesium ions with cations in the exchanger material such as sodium or potassium, thus converting low water solubility salts in whey to those of high water solubility. There is also a simultaneous adjustment of the whey pH to the range of 4.0 to 4.7 so that upon heating this whey, the proteins will readily coagulate and can be removed. The deproteinated whey is adjusted to pH 5.8-6.6 and condensed, and the lactose is crystallized and washed. It is claimed that the lactose so obtained is of high purity, and the yield is 73-85%, as compared to the customary 50-60% obtained by conventional methods.

Abrahamczic *et al. (3)* describe a process for treating cheese whey with ion exchange resins to remove flavor-impairing substances, sodium chloride, calcium chloride, and lactic acid. The product is concentrated to 60% solids, and the lactose is crystallized and removed. The residual product contains only 37% of the original lactose content. It is spray-dried and used as a dietetic product because of its excellent flavor.

A small scale process for lactose is described by Biswas *(8)* for using casein whey. The whey is heated to coagulate protein and filtered. It is concentrated to the consistency of molasses and allowed to crystallize. The crystalline mass is dissolved in water and treated with $MgSO_4$ (0.75%), glacial acetic acid (7

ml/liter), and charcoal (3%). It is then boiled and filtered. The filtrate is concentrated under vacuum and allowed to crystallize. The crystals are washed with a saturated solution of lactose and dried. Resulting product conforms to the British Pharmacopeia Standard.

Leviton *(23)* outlines a process at the laboratory level for preparing lactose from spray dried nonfat dry milk solids by means of methanol extraction. This is an extension of the earlier work of this author in which a similar process was applied to spray-dried whey powder *(24, 25, 26)*. The nonfat dry milk solids is treated with 62% methanol at –15° C. The lactose is completely extracted and is obtained in rather high purity. Lactose recovery may be brought to approximately 73% with an ash content of 0.02% and protein of 0.01%. A by-product of this process is a "soluble" protein fraction of which 81% is casein. There seems little doubt that this process should be thoroughly explored, at the pilot plant level, and the economics evaluated. The quality of the lactose obtained, as well as the comparative good solubility and quality of the protein by-products, warrants attention.

Hoover and Kokes *(21)* describe an aqueous extraction process for obtaining milk protein and lactose as a by-product from nonfat dry milk solids. The powder is leached with five times its weight of 0.25% sodium chloride at a pH of 4.1. The soluble extract contains 14% lactose, whey salts, added sodium chloride, riboflavin, and about 7% of the total protein. The residual extracted solids contain 86% protein, 2% ash, and 0.3% lactose. The lactose extract might be worked up by ion exchange methods to recover lactose. The extracted fraction contains 93% of the total milk proteins and should be excellent nutritionally, since both the casein and whey proteins are present.

McGlasson and Boyd *(28)* outline an effective process for making lactose of good purity by application of ion exchange procedures. Cheddar whey is adjusted with NaOH to an acidity of 0.10 to 0.13%. This whey is brought to near the boiling point and a $CaCl_2$ solution (1.3 lb. $CaCl_2$ per liter water) added at the rate of 1 ml. per pound of hot whey. The coagulated whey proteins are allowed to settle, and the supernatant liquor is siphoned off, cooled to 70° F., and clarified. The lactose solution is concentrated to 10° Bé., cooled to 40° F. and filtered. The temperature is then raised to 68-70° F. and the liquor is passed through cation (Amberlite IR-120) and anion (Amberlite IRA-400) exchange resins. The effluent is then spray-dried. The pH just prior to drying is 7.0. The dried lactose appears to be free of monosaccharides and has a lactose content ranging from 97-99%. Best results are obtained when the whey proteins are removed prior to ion exchange treatment.

Pratt *et al. (31)* describe a patented process for recovering lactose from milk sugar mother liquors. The mother liquor contains 35% solids, of which approximately one-half is lactose, more than one-fifth is ash, and more than one-seventh is nitrogenous matter calculated as protein. The liquor is diluted to 13-17% solids and treated with ion exchange resins so as to remove 85% of the ash and 50% of the nitrogenous material. The purified liquor is concentrated and subjected to crystallization to recover lactose.

A good resume of the application of ion exchange resins to lactose production is given by Genin *(17)*. This article provides six references on this topic. It is concluded that lactose having a purity in the order of 99% can be obtained by the suitable application of ion exchange procedures. The general trend in the recent literature on lactose refining is to emphasize the value of ion exchange procedures.

USES FOR LACTOSE

Lactose, when compared to sucrose or dextrose, is much less sweet, less soluble in water, and less hygroscopic. It has always lent itself to the formulation of tablets or pills for which some harmless and relatively tasteless product is needed to standardize and stabilize the drug being formulated. This has always been and continues to be a major use for lactose. Infant foods and other special dietary products also utilize large quantities of lactose. A recent patent *(7)* describes an infant food in which lactose is a supplementary carbohydrate.

The development of penicillin production rapidly created a large need for lactose so that by 1944, some 12-14 million pounds were required. This led to new plant facilities and a very rapid expansion in lactose production. Lactose serves as a slowly fermenting sugar, furnishing acidity to keep down the alkalinity of the penicillin broth and permit maximum production of penicillin which would otherwise be inhibited, since sufficient alkalinity causes the mold to cease growing. Lactose also may serve to provide a nutritional stress to the mold and thus force greater production of penicillin.

Dawson and Wood *(13)* propose the use of lactose as well as sucrose for improving the keeping and baking qualities of spray dried whole eggs. Untreated samples held at 100° F. retained good properties for only 1 week. A 10% lactose addition maintained the samples for 8 weeks. A 10% addition of sucrose was less effective than an equal addition of lactose. A 20% addition of sucrose was needed to obtain a shelf life of 8 weeks at 100° F.

A number of references may be found to the caramelization of lactose and its use as a caramel color. For this use, however, it must compete with less expensive carbohydrates. A typical procedure is described in a Dutch patent *(11)*. Lactose is hydrolyzed for 1 hour at 112° C. in the presence of HCl. It is neutralized to pH 7.3, and the solution is heated in an open vessel until the temperature rises from 105° C. to 140° C. It is then held at 142° C. for ½ minute, then cooled, and the pH again adjusted to 7.0.

Finely divided lactose appears to be rather extensively used for controlling lactose crystallization in some dairy products and thus preventing the defect of coarse texture or "sandiness." Recent patents *(14, 15)* describe the use of finely divided lactose to prevent "sandiness" in ice cream or condensed milk. In ice cream, 1 oz. of fine lactose crystals is added per 5 gal. of fluid mix. In sweetened condensed milk, lactose crystals of a particle size less than 2μ are used to the extent of 0.5% or less and the average lactose crystal size of the product is held to less than 10μ. The finely divided lactose in each case provides

nuclei for the crystallization of the lactose in the product in the form of very equal and small crystals which are not detectable on the palate. Buyze *(10)* describes very interesting studies on the production of seed lactose for the manufacture of condensed milk. He concludes that spray-dried lactose is in an amorphous state. Such a product when exposed to moisture crystallizes spontaneously, and the crystals are approximately 1μ in size, which is well suited for seed purposes. Spray-dried whey powder also can be used.

Whittier *(42)* in a recent U. S. patent outlines a process for making confections without the use of heat. Finely divided cane sugar is used to control the crystal size, and the firmness of the candy is determined by the ratio of added water to the lactose provided by a dried milk product. A wide variety of confections ranging from soft fondants to hard dry fudges can be made readily by this process.

Sharp *(34)* describes an interesting patented process for controlling the crystal size of lactose in sweetened condensed milk or whole condensed milk. The products are seeded with fine pulverized lactose crystals by impregnating the surfaces of a grooved drum with a lactose paste. The drum is rotated rather slowly through the product to be seeded, and a smooth final end product is obtained. Sharp and Hoeker *(35)* make use of seeding with fine lactose in a patented process for making cheese spreads and related products. Milk is concentrated to 40-70% solids, of which one part is milk solids-not-lactose per two parts of lactose. The product is cooled to 50-90° F. and seeded with fine lactose crystals to give an organoleptically smooth texture to the food product.

An interesting use for lactose is described in a recent patent *(28)* wherein lactose is used in solution for preserving microbial cultures in a high state of viability. In the case of a yeast, *Saccharomyces cerevisiae*, the lactose content is 10-20%, and it is stated that it will preserve the yeast for 6 months in a viable state. This yeast cannot ferment the lactose, as it is not adapted to handling this sugar. When such cultures are added to a suitable substrate (molasses), the fermentation begins very rapidly with a minimum lag period.

A recent Dutch patent *(12)* describes a tooth paste containing approximately 45% by weight of lactose or a calcium-lactose derivative in order to provide a polishing action but without leaving an insoluble residue between the teeth. It is also claimed that the lactose promotes the growth of microflora antagonistic to the acid-forming bacteria that produce cavities.

According to a recent Austrian patent *(20)* the addition of 1-3% lactose to butter increases the stability and improves the flavor. Two recent Swiss patents *(19, 33)* recommend the use of lactose for preserving the aroma of roasted coffee as it is obtained in extracts.

Weiss *(39)* reports that in the preparation of fruit conserves, replacing one-third of the sucrose with lactose gives a superior flavor; however, after 6 months, crystallization of lactose gives the product a gritty texture. This might be controlled by seeding the product with finely divided lactose.

Considerable attention has been given to the production of sweet noncrystallizing edible sirups from lactose. It is known that such products have been

marketed in Europe during periods of sucrose shortage. Their use in this country probably would require evidence that such products had some special nutritive value that sucrose or glucose sirups do not have.

Whittier *(43)* outlines a process for making a hydrolyzed lactose sirup according to the method of Ramsdell and Webb *(32)*. In this process 70 lb. of steam pressure is applied for 65 minutes to a solution containing approximately 23% lactose and a very small percentage of hydrochloric acid (approximately 0.1%). The lactose is nearly completely hydrolyzed. The sirup is treated with carbon, filtered, and condensed to 60% solids. It is then neutralized with sodium bicarbonate to a pH of 4.9-5.0. This product is stated to be suitable as a food sweetener or table sirup.

Bach and Spagenberg *(6)* describe a process for making an invert sirup from crude lactose by acid hydrolysis. The sirup is intended for use in the confectionery industry, pharmaceutical products, fruit conserves, and baby foods and as a substrate for fermentations. There appears to be no novelty in the process described except the use of relatively crude lactose, the requirement being that the ash content be less than 1%. The authors believe that a sirup of better flavor is obtained than when pure lactose is used.

Block *(9)* describes a process for making a noncrystallizing sirup from lactose suitable for use in ice cream, baby foods, bakery products, and confections. A lactose solution is treated at elevated temperatures with an ion exchange resin adjusted to the hydrogen cycle and the lactose is partially hydrolyzed, primarily into glucose and galactose. It is claimed for the process that it has advantages over customary acid hydrolysis of lactose in that the impurities resulting from the use of inorganic acids are eliminated and that the ash content is kept at a minimum.

In order for any of these "invert" lactose sirups to find a place in the food industry, it will be necessary to establish clearly the nutritional performance of the products or other advantages that will make them competitive with other sugar sirups.

A very interesting application of a hydrolyzed lactose sirup recently has been developed for the feed industry. A product named "Hidrolex" *(1)* is made by the enzymatic hydrolysis of lactose in whey (patent pending). It is used to provide a highly palatable whey supplement for mixed feeds. It can be fed at relatively high levels because the diarrhea that may result from the high lactose content of whey is controlled by hydrolyzing the lactose into its components, glucose and galactose. An interesting recent patent *(36)* describes the use of hydrolyzed lactose containing milk products to retard age thickening in frozen concentrated milk products. In a typical example whole milk is condensed to 35% total solids, homogenized, and pasteurized. One portion of this concentrate is treated with yeast lactase enzyme to convert the lactose to the extent of 85-95% into glucose and galactose. The enzyme is heat-inactivated and the treated concentrate is combined with regular concentrated whole milk in the ratio of 15% treated milk solids to 85% untreated milk solids. The product after freezing remains in good condition for at least 10 weeks at 15° F.

LACTOSE DERIVATIVES AND THEIR USE

A number of chemical derivatives of lactose have been made. However, it is believed that only a very few have been developed commercially. The primary reason for this is that lactose must constantly compete with less expensive sugars. This topic is discussed by Genin *(18)* in a recent article. Mention is made of the activities of Sheffield Chemical Company, Inc., Norwich, N. Y., which has initiated the pilot plant production of lactobionic acid from lactose by a process covered by a pending patent application. Galactose is also a product under development, and the commercial production of various salts of lactic acid such as the sodium, calcium, potassium, magnesium, iron, and copper salts has been carried on by this company for many years.

Some interesting properties of lactobionic acid are described by the above named company *(2)*. This compound, which is made by the electrolytic oxidation of lactose, is a useful nontoxic sequestering agent. The calcium salt is exceedingly soluble in water (70 parts per 100) and finds a place in pharmacy where calcium is needed.

Galactose can be obtained by the hydrolysis of lactose to glucose and galactose and the subsequent separation of the two sugars. However, the process has hitherto been too expensive to warrant development of a sizable market. It is expected that new techniques now under development will lead to cost lowering and a wider market in pharmacy and related areas.

Lactic acid and the lactate salts have been made for many years by Sheffield Chemical Company, Inc., by means of a whey fermentation which converts the lactose to lactic acid. Some of the salts have found a worthy place in pharmacy, as, for example, the calcium lactate. However, it is also true that many other low cost carbohydrates can be fermented to make lactic acid and its derivatives.

Lactose as it occurs in whey lends itself very well to fermentation by certain microorganisms. Since whey represents the lowest possible cost vehicle for lactose, it is the logical starting place for lactose derivatives. At the present time, it is believed that the only products actually being made commercially by whey fermentation *(16)* are butanol, acetone, riboflavin, lactic acid, alcohol, and vinegar. In accordance with a patented process described by Myers and Weisberg *(27)*, the production of alcohol and a valuable animal feed by-product is outlined. Milk sugar in whey is fermented by *S. fragilis*, a lactose fermenting yeast, to alcohol and carbon dioxide. The alcohol can be rectified and then converted to an excellent grade of vinegar by customary procedures. A general discussion of this development is given by Weisberg *(38)*. According to Fisher *(16)*, riboflavin, butanol, and acetone are made simultaneously by the fermentation of whey with *Clostridium acetobutylicium*.

SUMMARY

Indirectly, a major utilization of lactose is made by the feedstuffs industry. Several billion pounds of concentrated or dried whey are consumed each year as a supplement to feeds, and, unquestionably, the lactose content of this whey plays an important role in improving the nutrition of domestic animals and will

continue to do so. Much information is still needed on the role of lactose in both human and animal nutrition, and this is a field worthy of active study now and in the future.

The emergence of penicillin gave a major boost to lactose production, which on the whole has continued to be felt to the present time. Although no equally large new demand for lactose has yet appeared, there is substantial activity in the use of lactose, as in providing fine seed crystals for many condensed dairy products.

The development of lactose derivatives appears to have been most fruitful when whey was used as a fermentation substrate so that the lactose was employed in its least expensive form. Alcohol, vinegar, riboflavin, butanol, and acetone are being made by such fermentations.

Chemical derivatives of lactose which retain the unique structure of this sugar are likely to be more successful than those compounds which can be made equally well from less expensive sugars. An example of such a derivative is lactobionic acid.

Substantial improvements seem indicated in the production of lactose. Ion exchange methods appear to offer opportunity for greater efficiency when coupled with existing procedures.

Extraction methods for lactose applied to dried whey or nonfat dry milk solids appear to offer promise. These methods are based on the use of either methanol or a dilute saline solution. Such methods need pilot plant development and careful cost evaluation.

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THE FEEDING VALUE OF U. S. NO. 1 ALFALFA HAY AND U. S. NO. 2 ALFALFA HEAVY TIMOTHY MIXED HAY

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A considerable quantity of commercially available hay is bought, sold, and priced on the basis of the grade assigned to the hay by an official hay inspector using the official United States hay standards as a guide *(4)*. In spite of the importance of the role of official grading there are few data to indicate the relationships of feeding value and grade designation.

Data of this type reported by Van Horn *et al. (5)* demonstrated that the increased amounts of foreign material contained in U. S. Sample Grade Green Extra Leafy Lespedeza Hay as compared to U. S. No. 3 Leafy Green Lespedeza Hay resulted in lower palatability. Since the foreign material was to a large extent refused by the cows, the feeding value per ton of hay was decreased by the inclusion of increasing amounts of foreign material. Experiments previously reported by the authors *(2)* have measured the feeding value of No. 1, 2, and 3 Alfalfa in the rations of dairy heifers. These data demonstrated the higher feeding value of the higher grades, as well as an occasional overlapping of grade lines in the value of specific lots of hay. The experiment reported here is a continuation of that study in response to the demonstrated need for additional information. The objectives of this experiment were: *(a)* to compare the feeding value of U. S. No. 1 Alfalfa Hay with U. S. No. 2 Alfalfa Heavy Timothy Mixed Hay; *(b)* to test the effectiveness of alfalfa ash and purified protein as supplements to the heavy timothy hays should these hays prove to be of a lower feeding value.

EXPERIMENTAL PROCEDURE

An attempt was made to obtain three lots of U. S. No. 1 Alfalfa Hay (Lots A, B, and C) and three lots of U. S. No. 1 Alfalfa Heavy Timothy Mixed Hay (Lots D, E, and F). The six lots of hay were selected and purchased on the basis of the grade classification given by an official hay inspector. The final grade assigned to the various lots was made on the average analysis of samples taken each 10 days from each lot throughout the experiment, as the hay was fed to dairy heifers.

Eighteen Holstein and six crossbred heifers were divided into six groups. The groups were balanced as nearly as possible according to age and weight, there being three Holsteins and one crossbred in each group. Groups A, B, and C were fed hay Lots A, B, and C, respectively. Groups D, E, and F were fed hay Lots D, E, and F, respectively. The heifers ranged in age from 9 to 18 months.

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The average ages and body weights of the groups at the start of the experiment are shown in Table 1. Although there were small variations between groups in the average ages and body weights, it should be noted that the averages of these values for the groups fed the alfalfa hay were very close to the values for the groups fed the alfalfa-timothy hay.

TABLE 1

Average age and body weights of heifers at start of feeding experiment

No. of animals	Age	Body weight	No. of animals	Age	Body weight
	(Months)	*(lb.)*		*(Months)*	*(lb.)*
	Group A fed Lot A hay			Group D fed Lot D hay	
4	13.0	727	4	12.5	746
	Group B fed Lot B hay			Group E fed Lot E hay	
4	13.0	738	4	13.3	723
	Group C fed Lot C hay			Group F fed Lot F hay	
4	13.0	753	4	13.1	744
	Av. of groups A, B, and C			Av. of Groups D, E, and F	
4	13.0	739	4	13.0	738

All animals were on a hay and grain ration for about 1 month before the start of the experiment (November 13). Previous to that time the older animals had been on pasture and the younger animals on dry feed in the calf barn. The experimental ration consisted of hay only and was fed twice daily ad libitum for 120 days. The amount of hay fed and refused by the individual animals was recorded daily. The amount fed was frequently adjusted so that the refusal would be about 10% of that amount.

The hays were carefully sampled for grade and chemical analysis. The sampling procedure was as follows: Every 10 days during the experiment several bales from each lot were sampled, by boring. The bales from one sampling were fed during the period occurring before the next scheduled sampling. The dry-matter content of the sample was determined and a portion was reserved for grade determination, the remainder being then composited for three 10-day periods and analyzed for protein, crude fiber, ether extract, and ash according to the official methods of the Association of Official Agricultural Chemists *(1)*. The refusal from each heifer group was sampled once each 10 days and analyzed for dry matter.

The animals were housed in a stanchion barn and turned into an exercise lot twice daily. Water and a mineral mixture of equal parts bone meal and salt containing trace elements were available in the exercise lot. Wood shavings or sawdust was used as bedding.

The hay ration was supplemented with alfalfa ash or protein for a 30-day post-experimental period following the 120-day all hay period. Two animals in each of the groups B, C, D, E, and F were fed 0.4 lb. of ash daily and two were fed 0.7 lb. of a purified soybean protein[1] daily.

[1] Drackett Industrial Protein 220, 13.13% N or 82% protein (fresh basis).

RESULTS

The average grades finally assigned to the hay lots on the basis of the 10-day samples are presented in Table 2. Hay graded U. S. No. 1 Alfalfa must have a leaf content of 40-50%, at least 60% of green color, and a grass content of not

TABLE 2
Characteristics of grades of hay used

Lot	Alfalfa	Timothy	Grass	Clover	Foreign material	Leafiness of alfalfa	Color		Grade
	(%)	*(%)*	*(%)*	*(%)*	*(%)*	*(%)*	*(Hue)*[a]	*(% green)*	
A	92.4	0.0	7.6	Trace	1.8	50.5	7.13y	65	U. S. No. 1 Extra Leafy Alfalfa Light Grass Mixed Hay
B	97.6	0.0	2.4	Trace	3.4	32.2	6.16y	58	U. S. No. 2 Alfalfa Hay
C	96.3	0.0	3.7	Trace	1.3	51.1	7.58y	67	U. S. No. 1 Extra Leafy Alfalfa Hay
Av. A, B, C	95.4		4.6	Trace	2.2	44.6	6.96y	63	U. S. No. 1 Alfalfa Hay
D	54.4	45.3	0.0	0.3	1.2	27.1	4.78y	48	U. S. No. 2 Alfalfa Heavy Timothy Mixed Hay
E	35.7	62.7	1.3	0.3	2.9	35.2	5.20y	51	U. S. No. 2 Alfalfa Heavy Timothy Mixed Hay
F	75.6	24.4	0.0	Trace	1.6	28.5	5.14y	50	U. S. No. 2 Alfalfa Light Timothy Mixed Hay
Av. D, E, F	55.2	44.2	0.4	0.2	1.9	30.3	5.04y	50	U. S. No. 2 Alfalfa Heavy Timothy Mixed Hay

[a] Hue designation on the basis of the Munsell color system.

over 5%. The leaf contents of Lots A and C (50.5% and 51.2%) were slightly higher than the maximum allowed for the grade, and both lots were therefore graded extra leafy. Lot B with only 32.2% leaf failed considerably to meet the minimum leaf requirement and was graded U. S. No. 2 Alfalfa. The green color percentages of Lots A and C (65 and 67) were typical of the No. 1 grade, whereas Lot B with 58% green color was slightly below the required level. The grass content of Lot A was slightly above that allowed; hence light grass mixed was included in its classification. Lot B was the only one that differed appreciably from the originally intended grade. The average grade of the three lots, however, was U. S. No. 1 Alfalfa Hay.

Hay graded U. S. No. 1 Alfalfa Heavy Timothy Mixed Hay must have at least 60% green color, a timothy content greater than 30 and less than 70%, and the alfalfa must contain at least 40% leaf. Hay Lots D, E, and F were below the minimum color requirements with green color percentages ranging from 48 to

51. Lot F failed to meet the minimum timothy content requirement. Accordingly, Lots D and E were graded U. S. No. 2 Alfalfa Heavy Timothy Mixed Hay and Lot F was graded U. S. No. 2 Alfalfa Light Timothy Mixed Hay. The average grade of the three lots was U. S. No. 2 Alfalfa Heavy Timothy Mixed Hay, average leafiness and color (30 and 50%) being rather typical of this grade.

TABLE 3
Hay consumption and gain in body weight per heifer

Hay/day			Dry matter/day			Dry matter consumed per pound gain	Total gain	Average daily gain
Fed	Refused	Consumed	Fed	Refused	Consumed			
(lb.)	*(lb.)*	*(lb.)*	*(lb.)*	*(lb.)*	*(lb.)*	*(lb.)*	*(lb.)*	*(lb.)*
			Group A fed hay Lot A					
24.3	2.2	22.1	21.0	1.8	19.2	9.60	240	2.00
			Group B fed hay Lot B					
22.4	2.1	20.3	19.8	1.8	18.0	12.77	169	1.41
			Group C fed hay Lot C					
23.3	2.3	21.0	20.9	1.9	19.0	10.61	215	1.79
			Groups A, B, and C — Av. U. S. No. 1 Alfalfa Hay					
23.3	2.2	21.1	20.6	1.8	18.7	10.81	208	1.73
			Group D fed hay Lot D					
18.7	2.1	16.6	16.6	1.8	14.8	18.05	98	0.82
			Group E fed hay Lot E					
19.0	2.1	16.9	16.9	1.8	15.1	20.68	88	0.73
			Group F fed hay Lot F					
19.4	2.1	17.3	17.1	1.8	15.3	12.97	141	1.18
			Groups D, E, and F — Av. U. S. No. 2 Alfalfa Heavy Timothy Mixed Hay					
19.0	2.1	16.9	16.9	1.8	15.1	16.59	109	0.91

The body weight gains and feed consumption rates are presented in Table 3. The heifers fed hay Lots A, B, or C (alfalfa) made considerably greater live weight gains than the heifers fed hay Lots D, E, or F (alfalfa-timothy). Analysis of variance showed a highly significant difference between the average gain of these two main groups. Analysis of variance also showed the gains of group A to be significantly greater than those of any other group, and the gains of group B to be not significantly greater than those of group F. There was no significant difference in gain among groups D, E, and F, although the difference between E and F approached significance.

The hay dry-matter consumption rates for groups A, B, and C were consistently higher than those of groups D, E, and F. Efficiency of feed utilization expressed as dry matter consumed per pound of body weight gained, including maintenance (Table 3), shows that an average of 16.6 lb. of the alfalfa-timothy hay was required for each pound of body weight gained, compared to only 10.8 lb. of alfalfa hay. Supplementation of the hays with either ash or protein dur-

ing the 30-day post-experimental period produced no significantly different growth rates compared to each other or to the rate during the previously unsupplemented period.

TABLE 4

Average dry matter content and chemical composition of the dry matter of the hays fed

	Dry matter	Composition of dry matter					
		Protein	Ether extract	Fiber	N-free extract	Ash	Carotene[a]
	(%)	(%)	(%)	(%)	(%)	(%)	(γ/g)
Lot A	86.49	22.49	2.04	22.81	45.18	7.47	51.6
Lot B	89.07	16.94	1.73	30.76	43.17	7.40	11.6
Lot C	90.55	21.71	1.68	23.35	44.73	8.53	30.2
Lot D	89.09	12.69	2.21	36.71	41.09	7.44	10.2
Lot E	89.20	10.05	1.85	35.40	46.49	6.18	5.3
Lot F	88.95	15.79	1.81	33.85	41.26	7.29	6.2
Av. A, B, C	88.70	20.38	1.82	25.64	44.36	7.80	31.1
Av. D, E, F	89.08	12.84	1.96	35.28	42.95	6.97	7.2

[a] Carotene values from analysis of one sample from each lot.

The average dry-matter content and the chemical composition of the dry matter for the hays is presented in Table 4. The most outstanding differences in chemical composition appear in the average protein and crude fiber contents of Lots A, B, and C (20.4 and 25.6%, respectively) and of Lots D, E, and F (12.8 and 35.3%). The average calculated TDN content *(3)* of the dry matter was 59.4% for the U. S. No. 1 Alfalfa Hay (Lots A, B, and C) and 56.2% for the U. S. No. 2 Alfalfa Heavy Timothy Mixed Hay (Lots D, E, and F).

DISCUSSION

The relatively greater average gains of Groups A, B, and C demonstrated the superior feeding value of U. S. No. 1 Alfalfa Hay over U. S. No. 2 Alfalfa Heavy Timothy Mixed Hay. Although the reasons for this difference are not entirely understood, the correlation between certain factors and growth rate becomes apparent if the hays are arranged in the order of the growth they produced. This has been done in Table 5. It is noteworthy that, with few

TABLE 5

Relationship between body-weight gains, dry matter consumption and hay contents of timothy, leaf, protein, and crude fiber

Group and hay lot	Average daily gain	Dry matter consumed/ day	Timothy	Leafiness of alfalfa	Protein	Crude fiber
	(lb.)	(lb.)	(%)	(%)	(%)	(%)
A	2.00	19.2	0.0	50.5	22.49	22.81
C	1.79	19.0	0.0	50.5	21.71	23.25
B	1.41	18.0	0.0	31.5	16.94	30.76
F	1.18	15.3	24.8	28.2	15.79	33.85
D	0.82	14.8	44.1	26.8	12.69	36.71
E	0.73	15.1	62.9	35.5	10.05	35.40

exceptions, the order of decreasing daily gains is also the order of decreasing leaf and protein content and palatability as measured by dry-matter consumption. It is also the same order as increasing crude fiber and timothy content. The position of Lot E at the bottom of the list in spite of the relatively high leafiness of the alfalfa does not appear inconsistent when it is considered that alfalfa accounted for only 35.7% of the total forage present.

One might judge that the quality of timothy included in these mixed hays would have an important bearing on the total growth-producing ability of the hay since it accounted for up to 62.7% of the total forage (69% maximum allowable). However, under the present standards, color is the only quality factor of the timothy portion that affects the numerical grade designation of the hay lot.

The possibility that a carotene deficiency in some of the hays was responsible for growth differentials was considered. However, a carotene and vitamin A analysis of the blood from each animal did not indicate the existence of any deficiency, although there was a considerable range in values.

Since the supplementation of the hays with alfalfa ash or protein during the post-experimental period produced no significant changes in growth rate, it was assumed that a deficiency of neither the mineral elements in alfalfa ash nor protein was responsible for the growth differences.

The relative cash values of hays producing differing amounts of growth per ton of hay may be calculated if the cost of one of the hays is known or assumed. If the cost of U. S. No. 1 Alfalfa Hay such as that used in this experiment was $40.00 per ton (1,800 lb. dry matter), U. S. No. 2 Alfalfa Heavy Timothy Mixed Hay would be worth about $26.00 per ton. These hypothetical prices allow no extra value for more rapid growth, which although desirable is difficult to evaluate.

It is of interest that the individual hay lot producing the most rapid gains (Lot A) was one containing sufficient grass to warrant the term "Light Grass Mixed," yet the amount of grass was not sufficient to enable the demonstration of lower productive value under these conditions. This suggests the possibility that the existing standards as to the maximum allowable grass in alfalfa hay may be too strict.

SUMMARY AND CONCLUSIONS

Six lots of hay were purchased and fed to six groups of dairy heifers as a sole ration in a 120-day feeding trial during the winter of 1952-53. The hays were carefully sampled during the feeding period and the samples analyzed for the usual nutrient constituents and graded according to the official U. S. hay standards. Three lots had an average grade of U. S. No. 1 Alfalfa Hay and the other three lots had an average grade of U. S. No. 2 Alfalfa Heavy Timothy Mixed Hay. The heifers fed the former three lots consumed an average of 24% more dry matter and gained an average of 90% more body weight than the heifers fed the latter three lots. U. S. No. 1 Alfalfa Hay is much more satisfactory as a sole ration for dairy heifers than U. S. No. 2 Alfalfa Heavy Timothy Mixed Hay.

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EFFECT OF DEHYDRATED YOUNG GRASS AS A SUPPLEMENT IN DRY LOT FEEDING ON THE REPRODUCTIVE EFFICIENCY OF DAIRY BULLS[1]

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Development of the artificial breeding of dairy cattle has resulted in the housing of comparatively large numbers of bulls in artificial breeding centers, and managerial practices often make it difficult to maintain bulls under other than dry lot feeding conditions. Herdsmen often resort to such time-consuming practices as the feeding of freshly clipped grass or the feeding of sprouted grains in attempts to maintain and improve the reproductive performance of bulls kept in dry lot.

Grass silage has been shown to be an excellent source of succulent feed for bulls *(3, 9)*, but many breeding centers are so specialized that neither silos nor crops for ensiling are available. High quality dehydrated forages, long recognized as valuable feeds for milk production *(2, 4, 5, 6, 8, 15, 17)*, have been reported to influence certain reproductive functions favorably *(10, 11, 12)*. Such products, if of value for breeding bulls, would be particularly applicable in situations where silage feeding is not practical.

The study reported herein was undertaken to determine if the feeding of a supplement of dehydrated young grass would affect the reproductive efficiency of dairy bulls maintained under dry lot feeding conditions.

EXPERIMENTAL PROCEDURE

Eighteen bulls, including eight Holsteins, six Guernseys, and four Ayrshires, ranging in age from 2¼ to 14 years, were made available for the study at the Southeastern Pennsylvania Artificial Breeding Cooperative. The bulls were paired as closely as possible with regard to age, breed, and breeding efficiency. One member of each pair, selected at random, received the supplement of dehydrated grass, whereas the other bull of the pair received the control ration of hay plus concentrate. Bulls in the supplemented group received the same concentrate and hay as the control group, except that 2 lb. of dehydrated young grass replaced 2 lb. of hay daily. The manufacturers of the dehydrated young grass recommended its use as a supplement at the rate of 1 to 2 lb. daily per mature bull; the higher level of 2 lb. daily was adopted for the experiment. The concentrate fed was a commercial 15% protein bull ration containing the following ingredients in the per cent indicated: crimped oats, 30.00; course ground oats, 13.75; ground corn, 13.54; wheat bran, 12.50; beet pulp, 7.50; linseed oil

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meal, 7.50; soybean oil meal, 7.50; molasses, 5.00; bone meal, 1.00; iodized salt, 1.00; feeding oil A and D, 0.50; irradiated yeast, 0.20; and manganese sulfate, 0.01. The hay fed was a fair quality first cutting timothy-clover mixture consisting of 25 to 30% clover. The supplement was purchased from a commercial source and consisted of a finely ground dehydrated mixture of young grasses (principally oat and rye grass) analyzing 19.6% crude protein. The supplement was added to the concentrate at the time of feeding. No problem in palatability was encountered. Bulls were fed 100 to 110% of the recommended nutrient allowances for breeding bulls *(7)*. Supplementation was started in mid-January, 1953, and the amount increased gradually until the 2-lb. level was reached February 1. The experiment covered the 4-month period, February through May.

Results were evaluated primarily on breeding efficiency, as shown by the per cent of 60- to 90-day nonreturns to first services, although semen production

TABLE 1

Summary of fertility data for individual bulls for the pre-experimental (October-January), experimental (February-May) and post-experimental (June-September) periods

			Pre-exptl.		Experimental		Post-exptl.	
Bull	Group	Age	1st serv.	60-90 d. N.R.	1st serv.	60-90 d. N.R.	1st serv.	60-90 d. N.R.
		(yr.)	*(No.)*	*(%)*	*(No.)*	*(%)*	*(No.)*	*(%)*
A-1	C[a]	11½	117	70	111	71	65	79
A-2	S	14	288	65	312	70	334	58
A-3	C	9½	254	55	180	59	108	48
A-4	S	8	222	61	312	67	288	66
G-1	C	7	825	67	936	69	1,148	65
G-2	S	8	1,646	69	1,872	69	1,387	68
G-3	C	6½	647	63	697	57	274	54
G-4	S	5	244	66	57	61	62	53
G-5	C	2¾	91	78	122	73	93	54
G-6	S	3½	396	66	160	59	87	71
H-1	C	10	165	59	44	71	—	—
H-2	S	11	2,433	75	1,246	74	675	77
H-3	C	7¾	6,450	71	9,310	72	6,776	71
H-4	S	9¾	1,510	67	866	69	318	66
H-5	C	8¼	1,579	70	1,674	71	2,041	65
H-6	S	9	1,215	69	1,707	71	1,808	70
H-7	C	5	470	75	1,092	68	624	77
H-8	S	5	575	73	533	69	177	71
Group totals and averages:								
Services								
weighted	C		10,598	70	14,166	70	11,129	69
equally	S		8,529	70	7,065	70	5,136	69
Bulls								
weighted	C			68		68		64
equally	S			68		68		67

[a] C = control; S = supplemented.

data including volume, numbers of spermatozoa, and initial motility of the spermatozoa also were collected for all semen samples. Concentration of spermatozoa was determined by means of a colorimeter *(16)*. Heated homogenized milk diluter *(14)* was employed and semen samples were diluted to contain approximately 15×10^6 motile spermatozoa per milliliter of diluted semen.

RESULTS AND DISCUSSION

A summary of fertility data for individual bulls, together with the pairings and ages of bulls, is presented in Table 1. Fertility data for the 4-month period immediately preceding the experimental period are included to indicate the fertility levels of the bulls; similar data for the 4-month period immediately following the experiment are included to show any possible trends after supplementation was discontinued. The average nonreturn rates for the two groups indicate that the supplementation with dried young grass was neither harmful nor beneficial during the period tested.

TABLE 2

Summary of semen characteristics for individual bulls for the experimental period

Bull	Group	No. of ejaculates	Average			
			Vol/ejac.	No. of sperm	Initial motility	Total motile sperm/ejac.
			(ml.)	*($\times 10^9$/ml)*	*(%)*	*($\times 10^9$)*
A-1	C[a]	13	5.5	1.83	76	7.55
A-2	S	21	7.7	0.99	72	5.29
A-3	C	14	8.3	1.19	71	6.87
A-4	S	20	8.7	1.05	67	5.71
G-1	C	20	11.7	1.30	66	9.27
G-2	S	27	9.2	1.19	76	8.34
G-3	C	14	9.4	1.44	61	7.89
G-4	S	14	7.3	1.21	62	5.38
G-5	C	15	6.3	1.37	65	5.41
G-6	S	14	6.1	1.38	68	5.72
H-1	C	10	7.4	1.56	61	6.70
H-2	S	15	10.8	2.02	68	16.99
H-3	C	27	9.4	1.50	71	9.95
H-4	S	14	9.3	1.25	64	7.25
H-5	C	19	6.9	2.03	62	8.71
H-6	S	20	7.7	1.50	71	8.10
H-7	C	15	8.1	1.37	74	7.94
H-8	S	14	9.1	1.35	74	9.16
Group averages:						
Ejaculates						
weighted	C		8.4	1.51	68	8.10
equally	S		8.5	1.30	71	7.89
Bulls						
weighted	C		8.1	1.51	67	7.81
equally	S		8.4	1.33	70	7.99

[a] C = control; S = supplemented

As shown in Table 1, one control bull (H-3) accounted for about 60% of the services in the control group. This Gold Medal proved sire has been extremely popular with the breeders and has been collected 2 days each week for nearly 3 years. During that period he has been mated to 53,500 first service cows. One bull in the supplemented group, G-2, also was collected 2 days each week. Weighting bulls equally rather than services, however, did not affect the over-all results.

The supplemental period was of short duration, but no trends were detectable when nonreturn rates were analyzed on a monthly basis. When pairs of bulls were compared, six supplemented bulls showed higher nonreturn rates than their controls during the experimental period, as compared to four and five during the pre-experimental and post-experimental periods, respectively.

Semen characteristics for the control and supplemented bulls during the experimental period are summarized in Table 2. None of the differences in the means listed approached significance when tested by analysis of variance *(13)*.

These results indicate that at the level fed, dehydrated young grass is of little or no value to dairy bulls maintained under the dry lot feeding conditions of this experiment. While this study was being conducted, a report was published by Branton *et al. (1)* indicating that although pasture feeding was economical it provided no advantage over dry lot feeding insofar as the reproductive efficiency of the bull was concerned. Thus, the use of pasture or of dehydrated young grass as a means of improving the breeding efficiency of bulls used in artificial breeding does not appear to be justified.

These results do not, however, preclude the possibility that such supplementation may be of value for certain bulls of low fertility, or for bulls on a poorer basal diet or subjected to a heavier breeding schedule. Two bulls in the supplemented group had nonreturn rates of 65% or less during the pre-experimental period; both showed considerable improvement in nonreturn rates during the experimental period. However, two out of three control bulls with pre-experimental nonreturn rates of 65% or less showed similar increases during the experimental period.

SUMMARY

Eighteen bulls of the Holstein, Guernsey, and Ayrshire breeds and ranging in age from 2¼ to 14 years were paired on the basis of age, breed, and fertility. One bull of each pair received a concentrate mixture plus grass-legume hay (control group) while the other had 2 lb. of the hay replaced by 2 lb. of dehydrated young grass.

Over a 4-month experimental period the groups were compared on the basis of 60- to 90-day nonreturns to 21,231 first services and laboratory examinations of semen quantity and quality. No difference was found between the two groups, indicating that, under the conditions of this experiment, the supplementation with dehydrated young grass at the rate of 2 lb. daily was of no value in improving the reproductive performance of dairy bulls maintained on a conventional dry lot feeding regime.

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BACTERIOLOGICAL STUDIES OF CULTURED BUTTERMILK. II. PROGRESSIVE CHANGES IN THE NUMBERS OF LEUCONOSTOC CITROVORUM AND STREPTOCOCCUS CREMORIS AS ASSOCIATED WITH pH AND ACETLYMETHYLCARBINOL PLUS BIACETYL LEVELS[1,2]

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No studies have been reported in which progressive changes in the numbers of both the leuconostoc and lactic streptococcus types were followed throughout the course of the fermentation process. Such studies have not been undertaken, largely because of the lack of satisfactory cultural methods for the differentiation and enumeration of the leuconostoc types of starter bacteria growing in association with the lactic streptococci in dairy starters.

Recently, Prouty and Glenn *(7)* described a culture method which proved satisfactory for the differentiation and enumeration of a strain of *Leuconostoc citrovorum* present in two buttermilk starters designated as Hs and Fs, respectively. The lactic streptococcus growing in association with *L. citrovorum* in these starters was *Streptococcus cremoris.*

The present paper reports data obtained from many trials, using the Hs and Fs starters, in which progressive changes were followed during the fermentation in the numbers of *L. citrovorum* and *S. cremoris,* the pH level, and acetylmethylcarbinol plus biacetyl content.

METHODS

Culturing. A 1-liter portion of fresh nonfat milk was dispensed into a 2-liter flask, heated in flowing steam for 45 minutes, and cooled to 22° C. The fermentation was initiated by an inoculum of 0.1% from a mother culture which had been incubating at 22° C. during the preceding 24 to 30 hours. A temperature of 22° C. was maintained throughout the period of observation.

Usually the fermentation was started late at night. With the small inoculum used, the culture was at the proper stage for observation some 10 to 12 hours later. A sample was taken for bacteriological and pH examination immediately after inoculation. Beginning at 10 to 12 hours after inoculation, samples were taken at 1-hour intervals during the following 10 to 14 hours. This permitted

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[1] Scientific Paper No. 1296, Washington Agricultural Experiment Stations, Pullman. Project No. 1019.

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

a coverage of the early and rapid fermentation periods. Usually, additional samples were examined at the end of 35 hours of incubation and occasionally beyond this time.

Sampling. Samples for bacteriological examination were taken directly from the milk in the flask until the point of coagulation was reached. Prior to coagulation of the milk, 10 to 15 1.0-ml. samples were transferred aseptically to sterile screw-capped test tubes containing three or four glass beads. The tubes were tempered to 22° C. previous to receiving the samples. These tubed samples were then incubated adjacent to the flask of fermenting milk. Following the coagulation of the milk in the flask these tubed samples were withdrawn from the incubator and plated as needed. This procedure was followed to minimize the inherent variations associated with the measurement of small volumes of coagulated mlk. The presence of beads in the tube assisted in the disintegration of the curd during the preparation of the sample for plating.

Observations at each sampling period included plate counts for both *L. citrovorum* and total flora and determinations of pH value and acetylmethylcarbinol plus biacetyl content.

L. citrovorum count. The *L. citrovorum* count was made by the method described by Prouty and Glenn *(7)*. This consisted of a plate culture method using a medium capable of supporting the growth of *L. citrovorum* while retarding the growth of *S. cremoris*. Plate cultures, made in duplicate, were incubated at 25° C. for 5 days and the colonies counted with the aid of a wide field binocular microscope at a magnification of 7.0 ×. Although this medium proved highly satisfactory for differentiating the strains of *L. citrovorum* and *S. cremoris* present in the two starter cultures used in this study, such was not the case when applied to several other buttermilk starters. In this respect the limitation of its use is recognized.

Total count. The total count was considered to consist entirely of *S. cremoris* and *L. citrovorum*. The total count was made by the plate method using tomato juice–peptonized milk agar prepared as described by Turner and Nelson *(8)*. All plates were made in duplicate and were incubated at 26 to 27° C. for 5 days.

pH value. Determinations of pH values were made with a Beckman pH meter with glass electrode.

Acetylmethylcarbinol plus biacetyl. The acetylmethylcarbinol plus biacetyl content was determined using a modification of the King test *(3)* as developed by Beutler *(1)*. This test makes use of the Evelyn Photoelectric Colorimeter with a 540 filter and standard biacetyl curves to determine the milligrams of acetylmethylcarbinol plus biacetyl per 100 g. of the sample. The analysis was made on a 4.0-g. sample. This was done either at the time of sampling or within 24 hours when the sample was frozen immediately after being taken.

RESULTS AND DISCUSSION

Numerous trials were made in this study. Since the results obtained in all trials followed the same general pattern, summarizing data are presented to cover all trials and detailed data for only two trials. Figure 1 includes data

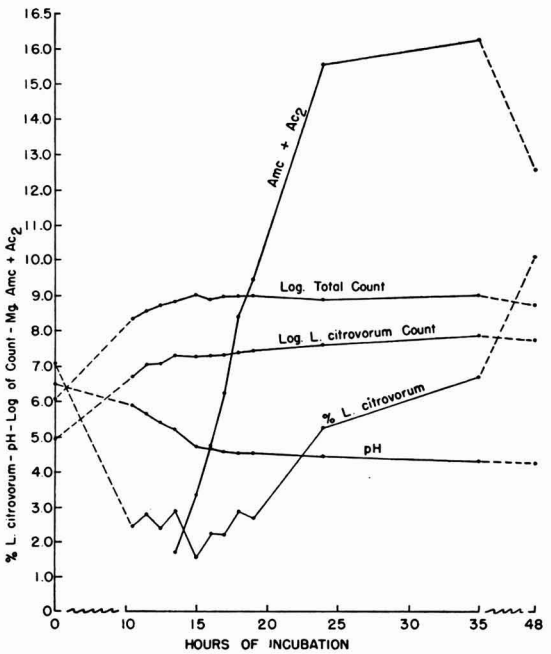

FIG. 1. Progressive changes in the total *(S. cremoris + L. citrovorum)* and *L. citrovorum* counts, percentages of *L. citrovorum*, pH and acetylmethylcarbinol plus biacetyl content. Hs starter.

for 13 sampling periods, using the Hs culture, over an observation period of 48 hours. Fortunately, for the clarity of this figure, the numerical values used fall in the common range for the expression of each of the five different types of data. Table 1 includes data of 14 sampling periods, using the Fs culture, over an observation period of 25 hours.

Some irregularity in results was occasionally encountered among the trials included in this study. However, the general trend in all trials was in the same direction. Occasionally the recorded counts of bacterial populations were lower or higher for some samples than would be expected from the immediate preceding sample during the early and rapid fermentation periods. Such variations are often encountered with the plate method of counting. In the two cultures used in this study, both types of starter bacteria were present in long-chain formation. Differences in the amount of agitation during the preparation of the samples for plating may have resulted in considerable variation in the breaking-up of these long-chain formations. The degree to which this occurred may have been reflected in the number of colonies developing on the plate cultures.

L. citrovorum. Maximum levels for this species were not reached until 35 hours of incubation and in some trials even beyond this point. Maximum counts ranged from 38 to 66 millions per milliliter at the 24-hour period and from 55 to 108 millions after 35 hours. At no time was there an explosive multiplication

TABLE 1

Progressive changes in the total (S. cremoris + L. citrovorum) and L. citrovorum counts, percentages of L. citrovorum, pH and acetylmethylcarbinol plus biacetyl content Fs starter

Hours	Plate Count 10^6 per ml.			pH	Amc. + Ac_2
	Total	*L. citrovorum*	*L. citrovorum*		
			(%)		*(mg/100 g.)*
0	1.18	0.062	5.2		0
10	69	0.375	5.4	6.23	0
13	290	6.2	2.1	5.75	trace
14	406	8.1	2.0	5.5	trace
15	890	13.0	1.5	.....	trace
16	1,270	13.4	1.01	4.85	1.25
17	1,200	17.1	1.4	4.60	2.75
18	1,140	16.5	1.4	4.50	4.35
19	1,750	21.6	1.2	4.40	5.70
20	1,320	17.1	1.3	4.40	6.55
21	1,420	26.5	1.9	4.40	8.20
22	980	30.5	3.0	4.40	8.70
23	1,500	47.0	3.1	4.40	10.15
25	1,360	52.0	3.8	4.35	13.90

of *L. citrovorum*. From Figure 1 it will be noted that the increase in numbers of this species was most rapid during the period up to 13.5 hours of incubation. Also, it will be observed that during the latter portion of this period the pH level was near 5.0, and measurable amounts of acetylmethylcarbinol plus biacetyl were in evidence for the first time. Likewise the data presented in Table 1 show a similar trend in regard to this species in the Fs culture.

According to Knudsen *(4)* the growth of the leuconostoc types is arrested when the pH value is reduced to about 5.0. The results of the present study show a reduced rate rather than an arrest of the growth of *L. citrovorum* at pH levels below 5.0.

Total count. The total count, of which *S. cremoris* comprised 90.0 to 99.0%, usually reached near maximum levels in 15 to 20 hours and remained at this level for an additional 10 to 15 hours. It then decreased more or less rapidly. Maximum total counts ranged from 800 to 1,770 millions per milliliter. The most rapid growth period for *S. cremoris* corresponded to that for *L. citrovorum*.

Relationship of L. citrovorum to S. cremoris. The numerical relationship of *L. citrovorum* to *S. cremoris* is best expressed in terms of percentages. In the numerous trials included in this study, the percentages of *L. citrovorum* to the total flora of the freshly inoculated samples ranged from 3.71 to 8.47. As the fermentation progressed, these percentages decreased, ranging from 1.0 to 2.79 at the time of coagulation (pH 5.1). As the fermentation progressed beyond coagulation, an increase occurred in the percentages of *L. citrovorum*. These rose to levels of 3.4 to 5.29 at the end of 24 hours of continuous incubation. In most trials, the percentage of this species continued to increase. At the 35-hour observation period, it approximated that of the initial period. In Figure 1, the initial, the low, and the final percentages are 7.09, 1.59, and 10.09, respectively. In Table 1, comparable percentage values are 5.2, 1.01, and 3.8; the last reading

being after 25 hours as compared with 48 hours in Figure 1. Hammer *(2)*, Orla-Jensen *et al. (6)*, and Knudsen *(4)* in their pioneer studies of the starter bacteria found the associate or leuconostoc types to constitute only a minor portion of the total bacterial population.

In most trials the numbers of *L. citrovorum* continued to increase after the *S. cremoris* population had reached a maximum level.

pH levels and acetylmethylcarbinol plus biacetyl. Michaelian *et al. (5)* and other investigators have reported that a definite relationship exists between the production of acetylmethylcarbinol plus biacetyl and the pH level of the fermenting milk. The data from these observations support this contention. Very little acetylmethylcarbinol plus biacetyl was produced prior to coagulation of the milk. Beginning at the time of coagulation, however, the production rate of these compounds accelerated rapidly, proceeded at a uniform rate to the end of the 24-hour period, and then continued to increase at a declining rate to the end of the 35-hour period. Beyond this point, there was a gradual destruction of the acetylmethylcarbinol plus biacetyl content.

In one trial, the *L. citrovorum* population had reached the level of 32 million prior to the point of coagulation without significant production of acetylmethylcarbinol plus biacetyl. In another trial in which coagulation had occurred a population of *L. citrovorum* at the 10 million level resulted in the production of appreciable amounts of these compounds. This example serves to show the relationship of the pH environment to the production of acetylmethylcarbinol plus biacetyl by *L. citrovorum*.

SUMMARY

An investigation was made, using two starters, in which progressive changes were followed throughout the fermentation period of cultured buttermilk in the numbers of *L. citrovorum* and *S. cremoris*, pH value, and acetylmethylcarbinol plus biacetyl content.

At all stages, *L. citrovorum* represented only a small percentage of the total flora as compared with *S. cremoris*. It was at the lowest level near the coagulating point of the milk and then increased beyond this point. The *L. citrovorum* population continued to increase after the *S. cremoris* population had reached a maximum level. Maximum levels for *L. citrovorum* were not reached until 35 hours of continuous incubation or longer.

No significant production of acetylmethylcarbinol plus biacetyl occurred prior to coagulation of the milk. After that time, the rate of increase was rapid to the end of the 24-hour period.

The results obtained with the two starter cultures were comparable.

ACKNOWLEDGMENT

This study was aided by a grant from the Stuart Foundation, Carnation Company.

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PEOPLE and EVENTS
in the Dairy Science World

Deaths of Prominent Dairymen

CARL A. VORHES, dairy technologist at the Quartermaster Food and Container Institute, died August 12, 1954. Mr. Vorhes was born September 23, 1892. He had been associated with the dairy industry since 1912. He attended Iowa State College, 1912-1916, receiving a B.S. degree in dairy industry. He was associated with the Blue Valley Creamery for 16 years, managing plants in Sioux City, Iowa, and Milwaukee, Wis. He operated a milk and ice cream business from 1935 to 1942 at Marshalltown, Iowa, and joined the Quartermaster Food and Container Institute staff in 1944. He had been a member of the Institute since that time.

Mr. Vorhes was known by many in the dairy industry for his work on products for the Armed Forces. He is survived by his wife, two sons, and a daughter.

BENJAMIN ANIXTER, a prominent Pacific Coast dairyman, died July 1 at his home in San Francisco at the age of 71. Mr. Anixter was president of the Dairy Dale Co., which was merged with the Borden Co. in 1928. He remained with the Borden Co. as head of the Borden's Dairy Delivery Co. until his retirement in 1944.

C. M. HIBBERT, 74, former manager of the Challenge Cream and Butter Assoc., died June 6 in Pasadena, Calif. He was a past president of the California Dairy Council. He retired from the Challenge organization in 1946 after 35 years of active management.

Mr. Hibbert was one of the nation's leaders in developing co-operative marketing. Under his supervision Challenge grew from a few members to be one of the world's largest dairy products marketing firms.

JOHN P. JONES, attorney and assistant secretary of the Isaly Dairy Co., a chain of dairy stores operating in Ohio and Pennsylvania, died August 10 of a heart attack. He leaves a widow and four children. Mr. Jones was associated with Isaly's for 21 years.

Sturtevant Sells to Borden

One of the largest independent milk and ice cream operations in Illinois, Sturtevant Dairy Products Co. of Rock Island, has been acquired by the Borden Co. The company was organized in 1913. KENNETH STURTEVANT, son of the founder, will continue as general manager of the company, which will be known as the Sturtevant Dairy Products Division of the Borden Co.

Foremost Continues Expansion Program

Foremost Dairies, Inc., a rapidly growing dairy corporation, has been authorized by its board of directors to acquire the Ives Ice Cream Co. and the DeSoto Ice Cream Co., both of Minneapolis. They have also purchased 21% of the outstanding stock of the Philadelphia Dairy Products Co. Other Foremost acquisitions since the first of the year include the Redwood Empire Dairies and the Golden State Co., both California operations, and the Moanalua and Rico Dairy Companies of Hawaii.

Borden Appoints Koenig to Chicago Post

R. D. WOOSTER, vice-president of the Borden Co. of New York City, has announced the appointment of O. N. KOENIG as chairman of the Chicago-Central district. He succeeds O. O. SMAHA, who was forced to retire from active duty because of illness.

Mr. Koenig has been associated with the Borden Co. since 1928 and has served as general sales manager of the Chicago Milk Division and more recently as president of that division.

Changes in N.D.C.

Two changes in the public relations program of the National Dairy Council have been announced by MILTON HULT, president.

REX THOMAS, a specialist in agricultural press and radio relations, has joined the staff, and the Department of Information Service, headed by W. S. EPPLE, has been merged with the Department of Public Relations, headed by L. H. GEIL. Both Thomas and Epple serve as assistant directors in the public relations department.

Thomas will be responsible for NDC relations with the agricultural press and radio. In addition, he will work closely with the 67 local Dairy Council offices located in major cities throughout the country to aid in the interpretation of programs and to assist the local directors in strengthening their press and radio activities. Thomas comes to NDC after 2½ years as director of promotion for the American Shorthorn Breeders' Assoc., purebred beef cattle registry organization. Prior to his last position, he served as publicity director of the

International Live Stock Exposition and handled other public relations projects for the Union Stock Yard and Transit Co. of Chicago from 1947 to 1951.

Epple will be in charge of a stepped-up program of information services to newspapers, magazines, radio, TV, and dairy industry processor and handler publications, and will continue as editor of the quarterly NDC publication, *The Dairy Councilor.*

North Carolina Dairy Products Association Scholarships

The North Carolina Dairy Products Assoc. and R. L. LOVVORN, Director of Instruction, School of Agriculture, N. C. State College, announce the awarding of two scholarships to dairy manufacturing students at the college. The funds for these scholarships are made possible by contributions of members of the North Carolina Dairy Products Assoc.

The $500 scholarship for a freshman enrolling in dairy manufacturing was awarded to R. JOHNNY MCGLAMERY, Statesville, N. C. Johnny was active in sports and other extra-curricular activities in high school. He was vice-president of his senior class. Since 1951, he has been working after school and during the summer months for Superior Dairies, Statesville, N. C.

The $300 scholarship for a rising sophomore in dairy manufacturing at N. C. State College was awarded to MAX I. LOYD, Statesville. Max, a veteran with a 1-year enlistment in the Air Force and a 2-year tour of duty in the Army, enrolled in dairy manufacturing in 1953. During his freshman year he achieved a high scholastic rating.

The Ohio State University

Representatives of the butter industry and the Ohio Dept. of Agriculture met in Columbus, Ohio, on August 19 to discuss the compliance with the Cream Regulation which went into effect May 1. This regulation establishes a maximum 4-day delivery period for producer cream for the summer period and 7-day for the winter.

A survey is being conducted by the department and Ohio Dairy Products Assoc. to ascertain the present status of bulk farm pick-up of milk and the anticipated developments within the next year.

The department, in cooperation with the Ohio Dairy Products Assoc., is planning a 1-day field program to be held at the University in October.

FRANK KOVAL, a graduate in dairy industry from The Ohio State Univ. in 1949, has been appointed a dairy technology extension specialist in the Dept. of Dairy Technology. Mr. Koval, whose home is in Shadyside, Ohio, was born and raised on a dairy farm in eastern Ohio. Since his graduation from The Ohio State Univ., he has been associated with the Evaporated Milk Assoc. as a member of the Sanitary Standards staff. He has been engaged in sanitary audits of evaporated milk plants and dairy farms and in assisting with the organization of fieldmen conferences. He will fill the position formerly held by P. R. Ellsworth.

Florida Events

"Mango Ice Cream" was the title of a report presented by W. A. KRIENKE of the Dept. of Dairy Science, Univ. of Florida, before the Florida Mango Forum at Miami on July 21, 1954. The new variegated mango ice cream which he prepared for sampling was received with enthusiasm by nearly 1,500 people in attendance.

H. H. WILKOWSKE, secretary-treasurer of the International Assoc. of Milk and Food Sanitarians, has been advanced in rank to associate professor in the Dept. of Dairy Science.

TOM G. LEE, who began dairying with one cow soon after graduation from the Univ. of Florida in 1917, is erecting a $250,000 dairy plant at Orlando, Florida, to house Lee's Dairy. He is a director of the Florida Dairy Assoc. and former president of Florida State Dairymen's Assoc.

Wisconsin News Items

P. A. BUCK has been appointed assistant professor of Dairy and Food Industries at the Univ. of Wisconsin. He recently completed his Ph.D. thesis at the Univ. of California. His new duties will be concerned with research and teaching of food processing.

K. G. WECKEL participated in a memorial program to the late Professor E. B. HART at the annual meeting of the food technologists in Los Angeles. Professor Hart was well known in the dairy industry field for his early work in cheese curing, his casein test, and many other contributions.

W. A. MCGUGAN, having completed his Ph.D. thesis at the Univ. of Wisconsin, has returned to Ottawa, Canada, where he will be a member of the dairy chemistry section of the new dairy technology research unit at the Central Experiment Farm in Ottawa.

ERKKI PELTOLA of the State Institute for Dairy Research, Jokioinen, Finland, who has been a Fulbright research scholar in the Dept. of Dairy and Food Industries for 5 months, has completed his study on problems relating to the rennet coagulation of milk and has returned to Finland.

SAEED GABALLAH has completed work on his Ph.D. thesis at the Univ. of Wisconsin and has returned to Egypt. He will teach and do research at the College of Agriculture, Shebin El Koom, Egypt.

H. E. CALBERT is the president-elect of the Wisconsin Milk Sanitarians Assoc. for the coming year. This organization, composed of milk sanitarians, regulatory inspectors, and dairy fieldmen, is active in promoting quality improvement work in milk production and processing in Wisconsin. Professor Calbert appeared on the program of the N. Y. State Milk Sanitarians Assoc. at their annual meeting in Albany on Sept. 20 and at the annual meeting of the International Assoc. of Milk and Food Sanitarians in Atlantic City in September.

Minnesota Happenings

During the past summer a series of meetings has been sponsored in various sections of the state by the Minnesota Milk Sanitarians Assoc. The subject matter covered included discussions, demonstrations, and interpretations of laboratory methods used in grading raw milk and in the evaluation of finished products. Members of the dairy bacteriology staff at the University and regulatory personnel from the Dept. of Agriculture, Dairy, and Food cooperated with the association in this work. The meetings have been open to all plant and field personnel.

The Univ. of Minnesota held the annual Dairy Products Institute on Sept. 15-17. On Sept. 16, a joint dinner of the Dairy Products Institute and the Minnesota Dairy Technology Society was held with H. A. MORRIS, assistant dean of agriculture, Utah State College, as guest speaker. On the evening of Sept. 17, the annual meeting and banquet of the Minnesota Milk Sanitarians Assoc. was held with H. L. THOMASSON, executive secretary of the International Assoc. of Milk and Food Sanitarians, as guest speaker.

DR. and MRS. T. W. GULLICKSON spent a portion of the summer in Europe. They sailed on the Queen Elizabeth from New York August 4 and visited England, Norway, Sweden, Denmark, Holland, Germany, Switzerland, and France. They returned by plane from Paris in September.

H. H. OLSON, who received his Ph.D. degree at Minnesota, has accepted a position as assistant professor at Southern Illinois Univ., Carbondale. He was formerly with the Curtiss Farms at Cary, Ill.

California Starts Important Studies

A special study is being made of quick dissolving powdered milk by A. H. BOCKIAN, who recently received his Ph.D. degree in agricultural chemistry. The study is being made possible by a grant from the Western Condensing Co. of Petaluma, Calif.

With funds supplied by the California Dairy Industry Advisory Board, R. W. THOMAS, a recent graduate of the Univ. of Florida, will study flow characteristics of H.T.S.T. pasteurizers and the seasonal variation of milk protein.

Western Division of A.D.S.A. Meets at Oregon State

The Western Division of the American Dairy Science Assoc. and the Western Section of the American Society of Animal Production held their annual joint meeting in July at Corvallis, Ore., with 150 members of the two societies present. A conference on milk pricing and marketing, with ROSS WURM, editor of the *Western Dairy Journal,* as moderator, and 40 scientific papers were on the program for the group.

G. M. DRUMM of California State Polytechnic College, chairman of the Western Division, presided at the business meeting. J. O. YOUNG of Oregon, vice-chairman, will automatically become chairman of the dairy division for 1955. I. W. SLATER, Wyoming, and T. A. NICKERSON, California, were nominated for secretary-treasurer, and L. R. HUNSAKER, Utah, and T. H. BLOSSER, Washington, were nominated for vice-chairman. Members will vote by mail to fill these offices.

A salmon barbecue, prepared by A. W. "AL" OLIVER and attended by 225 persons, was the highlight of the entertainment for the two groups.

Completed Theses

M.S. Degree:

DELMAR L. ANDERSEN—A comparison of freeze-drying milk and milk dried at temperatures between 0 and 10° C. Univ. of Wisconsin.

E. CHICOYE—Factors responsible for a hay-like flavor in vitamin A fortified low-fat milk. Univ. of Wisconsin.

A. FEDERER—A study of methods of sterilizing evaporated milks. Univ. of Wisconsin.

G. HADLAND—Solvent power as a measure of the total solids content of milk. Univ. of Wisconsin.

HAROLD RAPP—Studies on bulk handled milk. Univ. of Wisconsin.

H. J. SEYMOUR—Correlation of butterfat production predicted from single tests with butterfat yields calculated from monthly tests. Kansas State College.

GEORGE KLOSER—A study of the reliability of the Reichert-Meissl and Polenske methods for detecting substitute fats in ice cream. Univ. of Georgia.

RODDY PARKMAN—Effect of certain factors on quality of ice cream stored in home-type freezer lockers. Univ. of Georgia.

H. O. JAYNES—Studies of milk lipase. Univ. of Tennessee.

W. M. MASSEY—The economy of irrigating the University of Tennessee dairy farms. Univ. of Tennessee.

J. B. FREEMAN—Grazing habits of dairy cattle. Univ. of Tennessee.

Ph.D. Degree:

A. W. RUDNICK, JR.—Effect of heat during processing of nonfat dry milk solids for cottage cheese manufacture. Univ. of Minnesota.

ALAN THOMAS—A study of the mechanism of sulfhydryl action in milk. Univ. of Minnesota.

S. GABALLAH—Studies on the freezing point of milk. Univ. of Wisconsin.

WESLEY A. MCGUGAN—A study of the physical properties of spray-dried milk. Univ. of Wisconsin.

VINCENT L. ZEHREN—The isolation and properties of casein. Univ. of Wisconsin.

VIRGINIA F. ZEHREN—Denaturation of beta-lactoglobulin. Univ. of Wisconsin.

WHAT ABOUT MILK FLAVOR?

A Guest Editorial

It would seem that most of us in the dairy industry could agree that the only really basic solution to the present problem of surpluses is to sell more milk in the fluid form and to sell more butter. By better packaging, more advertising, and sales effort we can undoubtedly sell more cheese, more evaporated milk, and more ice cream. The possible increase in milk usage in these three products, however, will not solve the probelm. The potential market for fluid milk and for fine high quality butter could solve our problem.

It is true that we have a terrific long way to go in regard to butter flavor quality when we read of a meeting in one of our leading dairy states where they arrive at the momentous decision to receive no cream over 4 days old. Such a decision is certainly a sad commentary on that branch of our industry. Then, when you read a bulletin from a college in another state where they tell the operator how to standardize poor cream with good cream so as to meet mold mycelia standards, you begin to wonder how we sell as much butter as we do.

I believe the butter industry as a whole is very much aware of the need for flavor quality improvement. However, I have the feeling that the fluid milk people do not appreciate the need for improvement of flavor quality of the bottle of milk.

The Alfred Politz survey made for the American Dairy Association shows that flavor quality of milk sold as fluid is of great importance. My observations are that we have four periods of poor flavored milk in most areas of our country. In the spring we have the extreme grass flavor often in combination with various weed flavors. This flavor is very distasteful to many people. In many areas we have serious weed flavors again in the fall when pastures start to shorten up. In the very late fall when many cows are in late lactation, a slight rancid flavor can be observed in much fluid milk. In the early spring before grass, oxidixed flavors are quite common for a month or two. On one occasion when samples were accumulated for the International Students Contest in an eastern state, one-third of the samples carried a distinct wild onion flavor. Last year not a single sample of milk accumulated for the contest was of really fine flavor quality.

N. E. Fabricius

If we observe carefully I think all of us notice a difference in milk consumption by our families during each of these flavor change periods. My own family will change its milk consumption downward as much as 50% during such periods. It takes a long time to get them back to full consumption after they consume such defective flavored milk. Last spring two of us ordered milk in an eating place in Madison. I immediately recognized a combination weed and grass flavor that made the milk very distasteful. I didn't say a word, for naturally a dairyman is more particular than average in respect to flavor quality. The other person tasted his milk and immediately remarked, "Boy, this milk has a terrible taste!" When an average consumer makes such an observation, it is a serious matter. All milk delivered to this market is Grade A and undoubtedly was fine milk in regard to bacterial content and it was in all probability carefully processed.

Now I want to ask a question as one who has graded milk quality in nearly every state of the union, but one who has had little market milk experience. Why not subject all milk to be used as fluid to a mild vacuum treatment?

It would seem to me in periods of strong grass flavors and slight weed flavors, that even a mild vacuum treatment would level out these flavor defects. In the late fall it should remove some of the volatiles common in milk from late lactation cows. Certainly the undesirable wild onion flavor observed in much eastern and southern milk could easily be removed. The removal of some of the closely bound oxygen

from milk in early spring when oxidixed flavors are common also should be of value.

The fact that carefully processed 3-1 concentrate when recombined is superior in flavor to most ordinary fluid milk would seem to me to be of considerable significance. I know of one plant that is subjecting its milk sold as fluid to a mild vacuum treatment. I have interviewed a number of customers of this plant as to why they buy this milk even though it is often higher in price than locally processed milk. The almost unanimous answer is that the milk tastes better.

Certainly some research along this line is long overdue. My humble opinion is that if we could solve this fluid milk flavor quality problem we could regain our per capita consumption and even increase it greatly. If so it would, of course, solve our surplus problem.

N. E. Fabricius
Ladysmith Milk Producers' Cooperative Assoc.
Ladysmith, Wis.

LETTERS TO THE EDITOR

A Letter to Past-President Price

Receipt is acknowledged of your letter of May 25 addressed to the Secretary, and the cover containing the projects submitted by some 300 members of the American Dairy Science Association in response to the request made of you as president of that Association regarding what projects in research, extension, and education in the dairy field will do most to strengthen American agriculture.

This record of projects is most complete and interesting. You and the members of your Association are to be complimented on the fine response you have made to the Secretary's request. We are sure that all of the projects proposed are most worthy and would contribute greatly to solving many of the difficult problems currently troubling the dairy industry. We shall hope that many of them may come to fruition.

In reply to your comment about the creation of a study group to review these projects in order to develop a balanced program in research, education, and extension for the dairy industry, the Department has had in existence since 1947 a dairy research advisory committee that counsels and advises with the Department on its dairy research program. Your report will be made available to this committee for its consideration.

In addition, there exists in the Department a dairy research working group which is a counterpart of the research advisory committee. Members of this group along with appropriate officials in the various branches of the Agricultural Research Service, including the Office of Experiment Stations, and the Agricultural Marketing Service, and Federal Extension Service will also have an opportunity to see your report for use in planning their respective future programs.

In addition to this, should such a study group as you have indicated seem necessary, we shall not hesitate to form one. In this event we shall be happy to take advantage of your offer of assistance.

J. Earl Coke
Assistant Secretary of Agriculture

Liked Mack Gebert's Article

Please allow me to congratulate the *Journal of Dairy Science* on Mack Gebert's "The Self-Help Program" in the May issue. This and similar articles in your "Our Industry Today" section should increase reader interest among the nontechnical members of A.D.S.A.

Reed Shafer
Greenville, Ohio

A Report from Dr. Wilster

In Vol. XXXVII, No. 6, p. 762, there appears the following statement: "In 1950 Dr. G. H. Wilster of Oregon State College was appointed chairman of a committee to formulate detailed procedures for the test, and he continued as chairman until 1952 when he resigned because of illness."

In referring to my records I find that in March, 1948, the Association appointed a committee on "Standardizing Methods for Conducting all Phases of the Babcock Test." Mr. L. M. Lampert and Mr. R. P. Robichaux agreed to work with me on this committee. The committee was reappointed in 1949, 1950, and 1951. However, Mr. Robichaux was replaced on the committee by Dr. D. H. Nelson, who served for about three years. The committee presented a report at the 47th annual meeting. In the report of the manufacturing section printed in Vol. XXXV, No. 8, of the Journal of Dairy Science, it is stated, "G. H. Wilster, chairman of the committee on 'The Procedure and Equipment for Determining the Fat in Milk by the Babcock Method' presented a written report which was accepted after revisions were made with reference to centrifuge speeds, directions on pipette specifications and use and aliquot sampling. It also was voted to delete the last sentence of paragraph one, which reads, 'A mechanical stirring device shall be used with the weigh can in order to satisfactorily mix the milk.'

"It is recommended by the section that Chairman Wilster submit the revised report to the secretary of the section as a part of the minutes. The committee will be continued to receive addi-

tional comments and suggestions concerning methods recommended in the report."

The revised report was submitted to the Association and President H. B. Henderson approved it for inclusion in the proceedings of the 33rd annual meeting of the Western Division, American Dairy Science Association. This was done and I am attaching herewith a copy of the report as was published.

I would like to say at this time that I did not resign from the committee because of illness. As a matter of fact, my health was excellent and I did not resign. However, I was not advised by the association to continue as chairman of the committee.

I trust that a clarification of this matter can be made through the medium of the *Journal of Dairy Science*. I am pleased to be able to state that I am in fine health, I am enjoying my work, and I am enjoying life here in Oregon.

G. H. WILSTER
Professor of Dairy Manufacturing
Oregon State College

Special Committee Reports on Dr. Whitney's Proposal

A committee was appointed by President Price to study the possibility of developing uniform rules or principles for determining the order of authors applicable to all research publications in the *Journal of Dairy Science*.

In a letter published in the *Journal* it was stated that the authorship is now confusing and leads to misinterpretations. It was hoped that the committee would establish a policy which would be acceptable to all research agencies to relieve them and their investigators of the responsibility and effort of arranging the order of authors on publications.

After one meeting of two of the members and some correspondence your committee advises against such an empirical procedure to select and arrange authors of research articles. The American Dairy Science Association has neither the authority nor the right to inform colleges and commercial research institutions how they must make this decision. There may be reasons for different policies in the various educational and commercial research laboratories.

These two general considerations make it undesirable to formulate rules for arranging the order of authors of articles published in the *Journal of Dairy Science*.

F. E. NELSON
T. S. SUTTON
A. C. DAHLBERG, *Chairman*

Bulk Handling of Milk

We have noted with much interest B. L. Herrington's "Lipase, A Review" in the July *Journal of Dairy Science*.

The widespread interest in bulk milk methods and the possible relationship of this method of handling milk to lipase activity may make it worth while to review bulk milk history over recent years.

Bulk milk started on the large farms in California about 15 years ago. Previously milk was cooled on a surface cooler to temperatures of about 40° F. and collected in cans. When bulk handling was introduced, the surface cooler was raised to an elevated position, the cold milk discharging into a plain, insulated holding vat. Milkings were mixed without apparent detrimental effect, as temperatures in the holding tank were maintained around 40° F. The milk was under agitation for sampling purposes only at time of pick-up.

Elevating the surface cooler usually necessitated the installation of a dump tank with pump to transfer the milk to the elevated cooler.

On the smaller family-operated farms in the Midwest and East the pour-in tank with elevated surface cooler discharging into a holding vat did not appear to be the best answer to bulk handling. Recognizing this, on the first bulk route in the eastern part of the country established out of Hartford, Conn. in 1948, farm holding tanks were used with a cooler section suspended in the center of the tank. Milk was poured into a trough mounted on the tank which distributed the milk over this refrigerated section. An increase in the milk level in the tank had the effect of gradually submerging the cooler section and causing a drop-off in cooling efficiency.

The following year, 1949, a bulk route with all cold wall tanks was established out of Columbia, S. C. A refrigerated cold wall bottom with an agitator stirring the milk, cooled the milk. This combination of tank and cooling surface has made possible a farm unit requiring limited floor space and is well adapted to milkhouse sizes and practices on family-operated farms. Warm milk is added to the previously cooled milk and agitation is maintained during the cooling period. The temperature rise of the milk during the second or subsequent milkings is related to rate of milking, size of compressor and tank efficiency. On well engineered installations the milk temperature should not exceed 50° F. during the second or subsequent milkings, and on many installations the milk temperature seldom if ever exceeds 45° F. (As the milk is well below churning temperature we would not expect to find churned butter granules under these conditions. Where churned butterfat particles appear we would generally suspect a compressor too small in relation to refrigeration load or a tank design of such nature as to require too long an elapsed time to handle the cooling operation.)

Up until the present time the sanitarians have been primarily concerned with tank construction. Less attention has been paid to cooling performance, which in any event is not necessarily a problem involving milk sanitation.

In the 3A Sanitary Code on farm tanks it is required that the milk be cooled to "50° F. in one hour after the tank has been filled to rated capacity (per milking) with the compressor in operation during the filling period." This may be a low performance standard. It is altogether possible that tank designs which meet this standard, nevertheless permit a sufficient temperature rise during the second milking "to carry the milk across the threshold of organoleptic rancidity, especially if alternate day pick-up is practiced," as suggested by Dr. Herrington.

We feel that research work is needed to determine if possible the maximum allowable temperature rise on a bulk milk cooling tank during the second and subsequent milkings, as related to lipase activity.

H. G. MOJONNIER
Mojonnier Bros. Co.
Chicago

Inquiry from Israel

I should be grateful if you could publish the following letter in your *Journal.*

For several years now we have noted the appearance in various farms of certain abnormal symptoms in the cattle whose causes we have been unable to find. One of these is the lengthening of the gestation period which reaches 290-300 days and even more. As a result unusually large calves of 50-60 kg. are born and embryectomy or Caesarean sections have often to be carried out. The fluid at parturition is yellow in color and the placenta is generally retained. In most cases the cows show satisfactory health development during later pregnancy. The udder expands normally until shortly before parturition the swelling decreases and at calving time the udder is rather small. The milk yield, as might be expected, is low.

The second widespread phenomenon appears mainly among female calves and generally at the farms where the disturbances of fertility occur. There is an arrested development of horns. Up to the age of 6-7 months the horns hardly grow at all or, if they do grow, are weak and loose. With bull calves there is normal growth and development of the horns. We do not attribute this phenomenon to a genetic factor as it recurs in several farms in different parts of the country and as it is associated with other symptoms as weakness, diarrhea, and generally poor development of the young calves.

There is also no likelihood that the lack of horn development is the result of calfhood diseases because the bull calves, whose horn development is normal, are reared under the same conditions as the female calves and show the same retardation in growth.

We shall be greatly indebted to any of your readers who might be able to offer us explanation or advice with reference to the above phenomena and shall be only too glad to offer any further details which may be necessary for clarification of the position.

R. VOLCANI
Department of Dairy Husbandry
Agricultural Research Station
Rehovot, Israel

OUR INDUSTRY TODAY

Brief Reviews of Current Topics

BRUCELLOSIS IS BEING WIPED OUT

C. G. BRADT

Department of Animal Husbandry
Cornell University

Twelve years ago, the remaining ten of the 100 counties in North Carolina were placed upon the brucellosis-free, modified, certified list. North Carolina thus became on July 1, 1942, the first state in the nation to suppress this disease. Since that notorious event in farm livestock history, New Hampshire and Maine each have recorded similar achievements and many other states today are nearing their goals.

The brucellosis eradication work in North Carolina began in 1927 as a small research project carried on between the State Department of Agriculture, the Experiment Station and about 25 livestock owners with herds representative of those in the various regions in the state. The eradication program was completed with the aid of the U. S. Department of Agriculture, which in 1934 began paying indemnities for reacting cattle. From that time forward, the demand for herd testing in North Carolina grew rapidly in this state of low initial infection, which in 1934 was said to be but 4%. In Connecticut at that time, the rate of infection was 22%; in Massachusetts, 20%; in New York, 17%, and in California, 15%. At present, North Carolina still maintains its lead with its original 100 counties "certified."

Human Health Aspects Cited

Back in the thirties, Bang's disease, as it was then called, was viewed mainly as a disease responsible for heavy economic losses to the dairy and livestock industries. Breeding troubles and sterility, dead and weak calves at birth, retained placentas, drastically lower milk yields, and shorter productive life were the chief items of havoc wrought. Today, other considerations are coming to the front. Brucellosis in humans (undulant fever) and how to get rid of it is attracting attention. Also, many milk markets are accepting only milk and cream produced in brucellosis-free herds. Breeders of dairy and beef cattle and ranchers having feeder cattle for sale are finding it increasingly difficult to send their animals into other states unless they are tested and brucellosis-free. Definitely, the present trend is to wipe out this disease, instead of discovering ways to live with it forever.

According to Dr. Wesley W. Spink of the University of Minnesota Medical School, "There are over one hundred diseases of animals transmissible to man. From a world point of view, and certainly in the United States, brucellosis causes more human disease than any other animal disease. Human beings acquire the disease by drinking raw milk obtained from herds with Bang's disease. The disease is also acquired through the entrance of Brucella in small abrasions of the skin. This is one reason why brucellosis is considered an occupational disease occurring most frequently in farmers, packing house plant employees and veterinarians who handle cattle."

Extension Service and Sanitary Officials Cooperate

Two years ago, the author had occasion while on sabbatical leave from Cornell University to make a study of public livestock health programs in this country. The first month was spent in the U. S. Department of Agriculture at Washington reviewing reports in the Extension Service Office and in the Animal Disease Eradication Branch. The remainder of the 6 months was devoted to visiting state colleges and state and federal livestock sanitary officials. Stops were made in 24 states from New York to Florida and from Florida

Extension Service, local veterinarians, and state and federal livestock sanitary officials work together. Left: C. G. Bradt, the author, and Dr. G. E. Burch talking things over.

across the midwest to California and returning via Oregon, Washington, Idaho, Montana, North Dakota, Minnesota, Wisconsin, Michigan, Indiana, and Ohio.

Travel interests were centered on the kind of livestock health problems encountered in the different areas, the type of programs drawn up, the educational methods employed, and the relationships developed and enjoyed by the cooperating agencies, federal, state, and local.

On this survey it was found that all state extension services were doing educational livestock health work as a part of their programs. Brucellosis eradication was the featured project upon which greatest emphasis was being placed. Relationships between extension agents and state and federal livestock sanitary officials were cordial. However, progress was not equal in all areas. Lack of funds, shortage of veterinarians, particularly in the range states, and insufficient extension personnel to do all the jobs waiting to be done were the chief retarding factors noted. The dairy states and dairy areas of the range states were observed as making the greatest advances in eradicating brucellosis.

Four Plans Operating

Let us examine some of the plans for brucellosis control and eventual elimination. There are four plans recommended by the U. S. Department of Agriculture, Animal Disease Eradication Branch. These plans have the endorsement of the National Brucellosis Committee and the United States Livestock Sanitary Association.

Plan A. Test and remove reactors for slaughter with or without indemnities.

Plan B. Test and retain reactors; vaccinate calves.

Plan C. Vaccinate calves without testing the herd.

Plan D. Vaccinate adult cattle. This plan is advised only to meet emergency conditions.

Dr. F. N. Schafer, Saquoit, N. Y., takes a blood sample for brucellosis testing.

Practially all states have adopted this four-plan program or phases of it. Experience is proving that these federally recommended plans fit most situations — areas and herds badly infected and those lightly infected; dairy herds under close owner supervision and beef herds on range; owners who are willing to remove known reactors at once and those for economic reasons who wish to retain them. The man who wishes only to vaccinate his calves for the protection afforded has Plan C to enroll in. It seems that almost every cattle owner having an honest desire to free his herd of brucellosis can find a point of beginning in one of these four plans. The states give emphasis to those plans which meet their conditions best.

California, for instance, emphasizes Plan C. In that state, according to law, all dairy calves must be vaccinated. Colorado and Connecticut have similar compulsory calf vaccination laws. New York for 12 years has emphasized voluntary calf vaccination as a first step toward brucellosis elimination. Over 300,000 calves annually have been vaccinated in recent years.

This calf gets its protective shot of strain 19 vaccine.

Wisconsin vaccinated 541,000 calves last year; Illinois, 178,000; Minnesota, 117,000; South Dakota, 125,000; Mississippi, 108,000; Tennessee, 108,000; and Idaho, 104,000. Vermont vaccinated about 82% of its calf crop; New Jersey, 77%; Delaware, 54%; Maryland, 51%; Massachusetts, 67%; New Hampshire, 52%; Virginia, 29%; and Washington, 26%.

Adult vaccination, although included in the "4 Point" program, is not a practice generally advised except under unusual circumstances when active abortion threats occur. With a large percentage of the animals adult vaccinated retaining blood test titre reactions that obstruct clean-herd progress, this procedure is not being widely recommended. In some states, it is allowed on permit only. In recent years, the interest among farmers in vaccinating mature cows has been rapidly diminishing, the reason being that many of their cows were vaccinated as calves and possess some protection.

Vaccination Favored in Range States

In the range country of the South and Southwest, some ranchers claim that their brucellosis disease losses are so slight that control measures are unnecessary at this time. However, many cattlemen in these regions view calf vaccination as a good protection practice.

Cattlemen in the range areas of South Dakota, Nebraska, Colorado, Oregon, Washington, Montana, Idaho, California, and Wyoming have been vaccinating many calves. In Montana, Nebraska, and South Dakota, ranchmen may buy strain 19 vaccine at drug stores, farm supply houses, or by mail to do their own vaccinating, a practice not approved in most states. However, in the back country, 100 miles or more from a veterinarian, this owner-vaccination holds some justification. It is the abuses that may creep into the loosely controlled distribution of live vaccines that cause concern to livestock sanitary officials. Consequently, many states have laws restricting vaccination to licensed veterinarians or to persons operating under their direct supervision. Owner-vaccination does not carry official status when interstate cattle shipments are involved.

According to Thomas F. Arnold of the American Cattlemen's Association, the rancher's greatest need is for veterinarians in the remote regions of the cattle country.

In Ohio, North Dakota, Colorado, Alabama, Arkansas, West Virginia, and Maine, trained laymen under veterinary supervision are approved to draw blood or to vaccinate calves. States utilizing the milk ring test extensively in their programs employ many laymen as technicians for service at milk plants and in the testing laboratories. Some states, Minnesota and North Dakota, for example, have used successfully senior veterinary college students during their summer vacations to speed along the work.

Test and Remove Reactors, Best Plan

Test and remove reactors for slaughter (Plan A) has cleaned up many areas of light initial infection in this country. North Carolina, Maine, New Hampshire, North Dakota, Minnesota, Michigan, and Pennsylvania have made effective use of this procedure in their area work.

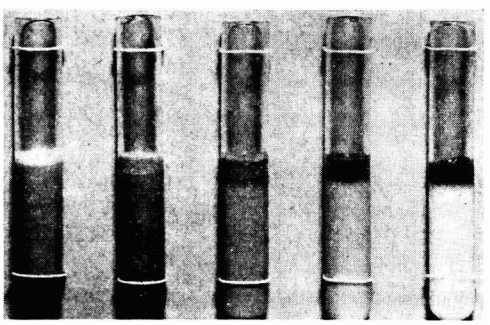
In counties and states where heavy infection is known to exist, a slower method of elimination has been applied. This is Plan B, which includes calf vaccination. Under this plan, the retention of reactors by owners is allowed while they are economically profitable. Usually, however, when these known reactors are sold, they must go for slaughter and not to other herds. Branding of these reactors is required in a few states. California passed such legislation effective July 1, 1953, applicable to all reactors not having been officially vaccinated.

Ohio has a law, Regulation No. 9, which makes it mandatory that all animals over 12 months old (some exceptions) which are to be "sold, disposed of or moved," pass a negative test within 30 days. Indiana, Michigan, Delaware, Minnesota, North Carolina, North Dakota, Washington, and Wisconsin have similar restrictions on the sale of untested cattle. Montana has a law requiring dairy cattle (but not beef cattle) to be negative to the blood test when sold for purposes other than for immediate slaughter. After January 2, 1956, in California, all dairy cattle sold must be officially vaccinated or be negative to a 30-day test previous to sale.

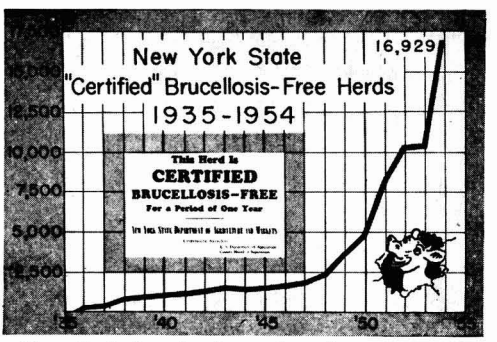
Nation-wide Progress Reported

Under the nation-wide brucellosis eradication program now under way, remarkable progress is being made. It is reported from Oregon that all dairy cows in that state are now tested and that the infection rate of tested herds has dropped to the all-time low of 0.78%. Similar low infection rates are recorded for Delaware, New Mexico, Rhode Island, and West Virginia. The states of Connecticut, New Jersey, Pennsylvania, South Carolina, and Arizona remain not far behind.

In the township of Randolph in Cattaraugus



The milk ring test for brucellosis is being used extensively in the dairy states of the Midwest. Left: Negative sample; extreme right, positive; intermediate reactions, in between.



Brucellosis-free herd numbers increase rapidly as herd testing expands. Latest figures for New York show 19,000 herds "certified."

County, New York, a brucellosis survey in 1933 of all farms disclosed a cattle infection rate of 17%. Twenty years later the survey was repeated and the rate had dropped to 8%. The clean herds increased from 31% in 1933 to 48% in 1953. Calf vaccination during these years was the chief control measure employed.

Another example of brucellosis eradication progress can be cited as coming from Jersey County, Illinois, where calf vaccination has been widely practiced. In 1942, the number of cattle infected was 5%. In 1953, it had dropped to 0.6%. Of the 56 reactors reported among 9,326 cattle tested, 20 were vaccinated reactors over 30 months old.

While these results emphasize the place and value of calf vaccination, it should not be overlooked that calf vaccination alone will never indicate when cows and herds are brucellosis-free. For this proof, tests are required. The milk ring test handled as a supplement to the blood test is a valuable testing aid. The states of Wisconsin, Minnesota, Ohio, Missouri, Indiana, Illinois, Iowa, and South Dakota are utilizing the ring test in their dairy areas, as are some other states, to reach more herds and to lower testing costs.

Dairy farmers throughout the country are noting that their cows calve more regularly and with fewer abortions since they eliminated brucellosis. They say breeding troubles have been reduced and that their cows produce milk more efficiently. The "abortion storms" of years gone by are no longer the nightmares that haunt them.

Fortunately, human brucellosis, too, in some areas is on the wane. Health authorities cite the steady progress made against the disease in farm animals as a major reason for this decline of brucellosis among humans in rural areas. In New York State, the Health Department reported about 250 cases a year in up-state New York prior to 1949. Last year, the number of cases dropped to 45. New York City recorded but five cases. The state records follow: 1948, 233 cases of human brucellosis; 1949, 138; 1950, 143; 1951, 87; 1952, 74; 1953, 45.

Continued Action Required

Eradication of brucellosis is the ultimate goal. Most states are pointing their programs in this direction. The safeguarding of human health, the saving of milk markets, and the protection of farm animals from this costly disease are the spurring incentives. In addition, many see visions after brucellosis in our dairy sections has been eliminated that "milk from healthy cows and healthy herds" will furnish one more argument to convince city consumers that they should drink more milk.

The problem of eliminating brucellosis among the swine population of the country still remains as a phase of the program needing additional attention, and in the dairy and range regions where calf vaccination alone is the chief control measure, more herd testing should be done. It is through testing, and testing only, that herds will be known as brucellosis-free.

"We are on the threshold of success in dealing with this serious livestock disease," says Dr. A. K. Kuttler of the Animal Disease Eradication Branch of the U. S. Department of Agriculture. Director of Extension Service, C. M. Ferguson, adds, "Although it is entirely fitting to take humble pride in the progress that has been made against brucellosis, it would be dangerous to relax the intensity of the drive. If we become satisfied, gains can quickly turn to losses. As all of you are aware, the work that still lies ahead is indeed formidable. There is real opportunity for extension workers to give further support to this campaign."

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ABSTRACTS OF LITERATURE

W. O. Nelson, Abstract Editor

ANIMAL DISEASES

800. The effect of mastitis on the carotenoids, vitamin A and phosphorus compounds of milk. R. CHANDA, Hannah Dairy Research Inst., Kirkhill, Ayr, Scot. Biochem. J., **54**, 1: 68. 1953.

Eight lactating Ayrshire cows—4 with mastitis and 4 normal controls—were used in this study. The normal ratio of nitrogen to phosphorus was markedly affected by mastitis. This was due to an increase in N and a simultaneous decrease in P. Total thiamine was decreased and the percent of carotenoids in the fat increased in infected quarters; however, there was a corresponding decrease in total fat secreted, hence, the total carotenoids secreted remained about the same in mastitic as in normal quarters. A. O. Call

BOOK REVIEWS

801. Industrial Fermentations. Edited by L. A. UNDERKOFLER and R. J. HICKEY, Chemical Publishing Company, Inc., New York, N. Y. 565 pp. $12.00. 1954.

This book is the first of a two volume series dealing with specific fermentation processes of actual or potential industrial significance. In the first chapter, the editors present an interesting discussion on the over-all aspects of industrial fermentation. The remainder of the book is divided into four major parts.

Part I deals with the alcoholic fermentation and its modifications and consists of seven chapters: Alcoholic Fermentation of Grain, by W. H. Stark; Alcoholic Fermentation of Molasses, by H. M. Hodge and F. M. Hildebrandt; Alcoholic Fermentation of Sulfite Waste Liquor, by J. L. McCarthy; Production of Alcohol from Wood Waste, by J. F. Saeman and A. A. Andreasen; The Brewing Industry, by R. I. Tenney; Commercial Production of Table and Dessert Wines, by M. A. Joslyn and M. W. Turbovsky; and Glycerol, by L. A. Underkofler.

Part II is concerned with the production of yeast: Commercial Yeast Manufacture, by R. Irvin; and Food and Feed Yeast, by A. J. Wiley.

Part III is one chapter: "The Butanol-Acetone Fermentations," by W. N. McCutchan and R. J. Hickey.

Part IV discusses the fermentative production of six organic acids: Lactic Acid, by H. H. Schopmeyer; Citric Acid, by M. J. Johnson; Gluconic Acid, by L. A. Underkofler; Fumaric Acid, by J. W. Foster; Itaconic Acid, by L. B. Lockwood; and Acetic Acid-Vinegar, by R. H. Vaughn.

Each chapter is presented in an authoritative manner, yet with sufficient detail to provide a complete over-all picture of the particular fermentation. A general style is followed throughout by the several contributors. The historical material and research background is summarized and the raw materials discussed. In most chapters there is a suitable description of the maintenance and cultivation of the microorganisms used in the fermentation. The process as carried out in the laboratory and in the plant is presented and in many cases is illustrated with flow-sheets and photographs of plant equipment. Appropriate consideration is given to by-products, process variations, and new developments. The economics of the process is discussed and the use of the products described. A selected bibliography concludes each chapter.

This book is carefully and attractively printed and with very few errors. It will be of value to all who are interested in fermentation and it will also serve as an excellent reference and text-book for students in Industrial Microbiology and in Bio-Engineering.

Z. John Ordal

802. Animal Breeding. LAURENCE M. WINTERS. Fifth Edition. John Wiley & Sons, Inc., N. Y. 420 pp. 1954.

Most of the first ten chapters of this book consists of an interesting discussion of the structure of the male and female reproductive tracts, gametogenesis, oogenesis, embryonic development, and introductory genetics. While all of these subjects are of importance to livestock men, it is questionable whether or not they warrant occupying such a large fraction of a book on animal breeding. Most students would have had courses in or courses covering these subjects before taking a course in animal breeding. Then too, there are many books devoted in their entirety to a more comprehensive coverage of these subjects. The discussion of these subjects, however, may be of considerable use to some breeders.

Chapters 11 and 12 are in the reviewer's opinion the weakest chapters of the book. In the last paragraph on page 154, the author of these chapters has stated, "In a herd in which the inbreeding of the animals is advancing, the heritability will decrease. After an outcross, the genetic variability and, therefore, the heritability will be raised." These statements need qualifying, for just as the author of the book suggests in the second sentence of the second paragraph on page 240, such statements cause confusion unless they are well qualified.

The author of Chapters 11 and 12 is in part recommending a kind of tandem selection that is supposed to be superior to using a selection index. The author gives no references or concrete evidence to substantiate this recommendation, while in the literature there is considerable evidence indicating that the selection index method is more efficient than tandem selection. The argument that the relative economic values must be known exactly for a fixed system of selection to serve well is not valid and even if it were, one is faced with the same problem when using the method suggested by the author.

On page 187 the author has the following to say about selection indices: "This would appear to be an almost foolproof method of selection if the information used in constructing the selection index could be known accurately. This information, however, is not known at the time that it is needed. Instead, average values covering a period of time must be used. This difficulty tends to defeat the use of selection indexes in breeding work. Average values of the importance of various traits are not good enough for particular situations. They do not permit the amount of selection for different characters to be varied as their importance to the line or herd varies. As a result, exceptional individuals may be overlooked in selection."

It should be obvious to the reader that if information on an animal is available it is known as accurately for one method of selection as it is for another and that this information is available as early in the life of the animal for one method of selection as it is for another. It is not clear as to what average values the text is referring in the third sentence of the quoted paragraph. The argument that the average values of the importance of various traits are not good enough for particular situations is not justified in that one of the strong arguments for a selection index is that the individual breeder can use relative economic values that are adapted to his own herd. It might be added that exceptional individuals may be overlooked by using any method of selection, but they are less likely to be overlooked by using a selection index.

The chapters on selection give the reader very little useful information on selection. In fact, they steer him away from one of his most powerful tools, the selection index.

Chapters 13 through 20 which are on inbreeding, crossbreeding, purebred breeding, grading, fertility, and artificial insemination are interesting, well written, and, in general, very informative. The main weakness of these chapters is that the author's discussion of population genetics on page 296 to 300 is entirely too brief, and it fails to indicate that the genetic improvement of farm animals is largely a problem of population genetics. The author's statement on page 300 about purebred breeding is good and worth quoting: "Modern research in genetics indicates that the purebred industry of the future will undoubtedly be different from what it is today. It also indicates that the over-all program of pedigree breeding has been sound, but that it is time to move on."

There are a few minor errors and ambiguous statements about which the reader should be warned. On page 106 the author states that the gamete can never contain more than one and the zygote more than two allelemorphs. This statement should be qualified. In Table 7 on page 119 the n's in the column heading should be exponents. On page 156 Norton's name is misspelled. On pages 192 and 193 the following statement is found: "It is therefore clear that, the higher the heritability of a trait, the more accurate is the selection." It is obvious that this statement needs qualification. On page 193 the author makes the following statement: "The method of weighting will vary with the investigator." The author should make it clear just what he means by this statement. On page 194 the author states that average values were used to compute the expected genetic gain. The author should make it clear as to what average values were used. The heading of the last column of Table 13 should read, "Within litter coefficient of variance."

This book should be of considerable use to students, research workers, and breeders.

R. W. Touchberry

803. Artificial Breeding and Livestock Improvement. G. W. Stamm. Edited by Dallas S. Burch, Windsor Press, Chicago, New York. 303 pp. $3.50. 1954.

The author has succeeded in his objective to present the principles of artificial insemination, sound breeding practices, and herd management in non-technical terms which the average farmer and breeder could easily understand.

Sections are devoted to reproduction, breeding management, artificial insemination, including the production and processing of semen, common breeding and calving problems, inheritance and systems of breeding, selection of dairy cows and bulls, breeds of livestock, and a directory of semen producers with the areas served by each and the breeds of bulls in their studs.

This book is a ready source of valuable information for livestock farmers, particularly

dairymen. It would also be an asset to the book shelf of vocational agricultural teachers.

E. E. Ormiston

804. Modern Chemical Processes. Vol. III. A Series of Articles Describing Chemical Manufacturing Plants. By the Editors of Industrial and Engineering Chemistry. 276 pp. $5.00. Reinhold Publishing Corporation. 1954.

A series of chemical plant process articles started seven years ago now in the third volume. Detailed information is presented even in some cases including aspects of materials of construction, safety practices, costs figures, material balances, and such discussions as philosophy of plant location. Carefully planned flow sheets are included. Three articles of interest in Vol. III are, Certified Food Colors, Chemicals from Milk, and Carboxymethylcellulose. Chemicals from Milk is a description of the plant operations of the Chemical Division, Sheffield Farms Co. Inc., at Norwich and Oneonta, New York, covering the manufacture of casein, milk protein powder, caseinates, whey proteins, milk sugar and protein hydrolyzates. L. M. Dorsey

805. Induction and Dielectric Heating. J. Wesley Cable. Reinhold Publishing Corporation. 576 pp. $12.50. 1954.

Induction heating is treated in the first half of the book and dielectric heating in the second. Application data are presented which are the accumulation of years of experience with actual installations of induction and dielectric heating equipment. Chapters are included presenting the mechanization of the equipment to adapt it to the production line. Three chapters of direct interest to workers in the dairy industry field are : 20. Dielectric Heating in the Food-Processing Field; 21. Dielectric Heating as Applied to Sterilization and Allied Processes; and 22. Dielectric Heating for Moisture Removal. L. M. Dorsey

BUTTER

806. A method for distinguishing between whey butter and ordinary butter and for their detection in mixtures. J. G. van Genkel and H. G. Homelink. Proc. 13th Intern. Dairy Cong., **3**, 1342. 1953.

Fifty to 60 g. of butter are melted at 40° C. and centrifuged as soon as protein flakes appear. The fat is washed with petr. ether. Not less than double the vol. of fresh raw milk is added to get a pH of 5.8 which is reduced at 5.4-5.5 with semisatd. $CaCl_2$ soln. If curdling of the milk occurs in 2 hrs. in a water bath at 35°, it contains whey butter. A curdling time of 5 min. to 2 hrs. was given by 50 samples of whey butter. A mixture of 10% whey butter in butter can be detected. H. J. Velander

CHEESE

807. Process of making grated cheese. E. Traisman and W. Kurtzhalts (assignors to Kraft Foods Company). U. S. Patent 2,683,-665. 7 claims. July 13, 1954. Official Gaz. U. S. Pat Office, **684**, 2: 406. 1954.

A noncaking grated cheese is made by mixing ground dried natural high-fat cheese with dried ground process high-fat cheese, and then tempering the mixture under controlled conditions of temperature and humidity.

R. Whitaker

808. Cheese packaging. D. P. Smith (assignor to Milprint, Inc.). U. S. Patent 2,682,-475. 2 claims. June 29, 1954. Official Gaz. U. S. Pat. Office, **683**, 5: 1135. 1954.

Freshly made cheese is coated with a flexible and tenacious layer of wax and then wrapped with an outer wrapper lined with a material possessing the property of sealing and adhering both to the wrapper and to the wax. When the wrapper is peeled from the cheese, the wax is also removed. R. Whitaker

809. The importance of the swelling capacity of the proteins and the moisture-retaining capacity of the cheese curd during ripening. B. Wauschkuhn. Proc. 13th Intern. Dairy Cong., **2**, 639. 1953.

The swelling capacity of proteins and the hydration of the curd affect the ripening. Milk heated to a high temp. contracts to a lesser extent. The whey should be firmly held before brining otherwise a slimy rind, a whitish to pale yellow color, and a tainted flavor develops. An unpleasant salty flavor accompanies a low pH. H. J. Velander

810. Amino acids in Gammelost. A paper chromatographic investigation. A. B. Svendsen and A. Jermstad. Neth. Milk Dairy J., **8**: 29. 1954.

In Gammelost (a Norwegian type of cheese) the following amino acids were found: glutamic acid, aspartic acid, serine, glycine, threonine, glutamine, alanine, proline, γ-amino butyric acid, valine, phenylalanine, leucine, and isoleucine. Lysine and ornithine and in some cases tyrosine and possibly α-aminobutyric acid were present. W. C. van der Zant

811. Report on dehydroacetic acid in cheese. L. L. Ramsey, F.D.A., Wash., D. C. J. Assoc. Offic. Agr. Chemists, **36**, 3: 744. 1953.

The results of a collaborative study of a qualitative test for dehydroacetic acid (DHA) in cheese were very satisfactory. No analyst obtained a positive test on cheese known to be free of DHA; all analysts obtained a positive test on cheese to which 20 p.p.m. DHA was added and all analysts obtained a positive test on cheese found to contain DHA by the quantitative method. F. J. Babel

CONDENSED AND DRIED MILKS; BY-PRODUCTS

812. Food product and method of making. G. H. STUART, H. HOWARD, J. T. WATSON, F. H. CLICKNER, and W. A. SOMMER (assignors to The Borden Company). U. S. Patent 2,682,469. 4 claims. June 29, 1954. Official Gaz. U. S. Pat. Office, **683**, 5: 1134. 1954.

A process for making a palatable dried food product is described, consisting chiefly of casein, having high water retention and being relatively free of glueyness. Freshly prepared casein is coated with lactalbumin and lactic acid and ground to produce a smooth slurry, and then spray dried at a temperature which will not denature the casein nor insolubilize the lactalbumin. R. Whitaker

813. High protein milk product. J. G. BRERETON, F. A. BALDAUSKI, and D. H. BORNOR (assignors to National Dairy Research Laboratories, Inc.). U. S. Patent 2,682,467. 11 claims. June 29, 1954. Official Gaz. U. S. Pat. Office, **683**, 5: 1134. 1954.

A high protein, water dispersible, dried dairy product is produced by suspending casein curd in water to yield not over 20% total solids, blending with water and skimmilk, adjusting to pH 6.0-6.2 with lime, pasteurizing and then drying. R. Whitaker

814. Conversion of lactose to glucose and galactose. E. G. STIMPSON (assignor to National Dairy Research Laboratories, Inc.). U. S. Patent 2,681,858. 42 claims. June 22, 1954. Official Gaz. U. S. Pat. Office, **683**, 4: 901. 1954.

Milk, skimmilk, and other milk products are pasteurized and concentrated to at least 20% solids, then incubated with a lactose hydrolyzing enzyme under conditions favorable for converting a large portion of the lactose to glucose and galactose. R. Whitaker

815. Oxygen uptake in starters. N. EVENHUIS. Neth. Milk Dairy J., **8**: 5. 1954.

The oxygen uptake of skimmilk, sweet cream, and ripened cream after shaken with air was studied. Skimmilk, sweet cream, and skimmilk and cream acidified with lactic acid did not take up oxygen. Ripened cream, however, took up a considerable quantity of oxygen (8.6-24.8 ml/kg cream). However, diacetyl formation was lowest in the container with the smallest amount of air, whereas the citric acid was decomposed faster in the flasks with the smallest amount of air. No direct relation was found between oxygen uptake and available oxygen. The oxygen uptake was lowest in the container with the smallest amount of air.

W. C. van der Zant

816. The determination of the density of butermilk, acidified skimmilk, and yoghurt milk at 20° C. with a lactodensimeter. The scientific committee for the standardization of methods for the analysis of milk and dairy products. Neth. Milk Dairy J., **8**: 34. 1954.

A detailed description is given of procedure and apparatus for the above determination.

W. C. van der Zant

DAIRY BACTERIOLOGY

817. Significance of selecting representative fields of milk films for accurate bacterial counts. F. E. CONIFF, Tuttle Instrument Co., Cumberland, Md. Milk Dealer, **43**, 10: 53. 1954.

Microscopic counts of milk films are generally accepted as the method of choice in purchasing milk on a premium basis because they are easy to perform and do not require a highly trained worker. Data are presented showing the wide variation in counts of the same sample of milk obtained by different technical workers. Data also are presented showing that consistent results can be obtained with the use of an improved mechanical stage that will move a slide specimen accurately from one field to the next, and by counting a complete cross section.

C. J. Babcock

818. The cultivation and metabolism of oligotrich protozoa from the sheep's rumen. B. SUGDEN, Rowett Research Inst., Bucksburn, Aberdeenshire. J. Gen. Microbiol., **9**, 1: 44. 1953.

Suspensions of protozoa were prepared from rumen liquid by settling and decanting. Settled protozoa were isolated by allowing gas produced by fermentation (with added maltose) to carry debris to top and removing debris by gentle suction. After repeated washing with buffer, various species of protozoa were cultured on powdered cotton wool and powdered hay at pH 6.8. Several species of protozoa could be cultured under bacteria-free conditions in the presence of streptomycin but reduced duration of culture life indicated possible dependence of protozoa on bacteria for some products or metabolic processes. There was considered to be a true symbiosis between organism and protozoa as well as between protozoa and host animal. J. J. Jezeski

819. A study of the mechanism of inhibition of bacteriophage multiplication by chymotrypsin. J. KLECZKOWSKI and A. KLECZKOWSKI, Rothamsted Experimental Station, Hertfordshire. J. Gen. Microbiol., **10**, 2: 285. 1954.

Multiplication of bacteriophage is prevented when 0.01% chymotrypsin has been added to mixtures of rhizobium bacteriophage and bacteria in liquid culture. Under these conditions the phage gradually became inactive: chymotrypsin did not interfere with the combination

of phage and bacteria. The phage particle combined with a bacterium becomes inactive and the bacterium is thus protected and is able to multiply. Multiplication of the phage is unaffected by previous incubation of either phage or host bacteria separately with 0.01% chymotrypsin and the chymotrypsin has no effect in an agar medium. J. J. Jezeski

820. The purification and electron microscopial examination of the structure of staphylococcal bacteriophage K. J. E. HOTCHIN, Nat'l. Inst. for Med. Research, London, N.W. 7. J. Gen. Microbiol., **10**, 2: 250. 1954.

The bacteriophage was concentrated by precipitation with 30% acetone in water; purified by further treatment with isobutanol and chloroform, and concentrated by pressure filtration. On the basis of examination with the electron microscope, a fine structure consisting of a removable solid rod or plug within the tail which terminates in the terminal blob is postulated. J. J. Jezeski

821. Some properties of cellulase from Myrothecium verrucaria. P. KOOIMAN, P. A. ROELOFSEN, and S. SWEERIS, Techn. Univ., Delft, Holland. Enzymologia, **16**, 4: 237. 1953.

The culture filtrate from this fungus contains thermosensitive cellobiase and transglycosidase, and a partially heat resistant cellulase. The latter attacks all 1,4-β-glycosidic polysaccharides down to cello-triose, producing glucose and cellobiose. Cellobiose is hydrolyzed very slowly. J. J. Jezeski

DAIRY CHEMISTRY

822. Ash determinations in foods with an alkaline balance. VI. Reaction of sodium carbonate with calcium phosphates in the ashing of milk. H. J. WICHMANN, F.D.A., Wash., D. C. J. Assoc. Offic. Agr. Chemists, **36**, 3: 979. 1953.

The water-insoluble ash of unneutralized milk contains mixed sodium-calcium and potassium-calcium phosphates with very little carbon dioxide. Sodium neutralizers introduce carbon-dioxide into the insoluble ash. This carbon-dioxide exists in the form of basic alkali-calcium phosphates or carbonated hydroxyapatite according to the degree of neutralization. Moderate neutralization may result in mixtures of hydroxyapatite, the above basic mixed phosphates and possibly other compounds such as calcium carbonate. The addition of still more sodium neutralizer produces an insoluble ash consisting of hydroxyapatite containing small intrusions of sodium and carbon-dioxide.

F. J. Babel

823. Report on the detection of algin and gums in cacao products. F. Y. MENDELSOHN, F.D.A., Los Angeles, Calif. J. Assoc. Offic. Agr. Chemists, **36**, 3: 599. 1953.

A method is presented for the detection of gums in chocolate-flavored milk. The method is based on hydrolysis of the gums, yielding reducing sugars and determination of reducing sugars using Benedict's solution.

F. J. Babel

824. The Weilrell-Stoldt method as a uniform method for fat determination in all milk products, and a standard method for the determination in sweetened condensed milk products such as sweetened condensed milk, ice cream, etc. W. MOHR and K. KOENEN. Proc. 13th Intern. Dairy Cong., **3**, 1245. 1953.

The method is described. The limit of error is $\pm$ 0.01%. H. J. Velander

825. A colorimetric method of determining the amount of reconstituted milk in normal milk. A. REINART and R. W. BROWN. Proc. 13th Intern. Dairy Cong., **3**, 1238. 1953.

A colorimetric method has been developed by modifying Evenson's color test for remade milk and cream. It is based on the fact that the curd of a sample of milk when treated with acetone and Et_2O during washings dissolves in NaOH soln. Upon filtering and heating, the soln. becomes clear. When a sample contains reconstituted milk, a yellow color develops. The depth of the color which varies with the amt. of reconstituted milk is measured by means of an electrophotometer or color standards.

H. J. Velander

826. Micromethods for the determination of trace elements in milk and milk products. H. HONNE. Proc. 13th Intern. Dairy Cong., **3**, 1272. 1953.

Procedures are given for the detn. of Cu, Fe, Co, Mg, nitrates, nitrites, citric and lactic acid. The difficulties due to products containing sugar, fat, albumen, amino acids, and mineral salts may be eliminated. H. J. Velander

827. The sensitiveness of the proteins in milk and their transformation due to aging processes. G. ROEDER. Proc. 13th Intern. Dairy Cong., **2**, 312. 1953.

This property may be detd. by a new method which is described. The sensitivity of proteins to alc. becomes greater as the milk ages. Individual samples vary widely. The temp. of storage affect the increase in sensitivity.

H. J. Velander

828. A comparison of the Schain and Babcock tests for the quantitative determination of butterfat in milk. R. HENNINGSON, Dept. of Dairy Ind., Cornell Univ., Ithaca, N. Y. Milk Dealer, **43**, 9: 56. 1954.

The Schain detergent method was compared with the Babcock method in two ways. Conditions of the Schain test were varied and the results compared with both Schain and Bab-

cock controls. Natural milks of varying m.s.n.f. content were tested by each method. By varying the conditions of the Schain test it was possible to obtain results from 0.2% higher to 1.5% lower than the Schain and Babcock control tests. Samples from individual animals did not deviate from the Babcock values in a straight line relationship. When the data from samples producing a clear and a cloudy solution in the bulk of the test bottle were plotted on a graph, the results were closer to the 45° line than to the Schain vs. Roese-Gottlieb (as abscissa) line. When the same values were corrected by means of the nomogram, they were less in agreement with the 45° line than before the corrections were applied. The cloudy, or cloudy and curdy, solution that sometimes appears in the bulk of the test bottle is reproducible. Dilution with distilled water of samples which showed this defect reduced, and in one case eliminated the condition. When samples from individual animals are used, the Schain method does not deviate from the Babcock method in a straight line relationship. The m.s.n.f. content of a milk sample apparently influences the value gained for the fat content, when using the detergent method. The Schain detergent method, in its present form, does not appear to be suitable for quantitative determinations of butterfat in milk. *C. J. Babcock*

829. A simple method for preparing crystalline rennin. (Abs.). N. J. Berridge and Cora Woodward, Univ. of Reading. Biochem. J., **54**, 3: 19. 1953.

Commercial rennet was saturated with NaCl and filtered through Whatman No. 3 filter paper. The filter papers (containing entrained precipitated proteins) were pulped and extracted with water. The filtered extract was adjusted to pH 5.4 and slowly saturated with NaCl by adding it through a rotating semipermeable membrane. The proteins formed a granular precipitate which was concentrated by centrifugation. Upon redissolving in water and standing refrigerated a good crop of crystals was obtained. The yield activity was 18%.
A. O. Call

830. The component acids of milk fats of balaenoptera physalus (finner whale). J. S. Cama and M. L. Meara, Univ. of Liverpool. Biochem. J., **55**, 2: 365. 1953.

The fat content of finner-whale milk is about 10 times that of land mammals. The fatty acid composition of the milk fat is rather similar to that of blubber fat, however, the milk fat contains more unsaturated fatty acids. Two tables are given showing detailed analyses of the four samples tested and also comparing blue whale milk, whale blubber, and grey seal milk and blubber. A. O. Call

831. Alkaline phosphatase of milk. 1. Association of the enzyme with a particulate lipoprotein complex. R. K. Morton, Univ. of Cambridge. Biochem. J., **55**, 5: 786. 1953.

Separated cream contains about 30% of the total phosphatase of the original milk. This is mostly removed in the raw buttermilk or it may be removed by washing the cream with water. The phosphatase appears to be associated with a lipoprotein complex adsorbed on the fat globules. Butanol was the only solvent found which not only dissolves the phosphatase-rich lipoprotein but also seems to release the enzyme into true solution. Details of methods are given. There are 6 tables of results and 31 references. A. O. Call

832. Alkaline phosphatase of milk. 2. Purification of the enzyme. R. K. Morton, Univ. of Cambridge. Biochem. J., **55**, 5: 795. 1953.

By the method described above (See abstract No. 831.) alkaline phosphatase of milk was purified to 5600 times the activity of the original milk, on a protein N basis. The yield was only 1.5% of the total. A step-wise procedure is given. In one of the purification steps an unidentified red protein fraction was separated. A. O. Call

833. Amino acid composition of a preparation of crystallized papain. J. Close, S. Moore, and E. J. Bigwood, Dept. of Biochem., Brussels Univ. Enzymologia, **16**, 3: 137. 1953.

Ion exchange chromatography of a crystalline, electrophoretically homogenous papain preparation yielded the following results: glycine was present on the largest molar basis, tyrosine on the largest weight basis. Methionine was the only common amino acid absent. The analysis accounted for 95% of the total nitrogen in this protein preparation.
J. J. Jezeski

834. Enzymic estimation of citric acid. S. Dagley and E. A. Dawes, Depts. of Biochem., Univs. of Leeds and Glasgow, Great Britain. Enzymologia, **16**, 4: 225. 1953.

The method depends on the use of cell-free extracts from a culture of *Aerobacter aerogenes* to convert citrate to pyruvate. The keto-acid was determined spectrophotometrically on ethyl acetate extracts of the 2:4-dinitrophenyl hydrazone. *Cis*-aconitic acid and *iso*-citric gave rise to varying amounts of pyruvate and the method was not satisfactory for systems containing large amounts of other keto-acids.
J. J. Jezeski

835. Food analysis. Detection of ethylvanillin extract. H. L. Janovsky and A. S. Filandro, Virginia Dare Extract Co., Broklyn, N. Y. J. Agr. Food Chem., **1**: 783. 1953.

The presence of ethyl vanillin is detected by use of alkaline 1% p-aminophenol solution to develop a purple color. Interfering substances such as coumarin and heliotropin are removed by ether extraction. S. Patton

836. Quelques remarques sur la biochemie des ferments d'arome (Some remarks on the biochemistry of flavor formation by starters). N. EVENHUIS. Neth. Milk Dairy J., **8**: 19. 1954.

An extensive discussion is presented on the question whether citric acid or lactose is the source of the formation of diacetyl. It is the author's opinion that there exists a very close relation between the decomposition of citric acid and the formation of diacetyl. When milk was sterilized a compound X (acetylcarbinol?) was formed. The ferrous salt of the dioxime of compound X is most likely identical with that of a compound X (methylglyoxal?) which is formed together with diacetyl. The reduction of the compound X formed during sterilization could not be proved. However, when a starter was kept at 20° C. a reduction of the biologically formed compound X occurred.

W. C. van der Zant

837. Quelques observations sur la biochemie des ferments d'arome (Some observations on the biochemistry of flavor formation by starters). R. COPPENS. Neth. Milk Dairy J., **8**: 14. 1954.

Experiments indicate that the main source of the formation of the C_4-compounds (diacetyl, acetylmethylcarbinol) is lactose and not citric acid. No formation of a substance X was observed with the starters employed in these experiments. A substance with identical colorimetric reaction was found in sterilized milk which was used as culture medium.

W. C. van der Zant

838. Rapid method for the estimation of water-insoluble fatty acids (WIA) in cream and butter. F. HILLIG, F.D.A., Wash., D. C. J. Assoc. Offic. Agr. Chemists, **36**, 4: 1077. 1953.

Since the official method for the determination of WIA in cream and butter does not meet the needs of convenience for a rapid sorting method, a more generally applicable method for the rapid estimation of WIA is outlined. The proposed method consists of separating the fat from cream or butter, washing the fat to remove water-soluble acids, and titrating an ether solution of the fat with 0.05 *N* sodium ethylate. The amount of WIA is calculated from the titration value using a molecular weight of WIA of 270. The method can be conducted in about 15 min. F. J. Babel

839. The chromatographic separation of progesterone and testosterone. J. CAROL, F.D.A., Wash., D. C. J. Assoc. Offic. Agr. Chemists, **36**, 3: 1001. 1953.

Testosterone is decidedly more soluble in dilute alcohol than progesterone because of the OH group at C_{17} in testosterone. When the two compounds are partitioned between 80% alcohol and isooctane, testosterone appears chiefly in the alcoholic fraction, and progesterone in the isooctane. A chromatographic partition, using 80% alcohol as the immobile solvent adsorbed on Celite, and isooctane as the mobile solvent, separated these two hormones sharply.

F. J. Babel

840. Report on quaternary ammonium compounds. Test for purity of bromphenol blue. J. B. WILSON, F.D.A., Wash., D. C. J. Assoc. Offic. Agr. Chemists, **36**, 3: 741. 1953.

A comparison was made of 5 lots of bromphenol blue for use in detection of quaternary ammonium compounds. One lot of indicator was satisfactory as received but 4 other lots gave low results. It is suggested that only the lots of bromphenol blue which pass the proposed test may be used as reagents for quaternary ammonium compounds. F. J. Babel

DAIRY ENGINEERING

841. Power washing cream separator and threaded drive member therefor. H. A. HECKENDORF (assignor to International Harvester Company). U. S. Patent 2,682,992. 12 claims. July 6, 1954. Official Gaz. U. S. Pat. Office, **684**, 1: 157. 1954.

A cream separator bowl which may be cleaned in-place is described. The shaft holding the bowl is threaded, and engaging these threads is a flexible collar. When the shaft is turned, the collar moves up or down to open or close ports in the outer periphery of the bowl, through which cleaning and rinsing solutions may be discharged from the bowl.

R. Whitaker

DAIRY PLANT MANAGEMENT AND ECONOMICS

842. The field of butterfat research. M. KEENEY, Univ. of Md., College Park. Sou. Dairy Prod. J., **55**, 6: 52. 1954.

Total consumption of milk in all forms has not kept up with our population during the past ten years. Butterfat research can play an important part in helping to maintain or even increase the consumption of dairy products. If the dairy industry conducted butterfat research on the same scale as the chemical industry in proportion to sales it would have to spend 200 million dollars annually. The present research budget related to butterfat in the United States probably does not exceed one million dollars per year. Research which would find methods to control oxidative deterioration in butterfat would greatly improve the flavor of milk and its products. Practical methods of concentrating milk which will allow it to be

reconstituted and still be competitive with fresh fluid milk would make it possible to move surpluses more effectively from surplus to deficit areas. More information on the composition and properties of butterfat is needed. The field of fat metabolism is wide open for exploration. More research is needed to improve the average quality of butter. The feasibility of reapportioning the evaluation of milk by lowering the price of butterfat and raising the price of solids-not-fat is an important economic problem. F. W. Bennett

843. Pitfalls and profits. ANON. Milk Plant Monthly, **43**, 7: 31. 1954.

The biggest concern milk plant operators have about vending is whether or not the machines will be profitable. Several examples are given showing where they have increased sales and profits. Among the points brought out are: (1) The outdoor milk vending machine will in time create a new buying habit for the consumer, (2) Purchases are made from the machines as a medium of convenience, (3) 50% of the week's sales are made on Saturday and Sunday, (4) 50% of the day's sales are usually made between 5 and 10 p.m., and (5) Complete visibility of machines on location is very important to their success. C. J. Babcock

844. Selling milk through vending machines. A. J. MORRIS and G. HOPSON, Utah State Agr. Coll., Logan. Milk Dealer, **43**, 8: 112. 1954.

The milk dispenser has grown in popularity throughout the country. It is another sales force which should receive continued analysis. The results of a study conducted at the Utah State Agricultural College from the operation of 53 automatic and hand-operated vendors located in Utah and Southern Idaho show that the type of machine to select is dependent upon: (1) number of people in a proposed location which determines the volume of sales per machine, (2) volume of the peak load, (3) ease of cleaning, servicing, and operation, (4) the sanitary characteristics of the machine, (5) the variety of products dispensed, (6) frequency of deliveries, (7) the type and attitude of the location owner, (8) appearance of machine in relation to its surroundings and (9) ease of operation and effectiveness of the coin changer. The number of units required to pay expenses of a machine is dependent on many factors, such as type and size of machine, capital investment, operation cost, number of machines operated and the location. In this study, the "break-even" point was 70 units ave. per day for the large automatic dispenser and 35 for the small hand-operated type. Operation practices are given. C. J. Babcock

845. Nine-route plant in Indiana finds volume and profit in outdoor vending. C. O. DAVIS, JR. Milk Plant Monthly, **43**, 7: 28. 1954.

Since last October, the Oak Ridge Dairy in Mishawaka, Ind. has installed 4 outdoor and 3 indoor vending machines. This is a 9-route milk plant having a market of 150,000 people in Mishawaka and South Bend. The vendors will handle 2 lines of merchandise but only ½ gal. of homogenized milk are used. The price is 35¢ per ½ gal. The machines hold about 208 ½ gal. in cartons. The plant operators are reluctant to discuss actual volume figures, but well over a thousand ½ gal. per week are going out through the vending machines. The volume is growing steadily. C. J. Babcock

846. In Fort Smith, everybody uses dispensers. T. D. LEATHERS. Milk Plant Monthly, **43**, 7: 25. 1954.

Fort Smith, Arkansas, a city of 53,000 persons, has a milk dispenser for every 500 persons. The Beckman Dairy Company has placed more than 80 dispensers at locations throughout the city. About 85% of the restaurants, drug stores, and other eating places have dispensers; they have also installed the machines in the three dime stores. Bulk type milk dispensers are used. The milk is delivered in 5 gal. cans. Milk sales have increased more than 20% by the use of the dispensers. C. J. Babcock

847. Vending ups profits. T. D. LEATHERS. Milk Plant Monthly, **43**, 7: 17. 1954.

A survey has shown that vending milk is a profitable operation when the vendors are placed in the right location. Milk plants in Utah, Idaho, Mich., N. Y., Mass., Wis., and other states have boosted not only their sales of plain milk but also have increased their chocolate and orange drink sales through vending. Several case histories of successful vending operations are discussed. C. J. Babcock

848. Location . . . the key to vending success. H. B. METZER. Milk Plant Monthly, **43**, 7: 13. 1954.

A one year study of the automatic vending of packaged milk conducted by the Bureau of Agr. Econ. at the Univ. of Me. showed that the dairy industry should make more room for the milk vending machine. It will create additional milk sales. Consumers like the mechanical convenience and the day-long availability of a fresh cold dairy product. Vending machines will be profitable when locations are carefully selected—locations depending not so much upon numbers of persons, but upon the income and occupation of persons, their opportunity to purchase other beverages and the total hours daily the machine can be used. Available data shows that a gross margin of 2.2¢ per pt. was available after allowing 0.5¢ for commission to location and 7.3¢ for product and container cost. Annual operating costs of about $500 were incurred. Where only a few machines are operated this means minimum sales of about 500 units per week for a profitable operation. C. J. Babcock

849. Vending hits big time. C. O. DAVIS, JR. Milk Plant Monthly, **43**, 7: 11. 1954.

There are more than 2,750,000 automatic machines of one kind or another in use in the United States. They are selling everything from soft drinks to ladies' hosiery. However, only 12,000 milk vending machines and 14,000 ice cream machines are estimated to be in use. Vending machines in 1953 are estimated to have done a sales volume amounting to $1,405,000,000 of which only $17,000,000 was grossed by milk machines, and $15,000,000 by ice cream machines. One-half of the concerns now active in vending milk were not doing so 2 years ago and a 25% increase is estimated this year. Homogenized milk and chocolate drink are the 2 most popular fluid items being vended. About 75% of all vended packages are either in ½ pt. or 1/3 qt. Prices range from 6 to 10¢ for ½ pt., from 9 to 12¢ for 1/3 qt., and from 12 to 18¢ for pt. In the ice cream field, about 85% of the 14,000 machines vend bar-type goods and the balance cup products.

C. J. Babcock

850. Experiences in machine vending of milk. S. JOHNSON, Univ. of Conn., Storrs. Milk Dealer, **43**, 10: 84. 1954.

Milk distributors' interest in vending machines is a simple matter of whether or not they will be profitable. Experiences are related with indoor machines, bulk dispensers, and outdoor vendors. These experiences are mainly in Conn. but also include Minn., N. Y., Wis., and Utah. In each instance, an increase in the quantity of milk sold is reported.

C. J. Babcock

851. Vending machines an opportunity for new milk plants. H. L. COOK, Univ. of Wis., Madison. Milk Dealer, **43**, 10: 92. 1954.

A discussion and description of the following types of vending machines: (1) the coin vended indoor package type; (2) the manually operated loose milk type, commonly called dispensers; (3) the coin vended loose, or bulk milk type, and; (4) the coin vended outdoor package type. Prices of vendors range from $250 to $850. Dispensers range in price from $185 to $285. An automatic coin vended loose milk type sells for around $1,100. Vending machines improve the availability of milk. Research in 1951 lead to the estimate that 510 million lb. of new fluid milk business could be obtained through indoor package type vendors, within a fairly short length of time. It seems reasonable to suppose that dispensers and outdoor vendors could push this to at least ¾ billion lb.

C. J. Babcock

852. Missouri taps the school market. O. E. ALLEN. Milk Plant Monthly, **43**, 7: 20. 1954.

Surveys in two counties in Mo. showed that 23% of the children drank no milk, 24% drank one glass daily, 38% two glasses, 9% three glasses and only 6% drank four glasses, the amount needed by a growing body. To help correct this situation and to help Mo. dairymen, milk was made available in schools through the use of milk vending machines. The results obtained in schools, offices, factories, garages, business houses, and Army posts are discussed.

C. J. Babcock

853. Why advertise dairy products? K. G. WECKEL, Univ. of Wis., Madison. Sou. Dairy Prod. J., **55**, 6: 28. 1954.

The changing nature of the market, the rapid increase in population, the low cost of milk as a food, the need for a favorable understanding of the dairy industry, high dollar incomes, the constant improvements of the nutritional quality of many processed foods, the need for information on selecting foods for balanced diets and better health and the creation of competitive stimulation for purchasing dairy products are eight very cogent reasons for advertising dairy products.

F. W. Bennett

854. Milk advertising needs a specialized approach. R. B. ROBERTS, Bozell & Jacobs, Inc., Indianapolis, Ind. Milk Dealer, **43**, 9: 58. 1954.

Following a discussion of milk advertising directed at (1) the infant-feeding market, (2) the child and youth market, (3) the teen-age market, and (4) the adult market, the following recommendations are made: (1) Start your campaign when you have assurance of continuity, (2) Direct your advertising primarily to the most responsive segment of the market—the adult, (3) Convince the housewife that milk is today's biggest food bargain, (4) Seek to improve milk-drinking habits but concentrate on improving milk-buying habits, and (5) Keep the campaign simple.

C. J. Babcock

855. How to advertise dairy products. C. A. MITTELSTADT, Campbell-Mithun Advertising Agency, Chicago, Ill. Sou. Dairy Prod. J., **55**, 6: 29. 1954.

There are two types of dairy products advertising: brand advertising and product advertising. In the dairy industry, the primary question is not the brand to be purchased but whether or not dairy products of any brand should be purchased. The more important question should be given the greater attention in advertising. Appeals should be made as directly as possible to the potential consumer rather than to distributors or dealers. Our advertising should be aimed at our more important prospects, the people over 20 years of age. Claims for dairy products should be as specific as possible. The advertising of individuals should be tied in with industry-wide advertising

campaigns, such as that of the American Dairy Association. Dairy products should be advertised much more aggressively.

F. W. Bennett

856. Marketing costs. R. E. Olson, A.M.S., U.S.D.A. Milk Plant Monthly, **43**, 5: 25. 1954.

Because of the desire to reduce milk marketing costs, the AMS has undertaken a comprehensive research program with this as one of its primary purposes. Data for the study are obtained chiefly from independently operated plants of medium to large size, each operating in a single market. Most of the plants are in cities of 5,000 population. The data collected each quarter include quantities and prices of raw milk and cream and manufactured dairy products purchased; prices received for the principal fluid milk products sold on retail and wholesale routes, by size and type of container; value of finished products purchased for resale; product inventories; unit and dollar sales on retail and wholesale routes and plant platform, by size and type of container; number of employees, average hours worked per week, and wages paid; number and average length of retail and wholesale routes; itemized expenses and the allocation of expenses to functions. The average costs by major expense categories for all the plants in the study for 1¾ years beginning with the first quarter of 1952, are presented in table form. The percentage summary of operating expenditures—July to September 1953 are also given. Reducing costs are discussed under 4 categories: (1) adjustments in price of cost items, (2) increases in volume, (3) changes in technique, and (4) changes in services. C. J. Babcock

857. Tank conversion route-by-route. C. O. Davis, Jr. Milk Plant Monthly, **43**, 5: 17. 1954.

About 30% of the milk supply of Sanitary Farm Dairies of Cedar Rapids, Iowa has been converted to bulk pick-up. Most of the producers have chosen one make of tank—a relatively inexpensive tank featuring bottom cooling by water. The 250 gal. size is most popular, and it can be purchased and completely installed for $1,895. The price of the tank seems to be the single most important consideration to producers. A description of the step-by-step conversion is given. Most of the haulers supplying the plant are using 1,700 or 1,800 gal. tanks on their trucks. The cost of these tanks is from $5,500 to $6,500. They are equipped with 50-gal.-per-min. pumps. At the plant, the milk is drawn out with a 100-gal.-per-min. pump. Samples for plate counts are taken twice a month from Sanitary Farms' producers. Those on tank pickup have shown low counts, and are showing lower counts as the months go by. C. J. Babcock

858. Problems in bulk pick-up. Anon. Milk Plant Monthly, **43**, 6: 23. 1954.

A discussion of the many problems that still exist in the bulk pick-up and handling of milk. The system, nevertheless, is doing an effective job of bolstering the economy of the dairy industry, and the industry has found that tank operation is far superior to the former hand operation, both from the standpoint of accuracy and economy. C. J. Babcock

859. Bulk milk transporting and dispensing apparatus. L. F. Norris, D. J. W. Timmersman, and T. L. Sage (assignors to Norris Dispensers, Inc.). U. S. Patent 2,681,747. 5 claims. June 22, 1954. Official Gaz. U. S. Pat. Office, **683**, 4: 867. 1954.

A milk can shaped vessel is described, suitable for bulk dispensing of milk. The bottom of the milk containing portion is rounded and provided with an outlet nipple at the lowest point, and is surrounded by a flange which serves as a base for the entire structure. Sufficient space inside the flange is provided to protect the nipple and allow for a coiled section of flexible conduit attached to the nipple.

R. Whitaker

860. What people think of your products. Anon. Milk Plant Monthly, **43** 6: 12. 1954.

An analysis of a survey of 3,905 interviews of which 2,189 were with persons who do the shopping for a household. These persons were selected from 400 sample areas in 110 counties widely spread throughout the United States. The following observations were made: (1) more men than women drink milk, (2) the use of milk declines as the average person gets older, (3) 37.1% who said they were drinking more milk than they used to, said they were drinking more for health reasons; 8.4% were drinking less for the same reasons, (4) an average of 17.6% of the people are drinking less milk because they think it is fattening and 5% of those who are drinking more milk are doing so because they think it isn't fattening, (5) the population is divided into 3 groups on the use of butter vs. oleo—30.4% use butter only; 40.0% use butter and oleo; 28.9% use oleo only, (6) 78.6% of the consumers express a favoritism for butter on the basis of taste; only 6.8% rank oleo first in that respect and 14.6% can see no difference, (7) consumers ranked oleo ahead of butter on the basis that it stays fresh longer than butter, (8) ice cream falls into three distinct buying patterns, those primarily concerned with a particular flavor, those who buy by brand and those to whom nothing in particular is important, (9) 23.1% preferred packaged ice cream because it was more economical; 21.3% because it was quicker and easier to get and 21.1% because it was easier to carry, and (10) a majority of those interviewed thought that milk was priced about right while butter is too high. C. J. Babcock

861. The subdealer. R. E. CLEARY, Welsh Farms, Long Valley, N. J. Milk Dealer, **43**, 9: 48. 1954.

A report of a survey of the subdealer method of milk distribution in which a subdealer is defined as "a person, firm, association, partnership, or corporation purchasing milk and/or cream from a dealer, processor or producer-dealer for resale to consumer and stores." The sales agent for milk operates no pasteurizing or bottling plant. The survey showed that the reasons why a processor may use subdealers are: (1) increased pressure from unions for higher wages without incentives for routemen to increase sales, (2) cannot handle volume with family help and hired help undependable or unavailable, (3) desire to eliminate worry of labor problems of selling, (4) small operator unable to properly supervise both plant operations and sales, (5) selling routes to drivers is sometimes an easy way out of problems with the union, (6) processor-subdealer arrangements make it possible for pasteurizing plants to increase in size while delivery units are small, so that the initiative of the drivers is maintained, (7) volume is usually larger on subdealer routes than on processor's own routes, (8) processor can pass on credit, collections, and customer relations to his subdealers but still work with them on a consulting basis, (9) savings in taxes, insurance, payrolls, and other employee benefits are believed sufficient to compensate for loss of net returns on direct sales to customers. The survey also showed that the following factors are against the development and expansion of the subdealer method of milk distribution: (1) processor's desire to control the quality of the product from the time he acquires it until it reaches the ultimate consumer, (2) opportunity to make more money in some other business, (3) large processor-dealers believe that if subdealers can make profits by selling their milk, they could too, (4) some milk control agencies restrict issuance of licenses in a marketing area, (5) some agencies grant too small a margin to the subdealer, (6) processor loses control of product at his platform. His customer-contact is sometimes very remote, (7) financial hazard to processor is great. If subdealers fail to pay promptly, they may undermine the financial position of the supplier, (8) processor may have difficulty in getting his product to a potential customer if his subdealer servicing that area does not care to handle the account. C. J. Babcock

FEEDS AND FEEDING

862. Digestion of cellulose by ruminants. (Abs.) A. T. PHILLIPSON, Rowett Research Inst., Bucksburn, Aberdeenshire. Biochem. J., **54**, 1: 3. 1953.

Several factors affecting cellulose (crude fiber) digestion are mentioned. Adding the ash of lucerne at the rate of 3.7% to a ration containing coarse fodder and a total fiber content of 17.8% increased digestion of fiber by some 10%. Further investigation of the role of the inorganic composition of the feed is suggested. A. O. Call

863. Minerals for dairy and beef cattle. R. B. BECKER, P. T. DIX ARNOLD, W. G. KIRK, G. K. DAVIS, and R. W. KIDDER. Fla. Agr. Expt. Sta. Bull., **513**: 5. 1953.

Mineral investigations with dairy and beef cattle at the Florida station over 27 years were summarized as regards calcium, phosphorus, iron, copper, cobalt, and molybdenum. Rates of mineral consumption were influenced by season and age of animals. Either bonemeal or marble dust corrected low-calcium rations to allow normal milk production, and bone strength (mineral storage) in dairy cows. Palatability tests with dairy cattle were conducted with defluorinated phosphate, disodium phosphate, and a fused superphosphate.

Functions, deficiency symptoms, and requirements of cattle are given for calcium, phosphorus, common salt, iron, copper, and cobalt. Relation of copper in nutrition of cattle on sand and two kinds of muck soils was discussed. Besides a relation to iron metabolism in hemoglobin synthesis, copper functions in color pigmentation of hair coat, skin healing, and offsets excess molybdenum in forages eaten. Cattle utilized copper sulfate, copper chloride, copper oxide, basic copper sulfate, or metallic copper. Toxicity from excesses of copper sulfate caused a purplish "blackberry jam" spleen, bloody urine, hemolysis, icterus, yellowish liver, thick bile, and darkened kidneys. Interrelations existed between molybdenum, copper, phosphorus, and cobalt in the bovine. Cattle depleted of both copper and phosphorus died suddenly under exertion before showing chronic copper deficiency symptoms. Heart muscle was affected.

Four mineral formulas are recommended for use with cattle under different soil-pasture conditions in the region. R. B. Becker

864. Vergelykende voederproeven met vetarm en meer vetryk kracktvoeder by melkvee (Comparative trials with low and high fat rations for dairy cows). N. D. DYKSTRA, J. DAMMERS, and A. M. FRENS, Versl. Landbouwk. Onderzoek., **59**: 6. 1953.

Two groups of cows were used. Group I (low fat) received a concentrate mixture composed of extracted coconut, groundnut, and linseed meal, with some products poor in fat such as barley meal, beet pulp, dried potatoes, tapioca meal or molasses. Group II (high fat) received the same mixture as I except that extracted oil seeds were replaced with pressed ones. The type of roughage had a marked effect on the milk and milk fat production when a low-fat diet was used. A fat content in the

concentrate of approximately 1.5% proved sufficient if the roughage consisted of good hay and good silage. If the roughage consisted of hay, beets, and straw an increase in milk and milk fat production was observed if the concentrate contained at least 4% fat.

W. C. van der Zant

GENETICS AND BREEDING

865. Hereditary female sterility in Holstein-Friesian cattle. J. F. KIDWELL, L. WALKER, and J. A. MCCORMICK, Univ. of Nev., Reno. J. of Heredity, **45**, 3: 142. 1954.

Data obtained from a 25-year-old Holstein-Friesian herd as well as from seven generations of females before the foundation herd support, and extend, the conclusion of the California group indicating that female sterility in this breed is conditioned by a single autosmal gene. Most bull calves were not saved for parents so the probable sex-limited nature of the inheritance could not be adequately checked. Neither were the data adequate to test Mendelian ratios or to obtain a reliable estimate of the frequency of the gene. It was suggested that females which produced only one calf (although rebred) might suffer some degree of genetic sterility if incomplete penetrance or variable expressivity of the causative gene were involved.

L. O. Gilmore

HERD MANAGEMENT

866. Milk can rack. R. G. FERRIS (assignor to Starline, Inc.). U. S. Patent 2,682,339. 2 claims. June 29, 1954. Official Gaz. U. S. Pat. Office, **683**, 5: 1096. 1954.

A frame structure for holding milk cans in an inverted position. R. Whitaker

867. Milk can hoist. R. G. FERRIS (assignor to Starline, Inc.). U. S. Patent 2,683,585. 7 claims. July 13, 1954. Official Gaz. U. S. Pat. Office, **684**, 2: 385. 1954.

A hoist for lifting cans of milk consisting of a wheel with a flared circumference, and a chain or rope attached to the axis of the wheel and fitting into the groove, so that the can is lifted by turning the wheel. R. Whitaker

868. Quality milk at lower costs. C. J. FENZAU and R. N. VAN ARSDALL. Milk Plant Monthly, **43**, 6: 25. 1954.

A three-year study of nine farms in the St. Louis milkshed indicates that with good practices, milk of consistently high quality can be produced with a relatively low investment in buildings, and with half or less labor than is commonly used on farms. Features contributing to efficiency of labor are: (1) ground-level storage of hay and silage, self-feeding and direct movement, (2) elevated stalls and an arrangement to bring cows to the operator, (3) minimum quantity of bedding and amount of labor for bedding, (4) pipeline milking, spray cooling, water heater and such equipment, and (5) work routines to reduce idle time.

C. J. Babcock

869. Dairy calves; their development and survival. P. T. DIX ARNOLD and R. B. BECKER. Fla. Agr. Expt. Sta. Bull., **529**: 5. 1953.

Records of Jersey calves born in the Florida station herd over 22 years were analyzed. Factors determined were rate of development of Jersey fetuses; relation of dam's age to birth weights of 832 calves; relation of gestation period to birth weights; sex ratio and multiple births among Jerseys. Av. gestation periods for 421 males were 277.9 days; and for 411 females 276.5 days. Losses of heifer calves included 4.9% stillborn, 1.2% freemartins, 12.2% from weakness or diseases while under 6 months old; 6.9% from various causes before calving, and 9.6% from low breeding efficiency or nonconception. There were 65.2% that conceived and freshened. There were 249 cows which dropped 450 heifer calves, of which 1.21 heifers per cow entered the milking herd . . . a slow increase when every living heifer was intended for replacement. R. B. Becker

870. Productive life-span of dairy cattle. R. B. BECKER, P. T. DIX ARNOLD, and A. H. SPURLOCK. Fla. Agr. Expt. Sta. Bull., **540**: 5. 1954.

Av. life-span of cows in 101 Florida dairy herds maintained largely with purchased replacements was 3.9 years after entering the herd, some as milking cows. With mainly home-raised replacements in 14 Florida herds, average useful life was 4.7 years, assuming entry of the milking herd at two years old. Udder trouble, low production and reproductive troubles, in order, caused 58% of removals from the latter group. There were 5.6% of deaths from diseases.

Data on dairy bulls were secured nation-wide. Many fertile bulls were discarded early to avoid inbreeding, disposition, low transmitting ability and for other reasons. The major turnovers of 5,177 desirable bulls were because of sterility, accidents, low fertility, age, foreign bodies, feet and legs and 15% for infectious diseases including actimimycosis, tuberculosis, and Johne's disease, pneumonia, and abscesses. The av. age at last effective service was 10.43 years for desirable bulls in natural service. Causes of turnover of bulls in artificial service were discussed. R. B. Becker

ICE CREAM

871. Mold for home frozen confection manufacture. N. T. BALDANZA (assignor to Curtiss-Wright Corp.). U. S. Patent 2,682,234. 6 claims. June 29, 1954. Official Gaz. U. S. Pat. Office, **683**, 5: 1066. 1954.

An open top mold for making individual frozen confections in home refrigerators. The

end wall is equipped with a flexibly mounted nipple which assists in removing the frozen piece from the mold. R. Whitaker

872. Container for frozen products. N. E. SPIESS, JR. (assignor to National Dairy Research Laboratories, Inc.). U. S. Patent 2,682,-987. 2 claims. July 6, 1954. Official Gaz. U. S. Pat. Office, **684**, 1: 155. 1954.

A flat package for ice cream, consisting of cardboard of relatively low thermal conductivity and so constructed that one of the flat sides can be easily peeled back to expose a metallic foil panel. The product is kept colder by laying the carton with the foil side down on the refrigerated surface of the freezing unit in home refrigerators. R. Whitaker

873. Ice cream package. E. L. TURNER (assignor to Robert E. Beckwith). U. S. Patent 2,681,144. 1 claim. June 15, 1954. Official Gaz. U. S. Pat. Office, **683**, 3: 640. 1954.

A carton for holding ice cream sandwiches. Partitions in the carton are so arranged that wafers are supported on edge with spaces between them. Ice cream directly from the freezer is filled into the spaces, thus producing a carton of completed sandwiches ready for hardening in the usual manner.

R. Whitaker

874. Exclusive analysis of 1953 packages by container sizes. ANON. Ice Cream Trade J., **50**, 7: 14. 1954.

About 150,000,000 gal. of ice cream was marketed in ½ gal. containers in 1953. This is equivalent to approximately 20% of the total gallonage. Quart containers have been losing ground and pts. have increased about 4% in 1953 over 1952. Gallon containers used remained about stationary, accounting for about 6,000,000 gal. of ice cream sold.

W. H. Martin

875. Self-service sales of pre-dipped bulk in super markets. E. R. LUCAS. Ice Cream Trade J., **50**, 7: 16. 1954.

Pre-packaged hand-dipped pts. and qts. for take-home use have increased ice cream sales 40% in one store and 100% in another in the Seattle, Washington area. The ice cream is dipped into cartons and wrapping of cellophane then is applied. The margin of profit to the grocer is 27 to 28% gross. Hand-dipped qts. retail for 73¢. The ice cream costs $1.54 per gal. and about 3 qts of 24 oz. each are dipped from a gal. W. H. Martin

876. Switch to self-service stores. ANON. Ice Cream Rev., **37**, 12: 44. 1954.

Highs Dairy Products Corp., Washington, D. C., which sells a million gal. of ice cream per yr. in the Washington area is rapidly changing over to self-service stores. Sixty % of the 75 stores now being operated by the company are self-service and all new stores being added are of this type. The best location for this type of operation is one with adequate parking near, but not directly on main traffic roads.

The stores currently carry 20 different flavors of ice cream prepackaged in attractive two-color design, round, liquid-tight containers. The round ½-gal. container has been popular with the housewife because it is not easily damaged and because the lid can easily be placed back on the container after it has been opened.

One of the big economic gains resulting from the self-service store is a lower labor cost at the retail outlet. W. J. Caulfield

877. Pre-dipping bulk ice cream. L. J. COUGHLIN, Slater Systems Inc., Philadelphia, Pa. Ice Cream Rev., **37**, 12: 48. 1954.

Pre-dipped No. 12 scoops of ice cream attractively displayed in sundae dishes in a variety of colors and flavors have resulted in a 53.6% increase in the portion sales of ice cream at one cafeteria. This new method of selling ice cream has resulted in as much as 30% of the customers choosing ice cream for their dessert.

Success with this method of selling ice cream depends upon choosing the right size portion for the type of customer served, maintaining uniformity in the portion sizes, keeping the pre-dipped portions at a suitable temperature to avoid partial melting, and educating the customer and employee on the use of pre-dipped bulk ice cream.

Pre-dipping when sales are slack is a means of making more efficient use of labor, keeps cabinets cleaner, and greatly facilitates the handling of customers during peak sale periods. W. J. Caulfield

MILK AND CREAM

878. Selectively hydrogenated butter oil for preventing development of oxidized flavor in recombined frozen milks. H. D. WEIHE and T. J. MUCHA. Proc. 13th Intern. Dairy Cong., The Hague, **3**, 1067. 1953.

All samples of butter oil hydrogenated in the presence of catalyst prevents the development of oxidized flavor in recombined milk stored at −17° for 83 days. Raney nickel catalyst was used at temps. from 96 to 130° at slightly above atm. pressure. There was no optimum due to temp. of hydrogenation. Complete hydrogenation is not desired. Off odors and catalyst were removed and the fat was recombined in fresh milk followed by storage. A butter oil heated to 125° in air just before hydrogenation showed superior flavor qualities as compared to other hydrogenated samples. H. J. Velander

879. The antibiotic action of polyunsaturated fatty acids in sterilized milk. H. CORNELISSEN, M. LONCIN, and D. JACQMAIN. Proc. 13th Intern. Dairy Cong., **3**, 1098. 1953.

Linseed oil soaps gave some inhibition by 100 p.p.m. and almost complete at 1,000 p.p.m., the action resulting from the linolenic acid in the oil. More activity was shown with acids having 4 or 5 unsatd. bonds. Broth and skimmed milk gave almost the same results while the inhibition in whole milk is less. The taste obviates this method as a means of preservation. H. J. Velander

880. Apparatus for manufacturing whipped cream. F. F. SUELLENTROP (assignor to Lemay Machine Company). U. S. Patent 2,683,558. 3 claims. July 13, 1954. Official Gaz. U. S. Pat. Office, **684**, 2: 376. 1954.

A description is given of a reciprocating device for filling cream to a predetermined level in containers and simultaneously engaging the partially filled containers with injectors for admitting a gas under pressure and then sealing the containers. R. Whitaker

881. Whipped cream dispenser. N. E. SPIESS, JR. and L. C. WIDDOES (assignors to National Dairy Research Laboratories, Inc.). U. S. Patent 2,682,977. 6 claims. July 6, 1954. Official Gaz. U. S. Pat. Office, **684**, 1: 152. 1954.

A push button type of valve is described for discharging whipped cream from a single service container of gas packed cream.
R. Whitaker

882. The solar activated flavor of homogenized milk. G. M. TROUT and B. R. WEINSTEIN. Proc. 13th Intern. Dairy Cong., **3**, 1061. 1953.

The sunshine flavor is referred to as the solar activated flavor. An oxidation process appears to be involved. Addn. of ascorbic acid does not prevent the flavor. The combination of ascorbic acid and nordihydroguaiaretic acid prevented the flavor after 60 min. exposure to solar radiation. α-tocopherol and hydroquinone added separately or in combination did not give complete protection. H_2O_2 added to destroy ascorbic acid rapidly did not develop the flavor. Heat treatment to 176° F. for 5 minutes did not prevent the flavor. A whey protein fraction was isolated which gave the flavor after being irradiated. Water solns. of this minor protein gave a pos. biuret, Million xanthoproteic and Molisch reactions, isoelec. zone, pH 4.1-4.3; min. mol. wt. was 70,300, and 12 amino acids were isolated from the minor protein.
H. J. Velander

883. Observation on cold milk separation. W. M. ROBERTS and L. F. BLANTON, N. C. State Coll., Raleigh. Sou. Dairy Prod. J., **55**, 4: 91. 1954.

Some advantages of cold milk separation are the elimination of preheating and cooling equipment, lower requirements for labor and utilities, and usually better products. Some possible disadvantages are an approximately 50% reduction in capacity, more difficulty in separation, more likelihood of cream plugging and tendency toward high butterfat loss. The viscosity of both raw and pasteurized cream was higher when separated at 42° than at 90° F. and the cream whipped in less time. Typical butterfat tests of skimmilk from cold separation were 0.01-0.05% higher than those from 70° F. separation. The separation efficiency at low temperatures is decreased as the cream tests increase above 40%. The efficiency of separation of homogenized milk generally is too low to recommend the practice. Fewer flavor defects have been noticed when the milk is not heated for separation.

Some operating suggestions are as follows: Feed the separator with a positive displacement pump. Install a 3-way valve on the suction side of the pump for a small tank for circulating hot water and sanitizing solutions. Start the separator with 80-90° F. water. Avoid tension on the cream control valve until cream is being discharged. Avoid, if possible, the necessity of separating partially churned milk. Keep the back pressure on the cream discharge below 5 lb. Skimmilk restriction discs can be used for light cream production. When the flow of milk is stopped release the tension on the cream control valve and flush with 115° F. water immediately. Stop the separator, disassemble and and clean. F. W. Bennett

884. Seriological detection of cow milk added to milk from the goat. P. SOLBERG and G. HADLAND. Proc. 13th Intern. Dairy Cong., **3**, 1287. 1953.

Sixteen different fractions of whey proteins were isolated from cow milk and used to obtain antisera. The fractions were injected into rabbits. Skimmed milk was also used to produce antisera. In known goat-cow milk mixtures, 10% cow milk gave pos. results in 1 min., 5% cow milk pos. in 1.5 min., 1% cow milk, pos. after 20 min., and 0% cow milk neg. after 60 min. H. J. Velander

885. The effect of light on the riboflavin in milk. T. K. KON and S. Y. THOMPSON. Proc. 13th Intern. Dairy Cong., **2**, 363. 1953.

Exposure of milk to direct sunlight in the summer causes a loss of 50% of B_2 and 20% the rest of the year. Covered trucks limit the loss from 4.1 to 8.0%. Loss of riboflavin is attributed to sunlight. H. J. Velander

886. The behavior of the phosphatase enzyme during the pasteurization of cream. W. RITTER. Proc. 13th Intern. Dairy Cong., **3**, 1014. 1953.

Reactivation of phosphatase activity in cream may occur in flash pasteurization. The phenomenon is not observed when the pasteurization is carried out by the holding method. Before making the test, addn. of NaCl, MgCl,

sucrose, and galactose accelerate the reactivation of the phosphatase. $K_2Cr_2O_7$ inhibits the reactivation. The cause of the reaction is not known. H. J. Velander

887. The content of tocopherol (vitamin E) in cow's milk. F. Kieferle and A. Seuss. Proc. 13th Intern. Dairy Cong., **2**, 264. 1953.

The detd. of tocopherol was by a modified method of Emmerie and Engel. A fraction of milk unsaponifiable in 48 hr. in cold 60% alc. KOH was extd. under N_2 with ether. The dried ether extract was dissolved in benzene and passed through an adsorption column of Floriden X Scortk to separate out vitamin A and carotenoids. The soln. contg. tocopherol was treated with a mixt. of α, α'-depyredyl and $FeCl_3$. After 10 min. the red color was measured. Summer milk contained more tocopherol than winter milk. During the first days of the colostrum period milk contained 300-400μ%. From the colostrum period the tocopherol falls to 76μ% in 13 months. The vitamin content varies in milk from different quarters of the udder. The variation bears no relation to fat content. H. J. Velander

888. The antibacterial substances of milk. J. E. Auclair. Proc. 13th Intern. Dairy Cong., **3**, 1130. 1953.

Lactenine and lactenine 2 are responsible for bacterial inhibition in milk, especially of *Streptococcus pyogenes*. They differ in pH and heat stability and can be separated by fractionation with acetone. Colostrum is high in lactenine, and lactenine 2 is found in milk at mid-lactation. They are more inhibiting under aerobic than anaerobic conditions as shown by action on various pathogens and on certain lactic acid bacteria. *S. agalactiae* is favored by a substance present in milk which is destroyed on autoclaving. It may affect growth of certain lactic organisms in raw milk.

H. J. Velander

889. Ultrasonic treatment of milk. F. R. Tobler. Proc. 13th Intern. Dairy Cong., **2**, 703. 1953.

A liter, 250 ml., 50 ml., and 25 ml. of milk were irradiated for 5-20 min. with a fixed frequency of 1,000,000 hertz and a variable intensity of 1.6, 2.8, 3.6, and 4 w/sq cm. The bacteria were reduced 50% with 3.6-4.0 w/sq cm. for 20 min. A greater breaking up of clumps occurred at lower intensities. The acid formation was slower. This may be due to a reduction in number of bacteria. There was no effect on creaming or curdling. The phosphatase activity was not impaired.

H. J. Velander

890. A method for continuous fortification of milk with concentrates of vitamins. K. G. Weckel, C. Vencia, and H. Maze, Univ. of Wis. and D. Beckerleg, Bancroft Dairy Co., Madison, Wis. Milk Dealer, **43**, 10: 58. 1954.

The usual procedure in the use of vitamin concentrates for fortification in milk is to add them "batch-wise." A measured quantity of the concentrate is added to a known weight of milk in a pasteurizing vat or in a storage tank. A metering device for adding vitamins to milk is described. It is adaptable to continuous flow operations such as the HTST pasteurizers which minimize or eliminate a tie-up of a bulk milk storage tank, the capacity of which normally is adequate for several products. The metering device is adaptable for the use of several types of concentrates, and for different operational capacities. It is necessary only to calculate and vary the extent of dilution of the canned vitamin concentrate to accommodate the several operations possible in any given plant. Commercial testing of the unit under normal operating conditions has indicated the procedure is economical and, on the basis of bioassay results, reliable. C. J. Babcock

891. Variations in the ionic and lactose concentrations of milk. J. M. Barry and S. J. Rowland, Univ. of Reading. Biochem. J., **54**, 4: 575. 1953.

Variations in Na, K, Cl, and lactose content of milk are reported. The influence of period of lactation and mastitis infection upon these variations as well as their correlation to the blood serum composition is discussed. As lactation advances or when mastitis is present the milk composition tends to become more nearly like the blood serum composition—i.e., an increase in Na and Cl with a decrease in K and lactose. These relationships are expressed in regression equations. A. O. Call

PHYSIOLOGY AND ENDOCRINOLOGY

892. The effect on mouse-liver catalase activity and blood-haemoglobin level of a milk diet deficient in iron, copper and manganese. D. H. Adams, London Hospital Med. Coll., London. Biochem. J., **54**, 2: 328. 1953.

A diet of milk supplemented with Fe, Cu, and Mn is equivalent to "normal cube diet" for liver catalase activity of weanling mice. Fe, either alone or in combination with other metals, had little effect on catalase level, but it was required for maintenance of normal haemoglobin level. There is a sex difference in the requirements of Mn and Cu to maintain normal catalase levels. A. O. Call

893. The source of antibody in rabbit milk. P. N. Campbell, J. H. Humphrey, and T. S. Work. (Abs.). Natl. Inst. for Med. Research, London. Biochem. J., **54**, 2: 12. 1953.

The results show that there is a direct transfer of antibody from blood plasma to milk.

A. O. Call

894. Observations on the oxidation of glucose and acetate by lactating sheep mammary tissue in vitro. W. G. DUNCOMBE and R. F. GLASCOCK, Univ. of Reading. (Abs.) Biochem. J., **55**, 2: 23. 1953.

Isotope labeled sodium acetate and glucose were used as substrates in 0.5 g. batches of tissue slices. Both glucose and acetate were metabolized. Each is oxidized at a greater rate in the presence of the other, rather than alone. The hormone, insulin, had no effect upon the respiration rate. A. O. Call

895. The synthesis of lactose from glucose in the mammary gland. O. K. REISS and J. M. BARRY, Univ. of Chicago. Biochem. J., **55**, 5: 783. 1953.

Glucose labeled uniformly with ^{14}C was injected intravenously into a lactating goat and the specific activities of blood glucose, of lactose, and of the glucose and galactose residues of lactose were measured in blood and milk samples taken up to 12 hr. after the injection.

The results show that blood glucose was the principal source of both the glucose and galactose residues of the milk lactose.

A. O. Call

896. Synthesis and oxidation of citric acid in mammary gland homogenates. (Abs.) C. TERNER, Univ. of Reading. Biochem. J., **55**, 5: 29. 1953.

Whole homogenates of lactating guinea pig mammary gland were found to synthesize citrate from pyruvate or acetate in the presence of fumarate and to be able to oxidize added citrate rapidly. This lends support to the possibility of the Krebs cycle being operative in the mammary gland since in this cycle citric acid is an intermediate. A. O. Call

SANITATION AND CLEANSING

897. Washing machine for cases, cans, and crates. J. J. BOCCHINO. U. S. Patent 2,682,881. 3 claims. July 6, 1954. Official Gaz. U. S. Pat. Office, **684**, 1: 123. 1954.

Construction details are given for a straight line washer which can be used for cleaning several types of cases, cans, and crates.

R. Whitaker

898. Valve mechanism for flushing pipe line milking systems. W. E. OZMINA (assignor to International Harvester Company). U. S. Patent 2,682,880. 11 claims. July 6, 1954. Official Gaz. U. S. Pat. Office, **684**, 1: 122. 1954.

A mechanism for alternately subjecting milking machine pipe lines and cans to vacuum and air and to a source of cleaning solution for flushing, cleaning, and sanitizing the equipment after use. R. Whitaker

899. The problem of waste water in the dairy industry. J. PIE. Proc. 13th Intern. Cong., **3**, 970. 1953.

The purification of waste water can be carried out by adding $Al_2(SO_4)_3$ and chalk followed by absorption on peat provided the water does not contain more than 2% milk serum. Otherwise the water must be subjected to two consecutive fermentations. In the first the lactose is fermented in 12 hr. at 37° C. in a slightly acid medium. The second breaks down 80% of the albuminoids into NH_3 in 24 hr. at 37°. With this treatment biol. purification is effected.

H. J. Velander

900. Investigations on the treatment of dairy waste water in septic tanks. K. P. ANDERSEN and R. POULSEN. Proc. 13th Intern. Dairy Cong., **3**, 986. 1953.

Exp. septic tanks each holding 5 l. were employed using waste H_2O which consisted of 2% skimmilk, 1% skimmilk + 1% whey, 2% whey, 5% skimmilk, and 5% whey. After a period of three days, composition of waste water is completely changed. The pH values at the intake were 7.2-7.3 but in 24 hr. decreased to 4.6-5.6, depending on type of waste. Protein breakdown was studied by detn. of total N and NH_3. The process is not an actual purification, except to detn. decrease in total carbon content. Greater part of carbon is present as ether-sol. cpds. which consist of the lower organic acids. After anaerobic treatment the waste water is more susceptible to a biological oxidation. H. J. Velander

901. Alkaline cleaners for HTST pasteurizers. T. LEWANDOWSKI and L. J. BROWN, Pa. Salt Manufacturing Co., Wyndmoor, Pa. Milk Dealer, **43**, 8: 61. 1954.

Satisfactory cleaning methods have been developed for the plate-type high-temperature, short-time (HTST) pasteurizer. The most widely used method consists of flushing with cold or warm water immediately after processing, circulating an acid cleaner, flushing with warm water, circulating an alkaline cleaner and then flushing thoroughly with warm and/or cold water. Soils obtained from HTST pasteurizers after processing whole milk and before cleaning have a high percentage of fat and protein with a relatively small amount of ash. Cleaning of a laboratory soil similar in composition to soils obtained from HTST pasteurizers, by a simulated acid-alkali HTST cleaning cycle, showed that with increase in pH of alkaline cleaner solutions, prepared in hard water, increased cleaning resulted. Alkaline cleaner solution concentration and possibly types of cleaner ingredients also influenced cleaning. Soil removal was largely due to alkaline cleaning although the preliminary cleaning with acid solutions contributes some soil removal and possibly removes mineral soil constituents. In the conventional acid-alkali cycle for cleaning HTST pasteurizers, superior results may be obtained through use of strongly alkaline cleaners. C. J. Babcock

902. Plant sanitation. C. W. ENGLAND, C. Y. Stephens Industries, Washington, D. C. Ice Cream Rev., 37, 11: 126. 1954.

It is important that every plant develop a cleaning and cleaning compound use manual. Such a manual should present detailed instructions on how to clean and sterilize each piece of equipment. The use of a planned system for cleaning procedures will ensure an efficient job of cleaning and also save on cleaning materials. To secure the active participation of employees in the program it is essential that they have full knowledge as to the reasons for performing each task in the prescribed manner.

In sterilizing equipment, after proper cleaning, the author suggests pumping a solution with 100-125 p.p.m. of available chlorine thru the assembled equipment for a minimum period of 5 minutes. The walls of vats and tanks may be effectively treated by spraying with a 400 p.p.m. quaternary ammonium solution. A new stable low pH chlorine sterilizer known as 1,3-dichloro-5,5 dimethyl hydantoin is now available which is claimed to be more effective than sodium and calcium hypochlorites.

In-place-cleaning of equipment when properly carried out will save time, labor, wear and tear on equipment, and do a better job than manual cleaning and will eliminate possible recontamination of equipment surfaces by personnel following cleaning and sterilization. The O-R system of cleaning in which a suitable acid cleaner is recirculated first and then an alkali added directly to the acid and recirculated is especially well adapted to cleaning vacuum pans, HTST units, and any other high temperature equipment.

Corrosion of stainless steel by acids and chlorine has been a serious problem in many plants. Inorganic acids are much more corrosive to stainless steel than organic acids. Thorough rinsing of equipment following the use of an acid cleaner is important in reducing possible corrosive effects of acid cleaners. Chlorine if used at too high a temperature or at too high a concentration may also be corrosive to stainless steel. The use of chlorine immediately before the equipment is put into service will do much to prevent possible corrosion of stainless steel due to chlorine. The addition of chlorine to the hot circulating wash solution when in-place-cleaning is practiced will oxidize the film of wetting agent that would otherwise remain on the interior of pipe lines and hence leave all surfaces in a bright condition. If too much chlorine is used, however, it can be very damaging to the equipment. To avoid this difficulty the wash solution should contain not over 35 p.p.m. of available chlorine.

Some specific types of bacteria, including coliforms, psychrophiles, thermophiles, and thermoduries, which may cause trouble in dairy plants are discussed with recommended control measures. W. J. Caulfield