JOURNAL OF DAIRY SCIENCE

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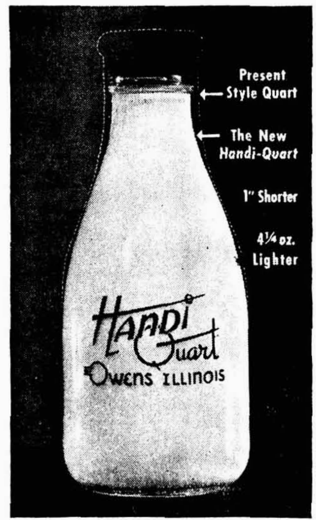

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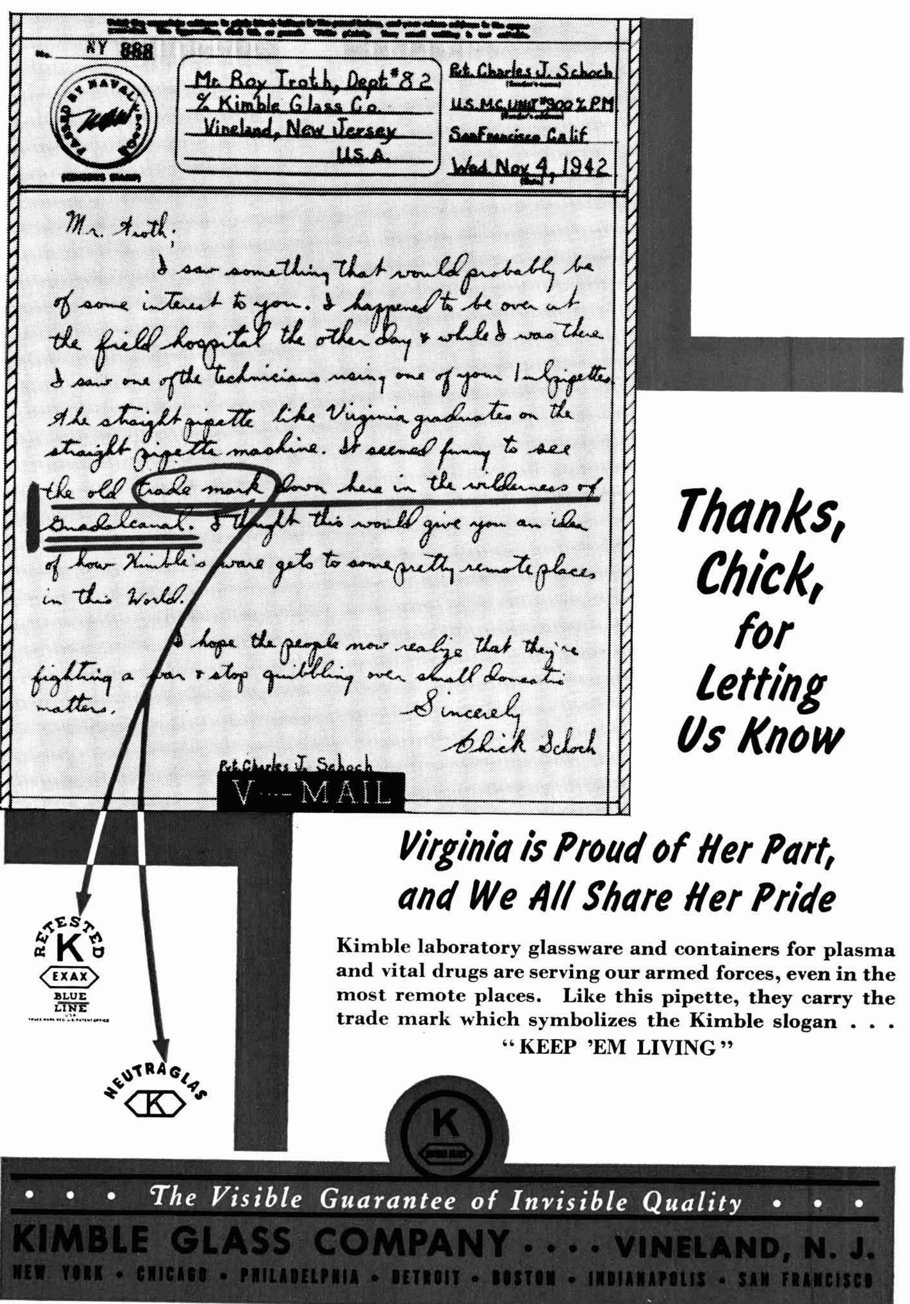

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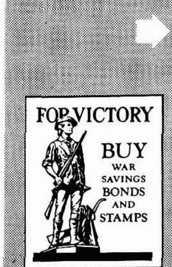

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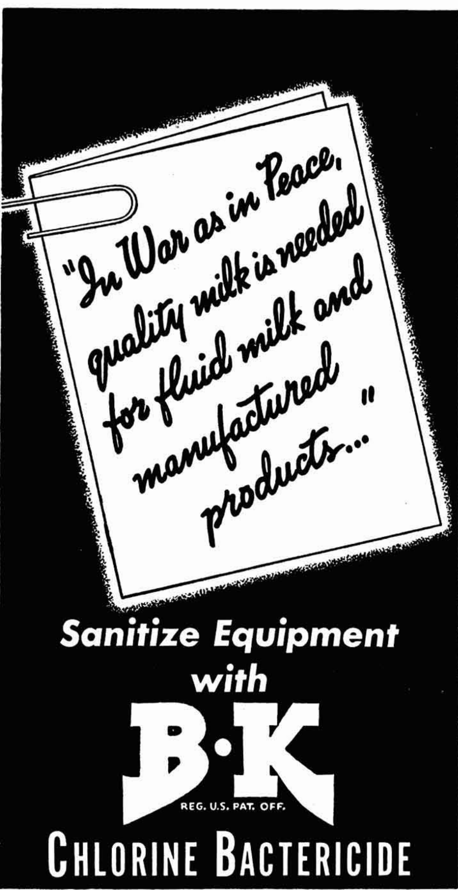

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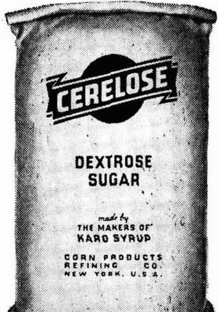

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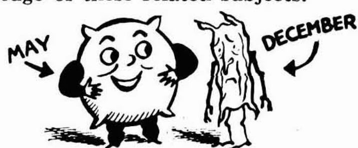

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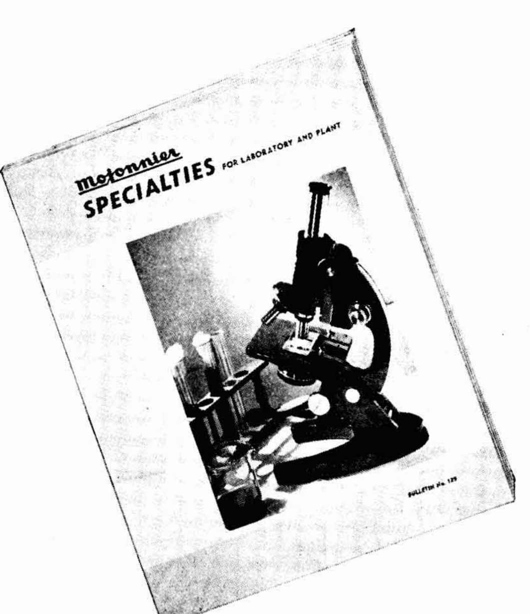

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JOURNAL OF DAIRY SCIENCE

VOLUME XXVI | APRIL, 1943 | NUMBER 4

THE EFFECT OF THIAMIN FEEDING UPON MILK AND FAT PRODUCTION*

J. K. LOOSLI AND H. L. LUCAS

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In view of the role which thiamin plays in the metabolism of carbohydrates (1, 2), and the evidence found in the recent literature that this vitamin also plays a role in the synthesis of fats from carbohydrate (3, 5, 6, 10, 11), it was thought desirable to study the possible effects upon fat production of feeding large amounts of thiamin to lactating dairy cows, which, especially on low-fat rations, produce considerable amounts of milk-fat from carbohydrates. It is realized that rumen synthesis of thiamin in large amounts has been demonstrated (4, 9), yet there is no clear evidence to show whether this source of supply is at all times adequate, especially since it has been reported that lactation increases the thiamin requirement of the rat enormously (8).

Four non-pregnant Holstein cows in middle lactation were placed upon a low-fat ration which consisted of timothy hay, dried beet pulp and a concentrate mixture containing approximately 1 per cent of ether-extract. Expressed on the basis of total digestible nutrients, the hay and pulp each supplied one half of the maintenance allotment which was approximately 120 per cent of the amount specified by Morrison (7) as "recommended for good cows." The concentrate mixture was fed at the rate of 1 pound of total digestible nutrients for each 3.1 pounds of four-per-cent fat-corrected milk. This amount supplied all of the production requirement according to Morrison's "recommended for good cows."

The concentrate mixture was made up of the following ingredients: solvent extracted linseed meal, 50 parts; solvent extracted cottonseed meal, 30 parts; solvent extracted soybean meal, 40 parts; solvent extracted corn distillers' dried grains, 20 parts; barley, 60 parts; wheat bran, 40 parts; cane molasses, 24 parts; corn starch, 30 parts; bone meal, 3 parts; and salt,

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3 parts. The chemical composition and nutrient content of the feeds used are shown in table 1.

TABLE 1

The chemical composition and digestible nutrient contents of the feeds used. (All values are expressed in per cent)

Feed	Crude protein	Crude fiber	N-free extract	Ether extract	Digestible nutrients*	
					Crude protein	Total
Timothy hay	4.74	34.88	46.25	1.79	2.42	49.77
Dried beet pulp	8.88	19.68	57.05	1.00	4.71	70.15
Concentrate	24.97	6.50	50.33	1.25	20.47	71.54

* Calculated using Morrison's coefficients of digestibility.

The effect of thiamin was tested by means of a double reversal trial with three periods of five weeks each. The design of the experiment was as follows:

Period	*No thiamin*	*Thiamin*
I	Cows 13 and 7	Cows 21 and 15
II	Cows 21 and 15	Cows 13 and 7
III	Cows 13 and 7	Cows 21 and 15

During alternate periods thiamin hydrochloride[1] was fed at the rate of 300 micrograms per pound of total digestible nutrients consumed. A freshly prepared water solution of thiamin, slightly acidified, was mixed with the beet pulp each day immediately before feeding.

The animals were milked twice daily throughout the experiment. The milk was weighed and aliquot samples were taken at each milking. Weekly composite samples were tested for fat by the Babcock method and the concentrate allowances were adjusted weekly on the basis of the fat-corrected milk production of the previous week. The cows were weighed once each week. The amounts of hay and beet pulp to be fed were determined on the basis of the preliminary body weight and condition of the individual cows. These amounts were not changed during the experiment.

All cows behaved satisfactorily with the exception of cow 21 which was "off feed" during the last three weeks of period I. This, of course, lowered slightly the overall average production during thiamin feeding. In interpreting the results a correction for this abnormality was considered. The corrected production was obtained by assuming that the behavior of cow 21 would have been the same as that of cow 15 which was receiving thiamin supplement during the same periods. Both corrected and uncorrected results are presented in table 2.

The figures indicated as "favoring thiamin" were obtained for each cow by comparing the production during period II with the average of the

[1] Thiamin Hydrochloride, Merck and Company.

productions during periods I and III. The average results for each treatment, presented at the bottom of the table, were obtained by taking the sum of the productions on the particular treatment during periods I and III plus two times the sum of the productions on the same treatment during period II, and dividing the grand sum by eight. The average figures indicated as "favoring thiamin" were obtained both by comparing the average productions on each treatment and by averaging the figures favoring thiamin for each cow. A negative sign indicates a lower production during thiamin feeding.

TABLE 2

The average daily production of milk, fat and fat-corrected milk, and the fat percentage during each of the three periods for each cow, and the overall results of the experiment

Treatment	Milk	Fat	Fat	Fat corrected milk
	lbs.	*%*	*lbs.*	*lbs.*
Cow 13				
Period I—no thiamin	29.95	3.80	1.139	29.06
Period II—thiamin	27.74	4.03	1.118	27.86
Period III—no thiamin	27.40	3.99	1.093	27.36
Favoring thiamin	- 0.93	0.13	0.002	- 0.35
Cow 21				
Period I—thiamin	26.01	4.50	1.172	27.98
Period I—thiamin*	28.06*	4.33*	1.215*	29.45*
Period II—no thiamin	26.72	4.32	1.154	27.99
Period III—thiamin	25.97	4.22	1.095	26.81
Favoring thiamin	- 0.73	0.04	- 0.020	- 0.59
Flavoring thiamin*	0.29*	- 0.04*	0.001*	0.14*
Cow 7				
Period I—no thiamin	25.47	4.29	1.092	26.56
Period II—thiamin	23.56	4.53	1.067	25.43
Period III—no thiamin	23.65	4.27	1.010	24.61
Favoring thiamin	- 1.00	0.25	0.016	- 0.15
Cow 15				
Period I—thiamin	24.92	4.53	1.128	26.89
Period II—no thiamin	23.73	4.51	1.071	25.55
Period III—thiamin	23.82	4.39	1.046	25.21
Favoring thiamin	0.64	- 0.05	0.016	0.50
Average—all cows				
Thiamin	25.41	4.33	1.101	26.68
Thiamin*	25.67*	4.31*	1.107*	26.87*
No thiamin	25.92	4.24	1.098	26.83
Favoring thiamin	- 0.51	0.09	0.003	- 0.15
Favoring thiamin*	- 0.25*	0.07*	0.009*	0.04*

* Corrected for "off feed" effects.

It is seen from table 2 that the feeding of thiamin had no significant effect upon the production of either milk or fat. Whether considered on the uncorrected or corrected basis, production trends were remarkably uniform among periods and animals. Body weight changes very slightly favored

thiamin feeding on the average, but when the variability of the changes among periods and animals was taken into account, the slight advantage for thiamin feeding very definitely became insignificant. In the present experiment, the cows produced more than a pound of fat daily at all times, whereas the feed supplied between 0.41 and 0.45 pounds of fat daily when expressed as ether-extract. Calculations show that the animals actually produced between 2.3 and 2.6 times more fat than they ingested. Thus, it is seen that although the synthesis of fat was considerable, under the conditions of this experiment, thiamin supplementation did not affect production.

It should be pointed out that the level of production in this experiment was only medium. It would seem that at higher production levels, perhaps only at maximum levels, where the metabolism of carbohydrate and the synthesis of milk fat from carbohydrate would be considerably greater, the effects of added thiamin might be more critically tested.

SUMMARY

By means of a double reversal trial, with periods of five weeks duration, and using four cows of medium production, the effect of thiamin supplement, when added to a low-fat diet, was studied. Thiamin was supplied at the rate of 300 micrograms per pound of total digestible nutrients ingested. No effect upon the production of milk or fat was observed.

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NATURE OF THE MATERIAL IN MILK RESPONSIBLE FOR THE MODIFIED WHITESIDE TEST FOR MASTITIS

H. O. DUNN, JAMES M. MURPHY AND O. F. GARRETT
New Jersey Agricultural Experiment Station, New Brunswick, N. J.

Recently an indirect test for the presence of mastitis was proposed by Whiteside (8), according to whom the formation of a viscid mass, when two ml. of normal NaOH are mixed with 10 ml. of milk, is a good indication that the cow is suffering with mastitis. Murphy and Hanson (6), however, have shown that the formation of the viscid mass has no real value as a test, since the reaction occurs only after the milk has become macroscopically abnormal. They modified the test in such a manner that different degrees of reaction could be detected and evaluated and showed that the degree of the reaction closely paralleled the leucocyte count of the milk. The modified test was found capable of detecting smaller degrees of udder irritation than was possible with other field tests.

Since the reaction which takes place in the Whiteside test seemed to have chemical and physiological significance in a study of udder infections, an attempt was made to discover the nature of the substance in mastitic milk which reacts with NaOH to give the test.

The modified Whiteside test was conducted in all cases according to the procedure given by Murphy and Hanson (6). Leucocyte counts were made by preparing films containing 0.01 ml. of milk, or of diluted slime from the cream separator, according to the Breed-Prescott (7) method and by staining with Newman-Lampert stain (formula No. 2). In the part of the study dealing with the various fractions of milk, separation was accomplished either by gravity or by use of a power-driven cream separator. Adherent milk and milk solids were removed from the separator bowl by draining and by careful wiping with absorbent cloth so as to leave the separator slime untouched. The weight of slime was found with a fair degree of accuracy by determining the increase in weight of the shell of the separator bowl.

The pH at which the reaction with NaOH took place was determined electrometrically with a small glass electrode and was found to average 12.6 (corrected for salt-protein error) for 25 samples. The reactions, with NaOH, of milk from cows suffering with various degrees of mastitis are shown in figure 1. The reaction depicted in the lower right corner is apparently typical of that originally described by Whiteside (8). This 4+ reaction results in the formation of a gummy viscid mass which adheres to a stirring rod when pulled away from the mixture.

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Modified Whiteside Reactions of Milk Fractions Obtained by Gravity and by Mechanical Separation. Samples of whole milk, obtained by the complete milking of two Holstein cows, Nos. 430 and 276, were each divided into two parts; part 1 was separated centrifugally into cream and skim milk; part 2 was allowed to separate by gravity. The whole milk and each fraction were subjected to the Whiteside test.

The udder of cow No. 430 was free of infection and yielded milk that was negative to the modified Whiteside test and contained few leucocytes. Every quarter of the udder of No. 276 was infected with *Streptococcus agalactiae* and was affected with advanced chronic mastitis. In all trials, milk from this cow gave a positive Whiteside reaction. Tests made on her quarter samples at 30- to 60-day intervals consistently showed high leucocyte counts.

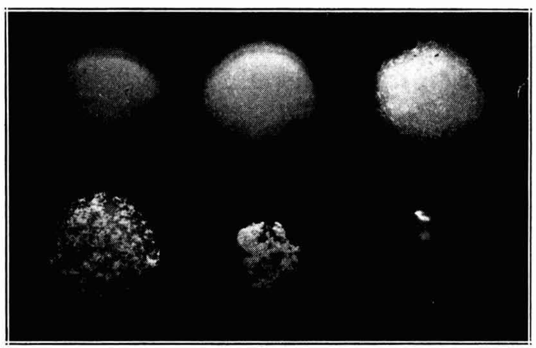

FIG. 1. Typical reactions obtained with the modified Whiteside test. Upper row (left to right): negative, ±, 1+. Lower row (left to right): 2+, 3+, 4+.

On standing a few hours, an epiphasic layer of flocculent curdy material formed on the centrifugally separated skim milk from No. 276. This layer contained from 3.7 to 5.8 per cent fat, and exhibited a strongly positive Whiteside reaction, but this was not due entirely to incomplete separation of fat from the whole milk. A thin, true cream layer formed on the centrifugally separated skim milk from No. 430, but the modified Whiteside reaction was never more than slightly positive. The intensities of the modified Whiteside reaction on different milk fractions from these two cows are presented in table 1.

The results showed that the material in milk responsible for the positive Whiteside reaction rose with the fat in gravity separation, thus concentrating the substance to give a stronger reaction in the cream layer than in the whole milk. Centrifugal separation produced cream that was free of the material. No separator slime was observed in the bowl after separation of the milk from No. 430, whereas some was always present after separation of the milk from No. 276. Four additional trials gave similar results.

TABLE 1

Intensity of the modified Whiteside reaction on milk fractions from mastitic positive and mastitic negative milk samples

Nature of milk fractions	Modified Whiteside reactions							
	Trial 1		Trial 2		Trial 3		Trial 4	
	276	430	276	430	276	430	276	430
Whole milk	2 +	–	2 +	1 +	2 +	–	2 +	±
Separator skim	±	–	1 +	–	±	–	1 +	–
Epiphasic layer	3 +	–	4 +	1 +	4 +	–	4 +	±
Hypophasic "	–	–	–	–	–	–	–	–
Separator cream	–	–	–	–	–	–	–	–
Gravity skim	–	–	–	–	–	–	–	–
Gravity cream	4 +	–	4 +	–	4 +	–	4 +	–

The cream layers of milk from No. 276 were always much deeper than those of milk from No. 430, although both cows gave milk of the same fat test on each day the samples were compared. The difference may have been due to the presence of material causing the Whiteside reaction.

In trial 3, leucocyte counts were made on whole milks, gravity-separated creams, and gravity-separated skim milks. Results are summarized in table 2 and show that the leucocytes were carried into the cream layer and that the intensity of the modified Whiteside reaction was proportional to the number of leucocytes present.

TABLE 2

Intensity of the modified Whiteside reaction and leucocyte counts in whole milk, gravity-separated cream, and gravity-separated skim milk

	Cow 430		Cow 276	
	Whiteside reaction	Leucocytes per ml.	Whiteside reaction	Leucocytes per ml.
Whole milk	–	50,000	2 +	4,000,000
Gravity cream	–	650,000	4 +	35,000,000
Gravity skim	–	0	–	0

Gravity-separated cream, which exhibited a positive Whiteside reaction, was thoroughly washed by diluting with an equal volume of warm (50° C.) water and centrifuging at high speed. This treatment rendered the cream negative to the Whiteside reaction. A sediment was obtained which reacted with NaOH to produce a gray, gelatinous mass. When observed under the microscope, the sediment, previous to treatment with NaOH, appeared to have a cellular structure, which disappeared upon the addition of NaOH.

The Relation of the Number of Leucocytes, the Amount of Separator Slime, and the Intensity of the Modified Whiteside Reaction. The milk from a Holstein cow, No. 298, was used to study the relationship of the amount and nature of separator slime, the leucocyte counts, and the intensity

of the Whiteside reaction. The right front quarter was non-functioning; the two rear quarters exhibited a mild chronic mastitic condition. Fore milk from these two quarters was consistently strongly positive according to the modified Whiteside test. One was infected with *Streptococcus agalactiae* and the other with hemolytic staphylococci. The left front quarter was infected with *Escherichia coli*, producing a Type AIII infection according to the coliform organism infection classification of Murphy and Hanson (5). In this instance, the infection was of the fluctuating kind which suddenly manifested a mild acute mastitis, lasting for several days, followed by gradual improvement to a non-clinical state, which was maintained for several days to several weeks, only to be followed by another clinical flare-up.

The minimum milk production of cow No. 298 at the time the experiment

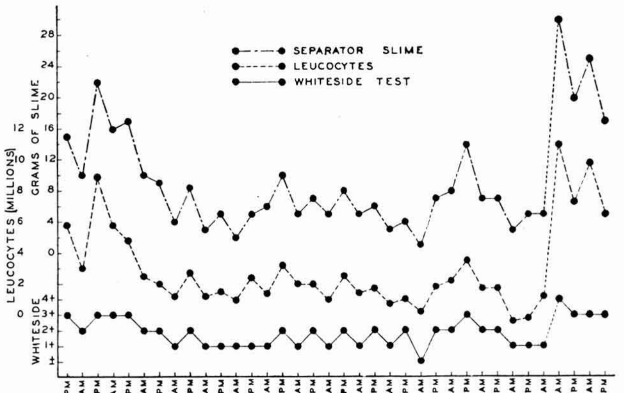

FIG. 2. Milking-by-milking relationship of number of leucocytes, weight of separator slime, and intensity of modified Whiteside reaction in milk from a cow affected with chronic mastitis.

was started was close to 16 pounds for the evening milkings; hence, this weight was used as the standard quantity of milk to be separated. Beginning on March 5th, samples were collected from each morning and evening milking for a period of 20 days with the exception that none were taken from the three milkings just prior to the major flare-up toward the end of the experimental period. The curves in figure 2, which show graphically the trend of the modified Whiteside reaction, the number of leucocytes, and the quantity of separator slime from these milk samples, closely parallel one another. The absence of results for the three milkings toward the end of the experimental period are indicated by dotted lines.

The sharp changes in the curves (figure 2) were caused by the coliform infection in the left front quarter, which was subject to periodical flare-ups of clinical mastitis. The infections in the two rear quarters were rather constant in their intensity.

Samples of slime, which exhibited a 4 + modified Whiteside reaction, contained from 700,000,000 to 900,000,000 leucocytes per gram. When 17 grams of slime from 9 pounds of milk from the mastitis affected cow, No. 298, were added to 9 pounds of milk from a Holstein cow, No. 463, which had a normal healthy udder, a positive Whiteside reaction in the mixture was obtained. The whole milk from No. 298 tested 4 + and the separator skim milk tested 2 +. Milk from No. 463 was negative to the Whiteside reaction and contained only 25,000 leucocytes per ml. After addition of the slime to this normal milk, the leucocyte count was 2,600,000 and the Whiteside reaction was 2 +.

When 17 pounds of milk with a modified Whiteside test of 2 + were separated, five grams of separator slime were obtained. Remixing of the skim milk and cream resulted in a modified Whiteside test of 1 + in the mixture. Upon a second separation one gram of slime was obtained. A second mixing of the skim milk and cream gave a mixture with a ± reaction. A third separation yielded 0.5 gram of slime but the Whiteside reaction in the reconstituted milk was completely masked by the clumping of fat globules.

These experiments show that the material causing the Whiteside reaction is contained in the separator slime and can be removed almost completely from milk by several centrifugal separations.

Effect of Adding Leucocytes to Milk That Is Negative to the Modified Whiteside Reaction. The milk used in this experiment was from the Holstein cow, No. 463. It was negative to the modified Whiteside test and contained very few leucocytes. Washed suspensions of equine and bovine leucocytes were prepared by diluting 500 ml. of blood with an equal volume of physiological saline solution containing 1.5 per cent of sodium citrate, according to Wright's method, which is described by Kelser (3). The washed leucocytes were centrifuged from the saline solution and were added to 4 ml. of the normal milk. A series of Kahn tubes were set up for two trials, equine leucocytes being used for one trial and bovine leucocytes for the other. By transferring one ml. of the suspension from the first tube into one ml. of milk in the second tube, one ml. of the suspension from the second tube to one ml. of milk in the third tube, and so on through all the tubes, a series of dilutions were obtained.

The results of the leucocyte count and the modified Whiteside test, presented in table 3, show that there is a direct relationship between the number of leucocytes added to normal milk and the intensity of the modified Whiteside reaction. The experiment presents strong evidence that the material involved in the reaction with NaOH is a constituent of the leucocyte cells and is not a free substance secreted into the milk or formed in the milk of udders affected with mastitis.

The addition of whole blood, blood plasma, blood serum, or erythrocytes from a horse or a cow to Whiteside-negative milk did not produce a mixture

that gave a positive reaction, even when the volume of these substances was equal to the volume of milk.

Normal bovine blood, which contained approximately 8,000,000 leucocytes per ml., reacted with normal NaOH in the ratio of 1: 5. The appearance of the reaction, however, could not be compared satisfactorily with that in mastitic milk because of the dissimilarity in composition and color.

A suspension of washed bovine blood leucocytes was mixed with normal NaOH on a glass slide. A transparent, viscid gel was formed that acquired the appearance of a typical Whiteside reaction in mastitic milk when one drop of normal milk was mixed into the mass.

TABLE 3

Modified Whiteside reactions on normal milk containing washed leucocytes from equine and bovine blood

No.	Horse blood		Cow blood	
	Leucocytes/ml. (thousands)	Reaction	Leucocytes/ml. (thousands)	Reaction
1	168,000*	4 +	126,000*	4 +
2	84,000*	4 +	63,000*	3 +
3	42,000*	3 +	31,500*	3 +
4	21,000*	3 +	15,800*	3 +
5	10,500	3 +	7,500	2 +
6	6,350	2 +	3,190	2 +
7	2,100	1 +	2,050	1 +
8	500	1 +	1,350	1 +
9	450	1 +	700	1 +
10	225	±	575	±
11	75	Faint	275	±
12	60	Very faint	225	Very faint
13	25	" "	175	Negative
14	50	" "	50	"
Control	None	Negative	None	"

* Calculated on the basis of dilution.

In further experiments, Whiteside-negative milk was heated at 98° C. for five minutes and then cooled before addition of a suspension of leucocytes to give a count of 56,000,000 per ml. The mixture gave a modified Whiteside reaction of 4 +. On the other hand, the addition of a saline suspension of leucocytes, heated at 98° C. for five minutes, to some of the same heated milk used above resulted in a negative Whiteside reaction.

Microscopic examination of milk containing large numbers of leucocytes showed that the cellular structure had been destroyed subsequent to the addition of NaOH. Boiling for five minutes the same milk before the addition of NaOH did not change the number and appearance of the leucocyte cells, yet such milk was incapable of reacting to the Whiteside test and showed no change in the appearance and number of cells after the addition of NaOH.

It seems clear from these experiments that heating of the normal com-

ponents of milk has no influence on the Whiteside reaction, whereas heating of the leucocyte cells produces a change, probably physical in nature, in some cellular material, which prevents the typical reaction with NaOH.

DISCUSSION

Material responsible for the Whiteside reaction in mastitic milk followed closely the path of the fat globules in gravity separation, indicating that the substance, or substances, involved is either less dense than water or is adsorbed on or encompassed by the fat globules. It has been shown that leucocytes rise in the cream layer upon gravity separation (table 2). Munch-Peterson (4) quotes Skar as saying that the leucocytes may become so loaded with fat that they rise with the fat globules into the cream layer. Centrifugal force appeared to separate the material from the fat portion of the milk. These observations showed a marked resemblance to the results obtained by Garrison and Gholson (1), who showed that the methylene blue-borax test for mold mycelia in cream, separated from milk from infected udders, was different for gravity-separated as compared to mechanically separated cream. Mechanically separated cream always gave a good methylene blue-borax test even when the gravity-separated cream from the same sample showed excessive sediment. These investigators explained this as probably due to the removal of body cells and mucous protein during centrifugal separation of the milk.

All of the results of this study clearly show that the substance responsible for the Whiteside reaction is some constituent of leucocyte cells. While no complete explanation of the chemistry of the reaction can be offered at this time, a reasonable supposition is that the substance which reacts with NaOH is a protein or protein-like material. This supposition is supported by the physical nature of the end-product of the reaction and by the fact that heat destroys the reactivity. Heat coagulates certain types of proteins thereby changing their physical nature and chemical activity. It is possible, therefore, that, in the Whiteside reaction, nucleic acid, a normal constituent of leucocyte cells, combines with NaOH to form the sodium salt. This salt is known to be liquid while warm but solidifies to a gelatinous mass on cooling (2). In the case of the unheated cells, the alkali breaks down the cell walls, as observed in this study, thus coming into contact with the cellular protein with which it immediately reacts. Heating of the leucocytes, however, results in coagulation of the cellular protein, the cells retain their form in the presence of alkali, and the typical Whiteside reaction is prevented.

Further, the fat globules and serum solids of the milk probably are adsorbed on the gelatinous material, the whole producing the characteristic precipitate of the reaction.

SUMMARY

The evidence obtained in this study that leucocytes are directly or indirectly responsible for the Whiteside reaction in mastitic milk may be summarized as follows:

The reaction in gravity-separated cream was stronger than in the whole milk; leucocytes have been shown to concentrate in gravity-separated cream.

Cellular material, which produced a viscid mass when mixed with NaOH, was washed from cream separated from mastitic milk by gravity.

Separator slime, which contained from 700,000,000 to 900,000,000 leucocytes per gram, always gave the most intense Whiteside reaction of any of the milk fractions obtained by mechanical separation.

The curves showing the trend of the number of leucocytes, the amount of separator slime, and the intensity of the modified Whiteside reaction in samples of milk from a cow affected with mastitis of varying severity were closely parallel to one another.

Addition of separator slime to Whiteside-negative milk produced a strongly positive milk and a large increase in leucocytes to about 2,600,000 cells per ml. The intensity of the Whiteside reaction of this mixture corresponded to that of other milk samples containing approximately 2,000,000 leucocytes per ml.

Addition of either bovine or equine leucocytes to Whiteside-negative milk samples resulted in a change to positive reactions, the intensities of which varied directly with the number of leucocytes added.

Addition of whole blood, blood plasma, blood serum, or erythrocytes to milk did not produce a positive test.

Normal blood, which contained approximately 8,000,000 leucocytes per ml., reacted with normal NaOH in the ratio of 1:5; the appearance of the reaction in blood, however, could not be satisfactorily compared with that in mastitic milk.

When a suspension of bovine blood leucocytes was mixed with normal NaOH on a glass slide, a transparent viscid gel could be detected. When one drop of normal milk was mixed into the gelatinous material, giving a white color to the mass, the appearance was that typical of the Whiteside reaction.

Addition of leucocytes to Whiteside-negative milk, previously heated to 98° C. and cooled, resulted in a mixture which was strongly positive; addition of a suspension of leucocytes, heated to 98° C. and cooled, to negative milk did not make the reaction positive; the heating of negative milk which contained a suspension of leucocytes gave a negative reaction.

It is postulated that the protein material of leucocytes in mastitic milk reacts with NaOH to form a gelatinous mass similar to that which is formed by the action of NaOH on nucleic acid from animal cells.

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THE CALCIUM AND PHOSPHORUS CONTENT OF COMMERCIALLY MADE COTTAGE CHEESE

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The increased use in the American diet of cheese, including cottage cheese, during the war period has called attention to the relatively small amount of information available on the calcium and phosphorus content of cottage cheese as made commercially in the United States. Blunt and Sumner (1) analyzed 38 samples of commercial cottage cheese, reported an average of 0.077 per cent calcium, and concluded that such cheese is not the rich source of calcium it is usually considered. Kramer and McCammon (3) found that about 20 per cent of the calcium and 37 per cent of the phosphorus of the milk are retained in soft cheese. McCammon, Caulfield and Kramer (4) presented analyses of 24 samples of cottage cheese, prepared in the laboratory by different methods, which showed that the calcium content varied between 0.091 and 0.128 per cent and the phosphorus between 0.134 and 0.186 per cent. Guittonneau and Chevalier (2), after experimenting with rennet coagulation, before and after lactic acid fermentation, reported that curd produced under the latter condition contained 11.8 per cent of the calcium and 36.6 per cent of the phosphorus of the original milk.

This is a report of the calcium and phosphorus analyses of 102 samples of cottage cheese made under ordinary commercial conditions by 11 different large dairies located roughly in the triangle formed by the cities of Washington, Detroit, and Hartford. A pound-sample of cheese, selected at random from the lot prepared for the market by each company, was received weekly at the laboratory and was analyzed for dry matter, ash, calcium, and phosphorus. Those samples not in good condition when received at the laboratory were discarded.

The mean percentages of total solids, ash, calcium, and phosphorus of the 102 samples as received at the laboratory and after calculation to the dry weight basis are presented in table 1. The magnitude of the standard deviation in each case indicates a wide variation in composition. The total solids varied from 14.89 to 28.71 per cent, the ash from 0.62 to 2.31 per cent, the calcium from 0.046 to 0.106 per cent, and the phosphorus from 0.172 to 0.359 per cent.

The mean composition of the cottage cheese samples from each of the companies is shown in table 2. It is apparent that the composition of the cheese varied considerably with the company. That of company 10 was

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TABLE 1

Mean composition of 102 samples of cottage cheese

	Mean and P.E.*		Standard deviation and P.E.*	
	Wet	Dry†	Wet	Dry†
	%	%	%	%
Total solids	22.80 ± .19	....	2.87 ± .14	....
Ash	1.22 ± .02	5.36 ± .08	0.31 ± .01	1.22 ± .06
Calcium	0.080 ± .001	0.356 ± .006	0.012 ± .001	0.084 ± .004
Phosphorus	0.230 ± .002	1.030 ± .015	0.035 ± .002	0.230 ± .011

* Probable error.
† Calculated to the dry weight basis.

consistently very low in total solids, but was high in ash and calcium. The calcium content of the cheese from companies 4 and 5 was consistently high whereas that of the cheese from companies 2 and 6 was consistently low.

There is some indication that the cheese of low solids content tends to have a high calcium content. This could be due to little washing and incomplete drainage of the curd in the manufacturing process used by the companies making such cheese. A statistical analysis of this factor, however, failed to reveal a highly significant relationship.

TABLE 2

Mean composition of cottage cheese samples from each of eleven companies

Co. No.	Total solids	Ash		Calcium		Phosphorus	
		Wet	Dry*	Wet	Dry*	Wet	Dry*
	%	%	%	%	%	%	%.
1	21.43	1.22	5.66	.076	.360	.223	1.067
2	25.14	1.40	5.70	.064	.256	.219	0.892
3	25.68	1.58	6.16	.083	.326	.222	0.872
4	21.81	1.12	5.15	.086	.397	.233	1.084
5	23.46	0.94	4.06	.089	.383	.226	0.974
6	23.72	1.57	6.67	.065	.274	.240	1.023
7	21.33	1.10	5.23	.085	.413	.234	1.122
8	22.15	1.09	4.95	.079	.358	.242	1.126
9	23.68	0.97	4.14	.073	.310	.246	1.044
10	18.83	0.95	5.06	.086	.479	.211	1.165
11	22.83	1.33	5.87	.083	.364	.230	1.014

* Calculated to dry weight basis.

The ratios of calcium to ash, calcium to phosphorus, and phosphorus to ash were calculated for each sample of cheese. The mean ratios of the samples from each of the companies are shown in table 3. The ratios varied greatly from sample to sample and with each of the companies. On the average the samples contained about 16 times as much ash as calcium, 3 times as much phosphorus as calcium, and 5.5 times as much ash as phosphorus. Normal whole milk contains about 6 times as much ash as calcium, 7.5 times as much ash as phosphorus, and 1.25 times as much calcium as phosphorus.

TABLE 3

Mean ratios of calcium to ash, calcium to phosphorus, and phosphorus to ash in cottage cheese samples from each of eleven companies

Co. No.	Calcium : Ash	Calcium : Phosphorus	Phosphorus : Ash
1	16.00	2.98	5.59
2	22.32	3.53	6.43
3	19.38	2.73	7.23
4	13.40	2.68	5.03
5	10.73	2.55	4.26
6	24.53	3.70	6.70
7	12.95	2.75	4.79
8	14.11	3.08	4.63
9	13.94	3.45	4.02
10	11.17	2.48	4.54
11	16.25	2.80	5.88
Average	15.88	2.95	5.42

The grams of calcium and of phosphorus in one pound of cheese were calculated on the basis of the percentages obtained in this study. The average results for the samples obtained from each of the companies are shown in table 4.

TABLE 4

Average weight of calcium and of phosphorus in cottage cheese samples from each of eleven companies

Co. No.	Calcium	Phosphorus
	grams/lb.	*grams/lb.*
1	0.345	1.012
2	0.291	0.994
3	0.377	1.008
4	0.390	1.058
5	0.404	1.026
6	0.295	1.090
7	0.386	1.062
8	0.359	1.099
9	0.331	1.117
10	0.390	0.958
11	0.377	1.044
Average	0.363	1.044

Average milk contains 0.535 grams of calcium and 0.422 grams of phosphorus per pound. On the basis of the values presented in table 4, one pound of cottage cheese contains about two-thirds as much calcium and 2.5 times as much phosphorus as one pound of milk. Comparison of these values in another way shows that about 3.2 pounds of cottage cheese would be required to equal the calcium and about 0.9 pound to equal the phosphorus in a quart of milk.

Cottage cheese has generally been regarded as a poor source of calcium and a good source of phosphorus in the human diet. This opinion possibly has been influenced by the fact that so much more of the calcium than the

phosphorus originally present in the milk is lost in the making of the cheese. Such an opinion does not seem entirely justified. Sherman (5) has listed the calcium and phosphorus contents of 147 human foods other than cottage cheese. Only 33 of these contained more calcium and 30 more phosphorus than cottage cheese on the basis of the results obtained in this study.

SUMMARY

Analyses of 102 samples of commercially made cottage cheese showed a mean total solids content of 22.80 per cent, a mean ash content of 1.22 per cent, a mean calcium content of 0.080 per cent, and a mean phosphorus content of 0.230 per cent.

The composition of the cheeses varied widely between samples and with the companies making the cheese.

There was some tendency for the cheese of low solids content to have a high calcium content.

Comparison with analyses given for other human foods indicates that cottage cheese is a good source of calcium and phosphorus in the human diet.

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BACTERICIDAL ACTION OF RADIANT ENERGY FROM SPECIAL TYPES OF LAMPS ON ORGANISMS FOUND ON DAIRY UTENSILS AND EQUIPMENT

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The availability of a new source of low-cost ultraviolet radiant energy has brought to the attention of industry the possibility of using this effective bactericidal agent in solving certain sterilizing or disinfecting problems not adequately solved by present methods. Rentschler, Nagy and Mouromseff (4) studied factors which influence the bactericidal action of ultraviolet radiation and reported that bacteria are killed when they have absorbed the required amount of radiant energy in the lethal range. Recently, Supplee, Flanigan, and Jensen (5) reported studies on the lethal effectiveness of ultraviolet rays when applied to milk. Other food industries have investigated the possibility of using this type of sterilization.

The studies reported in this paper deal with the bactericidal effect on microörganisms found on dairy equipment and utensils and are a continuation of those reported earlier (4).

Two types of lamps were used, both of which emit radiations mainly in a narrow band centered around the wave length of 2,537 Ångstrom units. Lamp A is a straight tube of corex glass about 30 inches in length. It is a cold cathode mercury vapor lamp operating at about 475 volts and 0.030 ampere from a transformer on a primary 110-volt, 60-cycle alternating current. Three such lamps were installed in series, parallel to one another, beneath a triangular hood lined with aluminum foil as a reflector. All references to lamp A, hereinafter, pertain to this unit of three tubes. Lamp B differs from A in that it is made of quartz and is bent into a U-tube fifteen inches long which permits insertion into rather narrow openings. The single lamp, B, operates from a larger transformer than the three lamps of A. When lamp B is in operation a considerable amount of ozone is formed.

Before any attempt was made to apply radiant energy from these lamps[1] to the sterilization or disinfection of dairy equipment, it seemed desirable to study various factors that presumably would have an influence on their effectiveness.

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* Now with the armed forces of the Nation.

[1] Lamp A is the ''Sterilamp'' manufactured by the Westinghouse Electric and Manufacturing Co. Lamp B is the ''Safe-T-Aire'' lamp manufactured by the Hanovia Chemical and Manufacturing Co.

In all cases the number of organisms was determined by the agar plate method. In specific instances the composition of the agar medium was varied to meet the requirements of particular organisms. Samples of the organisms were obtained by rinsing with sterile water or saline solution or by using the sterile swab technique.

Little difference in the bacteriological rates of the two lamps was found except when exposures were made at short distances. It was not logical, however, to compare the rates of the two lamps, since they differed in total emitting area, in electrical current used, and in general design.

ORDER OF DEATH

The logarithmic order of death of organisms which takes place in disinfection was reported by Madsen and Nyman (3) in 1907 and by Chick (1) in 1908. The order of death having been established as a constant, the death rate, or velocity constant, k, may be calculated from the equation:

$$k = \frac{1}{t} \cdot \frac{\log a - \log b}{.434}$$

in which t is the time in seconds, a is the initial number of organisms present,

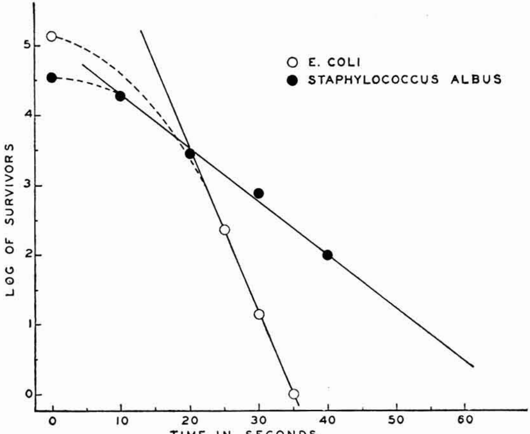

FIG. 1. Characteristic death curves of two microörganisms when exposed to radiations from lamp A at a distance of nine inches.

and b is the number of survivors after time, t, has elapsed. Disinfection rates have certain analytical value in a research problem, whereas, in practice, the total time required to produce sterilization is a more desirable measure. Any k values given in this paper are for purposes of comparison, are by no means absolute, and, therefore, should be understood to represent a range of values.

Numerous determinations showed that the radiant energy from the two

lamps did not cause a constant death rate, as evidenced by the curvilinear line that was obtained in every case when the log of the number of survivors was plotted against elapsed time. This is shown in figure 1 for two organisms, *Escherichia coli* and *Staphylococcus albus,* which were exposed to radiations from lamp A at a distance of nine inches. The curvilinear relationship, however, holds only in the early stages of irradiation. During the latter part of the exposure the death rate is greater and is constant, as shown by the straight line. No exact explanation can be offered at this time for the deviation from the logarithmic order of death in the early part of the exposure. This phenomenon, however, is exhibited in other types of disinfection.

DISTANCE OF LAMP FROM ORGANISM

From a theoretical viewpoint, it appears that the rate of disinfection should vary inversely as the square of the distance from the source of radiation, since the concentration of radiant energy per unit area varies inversely as the square of the distance. Chick (1) found that the logarithm of the death rate of organisms was inversely proportional to the square of the concentration of disinfectant.

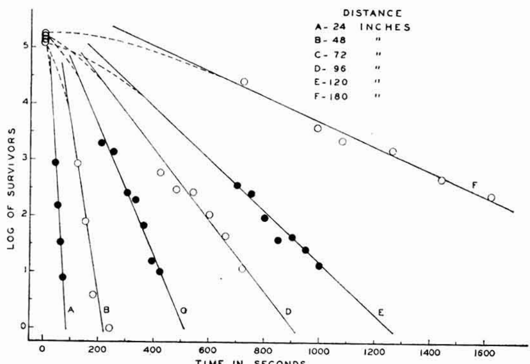

FIG. 2. Effect of distance on the bactericidal rate of a culture of *E. coli* when exposed to radiations from lamp A.

A culture of *E. coli* was used for studying the effect of distance of radiation on the bactericidal rate. The organisms were spread evenly on the surface of Petri dishes so as to give approximately the same number per dish, *i.e.,* about 146,000. The open dishes containing the culture were exposed to radiations from lamp A at distances varying from 9 inches to 25 feet. Determinations of the number of survivors at various time intervals were made for each distance.

The logs of the number of survivors at various time intervals for different distances were plotted against time. The results are shown graphically in figure 2. At each distance the logarithmic order of death is exhibited only after the initial stage of the exposure has passed.

The average death rates (k) of *E. coli* at the various distances were calculated by the formula previously given, and the values are shown in table 1. When these values were plotted against the distances, the logs of the distances, the squares of the distances, or the logs of the squares of the distances it was observed that an asymptotic curve was obtained in each instance. The points fitted so closely a smooth curve that existence of a

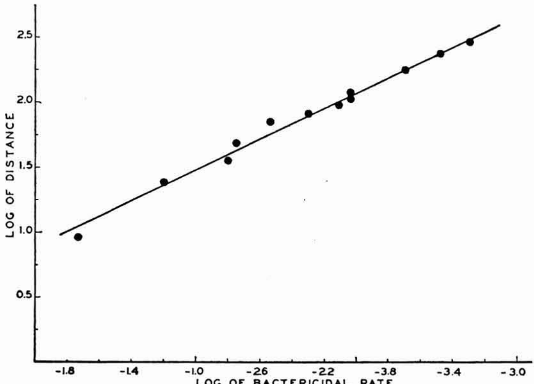

FIG. 3. Relationship of distance to bactericidal rate.

mathematical relationship seemed possible. Plotting of the logs of the death rates against the logs of the distances gave a slightly curvilinear line. When the death rates (k) were recalculated for only that portion of each curve which exhibited the logarithmic order of death, however, and the logs of the recalculated death rates were plotted against the logs of the distances, the straight line shown in figure 3 was obtained. The points fit the line about as closely as can be expected for biological data.

TABLE 1

Effect of distance on the death rate of E. coli exposed to radiations from lamp A

Distance	Death rate (k)	
	A	B
9 inches	.298	.544
24 "	.130	.157
36 "	.072	.063
48 "	.049	.056
72 "	.021	.035
84 "	.017	.020
96 "	.012	.013
108 "	.011	.011
120 "	.009	.011
180 "	.004	.005
240 "	.002	.003
300 "	.001	.002

A—Death rate for whole curve.
B—Death rate for part of curve exhibiting logarithmic order of death.

EFFECT OF INCLINATION OF EXPOSED SURFACE

In exposing most surfaces few of the rays from the lamp strike the surface perpendicularly. The effect of the inclination of the exposed surface on the bactericidal rate was studied by exposing, for 16 seconds at various angles of inclination, microscope glass slides which had been inoculated with a culture of *E. coli.* The amount of culture used per slide was 0.01 ml., which contained approximately 24,000 organisms. The droplets were so small and the surface tension so great that the droplets did not visibly change in size or shape with changes in angle of inclination. The results are graphically presented in figure 4. Essentially the same results were obtained with a culture of *Achromobacter viscosum.*

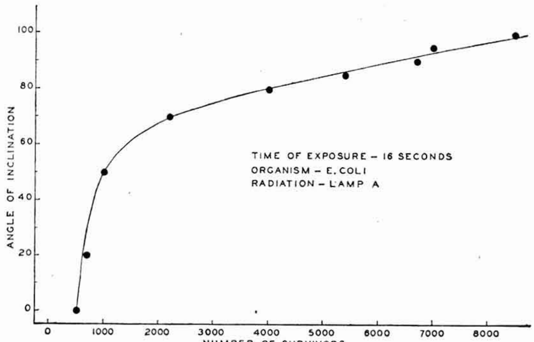

FIG. 4. Effect of inclining the exposed surface relative to the perpendicular of the beam of radiation on the bactericidal rate.

Increasing the angle of incidence of the radiant energy to the exposed surface did not greatly affect the bactericidal rate until the angle was greater than 50°. Some action was noted on surfaces inclined at 100°, this action being due in part to reflection and in part to the fact that a point source of radiant energy was not used.

INFLUENCE OF TEMPERATURE

Cultures of *E. coli* in open Petri dishes were exposed to radiations from lamp B at a distance of one foot and at temperatures of 5° C., 25° C., and 45° C. In this experiment the temperature of the lamp was kept constant at about 28° C. The numbers of survivors were determined after 4, 8, and 16 seconds of exposure. The results, presented in table 2, show little difference in the bactericidal rate at 5° C. and 25° C., but when the temperature was elevated to 45° C., the bactericidal rate increased considerably.

In a second experiment on the effect of temperature, both the lamp and the organisms, *Streptococcus lactis,* were held at the same temperature, which were 3° C. and 20° C. The data given in table 3 show that the bactericidal

TABLE 2

Influence of varying the temperature of the organisms on the bactericidal rate when the temperature of the lamp remained constant*

Temperature	Exposure time	Survivors after exposure
°*C.*	*seconds*	
5	0	750
	4	285
	8	19
	16	0
25	0	370
	4	245
	8	10
	16	0
45	0	720
	4	30
	8	2
	16	0

* *E. coli.*

rate at 20° C. was considerably greater than that at 3° C. The energy output of the lamp is greatly reduced at the lower temperature due to the slowed-up rate of mercury vaporization. Similar results were obtained with cultures of *Pseudomonas fluorescens, E. coli,* and *Staphylococcus albus.*

TABLE 3

Influence of varying the temperature on the bactericidal rate when the lamp and organisms were held at the same temperature*

Temperature	Exposure time	Survivors after exposure
°*C.*	*seconds*	
3	0	46,500
	30	33,000
	40	22,800
	50	12,500
	70	1,200
	90	250
	120	300
20	0	65,000
	30	550
	40	200
	50	1

* *Str. lactis.*

PENETRATING POWER

Radiations in the range of wave lengths of these lamps are known to have low penetrating power through opaque substances. They will, however, penetrate clear water. This was shown in an experiment in which a thin layer of agar containing a culture of *E. coli* was placed in the bottom of a Petri dish and covered with water at depths of 5 and 10 millimeters. The

organisms were readily destroyed when exposed to radiations from lamp A for 3 minutes. When a strip of common glass, 1.5 millimeters thick, was placed over the agar containing organisms, however, relatively few organ-

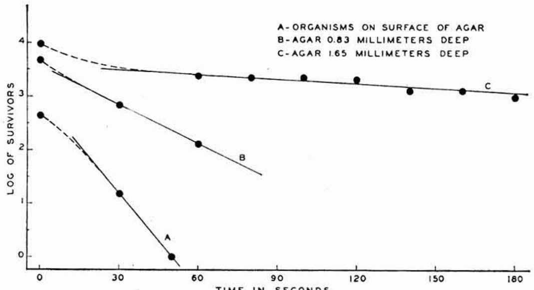

FIG. 5. Effect of the depth of the agar layer on the bactericidal rate of *Str. lactis* when exposed to radiations from lamp A.

isms were destroyed after an exposure of 2 minutes. Exposure of the unprotected agar for 2 minutes resulted in the destruction of 98 per cent of the organisms.

A culture of *Str. lactis* was mixed with agar and the mixture was poured into Petri dishes so as to give layers 1.65 millimeters and 0.83 millimeter deep. In a third series of plates, the culture was sprayed on the surface of a layer of hardened agar. The bactericidal rates of the organisms in the three series of plates on exposure to radiations from lamp A were studied. The results, presented in figure 5, show that the bactericidal rate decreased rapidly as the depth of the agar increased. The relatively rapid initial rate in the plates containing the deepest layer of agar probably was due to the destruction of organisms on or near the surface.

EFFECT OF CONCENTRATION OF ORGANISMS

Cultures of *E. coli* were spread on the bottom of sterile Petri dishes so as to give concentrations of 200,000 and 10,600 organisms per square centimeter, respectively. The results, depicted in figure 6, show that the cells were destroyed at a much faster rate in the lower concentration. This apparently happens when the organisms are numerous enough to shade one another from the radiant energy. Similar results were obtain when *Str. lactis* was used as a test organism. The formation of clumps of organisms would be expected to have a similar effect. The similarity of curve 1, figure 6, to curve C, figure 5, may be due to the same phenomenon, *i.e.,* absorption of a portion of the radiant energy by interposed opaque material which resulted in a more rapid death rate of the organisms on the surface than that of organisms protected by overlaying layers.

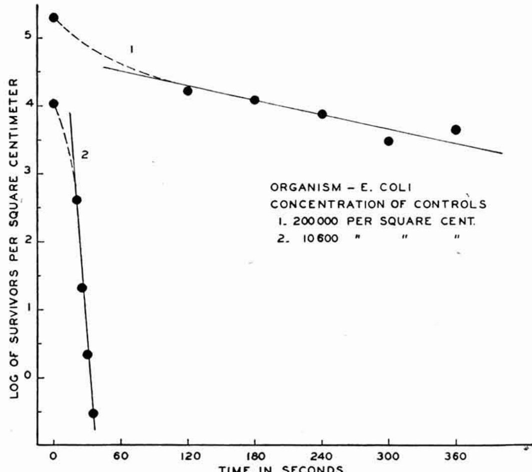

FIG. 6. Effect of the concentration of microörganisms on the bactericidal rate when exposed to radiations from lamp A.

INFLUENCE OF MILK IN THE SUSPENSION

A series of suspended cultures of *Achromobacter viscosum* containing various amounts of milk were placed in sterile plates where they were exposed for 30 seconds to radiations from lamp A. The time and distance of exposure, the concentration of organisms, and the thickness of the exposed suspension film were constant. The initial number of cells was 81,000. The number of survivors increased logarithmically with increased concentration of milk, as evidenced by the straight line which was obtained by plotting the

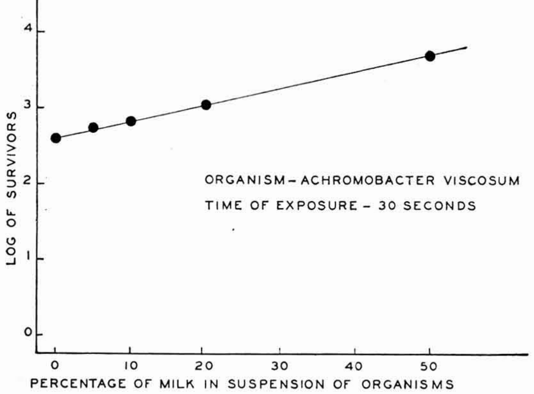

FIG. 7. Effect of the concentration of milk solids on the bactericidal rate produced by radiations from lamp A.

logarithm of survivors against the percentage of milk in the suspension (figure 7). This finding is in accordance with Beer's law, which states that the absorption of light at different concentrations of the same solute dissolved in the same solvent is an exponential function of the concentration. In this case the number of microörganisms killed is a measure of the amount of radiant energy transmitted through the suspension and bears an inverse relationship to the amount of light absorbed by the milk solids.

AGE OF CULTURE

A study of the effect of the age of the culture of organisms on the bactericidal rate was inconclusive. In one case the older culture was more resistant, and in another case the younger. In still another case, there was no difference between young and old cultures in death rates resulting from irradiation.

TYPES OF SURFACES

Smooth, clean surfaces free from cracks, pits, and shaded areas were effectively sterilized by radiations from both lamps if the distance was not too great and the time of exposure was sufficient. Concrete surfaces, either wet or dry, were not rendered sterile under the conditions of exposure used, although the numbers of organisms were greatly reduced. Shaded areas in equipment will prevent sterilization unless the radiant energy is reflected on the shaded surface. Radiation of unclean surfaces has little effect in destroying organisms.

APPLICATION TO EQUIPMENT

Two dozen bottles of milk were taken from the filler just after the plug caps had been set in place. Each bottle closure was rinsed with a stream of cold water. One dozen of these bottles were placed under lamp A at a distance of 2 feet for 5 minutes before the hood caps were put on, and the other dozen bottles were hooded without exposure. After the bottles had remained for 5 hours in a milk storage room, bacterial counts were made on the plug caps and the pouring lips by removing the hood and introducing upon the plug cap 5 ml. of sterile water. After the pouring lip was scrubbed with a sterile cotton swab, a 1-ml. portion of the water was plated out, and from this the count was made. On some bottles, both exposed and unexposed, a slight seepage of milk on the plug cap before the water was added increased the number of organisms present. The average number of bacteria on the exposed bottles was 20, whereas the average number on the unexposed bottles was 420, as shown in table 4.

Two- and four-quart dippers, such as are frequently used in dairies, were readily sterilized by radiations from lamp A for 1½ to 2 minutes. A typical example of the many sets of results obtained is shown in table 5.

TABLE 4

Influence on bacterial counts of irradiation of cap and pouring lip of milk bottles with lamp A for five minutes at two feet

Bottle No.	Number of organisms per bottle	
	Unexposed	Exposed
1a– 1b	42	5
2a– 2b	100	15
3a– 3b	110	45
4a– 4b	140	50
5a– 5b	125	10
6a– 6b	250	0
7a– 7b	35	5
8a– 8b	750	0
9a– 9b	105	0
10a–10b	125	63
11a–11b	500	45
12a–12b	2800	10
Average	420	20

The number of microörganisms can be greatly reduced in milk cans by radiations from lamp A when the lamp is suspended immediately above the open cans. Complete sterilization, however, does not occur under these con-

TABLE 5

Sterilization of a tinned 2-quart dipper by radiations from lamp A

Time in seconds	Number of organisms
0	250,000
60	33,000
75	2,000
120	0

ditions. Insertion of lamp B, which is in the form of a U-tube, into cans resulted in complete sterilization of the interior of the cans in a very short time. A part of the sterilizing effect from lamp B may be due to ozone

TABLE 6

Irradiation of cans with lamp A

Time in minutes	Bacterial count		
	10-gal. milk can	5-gal. ice cream cans	
		Can a	Can b
0	123,000	1,080,000	530,000
1	....	100,000	38,000
1½	....	90,000	....
2	25,000	2,400	3,500
2½	11,000	1,300	....
3	5,000	....	3,000
3½	7,000	....	....
4	10,000	....	....
5	7,000	....	....

formed by the action of this lamp. Sterility of the interior of the cans probably could have been attained with lamp A had its construction permitted insertion into the cans. Typical results with the two lamps are shown in tables 6 and 7.

TABLE 7

Irradiation of cans with lamp B

Time in seconds	Bacterial count			
	10-gal. milk can		5-gal. "shot-gun" can	
	Can a	Can b	Can a	Can b
0	5,500,000	82,000	3,500,000	60,000
2	......	......	500,000	30,000
4	......	6,800	1,000	400
5	1,400,000	......	......	......
6	......	......	1,800	0
8	......	250	......	......
10	75,000	......	......	......
12	......	250	0	......
15	9,000	......	......	......
16	......	0	......	......
20	2,000	......	......	......
25	1,000	......	......	......
30	3,000	......	......	......
35	0	......	......	......

A spray vat pasteurizer was seeded with a mixture of organisms by rinsing with milky water and draining off the water. After a measure was obtained of the number of organisms left in the vat upon seeding, the vat was sterilized with chlorine, was reseeded, and was exposed to radiations from lamp A, which was suspended above the vat, for periods of 4, 5 and 6 minutes. Sterilization with chlorine and reseeding took place between each exposure. The results, presented in table 8, show that the number of organisms was reduced enormously but that complete sterilization was never effected. This would be expected, since radiation could not reach all parts of the vat such as outlets and valves.

TABLE 8

Irradiation of spray vat pasteurizer with lamp A

Time in minutes	Bacterial count
0	5,800,000
4	180,000
5	110,000
6	42,000

SUMMARY

The bactericidal action of two lamps, both of which emit radiations mainly in a narrow band centered around the wave length of 2,537 Ångstrom units, has been studied. No attempt was made to compare the action of the two lamps.

The data show that the radiant energy from the lamps resulted in a logarithmic order of death of microörganisms only after the early part of the exposure period had passed.

The bactericidal rate decreased as the distance of the lamp from the organisms increased, but the relationship did not follow the law which says that the concentration of radiant energy per unit area varies inversely as the square of the distance.

Increasing the angle of incidence of radiant energy to the exposed surface did not greatly affect the bactericidal rate until the angle became greater than 50°.

Low temperatures had no appreciable effect on the bactericidal rate, provided that the lamp remained at room temperature. When the lamp was operated at low temperatures, however, a decided decrease in bactericidal rate occurred.

Films of water, 5 and 10 millimeters deep, over growing organisms did not decrease the bactericidal rate, but opaque substances such as milk and agar decidedly decreased the destructive power of the radiations. It is apparent that organisms must lie on or near the surface if they are to be destroyed by radiations from the lamps.

Increasing the concentration of organisms per unit area decreased the bactericidal rate. The formation of clumps of organisms probably would have a similar effect.

Unclean, rough, cracked, and shaded surfaces were not readily sterilized.

Organisms growing on the surfaces of such equipment as milk bottles, tinned dippers, cans, and pasteurizing vats were completely destroyed or greatly reduced in numbers in comparatively short times by the radiant energy of the lamps used in these studies.

ACKNOWLEDGMENT

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THE IDENTITY OF A STREPTOCOCCUS ASSOCIATED WITH FOOD POISONING FROM CHEESE

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In 1926, Linden, Turner and Thom (2) reported upon two outbreaks of illness among people which had been diagnosed as food poisoning—pains in stomach, severe vomiting, diarrhea, etc. Cheese appeared to be the one article of food which had been consumed by all of the affected persons in these two outbreaks. A sample of cheese from the same lot as that implicated in the first case and some of the actual cheese consumed in the second were studied. The same type of streptococcus was found in large numbers in both of these cheeses. When milk soured with this streptococcus was fed to cats a diarrhea developed, usually within 24 hours, and the stools did not return to normal for 5 or 6 days.

Linden, Turner and Thom stated, "The organism involved has not been identified certainly as any well-described species." It was noted that the organism had "much in common with the ordinary lactic types used in the preparation of 'starters' for butter and cheese making," and the cultures isolated from the cheeses were referred to as "members of the *Streptococcus lactis* type." However, they reported that milk soured with *Streptococcus lactis* did not produce the diarrhea nor other abnormalities in the experimental animals. Their study of the organism was careful and accurate and their conclusions were conservatively stated. It should be remembered that at the time they worked the *Streptococcus lactis* group had not been clearly differentiated from the so-called enterococcus group of streptococci, the belief that *Streptococcus lactis* and *Streptococcus fecalis* were identical being wide-spread. Unfortunately, the work of Linden, Turner and Thom has been referred to by others in more recent years as showing instances of food poisoning caused by organisms of the *Streptococcus lactis* group.

A strain of the organism associated with these cases of food poisoning has been preserved and we have had the opportunity to study it in connection with the identification of a number of unknown cultures of streptococci which have been deposited with the American Type Culture Collection. It proved to be a typical strain of *Streptococcus fecalis* and to be quite different from *Streptococcus lactis,* based upon all of the physiological and serological characteristics which are now known for the differentiation of these species and the so-called lactic and enterococcus groups (3, 4, 5, 7, 9). The organism has the basic characteristics of the enterococcus group, growing at 10° C. and at 45° C.; surviving 60° C. for thirty minutes in milk;

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having the characteristic tolerance for inhibitory substances, as shown by its ability to grow in the presence of 6.5 per cent sodium chloride, 0.1 per cent methylene blue, and 40 per cent bile; and it belongs to the Lancefield (1) serological group D, as shown by the precipitin technique. It also has the specific properties characteristic of *Streptococcus fecalis.* It produces slight greening of blood agar, hydrolyzes arginine and esculin, but not sodium hippurate. Litmus-milk is promptly reduced before curdling, showing that the organism has a strong reducing action. Milk is curdled in two or three days at 37° C. There is no visible evidence of peptonization of casein, nor is gelatin liquefied. The hexose sugars and disaccharides are fermented, as are arabinose, xylose, mannitol, sorbitol, glycerol, and salicin. Inulin is not fermented nor is starch hydrolyzed, raffinose being attacked only very slightly and weakly.

The fact that *Streptococcus fecalis* appears to have been the cause of gastro-intestinal disturbances emphasizes our lack of knowledge of the enterococcus group, especially in relation to dairy products. Whether or not the ability to cause such illness is common among strains of *Streptococcus fecalis* and of the enterococci generally is not known. Although enterococci are well known to occur commonly in milk and milk products, little is known about their quantitative distribution in these foods. More attention has been paid to the hemolytic varieties, *Streptococcus zymogenes* and *Streptococcus durans,* these being known to occur commonly in cheese (1) and in small numbers in fresh milk and spray-dried milk powder (6, 10). In a general way it is known that the non-hemolytic *Streptococcus fecalis* occurs more abundantly than do hemolytic enterococci in milk, and it is known to be present in large numbers in green Swiss cheese (8), but exact data concerning its quantitative occurrence in various dairy products are lacking.

SUMMARY

An unidentified streptococcus which was reported some years ago as the cause of food poisoning from cheese in human beings, and which caused diarrhea in cats, was studied in detail and found to be *Streptococcus fecalis.* The organism is typical of *Streptococcus fecalis* in its physiological characteristics and belongs to the Lancefield serological group D.

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MANGANESE IN COWS' MILK*

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In an earlier paper (1) on this subject the senior author reported that the manganese content of cows' milk could be just about doubled by feeding one ounce daily of anhydrous manganous sulphate. This finding was in contrast to the work of Kemmerer and Todd (7), who were unable to increase the manganese content of milk by feeding $MnSO_4$ to either cows or goats. In view of this conflicting evidence it was deemed advisable to repeat our work while at the same time conducting an investigation into the possible bearing of the higher manganese level on the development of oxidized flavor in the milk.

The experimental procedure was as outlined previously (1) with two exceptions; (a) the cows were not matched as breed pairs, and (b) the amount of manganous sulphate fed was increased to two ounces daily per cow during the second half of the season. Reasons for these changes are given later in this paper. The method of determining Mn was that of Sato and Murata (12) used in the previous year's work. The results of this second winter's work are shown in table 1.

The effect of the manganese supplement is very obvious and is even more marked than in our previous work (1). Some further increase in the Mn content of the milks occurred when the level of manganese fed was doubled during the second half of the season, but as usual with such increases it was nowhere near proportional to the higher intake.

MANGANESE AND OXIDIZED FLAVOR

It has been shown by Garrett (5) that divalent manganese added to milk contaminated with copper or iron completely inhibits or greatly retards the development of oxidized flavor. Since we have been able to increase so markedly the level of manganese in milk by feeding a divalent compound of the element, the question immediately arose, "Will this metabolized manganese have an effect on milk flavor similar to that reported by Garrett?"

In an attempt to answer this question eight cows were chosen from the college herd whose milk, according to preliminary tests made in November, 1941, developed oxidized flavor on standing for periods of from 24 to 72 hours. Because the number of cows in the herd whose milk showed this tendency was quite limited it was not possible to have matched breed pairs; three Ayrshires, three Holsteins, one Guernsey, and one milking Shorthorn

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TABLE 1

Effect on manganese content of the milk of feeding cows manganous sulphate (winter of 1941–42). Micrograms of manganese per liter of milk

Month	Cows receiving supplemental Mn					Cows on control ration				
	1st half of the season									
	A 319	G 632	H 457	H 548	Average all four	A 291	A 309	H 521	S 30	Average all four
December	17.8	29.8	47.4	52.1	36.8	17.5	19.3	32.1	17.8	21.7
January	379.4*	31.3	72.9	133.7	79.3	19.8	16.5	18.8	25.1	20.1
February	30.1	26.5	60.6	87.4	51.2	23.1	12.8	12.4	24.5	18.2
Average—1st half	......	......	......	......	53.6	......	......	......	......	20.0
	2nd half of the season									
	A 291	A 309	H 521	S 30	Average all four	A 319	G 632	H 457	H 548	Average all four
March	37.8	61.2	70.6	74.6	61.1	13.3	24.8	34.8	30.1	25.8
March–April	63.6	126.4	84.1	89.8	91.0	19.3	20.2	29.5	34.8	26.0
April	57.3	58.9	73.7	96.1	71.5	15.5	14.1	19.8	28.5	19.5
Average—2nd half	......	......	......	......	74.5	......	......	......	......	23.7
Average—entire season	......	......	......	......	64.5	......	......	......	......	21.9

* Not included in the average, contamination suspected.

NOTE.—The initial letter preceding each cow's number indicates the breed.

made up the group. These cows were divided according to the grouping and plan shown in table 1, and at the same time that composite milk samples were taken for the other work daily samples for flavor testing were taken from each cow during a three-day period. These were judged for flavor at the end of 24, 48, and 72 hours. Subsamples to which 2 p.p.m. of copper as $CuSO_4$ had been added, were also judged at the end of 48 and 72 hours. Results are summarized in table 2.

TABLE 2

Effect of metabolized manganese on the flavor scores of the milks

	1st day		2nd day		3rd day		Average—all three days	
	With Mn	Without Mn	With Mn	Without Mn	With Mn	Without Mn	With Mn	Without Mn
"Natural" milk	22.2	22.1	21.1	21.5	18.7	19.6	20.7	21.1
"Copper added" milk	......	......	18.7	18.9	15.5	15.7	17.0	17.3

Each of the daily values reported above is an average of 24 individual scores; the average values in the last two columns therefore represent 72 scores for the "natural" milks, and 48 for the "copper added" milks.

NOTE:—Scoring was on the basis of a possible 25.

Except on the first day in the "natural" milks, the flavor scores of the milks were lower whenever the additional metabolized manganese was present. It is true that the differences for the most part were slight and that none of them were significant, but the general trend is unmistakeable. Since flavor scores are influenced by the presence, not alone of oxidized flavor, but of other off-flavors as well, the incidence of these is also of interest (see table 3).

Although there are a few instances (notably "feed" flavor in the "natural" milks, and "unclean" flavor in both kinds of milk) where the manganese supplement seemed to effect some improvement, on the whole its effect was either negligible or adverse. It quite definitely increased the incidence of oxidized flavor in the "natural" milks, and did not inhibit it in the "copper added" milks.

DISCUSSION

The level of manganese in the control milks was in good agreement with that of other investigators (8, 9, 10, 11, 12, 13, and 14) except Drea (3) who did not find manganese present in spectrum analyses of the ash of either cows' milk or goats' milk. The increase in the manganese content of the milks when a manganese supplement was fed, is in agreement with the findings of Broek and Wolff (2) who noted an increase when feeds high in organic Mn (beets, squash and tulip bulbs) were given to cows. As already

TABLE 3

Effect of metabolized manganese on the incidence of oxidized flavor and other off-flavors in the milks

	Total observations	Feed	Bitter	Oxidized	Rancid	Malt	Unclean	Cowy	No criticism
"Natural" milks									
With Mn supplement									
Number	216	117	32	30	11	13	12	0	36
Percentage	100	54.4	14.8	13.9	5.1	6.0	5.6	...	16.7
Without Mn supplement									
Number	215	146	32	18	8	2	23	3	20
Percentage	100	67.9	14.9	8.4	3.7	0.9	10.7	1.4	9.3
"Copper added" milks									
With Mn supplement									
Number	144	38	25	103	7	2	7	0	2
Percentage	100	26.4	17.4	71.5	4.9	1.4	4.9	...	1.4
Without Mn supplement									
Number	144	41	13	103	4	0	12	1	0
Percentage	100	28.5	9.0	71.5	2.8	...	8.3	0.7	...

noted Kemmerer and Todd (7) did not find any increase. Possibly the difference is due to a somewhat higher level of manganese intake in our work—approximately ten grams of elemental manganese daily in most of our work and twice that amount for a time this year, as contrasted with about three grams daily in their work. In this connection it must be stated that the cows showed an increasing reluctance to eat their grain mixture when the larger amount of manganous sulphate (2 ounces daily) was mixed with it, finally refusing it entirely after they were turned out to pasture in early May.

In our earlier paper (1) the question was raised as to why the bovine mammary gland permits manganese to pass its barriers which so successfully exclude iron. About the time that question was raised Erf (4) reported that he had fed radio-active iron to cows and recovered part of it in their milk. Nevertheless it is still of interest to contrast the very different behavior of these two elements in this regard in their ordinary form. Although they are closely related,[1] it has been shown repeatedly that ingested iron, even in massive doses, fails to increase the iron content of cows' milk, while apparently additional manganese is readily introduced into milk by similar means.

The failure of metabolized manganese to retard or inhibit oxidized flavor in milks in a manner similar to that reported by Garrett (5) for additive manganese, cannot be readily explained. The only worthwhile suggestion regarding the discrepancy is that of Dr. Garrett himself (6), who has pointed out that the divalent manganese may have been changed to a higher, less active form in its passage through the cow's system.

SUMMARY

A second season's work on the effect of feeding supplemental manganese on the level of that element in cows' milk, has confirmed the finding previously reported (1) that the amount of manganese in milk can be doubled by this means.

This additional metabolized manganese did not retard or inhibit the development of oxidized flavor either in ordinary milk or in milk to which copper had been purposely added to accentuate the defect.

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ACTION IN CHEESE RIPENING OF AN ENZYME PREPARATION FROM CHICKEN PROVENTRICULI, INCLUDING MANUFACTURE OF A NEW TYPE CHEESE—SAVOUREUX*

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The Iowa Agricultural Experiment Station is attempting to develop cheeses that differ somewhat from the well-established types, with the idea that they will add to the growing interest in cheeses by consumers of the United States. In the attempts, effects of various enzyme preparations on cheese ripening are being investigated. An enzyme that is readily obtained from chicken proventriculi appears to have possibilities in this general connection; under certain conditions it actively curdles milk and rapidly breaks down the curd of cheese made with it.

PREPARATION OF THE ENZYME MATERIAL

Minced proventriculi from freshly slaughtered poultry showed some milk-coagulating power. Activity was greatly increased, at least 2 to 3 times, by adjusting to pH 1.5 to 2.5 with hydrochloric acid and holding at 40° C. until the material was digested.

Experiments on the preparation of an enzyme material were made on stomachs obtained from two large eviscerating plants (Omaha, Nebraska). The gland was detached from the gizzard and esophagus by means of scissors. Quantities were frozen in 30-pound tins and shipped to Ames where they were stored at – 23° C. until processed.

Three methods of preparing extracts from the stomachs were used. They are as follows:

1. *Crude extract.* Stomachs were thawed in running water, split with scissors, freed of excess fat and washed. They were put through a meat grinder, the first portion being rejected. One volume of water and enough concentrated hydrochloric acid to lower the pH to 1.5 to 2.5 were then added. The mixture was held at 40° C. until the tissue was digested. Two volumes of ice water and 2 per cent talc were added and the mixture allowed to settle in a tall vessel in the cold (5° to 10° C.) for 18 to 24 hours. The cloudy liquid at the top, containing most of the enzyme, was separated by decantation from the slimy mass at the bottom of the vessel. The extract was concentrated to the desired strength by evaporation at 40° to 45° C. in glass or enamelware dishes.

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2. *Purified extract.* The dilute crude extract (before evaporation) was filtered with aid of suction and a filteraid (Speedex) until relatively clear. The pH was adjusted to 2.7 to 3.0 and the solution saturated with sodium chloride. The enzyme separated first as a light coagulum which came to the surface and later, on holding at 5° to 10° C., as a flocculent precipitate. Both components actively coagulated milk. Coagulum and precipitate were combined and suspended in 2 to 3 volumes of water. By adjusting the pH to 5.0 with saturated trisodium phosphate, complete solution resulted. The material was adjusted, by dilution or evaporation, to the desired strength. Repeated precipitation at pH 3.0 with sodium chloride removed still more inactive material.

3. *Dried stomach extract.* The difficulty experienced in filtering extracts made from minced glands was greatly reduced by first drying the glands. After splitting and washing, the stomachs were salted and dried at 40° to 45° C. in rapidly circulating air. When dry they were extracted twice with gasoline to remove the fat and then steeped in water (1:4) at 5° to 10° C., the extracting liquor being kept at pH 4.5 to 5.0. About 1 week was required to extract the enzyme. At the end of the steeping period the whole mixture was filtered with aid of suction and a filteraid (Speedflow). A sparkling-clear extract resulted; this was concentrated to the desired strength by evaporation in glass or enamelware dishes at 40° to 45° C. in rapidly circulating air.

TESTING OF THE ENZYME MATERIAL

The following method was used to determine the strength of an enzyme preparation:

A quantity of dry skim milk was obtained as a uniform source of substrate. This was stored in an electric refrigerator to avoid changes.

The milk was prepared as follows: Water was brought to boil in the lower pan of an enamelware double boiler. Three hundred ml. of distilled water was placed in the upper pan. Immediately 30 g. of dry skim milk was added and the mixture stirred until it reached 92° C. The cover was then put in place and heating continued for 5 minutes. The milk was cooled immediately to 10° C. with ice water. Thirty ml. of a 5 per cent calcium chloride (anhydrous) solution was added slowly, with constant stirring. The milk was kept cold.

One-tenth of 1 g. of an enzyme preparation was diluted with distilled water until the dilution was found which caused incipient coagulation in $8\frac{3}{4}$ to $9\frac{1}{4}$ minutes at 42° C., using 5 ml. of the dilution to 5 ml. of the prepared milk. With commercial rennet extracts dilutions of approximately 1:18,000 coagulated the test milk in 9 minutes. The enzyme preparations usually were standardized to approximately the same strength for cheese making.

Although the stomach extracts commonly were standardized to the same coagulating power as commercial rennet extracts, they acted differently when used to coagulate milk in a cheese vat, and considerably larger quantities were needed than with rennet extract. In cheese making the firmness of the curd is a factor, and this is not taken into account in the testing procedure employed.

GENERAL PROPERTIES OF THE ENZYME MATERIAL

The enzyme preparations from chicken stomachs, in addition to possessing marked milk-coagulating powers, also showed great digesting powers (1). Repeated purification by iso-electric precipitation gave products with increased milk-coagulating and proteolytic properties. Preliminary studies indicate that the iso-electric point is between pH 2.5 and 3.0, as determined by solubility measurements.

The crude extracts appeared to lose strength very slowly at 5° to 10° C. Some purified preparations kept well, while others lost considerable activity after 2 to 3 months at 5° to 10° C.; studies on the factors controlling stability are under way.

CHEESE MADE WITH THE ENZYME PREPARATIONS BY THE CHEDDAR PROCESS

Effect of the enzyme preparations was first studied on cheese made by the cheddar process. Usually it was necessary to add 35 ml. of an enzyme preparation per 100 pounds of milk to obtain a rather firm curd in approximately 30 minutes. Acidity of the milk influenced coagulation to some extent; higher acidities caused the milk to coagulate in a shorter time, and the curd was somewhat firmer. Addition of soluble calcium salts to the milk also tended to cause the milk to coagulate in a shorter time and to form a firmer curd.

During the manufacture the curd behaved normally until the cheddaring process. Then it began to show evidence of proteolysis, and the slabs of curd flattened considerably. During cheddaring, acidity developed faster in cheese made with an enzyme preparation than in control cheese made with commercial rennet extract.

When the cheese were removed from the press, the body was much softer than that of normal cheddar cheese. After 1 month in the curing room (10° C.), the cheese had a soft, smooth, waxy body and a decidedly bitter flavor. With longer holding the body showed but little change, while the flavor continued to change. After 3 months the cheese was very bitter. The bitterness then seemed to decrease slightly; however, after 6 months the flavor still was decidedly bitter. Three lots of cheese cured at 7.2° C. for 1 year had a definitely bitter flavor.

SPECIAL TYPE CHEESE MADE WITH THE ENZYME PREPARATIONS

Since preliminary studies indicated that bitterness in cheese made with the enzyme preparations is influenced by the acidity developed in the cheese making, cheese were prepared with a modified edam method. In this, the curd was made up in a rather sweet condition and the lactose concentration was rather low due to replacing a part of the whey with water.

Pasteurized whole milk was ripened with 0.5 per cent regular cheese culture at 30° C. for approximately 30 minutes, or until the acidity of the milk increased 0.01 per cent. Then 35 ml. of an enzyme preparation was added per 100 pounds of milk. After about 30 minutes the milk was well coagulated. The curd was not as firm as that obtained when the milk was set at higher acidities. It was cut into cubes with $\frac{1}{4}''$ knives and further cut with the horizontal knife until the curd particles were about the size of wheat kernels. The curd was stirred for 20 minutes after cutting in order to expel the whey, and then approximately one-third of the whey was removed. Water at about 60° C. was added in three portions in sufficient quantity to raise the temperature of the vat contents to 38.9° C. The curd was stirred for approximately 20 minutes. The whey then was removed and the curd allowed to mat at the upper end of the vat. The matted curd was cut into the desired portions and placed in wooden hoops lined with muslin cloth.

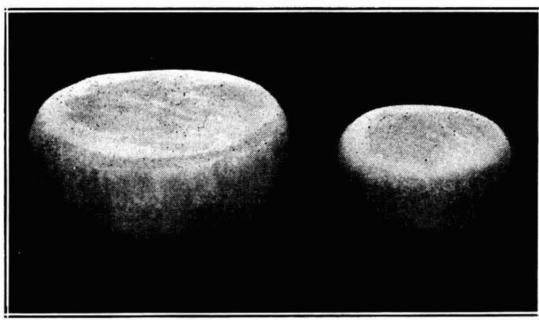

FIG. 1. Two styles that have been used with Savoureux cheese; weights about 5 and 2 pounds.

The cheese were pressed for 2 hours, turned, and then pressed an additional 2 hours. After pressing, the cheese were cooled over night at 10° C. and salted for 2 days in a saturated sodium chloride solution at 10° C. They were cured at 10° C. and examined for flavor and texture at the end of each 1-month period. After about 2 months the cheese were paraffined. When they were paraffined shortly after manufacture (1 week), there was a tendency for the cheese and paraffin to crack because of the soft body; allowing the surface of the cheese to dry for the longer period prevented such cracking.

After about 1 month the cheese showed considerable protein breakdown as was evidenced by the soft, smooth body. The cheese also were bitter. Protein breakdown continued for a time and the body became quite soft, smooth and waxy. As the cheese were held, the bitterness decreased until, with many lots, the bitter flavor was very slight, or had entirely disappeared. In some cases the cheese were satisfactory in as short a period as 6 weeks, but commonly from 2 to 3 months were necessary for satisfactory body and flavor development.

General characters and designation of the cheese. The special type cheese made with the enzyme preparations is rather distinct in its general properties. Perhaps the outstanding character is the soft, waxy body which, when the cheese is at room temperature, suggests butter and permits spreading. The cheese provides a natural cheese with rather satisfactory spreading qualities when it is not too cold. The flavor of the cheese has been variously described by persons who have eaten it for the first time. Some think it suggests aged cheddar cheese, some think it suggests mild brick cheese, while others consider the flavor to be quite distinctive.

The cheese can be made in various shapes and sizes. Figure 1 shows two styles that have been used; the larger one weighs about 5 pounds and the smaller one about 2 pounds.

Because the cheese is rather distinct in its general characters, a name for it seems advisable and SAVOUREUX is suggested.

TABLE 1

General composition of the special type cheese with no attempt to control it

Sample No.	Moisture	Fat	Sodium chloride	Per cent fat in dry matter
	per cent	*per cent*	*per cent*	
1	39.7	33.0	2.2	54.7
2	34.9	34.6	2.0	53.1
3	34.0	35.4	2.1	53.6
4	39.7	33.3	2.0	55.2
5	34.1	35.0	2.0	53.1
6	33.3	34.9	2.3	52.3
7	35.7	35.1	1.8	54.5
8	33.0	34.2	2.0	51.0
9	32.6	33.9	2.2	50.2

General composition of the cheese. The general composition of nine representative cheese, with which no attempt was made to control the composition, is shown in table 1. Perhaps the outstanding point in the composition is the relatively low moisture content of most of the samples. Because of the soft body, a comparatively high moisture content would be expected.

pH values were determined on a number of the cheese which were of various ages. The average pH values are given in table 2; they show that the average pH values increased as the cheese aged. With two lots of cheese

TABLE 2

Average pH values of the special type cheese at various ages

Age of cheese	No. of samples	Average pH value
Months		
1	4	5.19
2	8	5.42
3	4	5.49
6	6	5.69

the pH values were determined at regular intervals for 3 months; the results also show that the pH increased as the cheese aged.

Cheese made from partially skimmed milk. Three lots of cheese made with pasteurized, partially skimmed milk (2 per cent fat) by the general

TABLE 3

General composition of the special type cheese from partially skimmed milk

Sample No.	Moisture	Fat	Sodium chloride	Per cent fat in dry matter
	per cent	*per cent*	*per cent*	
1	33.5	25.9	1.7	38.9
2	34.3	24.9	2.1	37.9
3	35.8	24.1	1.9	37.5

procedure used with whole milk had a very firm body after 1 and 2 months; after 3 months the body was broken down only slightly. The cheese were not bitter at any stage of the curing but were rather lacking in flavor. The composition of the cheese is shown in table 3.

SUMMARY

Chicken proventriculi contain a milk-coagulating enzyme that is readily obtained in a form that can be used in coagulating milk for cheese. With a modified edam process, the enzyme preparations gave a type of cheese that is rather distinct in its general properties, the outstanding character being the soft, waxy body which permits spreading when the cheese is not too cold. The cheese is believed to have commercial possibilities, and the name SAVOUREUX is suggested for it.

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SOME FACTORS AFFECTING THE STABILITY OF CERTAIN MILK PROPERTIES. VI. RELATION OF THE CONCENTRATION OF DISSOLVED OXYGEN TO THE OXIDATION OF ASCORBIC ACID AND TO THE DEVELOPMENT OF OXIDIZED FLAVOR IN MILK*

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The destruction of ascorbic acid and the development of oxidized flavor in milk are oxidative reactions that are of paramount importance to the dairy industry. Research work has shown that dissolved oxygen is the principal agent (3, 4, 5, 7, 10) responsible for these chemical reactions. Research has shown further that both reactions are catalyzed by the presence of the metal ions, Cu^{++} and Fe^{++}, and perhaps by other types of catalysts probably organic in nature, and that these reactions may be inhibited or their speed decelerated by the presence of various types of antioxidants. It seemed desirable, therefore, to study quantitatively the relationship of dissolved oxygen to the oxidation of ascorbic acid and to the development of oxidized flavor in milk.

The adaptation of the voltammetric method (8) to the measurement of the concentration of dissolved oxygen in milk has provided a method of studying oxidative reactions in a more exact manner than hitherto has been possible. Since the method gives a continuous record of the oxygen concentration, the entire course of an oxidative reaction, or series of reactions, may be followed easily.

The amount of oxygen in milk may vary from nil to saturation, the amount at saturation depending primarily upon the temperature and pressure. The amount of oxygen that can be dissolved in milk at 1° C. and 760 mm. pressure by aeration is approximately 11.2 to 11.3 p.p.m. All market milk under the conditions of processing and packaging now in vogue contains some dissolved oxygen.

The voltammetric method of measuring oxygen concentration was used in studying the oxidation of ascorbic acid and the development of oxidized flavor in a number of milks. Each sample was collected in such a manner that little change in the original oxygen concentration occurred and was

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[2] This paper is based on results taken from a thesis submitted by G. H. Hartman to the graduate faculty of Rutgers University in partial fulfillment of the requirements for the degree of doctor of philosophy.

held in a brown glass bottle in an ice water bath at approximately 1° C., the temperature at which all oxygen determinations were made. Saturation with atmospheric oxygen was accomplished by passing washed air through a diffuser bulb into the milk. A layer of viscous mineral oil was placed over the sample to prevent absorption of oxygen from the air. Ascorbic acid was determined by the dye-titration method after the analytical sample had been acidified with a mixture of acetic acid and metaphosphoric acid. The intensity of the oxidized flavor was determined organoleptically, and numerical values, or plus signs, ranging from 0 to 5 were assigned to the various intensities.

Four different samples of commercial bottled milk, collected at different times, were treated in various ways and were periodically analyzed for oxygen, ascorbic acid, and intensity of oxidized flavor. Copper (copper sulfate solution), at the rate of 6.4 p.p.m., was added to all four samples. Sample A was not saturated with oxygen and contained only 8.00 mgs. of ascorbic acid per liter and 9.55 p.p.m. of oxygen when the tests were started. Sample B was saturated with atmospheric oxygen and at the beginning of the tests contained 11.20 p.p.m. of oxygen and 16.50 mgs. of ascorbic acid per liter. Sample C was saturated with atmospheric oxygen following which approximately 25 mgs. of synthetic levo-ascorbic acid per liter were added; this sample contained 11.30 p.p.m. of oxygen and 36.50 mgs. of total ascorbic acid per liter at the beginning of the tests. Sample D was treated like C except that approximately 50 mgs. of synthetic levo-ascorbic acid per liter were added; this sample contained 11.32 p.p.m. of oxygen and 59.50 mgs. of total ascorbic acid per liter at the beginning of the tests. The changes in oxygen concentration with time for the four samples are shown, respectively, by curves A, B, C, and D in figure 1.

The point at which all ascorbic acid had disappeared is marked by the letter, X, on each curve. The intensity of the oxidized flavor is marked at various points along the curves by plus (+) signs. It should be pointed out that the amount of copper used in these experiments is greater than is likely to be found in market milk. Since these are preliminary studies, the use of the high copper concentration seemed justified in order to obtain faster results.

All four curves exhibit the same general characteristics. In each case the rapid initial decrease in oxygen concentration was accompanied by a rapid decrease in the concentration of ascorbic acid. Immediately following the destruction of all the reduced ascorbic acid, the concentration of oxygen remained at a constant level for a period of time which varied with each milk from about two to five hours. The constant level period was followed in each case by a further but slower decrease in oxygen concentration accompanied by the development of oxidized flavor, which increased in intensity with further decreases in oxygen concentration. This final decrease

in oxygen concentration was not due to the growth of micro-organisms, since periodic bacteriological examinations showed an actual decrease in numbers with time. In no case did a perceptible oxidized flavor appear until the constant level period had been passed. The amount of oxygen, above that required for the oxidation of ascorbic acid, which was necessary to develop a given intensity of oxidized flavor apparently varied with the sample, as shown by the curves in figure 1. This may not be the true situation, however, since the error in judgment based on the organoleptic test is relatively large.

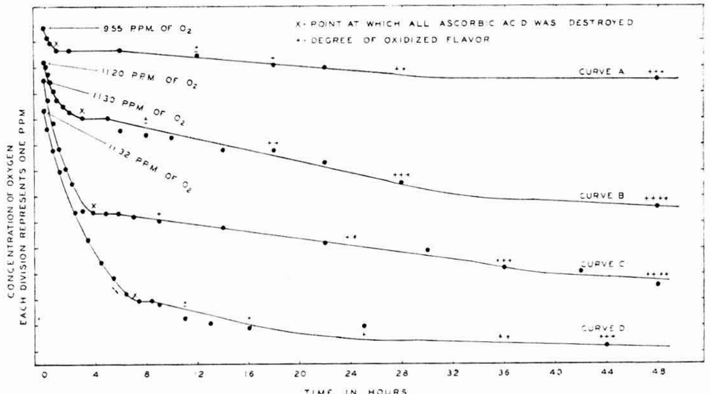

FIG. 1. Relation of the decrease in oxygen concentration in milk containing 6.4 p.p.m. of copper to the oxidation of ascorbic acid and to the development of oxidized flavor. Curve A—milk contained no synthetic ascorbic acid and was not saturated with atmospheric oxygen. Curve B—milk contained no synthetic ascorbic acid but was saturated with atmospheric oxygen. Curve C—milk contained 25 mgs. of synthetic levo-ascorbic acid per liter and was saturated with atmospheric oxygen. Curve D—milk contained 50 mgs. of synthetic levo-ascorbic acid per liter and was saturated with atmospheric oxygen.

A number of instances were observed in which the consumption of oxygen continued after an oxidized flavor of 5, the highest value assigned on the basis of organoleptic judgment, had been reached. This definite extended consumption of oxygen probably was accompanied by further oxidation of fatty components of the milk, which may have resulted in the formation of flavored compounds, but, if so, they could not be detected organoleptically. It is possible that the oxygen was being used in further oxidation of molecules already partly oxidized according to the postulation suggested by Greenbank (1, 2), that is:

$$\begin{array}{ccccc} R & \longrightarrow & RO & \longrightarrow & RO_2 \\ \text{Tasteless} & \longrightarrow & \text{Flavored} & \longrightarrow & \text{Tasteless} \end{array}$$

The oxidation of the reduced form of ascorbic acid to the dehydro-form theoretically requires one atom of oxygen for each molecule of ascorbic acid.

The data in table 1 show that this theoretical relationship does not hold throughout the course of the oxidative reaction in milk when the reaction is catalyzed by soluble copper. During the initial stages of the reaction the

TABLE 1

Ratio of atoms of oxygen consumed per molecule of ascorbic acid oxidized in the course of the reaction in milk when catalyzed with 6.4 p.p.m. of soluble copper

Time	Atoms of oxygen per molecule ascorbic acid		
	Sample No. 1	Sample No. 2	Sample No. 3
min.			
12	....	....	1.23
20	1.00	0.94	1.25
45	1.25	....	1.66
70	....	1.01	....
90	1.33	....	1.62
120	1.41	....	1.54
150	....	....	1.45
230	....	1.62	....
240	....	....	1.37
290	....	1.57	1.47

ratio is about 1:1, but as the reaction progresses the number of atoms of oxygen required per molecule becomes larger until a ratio of approximately 1.4:1 is reached at the end of the reaction. This ratio is in good agreement with those obtained by Hand and Chase (6) and by Noll and Supplee (9).

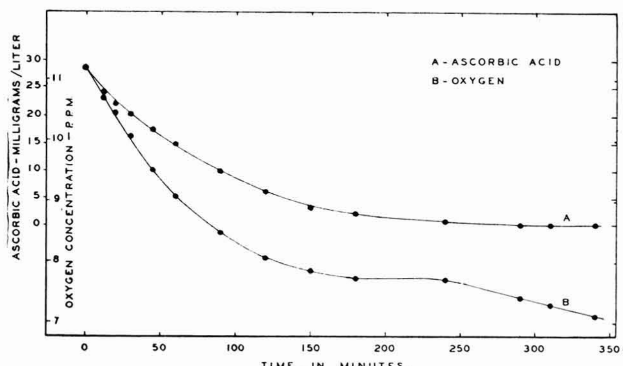

FIG. 2. Relation of oxidation of ascorbic acid to oxygen consumption in milk containing 6.4 p.p.m. of copper.

The concentrations of ascorbic acid and of oxygen in a sample of milk to which synthetic levo-ascorbic acid and soluble copper (copper sulfate) were added were plotted against time (figure 2). Since the ratio of the atomic weight of oxygen to the molecular weight of ascorbic acid is 1:11, the ordinate of the graph was constructed in such a manner that 1 p.p.m. of oxy-

gen was equivalent to 11 mgs. per liter of ascorbic acid. An inspection of the graph shows that the ascorbic acid curve and the oxygen curve diverge from the beginning of the reaction and do not parallel each other until the concentration of ascorbic acid and the consumption of oxygen approach zero. Had the ratio of one atom of oxygen to one molecule of ascorbic acid been maintained throughout the course of the oxidative reaction, the curves would have coincided. The failure to maintain a constant ratio may be due to the formation of hydrogen peroxide, to oxidation of the irreversibly formed hydrolytic decomposition products of dehydroascorbic acid, or to the oxidation of other components of milk which do not, at least immediately, impart a detectable flavor.

From the results of these experiments it may be postulated that:

The two oxidative reactions that occur in milk, involving ascorbic acid and the development of oxidized flavor, when copper ions are present as a catalyst, are independent of each other; that is, neither reaction is dependent on the other for the normal course of oxidation, even though copper is a catalyst for both reactions. Ascorbic acid, however, is more easily oxidized than the fatty substance involved in the development of oxidized flavor. Consequently, ascorbic acid is oxidized first and accordingly acts as an antioxidant in that its presence delays the onset of the second reaction.

Further, an induction period of several hours is required before the reaction that is responsible for the development of oxidized flavor occurs. This may be likened to the induction period preceding the onset of rapid oxidation in the case of such concentrated fatty substances as butter and lard. It is probable that the induction period in milk for the oxidized flavor reaction includes the period of ascorbic acid oxidation.

SUMMARY

The voltammetric method of measuring the concentration of dissolved oxygen has been applied to a study of the oxidation of ascorbic acid and the development of oxidized flavor in milk.

The results of the experiments on milk showed:

That the rapid oxidation of ascorbic acid, when catalyzed by soluble copper, was accompanied by a rapid decrease in oxygen concentration.

That the ratio of oxygen consumed to ascorbic acid oxidized progressively increased as the oxidative reaction proceeded.

That a period of constant level oxygen concentration followed the destruction of all reduced ascorbic acid and preceded the onset of oxidation which resulted in the development of oxidized flavor.

That the oxidation of fatty substances in milk resulting in the development of oxidized flavor was accompanied by a considerable decrease in concentration of dissolved oxygen in the milk.

That the speed of the oxidative reaction resulting in the destruction of reduced ascorbic acid is greater than the oxidative reaction resulting in the development of oxidized flavor in milk when both reactions are catalyzed by the same concentration of soluble copper.

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THE DEVELOPMENT OF A POSITIVE PHOSPHATASE TEST IN REFRIGERATED, PASTEURIZED CREAM

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Commercially pasteurized cream which had been negative to the phosphatase test immediately after pasteurization developed a positive reaction after three days at 10° C. This change in reaction made possible erroneous conclusions as to the previous treatment of the cream, since the cream was still in a salable condition and the change had taken place well within the normal marketing time of the cream. Shippers of pasteurized cream claimed that their product was known to have left their plant with a negative phosphatase test, despite the fact that the cream tested positive on arrival at distant markets.

These facts suggested the need for research on the development of a positive phosphatase test in refrigerated, pasteurized cream.

LITERATURE

There are numerous review articles concerning the uses and limitations of the phosphatase test (3, 4, 5, 7, 15). Only a few workers have reported the development of a positive test after a negative test had been obtained on the product. Hammer and Olson (8) showed that a positive test developed in butter after storage. Brown and Elliker (2) claimed that flash pasteurized cream developed a positive test after the cream had been held at refrigeration temperatures. Wiley, Newman and Whitehead (16) stated that cream, flash pasteurized by the Vacreator method, and used for making butter, at first gave a negative phosphatase test but several hours after the addition of salt or sugar or the drying of the cream gave a positive reaction. They explained the change by theorizing that there was a binding of small and varying amounts of the enzyme in the cream in such a manner that the enzyme escaped destruction during very short heat treatments. Later this bound phosphatase was released and gave a positive reaction.

The effect of bacteria on the phosphatase test has been studied by several workers. Paley (13) and Burgwald and Giberson (5) reported bacteria which gave positive reactions to the phosphatase test. Hammer and Olson (8) claimed that a number of species of *Pseudomonas,* species of *Aerobacter, Flavobacterium fecale,* some species of *Alcaligenes,* and *Oospora lactis* gave very strong phosphatase reactions. Leahy, Sandholzer and Woodside (11) found that species of *Staphylococcus, Aerobacter* and *Klebsiella* gave a true phosphatase test. However, they believed that unless large numbers of these

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organisms were present the interpretation of the test would not be affected by bacterial action.

Leahy, Sandholzer and Woodside (10) reported that certain Gram negative bacilli were capable of hydrolyzing the buffer substrate to produce phenol, when the substrate was buffered to a pH between 5.8 and 7.5. Later Leahy (9) claimed that four strains of thermophilic bacilli produced phosphatase which was detectable when the substrate was buffered between pH 5 and 6.2. He maintained that the phosphatase of these organisms would not interfere with phosphatase tests on milk since the optimal pH for milk phosphatase is 8.9 to 9, and that of bacterial phosphatase is between 5 and 6.2.

METHODS

The New York Rapid Field Test, as developed by Scharer, was used for tests of phosphatase activity, and was conducted as described in the Eighth Edition of Standard Methods for the Examination of Dairy Products (14). Incubation was at 37° C. for 20 minutes. A period of 30 minutes was allowed for color development after the addition of the indicator, and one milliliter of n-butyl alcohol was used for the extraction of the color which developed. Color standards for the Rapid Laboratory Test were used to obtain a wide range of standards.

Samples of raw cream 10 ml. in volume from the University Dairy were pasteurized in a water bath, the temperature of which was controlled within half a degree by adjustment of a Bunsen burner. Samples were held after pasteurization in either an electric refrigerator at 4° C. or in an ice refrigerator at 10° C.

Bacterial phosphatase was produced naturally in pasteurized cream when the samples were held at 4° or 10° C. for three or four days. Three methods were used to utilize the bacterial phosphatase obtained with pure cultures: 1—A loop of growth from an agar slant culture was transferred directly into the cream or skim milk. 2—A definite amount of a cell suspension, made by the addition of five milliliters of steamed cream to an agar slant culture, was transferred to the cream or skim milk by pipette. 3—A sterile-water suspension of growth from an agar slant was transferred to the cream or skim milk by pipette. Microscopic methods were used to detect the presence of the organisms in positive samples.

Attempts were made to detect bound phosphatase by extraction of the milk fat with ether and inhibition of bacterial growth by a three per cent solution of mercuric chloride.

Cream suspensions of an agar slant culture and uninoculated raw cream samples were subjected to various heat treatments and tested immediately for phosphatase activity to compare the heat resistance of bacterial phosphatase and milk phosphatase. The pH values for optimum phosphatase activity were obtained by means of buffers prepared according to the tables for

Clark-Lubs Standard Mixtures and Palitzsch's borax-boric acid mixtures as given in Clark's "The Determination of Hydrogen Ions" (6). A concentrated solution of disodium phenyl phosphate (0.4025 grams of disodium phenyl phosphate per 10 milliliters of distilled water) was added to five milliliters of the buffer to give a concentration of 0.5 grams of disodium phenyl phosphate per liter. A 0.5-milliliter sample of raw cream or a 0.5-milliliter sample of a cream suspension of the organism to be studied was added to the tubes of buffer substrate at different pH levels. The pH was determined by the use of a glass electrode and the phosphatase test continued in the usual manner. The intensities of the positive tests obtained on the samples were compared and the optimum pH for the phosphatase activity was determined.

Pasteurized cream was prepared by pasteurization in the laboratory at 63° C. for 30 minutes, and was used for experimental studies on the same day. Steamed cream was prepared by subjecting raw cream to free flowing steam for three hours. Stock cultures were carried on tryptone, glucose, beef extract, skim milk agar.

The organisms studied were identified by use of the Manual of Methods for Pure Culture Study of Bacteria (12) and the Fifth Edition of Bergey's Manual of Determinative Bacteriology (1).

RESULTS

Tests on Commercially and Laboratory Pasteurized Cream

Development of a positive phosphatase test in commercially pasteurized cream. During the routine run of phosphatase tests at an eastern dairy, a sample of cream, known to have been properly pasteurized, gave a positive reaction to the phosphatase test. In an attempt to locate the cause of this positive test, 47 bottles of cream containing from 16 to 50 per cent butter fat were selected at random to represent the output of the dairy on several days. These samples were negative to the phosphatase test. They were held at 10° C. and tested daily for four days. On the first day no samples were positive but on the second day 13 of the 47 had developed a positive reaction. This number was increased by 5 on the third day and 8 on the fourth day. At the end of this period 26 of these samples had developed a positive phosphatase reaction. The development of 2 units of color was recorded as a positive test, and with aging of the cream the tests increased in intensity to 5 and 7.5 units.

The butter fat content did not appear to exert any influence on the development of a positive test. Positive reactions were obtained in samples of cream with butter fat contents from 16 to 40 per cent.

The development of a positive reaction to the phosphatase test on refrigerated, pasteurized cream was evident while the odor and flavor of the cream were such that the cream was still in a salable condition. A comparison of

the titratable acidity and pH of samples immediately after pasteurization and after the development of a positive reaction showed that the titratable acidity and pH of the cream had not changed.

Sources of cream. Samples of raw cream from Wisconsin, New York, Vermont, New Hampshire, and Massachusetts were pasteurized in the laboratory at 62.2° C. for various lengths of time. These creams from different sources varied in the time of heat treatment necessary to obtain a negative phosphatase test. Some creams required a 34-minute heat treatment while others needed from 36 to 45 minutes at 62.2° C. before a negative phosphatase test was obtained. This difference in response to the test may have been due to the fact that there was more bacterial phosphatase in some creams than in others. As will be shown later, bacterial phosphatase requires a longer heat treatment for inactivation than does milk phosphatase, and therefore the presence of bacterial phosphatase might explain the difference in the heat treatment required to obtain a negative phosphatase test.

Effect of various heat treatments. Samples of raw cream were heated for 30 minutes at various temperatures and tested for phosphatase activity. A temperature of 64° C. or above for 30 minutes was necessary to obtain a negative phosphatase test. When the creams were heated at 62.2° C. for various lengths of time, as the time of heat treatment increased there was an increase in the period of storage at 10° C. before a positive phosphatase reaction developed in the cream. One sample gave a positive phosphatase test after 32 minutes at 62.2° C., a negative one after 34 minutes, but upon holding the sample in the refrigerator a positive reaction developed the next day. Similarly, the samples of cream removed after exposures for 36, 38, 40, 42.5 and 45 minutes were negative at first but developed a positive reaction after aging 3, 4, 6, 9, and 12 days, respectively.

Effect of various storage treatments. Samples of raw cream were pasteurized at 63° C. for thirty minutes, stored at different temperatures, and tested daily for phosphatase activity. The samples held at 25° C. became sour in 24 hours and no positive test developed. The 10° C. samples became positive in 48 hours while the 4° C. samples developed a positive reaction after 96 hours.

Presence of causal organisms. Whenever a positive test developed in cream there was always present a Gram-positive, spore-forming rod, growth of which on an agar slant gave a positive phosphatase test. No organisms isolated from negative samples gave positive phosphatase reactions. Microscopic examination of positive samples always showed a predominance of these Gram-positive rods.

Attempts to detect bound phosphatase in pasteurized cream. The theory has been advanced (16) that protected or bound phosphatase might cause the development of a positive phosphatase test on pasteurized cream. If this theory were true, it might be expected that excess agitation or disruption of

the fat globule immediately after pasteurization would cause the release of this protected phosphatase and the development of a positive phosphatase reaction at once. The fat was extracted from samples of pasteurized cream with ether and the extract and residue were tested for phosphatase activity. No positive phosphatase reactions were obtained, but samples of the same cream held at 10° C. for two days developed a positive phosphatase test and showed the presence of the phosphatase-producing rods. When bacterial growth was inhibited by three per cent mercuric chloride no positive tests were obtained even after storage for 14 days at 10° C., although mercuric chloride does not interfere with the test.

Tests on Cream with Added Pure Cultures

Isolation and identification of organisms. Different types of colonies were picked onto agar slants from plates of creams giving a positive phosphatase test. Those organisms that gave positive reactions when reinoculated into steamed cream were reisolated and identified. The phosphatase-producing organisms were all similar to two species which were identified as phosphatase-producing strains of *Bacillus cereus* and *Bacillus mesentericus*. Cultures of *Bacillus cereus* and *Bacillus mesentericus* from the stock culture collections of the University did not produce phosphatase.

Proof of phosphatase production. Growth from agar slants of the organisms giving a positive phosphatase test was tested by the substitution of buffered water (pH 9.0) for the buffer substrate. Negative reactions were obtained which showed that the organisms produced phosphatase and not phenol or phenol-like compounds.

Effect of inoculation of cream. Samples of pasteurized cream were inoculated with different-sized inocula of a suspension of a culture of the phosphatase-producing strain of *Bacillus mesentericus*. The samples held at 10° C. were tested daily for phosphatase activity and counted microscopically with results as shown in table 1. It is apparent that the rapidity with which a positive phosphatase test developed depended somewhat upon the number of organisms and the amount of bacterial phosphatase added by the inoculum. The samples which received the heaviest inoculum developed a positive test in 24 hours, while the samples receiving the lightest inoculum required 72 hours.

These results also show that the organism is capable of growth at 10° C., since the number of cells present increased appreciably when the cream was held at this temperature. Sample "a" increased in bacterial population from 6.34 millions per milliliter at the start of the experiment to 926 millions after 120 hours. The control, sample "d," increased from less than 0.45 millions to 7.79 millions per milliliter in 120 hours. Other experiments showed growth at 4° C.

Effect of different heat treatments on cream as a medium. Samples of

pasteurized cream and of steamed cream were inoculated with suspensions of phosphatase-producing organisms. In every case the pasteurized cream samples developed a positive phosphatase reaction in a shorter time and with fewer organisms present than did the steamed cream samples. Samples of cream were subjected to different heat treatments ranging from pasteurization for thirty minutes at 61.7° C. to a steaming treatment of two hours. These samples were inoculated and held at 10° C. Phosphatase tests showed

TABLE 1

Effect of size on inoculum of Bacillus mesentericus on the time required for the development of a positive phosphatase reaction in pasteurized cream held at 10° C.

Time	Rate of inoculation			
	0.5 ml. *B. mesentericus*		0.2 ml. *B. mesentericus*	
	Phosphatase activity	Bacterial count	Phosphatase activity	Bacterial count
hr.	*units*	*millions/ml.*	*units*	*millions/ml.*
0	–	6.34	–	3.2
24	2.0	7.90	–	4.3
48	2.5	75.40	2.0	4.7
72	10.0	92.20	2.0	8.1
96	10+	589.00	3.5	31.8
120	10+	926.00	3.5	39.6
	0.02 ml. *B. mesentericus*		No inoculum	
0	–	0.32	–	<0.45
24	–	1.07	–	<0.45
48	1.0	2.07	–	0.24
72	2.0	5.03	2	1.48
96	3.5	6.66	2	3.83
120	3.5	12.90	2	7.79

– = Negative reaction to the phosphatase test.
10+ = A more intense positive reaction than 10.

that as the severity of heat treatment increased there was an increase in the length of time required for the development of a positive phosphatase reaction. This would indicate that as the heat treatment was increased, the cream became less adaptable for use as a medium for phosphatase production by the organisms. The organisms increased in numbers in cream heated above 76.7° C. for 30 minutes, and in all steam treated cream, but did not produce phosphatase unless the pasteurization temperature was less than 76.7° C.

Comparison of Milk and Bacterial Phosphatase

Optimum pH and limits for milk and bacterial phosphatase. A series of buffers was prepared and substituted for the borate buffer used in the New York Rapid Field Test. Samples of raw cream, suspensions of the organisms in steamed cream, and loops of growth from agar slants were

tested for phosphatase activity at different pH values. Table 2 shows bacterial phosphatase activity to be evident between pH values of 8.13 and 9.72, with the greatest activity at pH 9.72 while milk phosphatase activity was evident between pH values of 7.87 and 9.72 with the greatest activity at pH 8.77 to 8.82. Milk and bacterial phosphatase are apparently active in the same general range and therefore the phosphatase produced by these organisms could interfere with the proper interpretation of the phosphatase test.

Heat resistance of milk and bacterial phosphatase. Raw cream and raw cream to which heavy suspensions of phosphatase-producing organisms had been added were subjected to 30-minute heat treatments at temperatures from 61.8° to 100° C. Milk phosphatase was inactivated after 30 minutes

TABLE 2

Effect of pH of buffer substrate on activity of milk phosphatase and of bacterial phosphatase

Reaction of substrate	Phosphatase activity	
	a	b
pH	*units*	*units*
3.11	–	–
5.01	–	–
7.36	–	–
7.69	10	–
7.87	10	–
8.12	10+	3.5
8.45	10+	5
8.62	10++	7.5
8.79	10+++	10
8.84	10++	10+
9.08	10+	10++
9.64	10	10+++
9.72	10	10++

a = raw cream.
b = steamed cream suspension of *Bacillus cereus.*
– = negative test.
+, ++, +++ indicate a progressively more intense positive test than 10 units.

at 62.8° C., but bacterial phosphatase was active after heat treatments as great as 79.4° C. for 30 minutes. This showed that the bacterial phosphatase was considerably more resistant to heat than milk phosphatase.

This difference in heat resistance makes possible a simple method of distinguishing between milk and bacterial phosphatase. If a sample of cream gives a positive phosphatase test, reheating the cream for 30 minutes at a temperature above normal pasteurizing temperatures but below 76.7° C. and testing for phosphatase activity will show whether or not there is bacterial phosphatase present in sufficient amounts to give a positive phosphatase reaction.

DISCUSSION

The production of phosphatase in refrigerated, pasteurized cream by strains of *Bacillus cereus* and *Bacillus mesentericus* is an explanation of the

change in the phosphatase reaction from negative to positive observed in stored cream. This bacterial phosphatase might also explain the different heat treatments required to obtain negative phosphatase tests on different creams as well as the various lengths of time required for the development of a positive phosphatase test after properly pasteurized cream has been stored at refrigeration temperatures. Since much emphasis is being placed upon the phosphatase test by health officials, it is important to realize in the interpretation of the test that positive reactions may be caused by bacterial growth in the refrigerated, pasteurized cream.

SUMMARY

The results of these studies on the development of a positive phosphatase reaction in refrigerated, pasteurized cream may be summarized as follows:

1. Commercially pasteurized creams, regardless of their butter fat content, changed in their reaction to the phosphatase test from negative to positive after storage for three to four days at 4° or 10° C. and while still in a salable condition.
2. Cream from different sources varied in the length of time of heat treatment at 62.2° C. required to obtain a negative phosphatase test.
3. When the temperature of the heat treatment for 30 minutes was increased, or the time of heat treatment at 62.2° C. was increased, the period of storage at 10° C. required for the development of a positive phosphatase reaction in the cream was increased.
4. Pasteurized cream samples which developed a positive phosphatase reaction after storage at 10° C. or 4° C. always contained Gram-positive, spore-forming bacilli that produced phosphatase and were identified as strains of *Bacillus cereus* and *Bacillus mesentericus*. These organisms were not detected in samples negative to the phosphatase test.
5. The positive phosphatase test on pasteurized, refrigerated cream apparently was not caused by bound or protected phosphatase adsorbed to the milk fat.
6. When mercuric chloride was used to inhibit bacterial growth, no positive phosphatase reactions developed in samples of pasteurized cream or in steamed or pasteurized cream inoculated with phosphatase-producing organisms.
7. When pure cultures of these strains of *Bacillus cereus* and *Bacillus mesentericus* were inoculated into cream, the rapidity with which a positive phosphatase reaction developed depended upon the number of organisms and the amount of bacterial phosphatase added by the inoculum.
8. The strains of *Bacillus cereus* and *Bacillus mesentericus* were able to grow and produce phosphatase at 4° and 10° C. in pasteurized cream.
9. A positive reaction was obtained on pasteurized cream when the micro-

scopic count of these phosphatase-producing organisms was between 1.48 and 7.8 millions per milliliter.

10. The production of bacterial phosphatase in cream increased in rapidity and less time and fewer organisms were required for the development of a positive phosphatase test as the severity of heat treatment of the cream decreased from steaming to 62.2° C. for 30 minutes.

11. Bacterial phosphatase was more active at pH 9.7 than was milk phosphatase; both kinds of phosphatase showed activity in the same general range of pH values.

12. In cream, bacterial phosphatase withstood heat treatments as high as 76.7° C. for 30 minutes, while milk phosphatase was inactivated in 30 minutes at 62.8° C.

13. Bacterial phosphatase could be distinguished from milk phosphatase by reheating a sample of cream for 30 minutes at temperatures above those normally used for pasteurization but below 76.7° C., and then testing for phosphatase activity by means of the New York Rapid Field Test.

14. The change of phosphatase reaction is of importance since it takes place within two or three days after pasteurization even though the cream is properly refrigerated and is still in a salable condition. Hence, erroneous conclusions may be drawn as to the previous heat treatment of the cream.

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PRELIMINARY REPORT ON PASTURE INVESTIGATIONS TECHNIQUE

Joint Committee of
American Society of Agronomy
American Dairy Science Association
American Society of Animal Production

Pasture research has had a phenomenal growth in America during the past 30 years. With this development a variety of methods and ways of expressing yields of pastures have been employed. Recognizing this fact as an obstacle in the way of maximum progress, committees of the American Society of Agronomy, the American Dairy Science Association, and the American Society of Animal Production have studied the problem and as a result, have proposed tentative procedures and methods of expressing yields as a guide for research workers. In formulating this report to supersede earlier ones (11) the committees recognize that the procedure employed may vary with the type of experiment conducted, whether it be a measure of growth response to particular fertilizer treatments, a comparison of different systems of grazing management, or some other important pasture problem. The committees also realize that not all investigators will be able to carry out each of the suggestions in full, and in some cases certain adaptations will be necessary.

It is not desirable to standardize every technique to the point that an investigator accepts it blindly as a stereotyped pattern. Such a practice prevents progress and improvements in methods of research. Many minor details of technique should be left to the good judgment and experience of the individual investigator to decide, and to make application to local conditions. Plants, soils, rainfall, day length, altitude, latitude, temperature, all cause variations which cannot be fitted to a completely standardized plan, but which require observation and the judgment of the local investigator.

The first principle of research is to attempt to hold conditions of an experiment constant, insofar as this is practical, in order to measure a single introduced variable at one time, and either to measure or eliminate all other differences between experimental groups or in procedures. Notes of all details should be recorded permanently at the time, and any changes of technique written down, together with the reasons therefore.

In publication of pasture research, these points should be stated specifically by the investigator, so that others may be free to give a different interpretation should they feel justified in so doing.

This report is particularly designed for humid and irrigated sections of the country, and for persons interested in fertilizers and in species and varieties of pasture plants. Workers in the field of pasture research are

urged to study these suggestions before outlining their particular pasture projects.

EXPERIMENTAL PASTURE

1. *Location of plots or paddocks.* The pastures should be located on a uniform soil type representative of large areas of grazing land, and if in rolling topography, up and down the slopes.

2. *Soil types and composition changes.* Sufficient soil samples to accurately represent the variations in each paddock or plot should be collected at the beginning and close of the experiment. The methods and equipment used in taking samples and making soil surveys should be used and the samples classified and analyzed by soil experts, analyses to be made at once, with possibly a duplicate of each. If the experiment covers a period appreciably longer than four years, the taking of samples at four-year intervals is recommended. It is recommended by soil chemists that a portion of soil samples taken at the beginning and during the experiment be held until the end in order that all factors affecting analyses may be the same. The methods used in making the analyses should be reported. From the samples obtained at various intervals the effect of pasture farming on the organic matter and other soil constituents may be determined.

3. *Size of pasture and number of animals.* From the experimental standpoint it is recognized that other things being equal, the larger the acreage and number of animals per plot and the greater the number of replications the smaller will be the experimental error. The acreage and the number of animals necessary for conclusive results in pasture experiments may vary with (a) kind of animals used, (b) type of experiment, (c) land and facilities available, and (d) productivity of the land. When small numbers of animals are used it may be necessary to conduct the experiment over a greater number of years.

4. *Shape of pasture.* Pastures in which the length is three or four times the width are preferable to square ones, being better suited to overcome soil variations.

5. *Fencing of pastures.* Experiments on permanent pastures should be securely fenced. Woven wire at least part way up is desirable. For annual or temporary pastures, where the fence has to be moved frequently, cheaper construction is allowable. Electric fences are especially suited for pasture experimental work.

EXPERIMENTAL ANIMALS

1. *Kind of livestock.* The kind of animal for which the results of the experiment are generally to apply should be used.

2. *Sex of animals.* In experiments with animals not primarily kept for milk, castrated males are preferable because of the disturbance of the females when they are in heat, when carrying or suckling calves, or when being milked.

3. *Size and uniformity of animals.*

a. Dairy animals. In the estimation of pasture yields through the use of grazing stock, mature animals are preferable to immature animals in that nutrient requirements for growth need not be considered. The animals used should be as uniform in size and condition as possible. In certain types of experiments dairy heifers may be employed. Heifers used in each experiment, or in different groups of an experiment, should be as nearly as possible the same age and weight, and in the same period of gestation if bred heifers are used.

b. Meat-producing animals. Older growing animals are better than the very young because the tables for calculating feed values can be applied with less adjustment. In the groups being compared there should be uniformity of age, sex, condition, inheritance, previous treatment, health, and breed.

MANAGEMENT OF PASTURES AND ANIMALS

1. *Management of pastures.* The pasture area used should be uniform at the start. It should have received the same fertilizer or cultural treatment for some time previous to the beginning of the experiment.

a. Management. For partial weed control, for the prevention of clumping, and for keeping the pasture sward in a vegetative state, it may be necessary to mow permanent pastures once or twice a year. Preferably, mowing should take place at the same date on test and check pastures, but in rotational grazing mowing is suggested immediately before the animals are removed. If pastures are mowed to remove excess herbage, this material should be weighed unless it is eaten by the grazing animals. Dragging to spread manure should take place after the field has been grazed closely and animals removed. In rotational grazing it may be done just after mowing. On some types of permanent pastures, rolling is desirable. Such operations should take place in early spring. Discing is sometimes recommended.

b. Rate of grazing. It is desirable to maintain a uniform rate of grazing throughout the summer in all experimental pastures. To promote this it is often desirable to cut one or more plots of the rotation series, as conditions warrant, early in the spring during the flush season while the herbage is immature. This practice not only aids materially in controlling the rate of grazing but also helps control clumping and minimizes the effects of tramping down forage. It is desirable that the manure produced from such herbage be returned to the plot from which the herbage was cut. Where continuous grazing is practiced it is less feasible but frequently practical to remove part of the early spring ungrazed forage. Where abundant spring growth is the rule and much tramping and uneven grazing results, the clipping of pastures as described under (**a**) is highly desirable. Proper irrigation and fertilization are also valuable aids in maintaining a uniform growth of pasture.

2. *Management of animals.*

a. Selection and allotment of animals. The animals used in an experiment, or in different groups of an experiment, should be uniform in species, age, sex, weight, conformation, condition, previous treatment, gestation, lactation (with dairy cattle), and productive ability. All animals should be maintained under uniform conditions and preferably on pasture for at least 7 to 10 days prior to going on the experiment. The animals should be free from disease and parasites, unless this is a part of the problem.

It may be desirable to stock all areas so as to run out of pasture at about the same time at the end of the pasture season. Grazing should cease in time to permit the storage of adequate root reserves. It is essential, however, to maintain uniformly grazed pastures and in some experiments this may require earlier spring grazing and/or later fall grazing on certain treated plots as compared with non-treated plots. In other words, it is more important to fit the degree of grazing to the growth of pasture than to maintain the same grazing period on all experimental plots. The length of grazing season may vary with the type of treatment that plots receive.

In maintaining a uniform rate of grazing it may be necessary to remove animals from the pastures during mid-season when less grass is available and to add animals later in the season as growth is again resumed. When removing animals from lots it is desirable to take out such animals that will leave the group most uniform; also that animals removed should occupy pasture which has received the same treatment as that from which the animals have come, in order to prevent the building up of mineral reserves. It is perhaps more important to maintain a uniform rate of grazing on experimental pastures than to maintain an equal number of animals in the various lots.

b. Rotation of groups. Unless rotational grazing is being investigated, it is best to keep the same animals continuously on one experimental area. Animals should be rotated between pastures and weights taken at the same hour of the day.

c. Weighing of animals. In pasture experiments, allowing time for the animals to become accustomed to the herbage and to obtain a normal fill previous to starting the experiment proper, is desirable. Weights should be taken on three successive days at the beginning and end of the experiment. If any major change, such as a transfer from permanent to supplemental pasture or the removal of one or more animals is made during the grazing season, weights of the animals involved should be taken at the time the change is made, in accordance with (**b**). During the course of the grazing season live weights should be recorded in 28-day or monthly intervals in order to indicate the condition of the animals, to obtain weight bases for the purpose of calculating the nutrient requirements, and to serve as a guide in determining the rate of supplementary feeding. Additional weekly

weights may be desirable. Weighings should be made at the same time each day, preferably early in the morning, since weights taken then appear to be more uniform. Occurrence of oestrus on weigh day should be noted, and weights interpolated, if obviously desirable.

d. Supplemental feeding. Supplying the animals during the grazing season with any concentrate or harvested forage should be avoided as far as possible, particularly in fertilizer experiments. Heavy feeding increases the returns of plant food to the pasture in the voidings of the animals and prevents an exact expression of the effect of fertilizer applications. In addition, any feeding of this kind adds to the difficulties in calculating results, especially when different kinds of pasture (different grasses or different mixtures of grasses and legumes) are being compared, because any deficiency of the needed element in the pasturage may be obscured by a supply in the supplemental feed.

In the case of dairy cattle it is generally desirable to provide some supplementary feed in addition to pasture to maintain a uniform rate of production and prevent drastic falling off in production during periods of drought. In pasture experiments it is desirable that the cows obtain as much of their nutrients as possible from pasture without losing in body weight.

Supplemental feed may be fed as a grain mixture, or as hay or silage. When grain is used the crude protein content need not exceed 12 per cent during spring and 16 per cent during summer. Such grains as corn, barley, and oats are recommended since they furnish high amounts of total digestible nutrients and are relatively low in nitrogen and mineral elements, thus keeping to a minimum the effect of adding fertilizing elements to pasture by way of supplementary feed.

Hay and silage in moderate amounts may at times be used as a supplement to pasture. As previously mentioned it is often advisable to clip the early spring growth as a means of controlling the rate of grazing. This harvested forage may well be made into grass silage for the purpose of feeding it to the cows later in the summer as supplementary feed. It is desirable that the silage or hay produced from a plot should be fed only to animals grazing the same plot. Grass silage makes an excellent supplement to pasture.

All supplementary feed should be weighed and analyzed for its chemical composition. Where possible, digestion experiments should be conducted in order to determine the total digestible nutrient content of the supplementary feed. Where it is not possible to conduct digestibility trials on the supplementary feed with the same class of animals, use coefficients reported by Morrison, "Feeds and Feeding," latest edition.

Unless a study of the value of supplementary feed is being considered it is desirable to keep the amount of supplement the same in all groups. This may not be possible, however, in cases where uneven numbers of cows are maintained in the different plots at various periods of the grazing season.

c. Supplemental pastures. The use of supplemental pastures is necessary wherever dry midsummer seasons are prevalent. Sudan grass, alfalfa, lespedeza, small grains, sweet clover, etc., or the aftermath of meadows may be used. However, where supplementary pastures are used the animals should not graze experimental and supplemental pastures at the same time or on the same days. The animals should be off the experimental pastures while grazing the supplementary pasture; otherwise it would not be possible to calculate the nutrient yield of the pasture under study. This recommendation would also apply in experiments where the value of supplemental pasture is being studied.

DETERMINATION OF YIELDS OF PASTURES

1. *Determination of yields by calculation of the amount of total digestible nutrients required by the grazing animals.* In expressing yields of experimental pastures in terms of the type of livestock for which the results are intended it is desirable to have some simple unit of expression based on nutrient requirements for maintenance, weight change, and production. This unit should be obtained from the nutrient requirements of the animals grazing the pasture. The standard cow-day, defined as an animal obtaining 16 pounds of total digestible nutrients from pasture per day, is an expression which seems to meet these requirements (9). Another expression which has been proposed is unit-days of grazing (14). The standard cow-day is more specific, and is adapted to work with dairy cattle and beef cattle. The standard cow-days per acre may be obtained by dividing the total digestible nutrient yield per acre by 16. The carrying capacity is obtained by dividing the standard cow-days per acre by the number of days in the grazing season. Actual time of grazing is equally essential in published results.

In the case of dairy cattle it is necessary to obtain accurate data on live weight, milk production, and the amount of supplementary feed given each animal, the number of days the pasture is grazed during the season and the acreage, in order to calculate the total digestible nutrient yield per acre. The nutrients fed in the supplementary feed are subtracted from the total nutrients required, the remainder being credited to pasture. When calculating the requirements for maintenance and production the latest edition of Morrison's (12) standards are used. For dairy cows monthly, weekly, or weekly composite butter-fat tests applied to daily milk weights should be secured. The milk yield is calculated to a 4 per cent fat-corrected milk basis by the Gaines (5) formula. In determining the nutrient requirements for change in weight, 3.53 pounds total digestible nutrients per pound gain in weight are added to the total requirements. For each pound loss in weight, 2.73 pounds total digestible nutrients are deducted from the total requirements (9). These factors have been developed from the nutrient requirements per pound caloric content of body gain and per pound caloric

content of 4 per cent milk and the relative value of feed energy for maintenance, milk production, and body increase. This method of calculating nutrient requirements of animals has been compared with the nutrients consumed by milking cows in dry-lot feeding where it was possible to measure the dry matter and nutrients consumed. For 42 observations extending over an average period of 161 days, the percentage of nutrients consumed of that calculated as required for maintenance, production, and weight change was $97 + 1.41$ per cent (8).

It is recognized that certain assumptions must be made in determining pasture yields in this way. Errors due to variation in live weight caused by fill may influence the results somewhat. The energy expended in grazing is not measured. It is believed, however, that the method perhaps offers the best available means yet devised of expressing what the animals have been able to net in the way of maintenance, weight increase and production as a result of grazing.

In the case of beef cattle, in addition to measuring pasture yields in terms of maintenance and weight gain, differences in market value and grading the live animals by use of descriptive terms should be practiced. To properly evaluate the gains made, the animals should, if possible, be slaughtered in order to grade the carcasses and to make possible a chemical analysis of the 9th, 10th, and 11th ribs.

Another way to make the expression, total digestible nutrients per acre of pasture, more practical, is to express it in terms of a common feed. This is termed feed replacement value. Price data obtaining in any area may then be applied to the feed replaced and a money value of the pasture indicated.

2. *Determination of pasture yields by agronomic methods.* In all grazing experiments it is recommended that a satisfactory system of pasture cuttings be employed from which may be obtained dry matter yields, chemical composition (and if facilities permit, sufficient forage for digestibility experiments). In many grazing experiments it is desirable to measure the yields by mowing plots or representative areas protected from grazing. Animals are all more or less selective in grazing and unless forced to graze the pasture closely they will not consume the herbage uniformly. Usually, therefore, the yield obtained by mowing and weighing all the herbage is larger than that indicated by the estimated digestible nutrients calculated from grazing results.

There are two methods of arriving at the yields from mowing or plucking protected plots: (a) attempts to measure the quantity of herbage consumed by the grazing animals; (b) measure the annual growth and seasonal distribution of herbage or that available for grazing without regard to whether it is all eaten by the animals. The quantity of herbage consumed is the most important but there are many difficulties encountered in measur-

ing this accurately. The annual growth may be measured with fair accuracy by agronomic methods but the relation of such yields to the actual yields obtained by grazing animals is undetermined. It will vary presumably with different kinds of pasture and different methods and rates of grazing.

a. To measure the quantity eaten the following plan is suggested. Protect plots of convenient size in each paddock by wire cages or movable fences and clip or mow these at regular intervals. Clipping dates should preferably coincide with the dates when the cattle are weighed every 28 days, or at intervals related to plant growth. An unprotected area of the same size and representative of the herbage left by the cattle is to be clipped at the same time and the yield subtracted from that of the protected area. Each time a clipping is made the cage is moved to a new location. The total of these calculated remainders should closely approximate the quantity of herbage eaten by the grazing animals. The protected plot should be replicated at least three times in each grazing unit or paddock.

Another method practiced and worthy of consideration is to hand-pluck the herbage in the protected areas, taking off as nearly as possible the same amount and character of forage that has been removed by the grazing animals, using as a model the unprotected areas near the cages. The personal factor in deciding just how closely to pluck the herbage gives to this method an element of possible error which may or may not be greater than that in the previous method in choosing the position of the unprotected area to be mowed. This method applies particularly to upright forage plants such as sweet clover, millets, Sudan and Napier grasses, and to decumbent grass such as centipede and carpet.

b. The method suggested for measuring the actual yield or seasonal growth of herbage is as follows: An area the same size as the cage is mowed and the yield recorded. Then place the cage on this mowed area to protect it from grazing until the date for the second cutting. When the second yield from this area has been obtained, move the cage to a new location in the paddock which likewise will be mowed before the cage is put in position. This process will be repeated, the cage being moved after each clipping of the protected area. The first clipping in the spring indicates the quantity of feed available for animals when they are turned on the pastures and this added to the yields from the subsequent mowings denotes approximately the quantity of herbage available for the animals. Each clipping after the first will represent only the growth made by the herbage during the period since the last clipping.

In either method, areas which have remained undergrazed should be avoided in placing the cages because the larger root reserves, or other conditions, in the under-grazed grass may affect the growth for a considerable period.

The areas used should be chosen carefully to secure representative vegetation and soil type and to avoid recent droppings. The residual effect of previous treatments is to be avoided unless that is the purpose of the experiment.

c. Methods of computing yields. The protected areas or plots should be cut when the herbage is free from dew or rain and the clippings weighed immediately. All unpalatable weeds should be separated from the palatable herbage and the weeds weighed separately and dried so that their dry weight may be recorded, after which they may be discarded. Since the percentage of moisture in pasture herbage varies considerably, dry-matter determinations are necessary for accuracy. When the green weight of the palatable herbage has been obtained a weighed sample of suitable size to avoid heating and moulding should be placed in a cotton bag to be dried. A preliminary drying room is desirable where the moisture in these samples can be reduced to 10 or 15 per cent when the samples can be stored safely until the end of the season or until a convenient time to reduce them to a moisture-free basis in a drying oven. If the chemical composition of the herbage is to be determined, aliquot portions of each sample should be reserved for chemical analysis after the preliminary drying.

If yields are not stated on a dry-matter basis, and only the preliminary drying or curing is attempted, then the approximate percentage of moisture in these "hay" yields should be stated. This can be ascertained by drying only a few representative samples to a moisture-free condition.

Information on the relation of pasture yields as determined by animal methods and agronomic methods described above, would be highly desirable. Some work has been done in this respect using dairy cows and heifers as experimental animals. In an experiment (7) in Washington, the yields of rotational and continuously grazed pastures were determined by means of pasture clippings and by the total digestible nutrient yield method. The average yield for a three-year period as determined by clippings was 17 per cent greater than that determined by the nutrient yield method. Another experiment reported (13) from West Virginia, covering a four-year period, showed that the yield of pastures determined by the clipping method was 19 per cent greater than that determined by the nutrient yield method. The Oregon Station (1) reported that where cows were grazed on pastures adjacent to the barn the digestible nutrient yield as determined by clipping was 5 per cent greater than the yield as determined by the animal method. Reporting results of a five-year experiment the Pennsylvania Station (6) observed that the yields obtained from clippings were 20–30 per cent greater than yields determined by grazing animals. In none of these experiments were there digestion trials conducted to obtain the digestibility of the pasture grass or supplementary feed given. There is need for additional work on this problem where digestion experiments are incorporated into the experimental procedure.

3. *Determining composition of herbage.* A knowledge of the chemical composition of herbage from the different paddocks or grazing units is useful, although not indispensable. Whenever the facilities are available, a feedstuff analysis of the samples should be made according to methods adopted by the American Association of Official Agricultural Chemists. In addition to a determination of the customary ash, crude protein, carbohydrates and fat, information regarding the calcium and phosphorus content of the herbage often will provide an explanation of results obtained by grazing. Where the soils are known to have a very low mineral content, special work should be undertaken to determine the percentage of iron, cobalt, copper, iodine, and other mineral elements less likely than calcium and phosphorus to be a cause of malnutrition. Where necessary to economize on time and expense, analyses may be made of composite samples representing the entire seasonal growth on a given paddock. Soil contamination and contamination while processing samples in the laboratory are to be avoided scrupulously.

4. *Digestion trials.* In order to apply effectively the results of chemical studies of pasture herbage, determinations of the digestibility of the herbage, especially in the green or uncured condition, are necessary. Recent work at several experiment stations with frequently cut pastures has shown the digestibility constituents to be higher than in earlier experiments. The pasture committee of the American Dairy Science Association has reviewed the results of 45 digestion trials, and suggests the following average coefficients of digestibility for mixed pastures: Crude protein, 75 per cent; crude fiber, 79 per cent; nitrogen-free extract, 80 per cent; and ether extract or fat, 50 per cent. These coefficients can be applied directly to the chemical analysis of the grasses to give the total digestible nutrient content. Coefficients of digestibility more specifically applicable to the pasturage being consumed may be used at the discretion of the investigator. That is, the average of three or more sets of figures from similarly-managed pasture tests might be desirable. Lacking the chemical analysis, the dry matter may be assumed as 72 per cent digestible. For dried grass hay containing 15 per cent moisture and 9 per cent ash, this is equivalent to 60 pounds of total digestible nutrients per 100 pounds. Lacking more specific data, this rough approximation can be used. However, there is need of more digestion trials with immature legumes and grasses, particularly those of the southern states, such as bermuda grass, carpet grass, and lespedeza.

It is suggested that digestion trials be conducted according to the recommendation of Forbes and Grindley (4). Due to the high nutritive value of pasture herbage, it is possible to conduct digestion trials with no supplement except salt, even to animals on limited milk production. Usually the protein supplied will be above the requirements of the animal. The detail that requires the greatest precaution in running digestion trials with young

pasture grasses fed in the green state as compared with digestion trials with dry feed, is in obtaining representative samples for the daily feeding and the accurate sampling of the grasses for determining the moisture and chemical composition. Some provision for drying a representative sample of the green forage quickly is necessary in order to feed the same quantity of dry matter each day.

5. *Use of pilot plots.* Small pilot plots subject to grazing conditions offer the most promise for furnishing preliminary data in determining which treatments, strains, etc., should be included in the final critical experiment. These plots do not require refined methods of technique, since the differences may be large and the main objective is to eliminate the poorest rather than to determine the best.

Insofar as possible, plots of pure stands of forage crops for preliminary comparative grazing trials should be long and narrow (3 : 1 to 7 : 1) rather than square, placed side by side, and not too large. The plots should be replicated in the series under grazing test. A standard plot may be repeated at definite intervals throughout the series.

The plots of forage crops used in preliminary comparative grazing trials also may serve for observations concerning the months during which different crops may be grazed, the responses of plants during grazing, growth subsequent to grazing, studies of composition in relation to stage of maturity, or to palatability, successions of grazing crops, possible toxicity of plants, and other related subjects, using a minimum of acreage, time, and funds. Forage yields may be obtained from caged quadrats. With sufficient observations throughout the season, the relative grazing capacity between plots may be estimated in cow-grazing-hours per plot.

6. *Palatability determinations.* The animals selected for experimental use should be those to which the crops are adapted best, and to which the results are to be applied. They should be tractable and accustomed to presence of the observer, who in turn should understand the animals of that kind. These animals may be used to determine relative palatability between different pasture forages, between fertilizer treatment of crops, grass management practices such as mowing, relative grazing capacity of crops, etc. Previous access of animals to certain forage plants, such as biennial white sweet clover, may affect their preference for it. This fact should be considered when selecting the individual animals for use in palatability trials, and interpreting the observations.

In relative evaluation, including palatability trials, the animals should be allowed a preliminary period of five or more minutes to start grazing regularly before other than preliminary observations are recorded. An animal not grazing should be omitted from the count, and added when grazing is resumed. If vegetation is luxuriant and palatable, animals may

graze their fill within an hour, and be removed from the plots when the majority of the animals have ceased grazing. Because of the individual peculiarities in appetites, conclusions concerning palatability of forages should not be based on a few animals.

The relative palatability of forages may be determined by several methods such as (a) tabulating the number of animals grazing on individual plots at definite intervals of time; (b) with row crops, daily estimations of the lineal footage of rows grazed; and (c) interval of time required per animal unit to graze a given area completely. Palatability observations are of value mainly when considered along with yields and growth characteristics of a crop.

7. *Cooperative demonstrations.* Field tests should be conducted with farmers to obtain yields under variable soil and management conditions, with less detailed observations than are possible at experiment stations. For example, yields may be determined only the earlier part of each pasture season; botanical composition and growth estimates determined at the same time and stage of growth, and by the same person. Replicate plots may not be obtainable, and the areas harvested will vary with circumstances. Such tests approach the ideal, in so far as practical under local farm conditions.

STUDY OF PASTURE FLORA

A botanical survey of the plant population in pastures should be made at least once each year, and preferably twice, spring and fall. The survey should be made the same time of year, each succeeding year. The object of such surveys is to determine the relative contribution of each plant species to the feed consumed by the animals and in addition to record the changes which take place in the pasture flora from year to year under different methods or rates of grazing, with differing treatments such as clipping or the application of fertilizers. Most of the identifications must necessarily be made from vegetative characteristics but the cooperation of trained taxonomists should be obtained in this work.

Where weeds are present in considerable amounts, the family or genera and species, if possible, should be listed, together with percentage present.

Several methods of analyzing the flora of pastures have been tried. The ideal method is one in which the herbage from small plots is cut and brought into the laboratory where trained workers separate the species and weigh each lot, thus obtaining an accurate picture of the percentage contribution of each plant species to the total yield of herbage. This method, however, has largely been discarded because it is too expensive and time-consuming. It may be resorted to occasionally, however, as a check upon the accuracy of a simpler method which is being used.

Wm Davies (3), Aberystwyth, Wales, suggests methods of pasture analy-

sis and fodder sampling. Davies and Trumble (2) discuss work done at the Waite Agricultural Research Institute, University of Adelaide, Australia, and present a very useful comparison of the different methods. In addition there is the "point quadrat method" used extensively by Bruce Levy (10) in New Zealand. The inclined point quadrat method is satisfactory in the north humid region of Wisconsin. About 6 to 10 random counts are made on each of 3 to 5 random selected 1/100-acre plots on 3 comparable random $\frac{1}{4}$- to $\frac{1}{2}$-acre areas in each grazed pasture. Additional $\frac{1}{4}$- to $\frac{1}{2}$-acre areas may be used for each variable condition in a pasture.

No investigations in experimental technique of any magnitude have been conducted in the United States. Until more definite information is available it is suggested that the following course of procedure be adopted:

1. *The pastures east of the 97th meridian* in the northern states, those on the Pacific Slope of Washington and Oregon, and irrigated pastures in the interior, all are more or less uniform mixtures. On such pastures a light but firm frame 10 inches square divided by cross wires two inches apart each way, into two-inch squares, is tossed at random onto the turf and a reading made of the ground-cover supplied by each species of plants useful for grazing, and of the weeds. The percentage of bare ground is estimated also. Readings with this frame are quickly made because of the division into two-inch squares on line charts. In a paddock of 4 to 5 acres 15 or 20 individual readings when averaged will give a fairly accurate picture of the plant population.

2. *For pastures in the Cotton Belt* where bermuda grass and carpet grass prevail, the above method is not satisfactory. In such pastures considerable areas are occupied by one grass exclusively and there is no uniform mixture of the various species. For such pastures the following procedure is suggested. Mark off an area of approximately 1/200 acre, $6' \times 36'$, using a light rope or heavy cord. In this enclosure, estimate by observation the percentages of cover supplied by different species of grazing plants and weeds and the percentage of bare ground. In a 4- to 5-acre paddock repeat this process a sufficient number of times so that every floral condition in the paddock is represented. Average the percentages as in the use of the small square. Usually five readings of this size in one paddock will be sufficient, but more may be necessary where the distribution is distinctly not uniform. These larger plots it is believed, by extending across the areas of pure stands, will give a better average of the herbage characteristics.

3. *For range pastures in the West,* quadrats one or two meters square have been used effectively. Small frames are not well suited to range pastures of bluestems, buffalo grass, or wheatgrass. The usefulness of fixed quadrats one meter square is confined largely to registering the change which takes place in the flora from year to year.

In any study of the pasture flora, and especially studies made with a small frame, it is obviously necessary to avoid situations where the herbage is affected by the excrement of animals, by a disturbance of the soil by rodents, or other unusual conditions.

DURATION OF EXPERIMENT

In a pasture set-up several conditions, including variability among animals and favorable and unfavorable seasons, are bound to be disturbing factors. These, together with the cumulative effect of the animals on the pasture and the fertilizer application or management on the pasture, may require years for the establishment of significant differences in experimental pastures. Therefore, a period of six to ten years for a permanent pasture experiment is to be preferred to one-half or less of that time. In areas where climatic conditions are more uniform and where there is less variation from season to season, reliable results may be obtained in somewhat less time.

USE OF FERTILIZER AND SOIL AMENDMENTS

In the application of fertilizers to pastures careful consideration of the amount is imperative, since the return per acre may be lower from pastures than from some cultivated crops. In general, it has been found best to apply lime, phosphate, potash, and other minerals in the fall; nitrogen, because of its ready solubility, may also be applied then, or in early spring, or as a top-dressing during the growing season. The time and rate of application should be governed, however, by local conditions. The quantity of lime should be determined largely by the pH value of the soil and the lime requirements of the prevailing plants in the pasture. Because of the cost of nitrogen fertilizers, the quantity applied should be regulated in accordance with the probable value of the products to be obtained by grazing the pasture and the percentage of legumes present. Solid manure may be applied late in the fall or early spring with good results. Liquid manure may be applied late in the fall, early in the spring, and, in the case of rotationally grazed pasture, throughout the grazing season immediately after the plots have been grazed.

ANIMAL PARASITES

The prevalence of parasites is a problem that must be given careful attention in planning and carrying out grazing experiments. The more intensive the grazing, the more dangerous parasites are likely to be. The true difference between an unimproved pasture of low production and a highly improved and fertilized pasture may be largely obscured by parasites. Permanent pastures for swine should be used only in alternate years.

Sheep alone should not be used in pasture experiments where they must

be kept on the same intensively grazed area throughout the grazing season unless adequate precautions are taken to control internal parasites. The pastures should be established on land which has been neither pastured by sheep nor dressed with sheep manure. It may be possible to select established pastures which had no sheep on them for several years and no opportunity to become contaminated through the droppings of the sheep.

Sheep found free from stomach worms by fecal examination should be secured and dosed with appropriate anthelmintics before they are put on the pasture. By following this method of securing sheep each year and repeating the dosing at intervals of three weeks throughout the grazing season, the pastures may be kept practically free of parasites. Under such conditions, sheep may be used as experimental animals on highly productive and extensively grazed pastures for several years.

INSECTS AND RODENTS

Damage by insects and rodents should be recorded with the aid of entomologists and biologists.

DETERMINATION OF COSTS

Cost data from pastures are of little value except for comparison of methods within the experiment, such as: The cost of supplemental feed combined with pasturage compared with feed obtained wholly from grazing; or the additional cost of fencing for rotation grazing with that needed for continuous grazing.

The cost of materials, with the possible exception of fertilizers, and operating costs are always excessive when calculated from small plots. Such cost data, if given, should be calculated on the average cost per acre of large fields.

CONTINGENT OR CONTRIBUTING DATA

The following data should accompany the grazing results:

1. Previous history of the pasture area.
2. If irrigated, dates and quantity of water applied.
3. Condition of pasture, including stage of growth of principal flora and date when grazing began and ended.
4. Rainfall distribution and amount compared with normal.
5. Mean, maximum, and minimum temperatures for each month.

PHOTOGRAPHY

Photography may be a very valuable tool in pasture experimentation. A series of well-planned photographs of the different pasture plots at various periods of the year and from year to year, effectively portray the changes in the appearance of the pasture flora as the experiment progresses. It is also

an aid in describing methods employed in pasture research. Finally, colored photographs are very helpful in presenting the results of pasture research.

Respectfully submitted,

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ABSTRACTS OF LITERATURE

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ABSTRACTS OF LITERATURE

BOOK REVIEWS

135. Advances in Colloid Science. ELMER O. KRAEMER. Interscience Publishers, Inc., New York. 12 papers, index, 434 pages. $5.50.

This book is planned to be the first of a series of volumes "intended to provide a medium in which recent significant discoveries or advances in Colloid Science, either experimental or theoretical, may be presented in a more comprehensive and unified fashion than is possible in the regular technical periodicals." The present volume, under the able editorship of Elmer O. Kraemer, consists of twelve papers. Each paper is written under separate authorship; four involve co-authorship, making a total of sixteen contributors.

This compilation of papers is in no sense a text book on Colloids. It is intended for advanced students of colloid science, for whom this and later volumes of the series will be very useful and essential. In the present volume the following papers may be of special interest to dairy research workers: "Solubilization and Other Factors in Detergent Action" by James W. McBain, "The Creaming of Rubber Latex" by G. E. Van Gils and G. M. Kraay, "The Study of Colloids with the Electron Microscope" by Thomas F. Anderson. H. H. Sommer.

136. The Infectious Diseases of Domestic Animals with Special Reference to Etiology, Diagnosis, and Biological Therapy. WILLIAM ARTHUR HAGAN. New York State Veterinary College, Cornell University. Comstock Publishing Company, Inc., Ithaca, N. Y. 665 pages. Illustrated. Price $6.00.

This work brings together in a single volume discussions of all of the important infectious diseases of domestic animals. In addition to bacterial agents; fungi, protozoa and viruses that are pathogenic to animals are included.

One of the unique features of this book is the manner in which the etiological agents of animal disease are dealt with. Without omitting any of the important features of microbiology, the disease—pathology, immunology, diagnosis, therapeutics—forms the main subject matter of the text material. Sanitary science and its practical application is included.

The subject matter is divided into 6 parts and 44 chapters. Part one deals with the mechanisms of infections and resistance; part two the pathogenic bacteria; part three the bacteria like pathogenic organisms of uncertain classification; part four the pathogenic fungi; part five the pathogenic protozoa; and part six the viruses. A list of selected references is given at

the end of each chapter for those who wish to read the more important papers in the literature.

While written to serve as a college text for students in Veterinary Medicine, this book will be welcomed by many among our readers who are engaged in animal research and teaching. T.S.S.

BREEDING

137. Hydrocephalus, A Lethal in Cattle. C. L. COLE AND L. A. MOORE. Jour. Agr. Res., *65*, No. 10. Nov., 1942.

There are two types of hydrocephalus, internal and external. The internal type is a collection of fluid in the cerebral ventricles and the external type a collection of fluid outside the brain substance. The lethal was discovered in a herd of grade and purebred Holstein-Friesan cattle. The authors' conclusions are as follows: A new lethal in cattle, internal hydrocephalus, is described and shown to be probably a simple recessive in its mode of inheritance. Asymmetry and ''jumpy'' conditions are described and may be recessive and not linked with each other or with the lethal gene. The data suggests that the sire used carried three rare recessive genes but since the probability that any one animal would carry three rare factors is very small the problem should be studied further. H.P.

CHEESE

138. Contributions to the Study of Rancidity in Canadian Cheddar Cheese. II. The Growth of Butyric Acid Anaerobes in Cheddar Cheese. C. H. CASTELL AND O. R. IRVINE, Ont. Agr. Col., Guelph, Canada. Sci. Agr., *23*, No. 3: 176. 1943.

A series of experiments was performed to determine to what extent butyric acid anaerobes are able to grow, to form spores, and to germinate spores when inoculated into Cheddar cheese. Inoculations were made with single cultures as well as in combination with several aerobes which were known to be symbiotic types. In addition, milks were inoculated with washings with good silage, sour silage, sour whey-soaked soil, and ordinary soil.

The numbers of organisms in the cheese were estimated by making serial dilutions in corn-liver and grass media.

The results indicate that in no instance was there an increase in the number of cells, sporulation, or germination of spores during a period of two months. The addition of various aerobes and reducing the rate of salting made no difference in these results. In no instance was a rancid flavor produced in cheese by these inoculations. O.R.I.

CONCENTRATED AND DRY MILK: BY-PRODUCTS

139. Influence of Moisture on Browning of Dried Whey and Skim Milk. HUGO DOOB, JR., ALFRED WILLMANN, AND PAUL F. SHARP, Cornell Univ., Ithaca, N. Y. Jour. Indus. Engin. Chem. Indus. Ed., *34,* No. 12: 1460. 1942.

Dried whey darkens more rapidly and to a higher degree than dried milk during storage. This report deals with some of the moisture relations of dried milk and dried whey as they affect the rate of development of the brown color defect. Two methods of extraction for determination of brown color are described. In the first, applicable to whey only, an aqueous suspension of the products is clarified with alcoholic zinc chloride at 50° C.; in the second, applicable to both dried milk and whey, a clear extract is obtained by extracting the product at room temperature with a solution containing 300 grams sodium chloride and 10 grams $Na_3PO_4 \cdot 12\,H_2O$ per liter of water. Slightly more color is extracted from dried whey by the alkaline solution. In a set of experimental and commercial dried wheys, the color developed on aging was found to be associated with high osmotically held moisture, particularly with water not present in α-lactose hydrate crystals. Other variables which had less effect than moisture content were high titratable acidity, low pH and low lactose content. The relative humidity of the atmosphere had a marked effect upon the browning of dried milk and whey in storage. At 25° C. browning was inhibited when the relative humidity was below about 30%, while color increased at humidities above 30% with a maximum extractable color being obtained at 55 to 65% relative humidity. Browning was accelerated at storage temperatures above 30° C. while the rate first increased and then decreased with time of storage. At least one stage of the browning reaction appeared to be autocatalytic. B.H.W.

DISEASE

140. Johne's Disease in Farm Animals. D. F. EVELETH AND REBECCA GIFFORD, Fayetteville, Arkansas. Jour. Amer. Vet. Med. Assn., *102,* No. 790: 27. Jan., 1943.

In a survey and study of Johne's disease (para-tuberculosis) in farm animals in Arkansas, approximately 38% of the cattle, 43% of the sheep, 24% of the goats, 32% of the swine, 39% of the horses, and 94% of the mules tested reacted to the intradermal Johnin test. Clinical symptoms were found only in cattle and sheep. The causative organism has not been found in all reactors when examined on autopsy, possibly due to the fact that a light infection may cause a reaction to the test, but due to the fact that these few organisms infect such a large area along the digestive tract the finding of these is somewhat a matter of chance. In several instances a high per cent of reactors was found in cattle and sheep imported into the state.

In addition to the usual clinical symptoms, the disease is suspected of causing uterine inertia in pregnant animals, particularly cattle, sheep, and swine. Disposal of clinical cases apparently helps to keep infection at a low level, but the possibility remains that non-clinical cases may spread the disease. S.A.F.

141. Laboratory Methods for Differentiating *Trichomonas Foetus* from from Other Protozoa in the Diagnosis of Trichomoniasis in Cattle. BANNER B. MORGAN AND LOWELL E. NOLAND, Madison, Wis. Jour. Amer. Vet. Med. Assn., *102*, No. 790: 11. Jan., 1943.

The difficulties of differentiating infections of *Trichomonas foetus* from other flagellates found in the genital tract by other than a trained protozoologist and microscopist are pointed out. Cultural and identification methods are given in detail for *Tr. foetus* and for the other flagellates commonly found. One plate shows detailed drawings of these protozoa. Those who have occasion to make diagnostic examinations for trichomoniasis should find the article of considerable help. S.A.F.

142. Infectious Bovine Mastitis. W. N. PLASTRIDGE, E. O. ANDERSON, AND F. J. WEIRETHER. Conn. Agr. Expt. Sta., Storrs, Conn. Bul. 240. Jan., 1942.

A procedure for the routine laboratory testing of milk samples for mastitis, and identification of mastitis organisms is described. Observations on five experimental herds which were employed in developing a control program based on segregation showed that: one herd which was started in 1933 with eight bred heifers, has always been free from *Str. agalactiae* infection; *Str. agalactiae* infection was completely eliminated from the four originally infected herds by the gradual replacement of infected animals with first-calf heifers raised on the premises. The time required for complete eradication by segregation and gradual disposal of infected animals was from two to five years. No decrease in the size of the herds was made necessary by the eradication process; once freed from *Str. agalactiae* infection, the herds remained that way.

The results of observations on 74 herds tested under the Connecticut state program for periods of two to four years showed that: the average initial incidence of *Str. agalactiae* infection was 40.9% the annual rate of new infection in 53 segregated herds was 12.5, 8.2 and 7.9% for herds tested one, two and three times per year, respectively; the average annual rate of new infection was 27.0% in 21 herds which were not segregated; *Str. agalactiae* infection has been eradicated from 24 of these herds. All have remained free from infection except four in which infected animals from outside sources were added.

Observations on *Str. agalactiae* infection in 181 first-calf heifers showed that seven were infected during the first three months of lactation, eight became infected between the third and sixth month, and seven after the sixth month. It is concluded that with proper segregation, sanitation, herd management and laboratory tests made at intervals of three to four months, the annual spread of *Str. agalactiae* infection may be kept below 10%, and that if this is done the disease can eventually be eliminated from the average herd.

An appendix to the bulletin contains practical information on a segregation program for the control of the disease and suggestions for conducting such a program. Three pages are devoted to suggestions on general management and sanitation for preventing the spread of infectious mastitis.

J.G.A.

143. The Response of "Ceased" Reactors in Bang's Disease to Reexposure. B. A. BEACH, M. R. IRWIN AND L. C. FERGUSON. Jour. Agr. Res., *65*, No. 11. Dec., 1942.

In this study two herds were used comprising approximately 64 cows and heifers to determine the response of ceased reactors in Bang's disease to re-exposure. The results show that only 1 of 16 ceased reactors in two different herds aborted its fetus when subjected to a second exposure. *Brucella abortus* was isolated at parturition from only one other ceased reactor following a re-exposure. An active immunity to *Brucella abortus* is engendered by an infection with virulent organisms. The immunity thus engendered is of relatively long duration. The agglutination titers of the sera of the different ceased reactors were either lower or approximately the same during the second as during the first infection. H.P.

FOOD VALUE OF DAIRY PRODUCTS

144. Ice Cream—The Protective Food. W. H. MARTIN, Dairy Dept., Kansas State Col., Manhattan. Ice Cream Trade Jour., *38*, No. 10: 44. Oct., 1942.

One of the best solutions of the problem of increasing the amount of milk in the diet of many people is through the greater consumption of ice cream. It has been calculated by the National Dairy Council that an average serving of vanilla ice cream contains the following constituents: calories, 200; iron, 0.34 gm.; protein, 3.9 gm.; vitamin A, 339 I.U.; calcium, 0.131 gm.; thiamin, 0.038 mg.; phosphorus, 0.104 gm.; riboflavin, 0.105 mg.

This serving of ice cream furnishes 1/5 of the calcium needed by an adult daily and generous quantities of other minerals found in milk. It also contains 1/5 of the day's need of vitamin A and about 1/10 of the relatively scarce vitamin G. W.H.M.

ICE CREAM

145. Cocoa. Oliver P. Petran, Robert A. Johnston Co. Ice Cream Trade Jour., *38,* No. 12: 16. Dec., 1942.

Even though 100% of the cocoa bean production is in allied hands, the big problem is to get the products transported to the United States. The inventory on cocoa beans on hand Sept. 30, 1942, was 50% less than it was Jan. 1, 1942. Rationing started in May at 70% of the corresponding months in 1941 and dropped to 60% in July, through Dec. and may go to 50% Jan. 1, 1943. The present stock may last 8 months. Now that the Allies have occupied French African ports the situation may change after the first of the year depending on the amount of beans received after Jan. 1, 1943. W.H.M.

146. Milk Products Rationing for Ice Cream. Ice Cream Trade Jour., *38,* No. 11: 8. Nov., 1942.

The following methods for rationing milk products for use in the manufacture of ice cream have been suggested: (1) A downward revision of ice cream standards placing a ceiling on butterfat going into ice cream at possibly 10.5%. This would involve a reduction of slightly under 2% in butterfat use inasmuch as the national average on ice cream is said to be slightly above 12%—about 12.4%. (2) Limiting gallonage generally. (3) A blanket reduction on the quantity of butterfat and milk-solids-not-fat available for the ice cream industry, the mechanics of which may be worked out similar to sugar rationing. (4) A combination of any two or all three of the foregoing points. (5) The Canadian plan—the method by which Canada is already controlling its ice cream industry. Since this article was written, plan No. 3 with certain modifications became effective, February 1, 1943. Ice cream manufacturers may use 65% of the fat and milk solids used in the corresponding month in 1942. W.H.M.

147. What the Milk Shortage May Mean. Dan A. West, U. S. Dept. Agr., Washington, D. C. Ice Cream Trade Jour., *38,* No. 11: 10. Nov., 1942.

The ice cream industry is faced with a shortage of milk fat and milk solids in 1943. While steps are being taken to keep milk production from declining, the demand for dairy products due to the war, is far in excess of the supply. It will be necessary for ice cream manufacturers to make certain changes in their manufacturing and selling techniques. W.H.M.

PHYSIOLOGY

148. Mammary Gland Growth in Hypophysectomized Castrated Guinea

Pigs. E. T. Gomez, U. S. D. A., Washington, D. C. Endocrinology, *31*, No. 6: 613. Dec., 1942.

Growth of the duct system of the mammary glands of hypophysectomized castrated male and female guinea pigs was induced either by implanting or injecting macerated fresh anterior hypophyseal tissue of adult male or female guinea pigs. The minimal effective dosage was found to be approximately 20 mg. An alcohol-ether extract from the same weight of fresh tissue was more highly active than the fresh tissue. Extracts which induced mammary growth equal to that of early pregnancy were without demonstrable activities of all established anterior hypophyseal hormones. A slight mammary duct growth was induced in hypophysectomized castrated animals with the injection of a lactogenic hormone preparation. Duct growth was increased when estrogen was injected simultaneously with the lactogenic hormone. The lactogen preparation possessed appreciable growth, thyrotropic, adrenotropic, and gonadotropic activity. Extraction with a hot alcohol-ether mixture rendered the lactogen preparation only mammogenically inactive. The results were believed to further the concept that anterior hypophyseal mammogen(s) is the direct agent of mammary growth in hypophysectomized castrated guinea pigs.

R.P.R.

149. The Direct Mammotrophic Action of Lactogenic Hormone. Wm. R. Lyons, University of California, Berkeley. Soc. Exp. Biol. and Med. Proc., *51*, No. 2: 308. Nov., 1942.

Thirty hysterectomized, ovariectomized, virgin rabbits, 4 to 5 months old, were injected subcutaneously 5 days weekly for 4 weeks with 200 I.U. of estrone and one I.U. of progesterone. Such treatment induced mammary growth approximating that of a rabbit pregnant for 3 weeks. Three days after the last of these injections 5 different levels of lactogenic hormone were tested on groups of 6 rabbits. The hormone was injected intraductally so that an individual sector in each animal received a constant volume of fluid. Six and 3 I.U. caused localized sector lactation in all 6 animals, 1.5 I.U. in 5 of 6, and 0.75 I.U. in 3 of 6. None of the 6 animals injected with 0.37 I.U. lactated. That the lactogenic hormone should be considered a mammary growth-promoting hormone was maintained for the following reasons: the number of epithelial cells forming the circumference of alveoli under the influence of the lactogenic hormone was several-fold that of the control alveoli; a large number of alveolar cells were cast off in the formation of the first milk and were replaced by newly-proliferating cells; mitotic figures were observed in the alveolar epithelium during lactogenic treatment; there were not only more cells per alveolus under lactogenic stimulation but the cells were, at certain stages of their

cycle, larger than the cells of the resting alveoli; and, when full lactation had set in the secretory cells had to be constantly renewed either in their entirety or, as was more usually the case, in their supra-nuclear cytoplasm only. R.P.R.

150. Role of Inositol and p-Aminobenzoic Acid in Normal Lactation. DAVID R. CLIMENKO AND EVAN W. MCCHESNEY, Winthrop Chemical Company, Rensselaer, N. Y. Soc. Exp. Biol. and Med. Proc., *51,* No. 1: 157. Oct., 1942.

Young female rats were divided into groups of 12 and placed on the following diets shortly after weaning: a normal breeding diet containing all known dietary elements; a deficient diet plus B-complex supplement plus 15 mg. of p-aminobenzoic acid daily; a deficient diet plus B-complex supplement plus 15 mg. of inositol daily; and a deficient diet plus B-complex supplement plus 15 mg. of inositol and 15 mg. of p-aminobenzoic acid daily. The critical role of inositol in the maintenance of normal lactation was confirmed. P-aminobenzoic acid did not seem to increase lactation directly but when given in conjunction with inositol it slightly decreased mortality rates of the newborn. R.P.R.

151. Effects of Testosterone Propionate on the Mammary Glands of Female Albino Rats. G. L. LAQUEUR, Stanford University School of Medicine. Endocrinology, *32,* No. 1: 81. Jan., 1943.

A total of 73 adult virgin rats was used in the study. Testosterone propionate injected for the first time during late estrus produced development of alveoli in the mammary glands within 20 days. Such mammary glands were capable of lactating when exposed to a suckling stimulus. Continuation of treatment beyond 20 days resulted in a regression of hypertrophied corpora lutea which was accompanied in the mammary glands by a marked increase in stored secretion. R.P.R.

152. Lactogenic Hormone Prolongs the Time During Which Deciduomata May be Induced in Lactating Rats. ROBERT LYON, University of California, San Francisco. Soc. Exp. Biol. and Med. Proc., *51,* No. 1: 156. Oct., 1942.

Fifteen lactating rats were subjected to uterine stimulation from 20 to 24 days after parturition and were then injected subcutaneously daily for 4 days with 100 I.U. of commercial lactogenic hormone. Thirteen of the 15 animals sacrificed on the 5th day showed deciduomata and large robust-appearing corpora lutea. Eight uninjected control animals showed small regressive corpora lutea and no deciduomata at the uterine sites previously stimulated. R.P.R.

153. Electrophoretic Studies on New-Born Calf Serum. E. Jameson, C. Alvarez-Tostado, and H. H. Sortor, Stanford University, Palo Alta, Cal. Soc. Exp. Biol. and Med. Proc., *51,* No. 1: 163. Oct., 1942.

The serum of new-born calves and before the ingestion of colostrum was found to contain no gamma globulin and only small amounts of beta globulin. During the nursing period the composition of the calf serum changed rapidly. The gamma globulin increased in concentration and then decreased. The concentration of alpha globulin and albumin decreased during nursing with the latter finally increasing. During the period of greatest change in serum composition the protein fractions exhibited an abnormally high mobility. R.P.R.

154. Evidence Against a Progesterone-like Action of Ascorbic Acid. Philip C. Pratt, Carnegie Institution of Washington. Endocrinology, *32,* No. 1: 92. Jan., 1943.

The injection of ascorbic acid, either subcutaneously or locally into the uterus, in varying dosages during periods of 3 to 10 days into infantile rabbits primed with estrogen failed to induce progestational proliferation of the uterine endometrium. R.P.R.

155. Relation of Thyroid to Mammary Gland Structure in the Rat with Special Reference to the Male. J. Frederick Smithcors and Samuel L. Leonard, Cornell University, Ithaca, N. Y. Endocrinology, *31,* No. 4: 454. Oct., 1942.

The removal of the thyroids from normal immature male rats resulted in inhibition of mammary duct growth and stimulation of alveolar development when compared with controls. In castrated males thyroidectomy brought about similar structural changes, but to a lesser degree, when compared with castrated controls. The treatment of thyroidectomized, castrated males with testosterone propionate induced lobule-alveolar growth of the mammary glands but there appeared to be an inhibition of duct extension when comparison was made with appropriate controls. Thyroidectomized, castrated males injected with α-estradiol dipropionate showed mammary development similar to that observed in the group treated with testosterone propionate. R.P.R.

156. Relation of Mammogenic Lobule-Alveolar Growth Factor of the Anterior Pituitary to other Anterior Pituitary Hormones. J. P. Mixner, A. J. Bergman, and C. W. Turner, University of Missouri, Columbia. Endocrinology, *31,* No. 4: 461. Oct., 1942.

A group of anterior pituitary materials and extracts which varied

widely as to the kind and amount of hormones contained therein were assayed for their properties in promoting mammary lobule-alveolar growth. The ovariectomized mouse was used as the assay animal and 75 I.U. of estrone per mouse per 10 days were injected in addition to the extracts to be assayed. The mammogenic lobule-alveolar growth factor was found to be protein in nature as are the other anterior pituitary hormones. Comparative assays indicated that the mammogenic lobule-alveolar growth factor was not identical with either the lactogenic, thyrotropic or gonadotropic hormones. R.P.R.

157. Effect of Diethylstilbestrol on Mammary Gland Development in Dairy Animals. A. A. LEWIS AND C. W. TURNER, University of Missouri, Columbia. Endocrinology, *31,* No. 5: 520. Nov., 1942.

The mammary glands of virgin or dry goats were greatly increased in size over the control condition by treatment with diethylstilbestrol and diethylstilbestrol diproprionate pellets. In 5 cases histological examination indicated fairly normal, although not complete, lobule-alveolar development after extended treatment as compared to the complex duct system found in control virgin goats. The histological structure of the mammary glands in 3 goats treated with diethylstilbestrol was not that of normal alveolar lobules but consisted of solid masses of cells. The mammary glands of male goats in spite of heavy dosage and prolonged treatment failed to respond to diethylstilbestrol dipropionate pellets placed subcutaneously. A mature multiparous goat which gave as much as a liter of milk a day from one mammary gland showed comparatively little epithelial development following treatment. Small isolated clusters of alveoli or ducts and hypertrophied main ducts filled with secretion were present. An aged sterile cow showed fairly well-developed lobule-alveolar systems in a normal as well as in 3 abnormal quarters of the udder after prolonged diethylstilbestrol treatment and lactation. R.P.R.

158. Effect of Adrenalectomy on the Lactogenic Hormone and Initiation of Lactation. JOSEPH MEITES, J. J. TRENTIN, AND C. W. TURNER, University of Missouri, Columbia. Endocrinology, *31,* No. 6: 607. Dec., 1942.

One hundred and eleven albino and white-hooded female rats and 29 male and female guinea pigs were used in this study. In two groups of female albino rats adrenalectomy caused a reduction of 27.5 and 25.7%, respectively, in hormone content on a body-weight basis when compared with normal intact rats of the same species. The injection of 1000 I.U. of estrone into 10 intact female albino rats increased the average lactogen content of the pituitary gland by 216.9% on a body weight basis. Similar injections into 14 adrenalectomized rats increased the pituitary lactogen con-

tent by only 104.5%. The injection of estrone is adrenalectomized male and female guinea pigs caused increases in pituitary lactogen content equal to those obtained in intact guinea pigs. Most of the guinea pigs survived but a few days after adrenalectomy and it was impossible to conclude that the full effects of adrenalectomy were operative on the pituitary. The pituitary lactogen content of female albino and white-hooded rats adrenalectomized during the last week of pregnancy and killed 48 hours after parturition was found to be equal to that of intact rats killed 48 hours after parturition. Milk was present in the mammary glands of the operated rats and in the stomachs of their living young but not in amounts equal to that of intact rats and their young. It was concluded that the failure of rats, adrenalectomized during pregnancy, to lactate sufficiently following parturition was not due to a deficient pituitary lactogen content. R.P.R.

159. Effect of Pregnancy and Lactation upon the Thyrotropic Hormone of the Rabbit. A. J. BERGMAN AND C. W. TURNER, University of Missouri, Columbia. Endocrinology, *32,* No. 1: 59. Jan., 1943.

Normal New Zealand White rabbits ranging in body weight from 3000 to 4000 gm. were mated and then killed at 10, 20, and 28 days of pregnancy and at 2, 5, 10, 20, and 30 days post partum. Pituitaries were removed, weighed, and kept in a frozen condition until assayed on one-day-old White Leghorn male chicks. On the basis of thyrotropic hormone content per pituitary, per gm. of pituitary tissue, and per 100 gm. of body weight the values for the pregnant animals were within the range of the corresponding values for normal non-pregnant females. There was a tendency for the pituitaries to be slightly heavier during the first 10 days post partum and as a result there was a slight increase in thyrotropic hormone content per anterior pituitary without a corresponding increase in the concentration per gram of tissue. Neither the removal of the young from the mothers for a period of 15 hours before sacrifice nor a 3-hour nursing period just before killing the mothers influenced the concentration of the thyrotropic hormone in the pituitary. R.P.R.

160. Effect of Hypophysectomy on the Concentration of Ascorbic Acid in the Adrenals of the Rat. R. TYSLOWITZ, Harvard Medical School and Peter Bent Brigham Hospital, Boston. Endocrinology, *32,* No. 1: 103. Jan., 1943.

The weight of the adrenals and the concentration of ascorbic acid in the adrenals of male rats hypophysectomized at 21 to 40 days of age were determined one to 56 days after hypophysectomy. A gradual decrease was observed, however, this was not specific for the adrenals as ascorbic acid concentration in the testis, liver, kidney, and blood serum showed a similar

decrease. The starvation of normal rats for 6 days reduced the ascorbic acid in the liver and kidney to a greater degree than in the testis, adrenal, or blood serum. Adrenals of normal and hypophysectomized female rats showed values for ascorbic acid similar to those in males. The injection of suitable pituitary extracts increased the concentration of ascorbic acid in the adrenals of hypophysectomized rats. Adrenal ascorbic acid concentration was found to be high in instances of incomplete hypophysectomy. Removal of the thyroid in addition to the pituitary gland in rats did not alter the results observed following the injection of pituitary extracts into hypophysectomized animals. R.P.R.

161. In-vivo Activity of Streptothricin against *Brucella abortus*. H. J. METZGER, SELMAN A. WAKSMAN, AND LEONORA H. PUGH, N. J. Agr. Exp. Sta., New Brunswick. Soc. Exp. Biol. and Med., Proc., *51,* No. 2: 251. Nov., 1943.

An antibiotic substance, streptothricin, obtained from a soil Actinomyces was tested against *Brucella abortus* in vitro and in vivo with favorable results. Ten mg. of crude streptothricin, administered 24 hours after the inoculation of 15-day incubated eggs with approximately 2000 *Br. abortus* cells, was sufficient to bring about complete destruction of this organism in the living chick embryo. Guinea pig studies indicated that streptothricin offers considerable promise as an antibiotic agent against brucellosis in animals. R.P.R.

162. Normal Development and Experimental Treatment of the Opossum Mammary Gland Primordium. DOROTHY WELLS PLAGGE, University of Chicago, Chicago, Ill. Soc. Exp. Biol. and Med. Proc., *51,* No. 2: 219. Nov., 1942.

A study was made of the normal mammary gland development in the male and female opossum up to the age of 100 days. The normal growth pattern of the mammary primordia was modified by treatment with estradiol testosterone, and testosterone propionate. Equine gonadotropin in the dosages and ages used had no effect on mammary gland development.

R.P.R.

163. The Antagonistic Effect of Lipocaic and the Anterior Pituitary on Fat Metabolism. ORMAND C. JULIAN, DWIGHT E. CLARK, JOHN VAN PROHASKA, C. VERMEULEN, AND LESTER R. DRAGSTEDT. Department of Surgery, University of Chicago. Amer. Jour. Physiol., *138,* No. 2: 264–268. January, 1943.

It seems probable that the anterior pituitary liberates a substance into the circulation which causes a migration of body fat to the liver. Excessive

breakdown of this fat results in hyperketonemia and ketonuria. Lipocaic, on the other hand, opposes at least a part of this effect and causes the migration of fat to the body depots. Evidence with respect to the effect of lipocaic on the ketonemia and ketonuria resulting from the injection of the ketogenic hormone is not available. In an accompanying article from this laboratory (*Ibid.*, pp. 352–356) one finds this statement: "The total loss of pancreatic juice from the body does not produce hypolipemia or fatty infiltration of the liver. The conclusion that lipocaic is not present in pancreatic juice to any significant extent is confirmed." D.E.

164. Effect of Ultraviolet Radiation on Body Weight of Mice. Harold F. Blum, Hugh G. Grady, and John S. Kirby-Smith. National Cancer Institute, National Institute of Health, U. S. Public Health Service, Bethesda, Maryland. Amer. Jour. Physiol., *138*, No. 2: 378–384. January, 1943.

Ultraviolet radiation of wavelengths shorter than 3200Å slows normal gain or may reduce the body weight of mice. Below a certain dosage body weight is not affected. There is no evidence of growth stimulation at any dosage. Wavelength 3130Å is almost without effect in the production of vitamin D, whereas 2537Å is quite effective, and this is the reverse of the relative effectiveness of these wavelengths in reducing body weight. In one experiment the food consumption of irradiated and control animals was followed daily, and found to be distinctly less for the former. D.E.

165. An Androgenic Substance in Feces from Cattle as Demonstrated by Tests on the Chick. Gardner M. Riley and John C. Hammond, Beltsville Research Center, Beltsville, Md. Endocrinology, *31*, No. 6: 653. Dec., 1942.

Urine-free feces of cows in various stages of gestation and of unbred heifers when dried and fed to one-day-old Rhode Island Red chicks as a supplement to their diet stimulated comb-growth. Testicular and ovarian development was retarded when chicks were fed material containing the active factor. One or more androgenic principles was thought to be present. Both alcohol and chloroform extracts of cow feces showed androgenic activity. A chloroform extract of 1.0 gm. of dried cow feces had at least as much androgenic activity as 16 micrograms of testosterone acetate. Feces from mature bulls showed no effect on either comb or gonadal development. R.P.R.

166. Electrocardiograph Studies in Normal Dairy Cattle. B. V. Alfredson and J. F. Sykes. Jour. Agr. Res., *65*, No. 2. July, 1942.

Three serial electrocardiograms, taken approximately one month apart,

were obtained on each of 97 normal dairy cattle. A brief summary of the data derived from the analysis of these records is as follows:

(1) The heart rate of the cattle studied averaged 71.6 beats per minute. The rate for animals up to $1\frac{1}{2}$ years old was significantly greater than this (82.5) while for animals over $1\frac{1}{2}$ years of age the rate was lower (67.8). (2) The duration of the PR interval ranged from a minimum of 0.10 second to a maximum of 0.30 second, with an average of 0.19 second. The QRS interval averaged 0.09 second. (3) The systolic index as determined by Bazett's formula ranged from 0.34 to 0.48 with an average of 0.418. (4) The form and occurrence of the various deflections is discussed. (5) The potential of the several deflections was generally small. (6) The electrical axis of QRS ranged from $+30°$ to $+180°$ and from $-30°$ to $-170°$. (7) The electrocardiograms of the various individuals were apparently unaffected by the breed of the animal.

The authors present the following conclusions: (1) The bovine electrocardiogram resembles that of the human subject only in one respect and that is the interval lengths are nearly alike in the two species. (2) Bovine electrocardiograms can be classified into four main groups. (3) Variations in interval length and in the form and potential of the various deflections occur much more frequently in the bovine than in the human electrocardiogram. (4) The electrical axis QRS varied within a very wide range and with the methods used no very definite limits could be set. H.P.

167. The Cow's Udder. W. E. PETERSEN. Minn. Agr. Expt. Sta., St. Paul. Bul. 361. June, 1942.

An attractive and effectively illustrated treatise on the growth, development, structure, and functioning of the cow's udder, written in a style that will be pleasing, and readily understood by the non-scientific reader. Eleven simply stated rules for good milking practice are prominently featured.

J.G.A.

168. Arrangement of the Tissues by Which the Cow's Udder is Suspended. W. W. SWETT, P. C. UNDERWOOD, C. A. MATHEWS, AND R. R. GRAVES. Jour. Agr. Res., *65,* No. 1. July, 1942.

This study was undertaken to obtain a more comprehensive knowledge of the supporting tissues of the udder and to determine the nature of the structures that support and maintain the udder in a well-balanced suspension. Twelve figures are included, eleven of which are excellent photographs of the cow used and dissections of structures suspending the udder in their normal position. In the illustrations that accompany the discussions are clearly shown the fine areolar subcutaneous tissue, the cordlike coarse areolar tissue, the superficial lateral sheets, the deep lateral sheets,

the subpelvic tendon from which the superficial and deep lateral layers arise, and the heavy yellow elastic sheets which arise from the abdominal wall. H.P.

MISCELLANEOUS

169. Maintenance and Care of Refrigeration Plants. FRED D. MOSHER. Ice and Refrig., *103*, No. 4: 194. Oct., 1942.

Regular inspections should be made; daily in large plants, weekly in small plants, and monthly for small automatic units. Records should be kept of temperatures, pressures, flows, etc. In this way changes are readily noted, and corrective measures employed. Water going through the condenser should be checked for pH. Regular oil purging of the condenser is necessary. Brines should be tested regularly and treated when necessary. Air vents for purging should be located at high points on the system. The entire system should be checked for leaks of all kinds, such as oil, water, brine, steam, and refrigerant. Belts should be checked regularly. Slack belts are inefficient, and tight belts waste power and belts. All electrical apparatus should be checked once a month. Compressors should be opened and inspected once a year. L.C.T.

170. Reclaiming Refrigeration Equipment with Metallizing. WILLIAM C. REID, Metallizing Engin. Co., Inc., Long Island City, N. Y. Ice and Refrig., *103*, No. 6: 347. Dec., 1942.

In the past metallizing for repair was not widespread because such parts were readily obtainable. Since this is not now the case the process will become more important for the repair of pump plungers, crank shafts, pistons, plates of equipment to make them corrosion resistant, etc. An outstanding advantage of the process is the fact that any metal can be permanently bonded to any similar or dissimilar base metal. There is no heat distortion or danger of crystallization or disturbance of physical characteristics of the base metal.

There are three steps in the metallizing process: (1) The surface to be built up must be roughened. This may be done by placing the object in a lathe and undercutting below the point of wear, after which it is roughened by grit blasting, threading or grooving. (2) The surface is then sprayed with the desired molten metal. Oxygen and acetylene flames generally provide the heat. (3) The sprayed surface must be machined or ground to the desired dimension and shape. L.C.T.

171. Conservation of Power in Refrigeration. L. C. THOMSEN, Dairy Inds. Div., Univ. Wis., Madison. Ice Cream Trade Jour., *39*, No. 1: 20. Jan., 1943.

Examples are given to show that compressors should be operated at the maximum possible suction temperature (pressure) which will give the desired temperature to the product to be refrigerated. A ten-ton machine operated for 10 hours at 35 pounds suction pressure may cost about $1.00 and if the same machine were operated at 15 pounds suction pressure it would have to operate 17 hours at a cost of $2.50.

It is not always possible to operate a machine at its optimum suction pressure because the surface area of the expansion unit is too small, or the velocity of the air; the water, the brine, or the dairy product over the expansion surface is too low, or the refrigerant within the expansion unit is moving too slowly, or the condition of the heat exchanging surface may be unsatisfactory because of oil, scale or frost which may be present.

If the surface area of the expansion unit cannot be increased, the velocity of the air, water, brine or dairy product may be increased. Low suction pressures sometimes are the result of a lack of refrigerant. Leaks of ammonia are easily detected and steps should be taken to overcome them. Corroded valves which sometimes stick should be cleaned carefully with fine emery cloth and the right kind of oil applied. Care should be taken to draw up packing ring on stuffing boxes evenly and not too tightly to avoid damage to the crank shaft.

It is not easy to detect Freon leaks, and after a system has been installed for 8 or 10 years all joints should be carefully inspected for leaks. The addition of anti-freezing compounds to Freon system to prevent the freezing of expansion valves due to moisture is not recommended.

High head pressures may be due to the presence of non-condensable gases in the system, oil coatings on the condensing surfaces, scale or sludge on the water side of the condenser, location of the condenser in a warm room condensing water too warm, an insufficient supply of condensing water or a condenser that is too small.

Proper purging should remove non-condensable gases from the system. Thorough cleaning of the condenser surfaces will remove scale and oil. By checking the temperature of liquefaction of the refrigerant and the discharge water it is possible to tell if non-condensable gas, oil or scale are responsible for high pressures. The temperature of refrigerant should be within 5° F. of that of the water.

Brine should be checked at regular intervals for quantity, concentration and chemical reaction. The brine level should not be below that of the expansion coils. Brine should have a pH of between 7 and 8.

The author also presented table giving the properties of saturated ammonia and Freon and formula for calculating the valid capacity of a refrigeration system.

$$\frac{\pi \; r^2 \times L \times C \times N \times E}{D}$$

$\pi = 3.1416$, r = radius of cylinders expressed in feet, C = number of cylinder in compressor, N = number of strokes, E = volumetric efficiency of compressor expressed decimally and D = cubic feet of saturated refrigerant which must be displaced per minute per ton of refrigeration at standard conditions.

For an ammonia machine operating at standard conditions

$$D = \frac{200 \times 8.15}{613.3 - 138.9} = 3.43 \text{ cu. ft. per minute}$$

For a Freon machine operating at standard conditions

$$D = \frac{200 \times 1.49}{78.8 - 27.7} = 5.83 \text{ cu. ft. per minute.}$$

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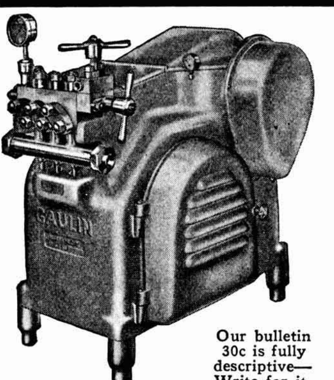

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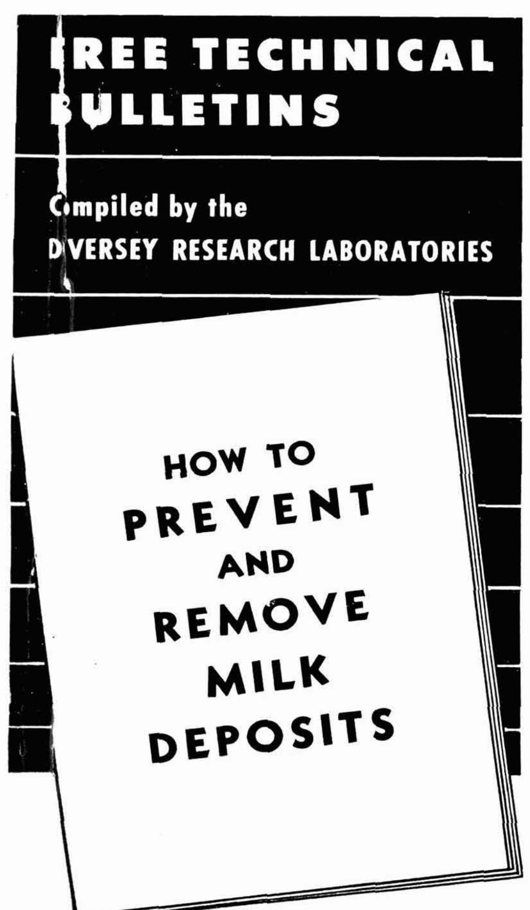

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Definition of milkstone . . . four general conditions under which milk deposits are formed on dairy equipment . . . chemical composition of milkstone . . . four major objections to milkstone . . . factors affecting the composition and amount of milkstone formations . . . how to prevent the formation of milkstone . . . removal of milk deposits and other contamination present . . . treatment of plate type equipment . . . treating milk cans . . . the *"coin test" for detecting the presence of milkstone* . . . bibliography of references.

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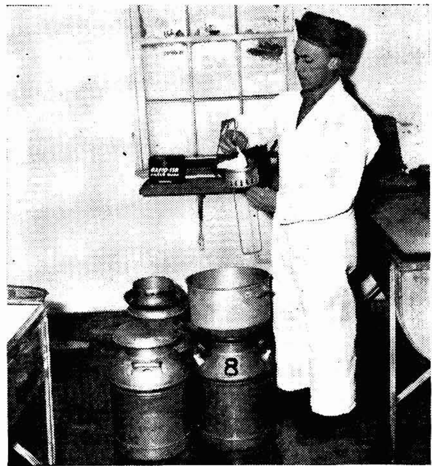

Acute farm labor shortages are placing added burdens upon milk sanitarians, already faced with the urgent need for quality milk products in 1943. Johnson & Johnson, through its nationwide field educational service, is maintaining an effective program to aid quality milk production that began with the introduction of Rapid-Flo filter disks.

Painstaking research in the laboratory and on the farm, a basic and continuous procedure in the development of Rapid-Flo—high quality Virgin Cotton—full weight and uniformity—these are the quality safeguards which assure maximum filtration efficiency.

Dairy sanitarians, recognizing the value of technical skill in quality control, know that Rapid-Flo filter disks and quality dairy products go hand in hand. Filter Products Division, Johnson & Johnson, 4949 West 65th Street, Chicago, Ill.

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RAPID-FLO

FILTER DISKS—ROLLS—STRIPS