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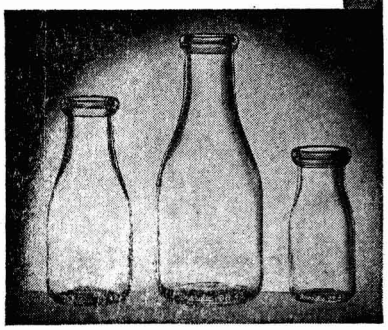

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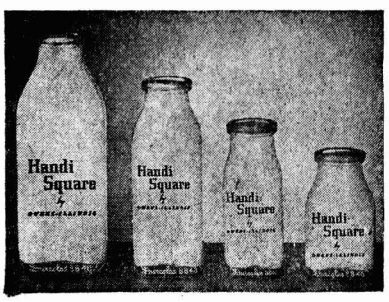

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MODIFICATION OF THE PHOSPHATASE TEST AS APPLIED TO CHEDDAR CHEESE AND APPLICATION OF THE TEST TO FLUID MILK

GEORGE P. SANDERS AND OSCAR S. SAGER

Division of Dairy Research Laboratories, Bureau of Dairy Industry, Agricultural Research Administration, U. S. Department of Agriculture

A description of a phosphatase test applicable to Cheddar cheese, to determine whether or not the milk used in making the cheese had been pasteurized, was published by us in 1945 (1, 2). Recently, as a result of additional research on the chemistry of (a) the enzymic hydrolysis and of (b) the indophenol color reaction, the test has been improved further to make the results more precise and quantitative. This report is a description of the modified test applied to Cheddar cheese and also to fluid milk. The modifications are described briefly as follows.

(a) The optimal pH of hydrolysis for the production of phenol in the presence of barium buffer substrate, with the concentration of buffer used in this test on milk, has been found to be 10.0 to 10.05, rather than a lower value found previously with a buffer substrate containing a relatively high concentration of sodium tetraborate. The composition of the buffer has been adjusted accordingly, and it yields relatively uniform pH values with different samples. The increased precision of the pH adjustment with the buffer described herein has resulted in an increase in the quantity of phenol liberated in definitely positive tests over that liberated in the test described earlier, and the additional advantage in the use of barium in the test, to reduce the effects of phosphates and of other interferences, has been verified.

(b) Results obtained in research on the indophenol color reaction have shown that the use of an auxiliary protein precipitant makes it possible to increase the sensitivity, precision, and quantitative accuracy of the test. With an auxiliary precipitant, the need for boiling the test after incubation has been eliminated—the test is heated only to 90° C. (194° F.), sufficiently to inactivate the enzyme. The elimination of boiling has been found advantageous since boiling causes some hydrolysis of the disodium phenyl phosphate and thus results in the formation of a trace of blue color in the blank. Moreover, prolonged boiling may result in some caramelization. Thus the use of an auxiliary protein precipitant results in complete protein precipitation without boiling, yielding a perfectly clear, colorless filtrate.

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Numerous protein precipitants were investigated, and it was found that BQC produces considerably more blue color, with excess phenol, in the presence of zinc than in the presence of lead, and that under such conditions copper has a remarkable catalytic effect on the rate of formation and the final amount of blue indophenol color produced and also aids in reducing the amount of visible yellow color in the blank. Therefore, a combination of zinc and copper salts was introduced as an auxiliary precipitant in the modified test.

Coincident with the demonstration of the value of copper in the development of blue color in the test, it became necessary to determine the quantity of copper to use in testing different products. A difficulty in the use of copper is the fact that an excess quantity of it imparts a copper-blue color to the filtrate. This difficulty was overcome by determining the optimal quantity of copper that would produce the desired catalytic effect without causing appreciable interference. The required quantity of the copper salt—different for milk than for cheese—was then combined with a quantity of zinc salt sufficient to complete the precipitation of the proteins and reduce the pH to 9.1 to 9.2.

The modification includes also the development of an additional buffer, made with sodium metaborate and sodium chloride and referred to as the color-development buffer, added to the test filtrate to automatically adjust the pH correctly. The optimal pH for the complete BQC-phenol coupling reaction in the improved test has been found to be within a range of 9.1 to 9.5. More yellow off-color is produced at still higher pH values, and slightly more blue and less yellow at pH 9.1. However, the reaction is slower at pH 9.1 than at 9.5. This improved color-development buffer adjusts the pH at 9.3 to 9.4—suitable for complete color development in the time specified, with a minimum of off-color. The use of it performs the additional function of diluting the interfering substances present, thus further reducing the reading of the blank.

One of the aims of the development work has been to simplify the phosphatase test. For example, preliminary preparation of the sample with the use of a mortar and pestle or with a blender has not been found necessary; the alkalinity and the strength of the buffers have been adjusted to give results that are reliable for all samples without the need of determining and adjusting the pH when testing; and all reagents are prepared by weighing, hence the need for primary standards or for making solutions of designated normalities for the test has been eliminated.

I. APPLICATION TO CHEDDAR CHEESE

Reagents

Barium borate-hydroxide buffer for Cheddar cheese. Dissolve 25.0 g. of C.P. barium hydroxide ($Ba(OH)_2 \cdot 8H_2O$, fresh, not old) in distilled water

and dilute to 500 ml. Dissolve, in another flask or cylinder, 11.0 g. of C.P. boric acid (H_3BO_3) and dilute likewise to 500 ml. Warm each to 50° C. (122° F.), mix the two solutions together, stir, cool to approximately 20° C. (68° F.), filter, and stopper the filtrate tightly. The pH of the barium borate-hydroxide filtrate is approximately 10.6, and a mixture of 9 ml. of it with 0.5 g. of Cheddar cheese yields, with different samples, pH values of 10 to 10.05 $\pm$ 0.15.

Disodium phenyl phosphate substrate. Specify phenol-free, crystalline disodium phenyl phosphate.[1] Prepare a stock solution by dissolving 1.0 g. in 9.0 ml. of the color-development buffer described below. Before use, remove any free phenol by adding 1 drop of BQC, developing the color for 30 minutes at room temperature or 15 minutes at 37–38° C. (99–100° F.) and extracting the color with 5 ml. of n-butyl alcohol (Scharer extraction method). Draw off and discard the alcohol. The extraction should be repeated if there is much blue color in the alcohol layer, but usually one extraction is sufficient. This stock solution should be kept in a refrigerator and, if used again after standing for several days, should be re-extracted before use.

To prepare the buffer substrate for several days' use, make up this entire stock solution of purified disodium phenyl phosphate to 1 liter with the barium buffer; or for daily use, prepare fresh buffer substrate by adding 1 ml. of this stock solution of purified disodium phenyl phosphate to 100 ml. of the barium buffer. Store the buffer substrate in a refrigerator.

It has been found that 1 g. of disodium phenyl phosphate per liter, as described above, is sufficient for detecting under-pasteurization. However, for quantitative tests on raw or definitely under-pasteurized samples, somewhat more phenol is liberated if the concentration of purified disodium phenyl phosphate is increased to 2 g. per liter or to 2 ml. of the stock solution per 100 ml. of the buffer.

Zinc (or zinc-copper) precipitant for Cheddar cheese. Weigh 6.0 g. of zinc sulfate ($ZnSO_4 \cdot 7H_2O$) and make up to 100 ml. with distilled water. For testing non-ripened cheese, not more than a few days old, for use in the rapid field test, and for use in all tests in which the butyl alcohol extraction method is used, add 0.1 g. of copper sulfate ($CuSO_4 \cdot 5H_2O$) before making up with water.

The optional use of either zinc or zinc and copper is suggested for the test on cheese, since it has been found that, in tests on ripened cheese, copper catalyzes also the formation of a violet- or purple-colored compound resulting from chemical reaction of BQC with decomposition products (probably amino acids) present in ripened cheese. In tests that are to be read in aqueous solution, the use of the zinc-copper precipitant is suggested for testing

[1] This compound, relatively pure, is obtainable from Applied Research Institute, 139 Fifth Avenue, New York 10, New York.

green (non-ripened) cheese—not more than a few days old—and the use of the zinc precipitant without copper is suggested for testing ripened cheese.

The violet-colored compound produced in the presence of copper in tests on ripened cheese is not sufficiently soluble in butyl alcohol to cause appreciable interference in tests in which the butyl alcohol extraction method is used. Therefore, the use of the zinc-copper precipitant is suggested for all tests in which the color is to be extracted.

Color-development buffer. Mix 6.0 g. of sodium metaborate ($NaBO_2$)[2] with 20.0 g. of sodium chloride and make up to 1 liter with distilled water. The pH of this buffer is approximately 9.8, and that of a mixture of 5 ml. of it with 5 ml. of the test filtrate is 9.3 to 9.4.

The color-development buffer is used also for adjusting the pH in the following steps: extracting free phenol from the disodium phenyl phosphate; adjusting correctly the alkalinity of the butyl alcohol; and preparing the phenol standards. Also, a solution containing 1 part of it in 9 parts of water is used for preparing dilutions of the test, in order to reduce the color of strongly positive tests so that they will be in a readable range for colorimetric analyses.

2,6-dibromoquinonechloroimine (BQC) solution (Gibbs' reagent). Dissolve 40 mg. of the pure substance in 10 ml. of methyl or ethyl alcohol and transfer to a dark-colored dropper bottle. This reagent remains stable for at least a month if kept in the ice tray of a refrigerator. Do not use it after it begins to turn brown.

To prepare the chloroimine solution from INDO–PHAX tablets,[3] dissolve 1 tablet in 5 ml. of methyl or ethyl alcohol and keep in a dark bottle as indicated above.

The 2,6-dichloroquinonechloroimine reagent (CQC) can be substituted for the dibromo reagent, if desired, provided the standards are prepared with it also, but it requires a slightly longer time for full color development. It, as well as the tablets mentioned above, produces blue color at a slower rate than BQC, but a given quantity of it eventually produces more blue color in strongly positive tests than the same quantity of BQC.

It is recommended that CQC, rather than BQC, be used in the field test, because CQC is relatively stable and is affected less by protein interference in the test.

Butyl alcohol. Specify n-butyl alcohol, B.P. 116–118° C., for making standards and for quantitative work. The cheaper grade of n-butyl alcohol can be used for routine testing. To adjust the pH, mix 50 ml. of the color-development buffer with 1 liter of the butyl alcohol.

Phenol standards. Weigh exactly 1.0 g. of U.S.P. phenol and make up

[2] Obtainable from Amend Drug and Chemical Company, 117 East 24th Street, New York 10, New York.

[3] Obtainable from Applied Research Institute.

to about 700 ml. with distilled water in a liter flask. Add 150 ml. of the color-development buffer to form a solution of sodium phenolate with a pH of approximately 9.25, which is more stable than phenol. Add 3 ml. of chloroform and water to the 1,000-ml. graduation, and mix. One ml. of this stock solution contains 1 mg. (0.001 g.) of phenol. Pipet 1.0 ml. of the stock solution into a liter flask, add water to the 1,000-ml. graduation, and mix. One ml. of this standard solution contains 1 mmg. (0.000001 g.—1 microgram, gamma, or unit) of phenol. Prepare additional solutions as needed, containing, for example, 2, 5, 10, 20, and 40 mmg. of phenol per ml. by diluting 2.0 ml. of the stock solution to 1,000, 5.0 to 1,000, 5.0 to 500, 10.0 to 500, and 20.0 to 500 ml., respectively. From these, measure appropriate quantities into a series of tubes, preferably graduated at 10.0 ml., to provide a suitable range of phenol standards containing 0 (blank) 0.5, 1.0, etc., to 40 units.

A solution containing copper is added at this point to increase the brightness of the blue color and to improve the stability of the standards. Prepare a 1 per cent solution of copper sulfate in distilled water, dilute 5 ml. of it to 100 ml., and pipet 1 ml. of this 0.05 per cent solution into each tube.

Add color-development buffer to each tube to bring the volume to 10.0 ml. Add 4 drops (0.08 ml.) of BQC to each, and mix. Allow to develop for at least 30 minutes at room temperature. If the butyl alcohol extraction method is to be used in the test, extract the standards at this point with butyl alcohol as described in the laboratory test.

Read the color intensities with a photometer with the proper filter, subtract the value of the blank from the value of each phenol standard, and prepare a standard curve; or, for use in visual comparisons, store the standards in a refrigerator.

SAMPLING

Take a sample from the interior of the cheese with a *clean* Roquefort trier, place it in a small tube, stopper the tube, and keep it in a refrigerator.

LABORATORY TEST

Weigh carefully, on a *clean* watch glass, 0.5 g. of cheese and place it in a culture tube 16 or 18 × 150 mm. Macerate the sample with a glass rod about 8 × 180 mm., pipet in 1.0 ml. of buffer substrate, complete the maceration, pipet in 8.0 ml. more of buffer substrate (total 9.0 ml.), and mix thoroughly with the rod. To include in the test the fatty material adhering to the rod, cut a piece of filter paper approximately 1 × 1¼″, wrap and hold it tightly around the rod, rotate the rod while withdrawing it from within the tube in such a manner as to wipe the rod clean, and insert the paper with the fat into the test. Stopper the tube and mix the contents thoroughly by shaking. Incubate in a water bath at 37–38° C. for 1 hour, preferably shaking the tube occasionally. Place the tube in a beaker of boiling water and leave it for 1

minute, or heat the contents to at least 90° C. (194° F.). Cool to room temperature. Pipet in 1.0 ml. of the zinc precipitant (for cured cheese) or the zinc-copper precipitant (for green cheese), mix thoroughly, and filter (5-cm. funnel, 9-cm. Whatman No. 42 paper recommended). Pipet into another tube (preferably graduated at 5.0 and at 10.0 ml.) 5.0 ml. of the filtrate and add 5.0 ml. of color-development buffer. Add 4 drops of BQC, mix, and let stand at room temperature for 30 minutes or at 37–38° C. for 15 minutes.

At this point, the blue color in tests with phosphatase values of about 5 or more units can be detected visually in the aqueous solution.

For detecting under-pasteurization in border-line instances—*e.g.,* tests yielding 0.5 to 5 units of color—and for more quantitative results with all samples, the intensity of the blue color is increased and that of the off-color in the blank is diminished by extracting the color with n-butyl alcohol. Add 5.0 ml. of the alcohol (Scharer extraction method) and invert the tube slowly several times. Compare visually the blue color in the alcohol layer with the colors of standards, or draw off and filter the alcohol and measure the color in it by means of a colorimetric instrument.

A control (blank) determination should be conducted with every sample tested, using 0.5-g. of cheese, macerating it in the tube and heating it to at least 90° C. before adding the buffer substrate. Subtract the value of the blank from that of each test.

Alternatively, to avoid gumminess of the blank when it is heated, macerate it in the tube, pipet in 1.0 ml. of the barium buffer (without substrate added), then mix and heat to 90° C., cool, pipet in 8.0 ml. of the barium buffer substrate, and proceed with incubation and subsequent steps as described above.

Since 0.5 g. of cheese is used and the quantity of filtrate used is equal to one-half the total quantity of solution (buffer substrate plus precipitant), the result obtained as described above is recorded as units of color or phenol equivalents in mmg. per 0.25 g. of cheese. However, the result may, if desired, be converted to phenol equivalents in mmg. per 1 g. of cheese.

If it is more convenient to add a total of 10.0 rather than 9.0 ml. of buffer substrate, this may be done and it will not affect significantly the pH of the mixture. The additional dilution of the test will increase slightly the quantity of phenol liberated. In calculating the result obtained when using 11.0 ml. of liquid in the mixture and 5.0 ml. of filtrate, multiply the value obtained by 1.1 to obtain the result in phenol equivalents per 0.25 g. of cheese.

In testing samples that are observed during color development to be strongly positive—*e.g.,* approximately 20 units or more—in which the quantity of BQC specified is not sufficient to combine with all of the phenol, quantitative results may be obtained by means of dilutions made as follows: While the color is developing, pipet out exactly half the contents (5.0 ml.) into another tube, pipet in with it 5.0 ml. of a 1 to 9 dilution of the color-

development buffer, add 2 drops more of BQC, and allow to develop. To reduce the color at the end of the development period to conform with the range of standards or of the photometer, repeat this one-half dilution procedure as many times as necessary, allowing the full specified time for color development after the last addition of BQC. With each test diluted thus, conduct a blank determination in the corresponding manner.

Samples of cheese made from raw milk may yield values as high as 1,000 units of color per 0.25 g. of cheese. Therefore, to save time when testing such cheese, the dilutions may be increased. For example, a first dilution of one-fifth (2.0 ml. of test solution plus 8.0 ml. of diluted color-development buffer) rather than one-half may be used, followed by a second dilution of one-fifth (2.0 ml. of the diluted test solution plus 8.0 ml. of the diluted buffer), thus diluting the solution one-twenty-fifth in only two steps. Develop the color as indicated above.

Photometric Determination

To read the color in aqueous solution, use a filter with maximum light transmission in the region of 610 mμ wavelength.

To read the color in butyl alcohol, extract the color as described above and centrifuge the sample for 5 minutes to break the emulsion and to remove the moisture suspended in the alcohol layer. A Babcock centrifuge can be adapted for this purpose by making special tube holders as follows: Slice a section ¼ inch thick from a rubber stopper of suitable diameter to fit in the bottom of the centrifuge cup. Glue together two cork stoppers of appropriate diameter, bore through the center a hole of proper size to hold the tube snugly, and insert the double cork section in the cup. After centrifuging, remove nearly all of the butyl alcohol by means of a pipet with a rubber bulb on the top end. Filter the alcohol into the photometer cell and read with a filter with maximum light transmission in the region of 650 mμ wavelength.

If more than approximately 4 ml. of butyl alcohol is required for the photometer used, the test is conducted in a larger tube and the color is extracted with the necessary quantity of butyl alcohol rather than with 5 ml. specified above.

Rapid Field Test

Macerate 0.5 g. of cheese with the buffer substrate, as described in the laboratory test, adding a total of 9 ml. of buffer substrate. Mix thoroughly and incubate for 30 minutes at 37–38° C. Add 1.0 ml. of the zinc-copper precipitant. Add 8 drops of CQC, mix thoroughly, and allow the color to develop for 15 minutes at room temperature. Conduct a control (blank) determination using a 0.5-g. sample of the cheese, macerating it in the tube and heating it to at least 90° C. before adding the buffer substrate.

To increase the precision, especially for border-line tests, the color may

be extracted with 5 ml. of the n-butyl alcohol before making comparisons with the colors of standards. When it is necessary only to detect under-pasteurization, a few standards, *e.g.*, 1, 2, 5, and 10 units, may be sufficient; under these conditions, extraction with butyl alcohol is necessary for only those tests in which the results are doubtful.

Precautions

The presence of free phenol in the buffer substrate is frequently the cause of a trace of blue color in the blank determination. It is therefore desirable to purify the stock solution of disodium phenyl phosphate by extracting it immediately before use.

The length of time that the crystalline disodium phenyl phosphate and the BQC powder will remain stable can be increased greatly by keeping them in the freezing chamber of a refrigerator.

The glassware and stoppers should be scrupulously clean and it is desirable to soak them in hot, running water after cleansing.

The solid barium hydroxide and the barium buffer must be kept stoppered tightly to prevent absorption of carbon dioxide.

Phenolic contamination from plastic closures on reagent bottles has been encountered, and the use of plastic closures should be avoided. Rubber stoppers should not be used in flasks in which butyl alcohol is stored. Glass or cork stoppers should be used.

Interpretation

Results obtained with this test on cheese made from milks containing small proportions of raw milk in pasteurized milk, and also corresponding results published earlier, show that the enzyme is more concentrated in cheese than in the milk from which it was made. The earlier results indicate also that the enzyme is not completely inactivated under the temperature and time conditions specified in pasteurization standards. The modified test described here yields less blue color in the blank and also less yellow off-color in the blank than earlier tests, and therefore yields results that are more precise for detecting under-pasteurization. On the basis of our results, it is suggested tentatively that values greater than 3 mmg. of phenol per 0.25 g., or 12 mmg. per 1 g., of cheese be considered as indicating under-pasteurization.

Results

Results obtained in using the modified test on samples of cheese of different ages show that the enzyme is much more stable in cheese than has been generally supposed. Data obtained on raw-milk cheese 18 months old, illustrated in table 1, show phosphatase values more than half as great as those obtained in tests on raw-milk cheese 2 days old. Moreover, analyses made with definite quantities of phenol added to test filtrates prepared with sam-

ples of cheese of various ages, without disodium phenyl phosphate substrate added, show that the color-reaction inhibition or interference increases with the age of the cheese, and that the increased interference is the result of decomposition products accumulating in the cheese as it ripens. As the interference (less blue and more yellow color in the test) increases with the age of the cheese, the amount of off-color in the blank increases, the efficiency of the BQC reagent in the test diminishes, and the results for detecting under-pasteurization become slightly less precise. Finally, phenol present in the cheese-test filtrate cannot be determined quantitatively regardless of how much BQC is added. The failure to determine the phenol quantitatively becomes more and more marked as the age of the cheese increases.

TABLE 1

Phosphatase values of Cheddar cheese of different ages and of the milk and whey

Lot No.	Age of cheese when tested	Description of milk	Phosphatase value, phenol equivalent		
			Cheese	Milk	Whey
			mmg./g.	*mmg./ml.*	*mmg./ml.*
1031	18 months	Raw	1640	.....	.....
1107	18 months	Raw	1960	.....	.....
514–1	2 days	Raw	3200	2060	920
514–2	2 days	2.5% raw milk in past. milk	134.4	56.6	23.2

The earlier results demonstrated that the enzyme present in milk is concentrated in the cheese at the expense of the whey. This fact has been verified in the present work with the modified test, as indicated by illustrative results shown in table 1. The values obtained on samples of cheese made from milk containing small proportions of raw in pasteurized milk are generally two to three times as large, per unit of volume or of weight, in the cheese as in the milk, and considerably smaller in the whey than in the milk.

II. APPLICATION TO FLUID MILK

Reagents

Barium borate-hydroxide buffer substrate for milk. Dilute a definite quantity of the barium buffer described under the cheese test with an equal quantity of distilled water. A mixture of 8 ml. of this diluted buffer with 1 ml. of milk yields, with different samples, pH values of 10.0 to 10.05 $\pm$ 0.15. Prepare and purify the disodium phenyl phosphate substrate and add it to the diluted buffer in the manner described under the cheese test.

This buffer substrate for milk is suitable also for testing cream and whey.

Zinc-copper precipitant for milk. Mix 3.0 g. of zinc sulfate ($ZnSO_4 \cdot 7H_2O$) with 0.6 g. of copper sulfate ($CuSO_4 \cdot 5H_2O$) and make up to 100 ml. with distilled water.

Other reagents. The color-development buffer, the BQC solution for use in the laboratory test, the CQC solution for the rapid field test, the butyl alcohol, and the phenol standards are prepared in the manner described under the cheese test—except, however, that 2 drops of BQC is sufficient for the low-phenol or border-line standards.

Laboratory Test

Mix the sample of milk well and pipet 1.0 ml. into a tube 16 or 18 × 150 mm., preferably graduated at 5 and at 10 ml. Pipet in 8.0 ml. of the buffer substrate and mix the contents thoroughly by shaking. Incubate in a water bath at 37–38° C. (99–100° F.) for 1 hour, preferably shaking the tube occasionally. Place the tube in a beaker of boiling water and leave it for 1 minute, or heat the contents to at least 90° C. (194° F.). Cool to room temperature. Pipet in 1.0 ml. of the zinc-copper precipitant for milk, mix thoroughly, and filter as described under the cheese test. Pipet into another tube 5.0 ml. of the filtrate and pipet in 5.0 ml. of color-development buffer. Add 2 drops (0.04 ml.) of BQC, mix, and let stand at room temperature for 30 minutes or at 37–38° C. for 15 minutes.

In making quantitative tests on samples that are observed during color development to be strongly positive, use 4 drops of BQC and prepare color dilutions by the method described under the cheese test.

In the test as modified for milk, a quantity of phenol as small as 1 mmg. can be detected visually and extraction with butyl alcohol is not necessary. If it is desired to use the butyl alcohol extraction method for a still greater increase in sensitivity, perform the extraction as described under the cheese test.

A blank determination should be conducted every time a new lot of buffer substrate—or of any of the other reagents—is used, using 1.0 ml. of milk that has been heated to at least 90° C. before adding the buffer substrate. Subtract the value of the blank from that of each test.

Since 1 ml. of milk is used and the quantity of filtrate used is equal to one-half the total quantity of liquid, the result obtained as described above is recorded as units of color or phenol equivalents in mmg. per 0.5 ml. of milk. However, the result may, if desired, be converted to phenol equivalents in mmg. per 1 ml. of milk.

If it is more convenient to add a total of 10.0 ml. rather than 8.0 ml. of buffer substrate, yielding a total of 12.0 ml.in the test mixture, this may be done and it will not affect significantly the pH of the mixture. In calculating the result obtained when using 12.0 ml. of liquid in the mixture and 5.0 ml. of filtrate, multiply the value obtained by 1.2 to obtain the result in phenol equivalents per 0.5 ml. of milk.

When using either phenol standards or a photometer, make the readings as described under the cheese test.

Rapid Field Test

Mix the sample of milk well, pipet 1.0 ml. into a tube, pipet in 8.0 ml. of buffer substrate for milk, and mix thoroughly, as described under the laboratory test for milk. Incubate for 30 minutes at 37–38° C. Pipet in 1.0 ml. of the zinc-copper precipitant for milk. Add 6 drops of CQC, mix well, allow the color to develop for 15 minutes at room temperature, and complete the determination as described under the rapid field test for cheese.

Precautions

The same precautions apply in the milk test as described under the cheese test.

Interpretation

On the basis of our results, it is suggested tentatively that values greater than 2 mmg. of phenol per 0.5 ml., or 4 mmg. per 1 ml., of milk be considered as indicating under-pasteurization.

Results

In the experiments on cheese it was found that barium reduces the inhibition of enzymic hydrolysis caused by the presence of phosphates and some other anions. These results have been verified in tests on milk, wherein the use of a barium borate substrate yielded an average of approximately 12 per cent more hydrolysis than did a sodium tetraborate substrate under comparable conditions.

Spectrophtometric data obtained on milk-test filtrates are shown in figure 1.

The curves indicate the increase in intensity of the blue color at wavelengths in the region of 610 mμ and the decrease in intensity of the interfering color in the region of 450 mμ, when 0.04 ml. (2 drops) of BQC is used with the zinc-copper reagent instead of with the lead reagent. With the use of the greater quantity of BQC, the interfering color is increased greatly, especially when the lead reagent is used. This interferes with visual detection of a blue color of low intensity and increases the photometric reading of the blank in the region of 610 mμ wavelength.

The improvement produced with the zinc-copper reagent has made it possible to reduce the quantity of BQC from 4 to 2 drops in the test on milk. Thus the difference between a control test and a test of 1 unit of blue color can be clearly detected visually, making the extraction of the color with butyl alcohol unnecessary.

It was found in this work, and also in experiments conducted with Horwitz (3), that the results of the test are a straight-line function of the concentration of the enzyme present, provided that a sufficient excess of disodium phenyl phosphate is present. However, the concentration of disodium phenyl phosphate specified for detecting under-pasteurization is not suffi-

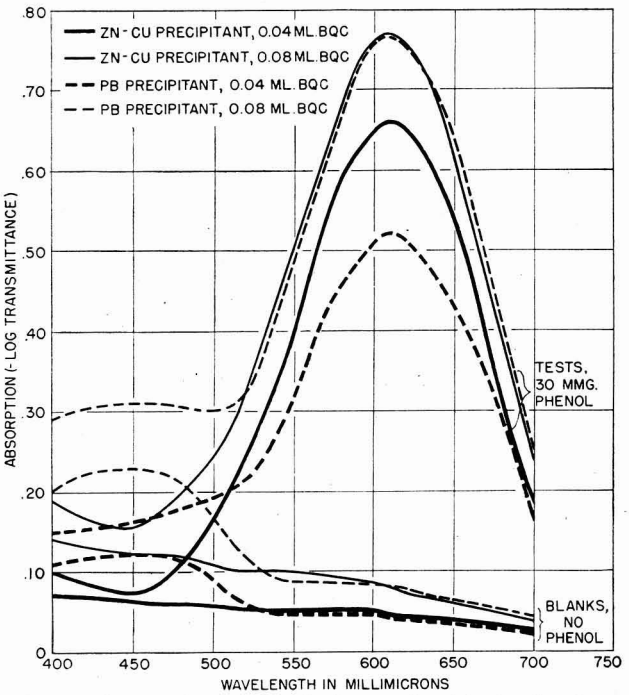

FIG. 1. Spectrophotometric analyses of colors produced in the phosphatase test with zinc-copper (new method) compared with lead (other earlier methods) precipitant (Beckman spectrophotometer used).

cient to determine quantitatively the enzymic activity in raw samples. Therefore, the optional use of twice the usual quantity of disodium phenyl phosphate has been specified as an alternative for obtaining more quantitative results on raw samples.

Tests to determine the precision of the modified method were conducted on standardized 4 per cent milk, on the cream separated from it and stand-

TABLE 2

Phosphatase values of standardized milk and of the corresponding skim milk and standardized cream, with various proportions of raw added to the boiled product (averages for four sets of samples)

Proportion of raw added to boiled product	Phosphatase value, phenol equivalent		
	Whole milk (4.0% fat)	Skim milk (0.01% fat)	Cream (32% fat)
per cent	*mmg./ml.*	*mmg./ml.*	*mmg./ml.*
0.1	2.6	1.2	5.6
2.5	63.0	31.2	136.6
100	2080	1320	2630

ardized to 32 per cent fat, and on the skim milk. The averages of the data, shown in table 2, indicate that the enzyme is highly concentrated in cream and considerably diluted in the skim milk. It was found also in numerous tests that the enzymic activity varies considerably in different milks, some whole milks yielding values as high as 1500 units of color per 0.5 ml.

The method is suitable for detecting phosphatase activity as low as 0.5 unit of color in the test, by means of a photometer or by comparison with standards in butyl alcohol. Therefore, on the basis of comparisons with values found for 0.1 per cent raw in a boiled product, illustrated in table 2, the sensitivity of the test is such that approximately 0.05 per cent (1 pound in 2,000) of a raw in a boiled product can be detected in the case of 4 per cent milk, 0.02 per cent (1 pound in 5,000) in the case of 32 per cent cream, and 0.1 per cent (1 pound in 1,000) in the case of skim milk. The proportion that can be detected in non-ripened cheese (table 1) is approximately the same as that in whole milk.

SUMMARY

A modification of the phosphatase test for detecting under-pasteurization of milk has been developed for testing Cheddar cheese and this modified method has been applied also to fluid milk.

Research on the conditions and substances that cause interferences in the test has been conducted, and the modifications found effective in minimizing the interferences are described. The resulting test has been found to yield results that are more precise for detecting under-pasturization than those obtained with the tests available heretofore.

In tests on whole milk and on non-ripened cheese, contamination of the pasteurized milk with as little as 0.05 per cent of raw milk (1 pound in 2,000) can be detected. The test applied to cheese has been found reliable for detecting under-pasteurization, regardless of the age of the cheese.

REFERENCES

(1) SANDERS, GEORGE P., AND SAGER, OSCAR S. A Phosphatase Test for Cheddar Cheese. U. S. Bur. Dairy Indus. BDIM-Inf-22, 5 pp. 1945 (Processed); Jour. Milk Tech., 8(4): 223–226, 1945; Natl. Butter & Cheese Jour., 36(7): 42–48, July, 1945.

(2) SANDERS, GEORGE P., AND SAGER, OSCAR S. Development of a Phosphatase Test Applicable to Cheddar Cheese. Jour. Assoc. Official Agr. Chem., 28(3): 656–675. 1945.

(3) HORWITZ, WILLIAM. (Food and Drug Admin., Fed. Security Agency, Minneapolis 1, Minn.) A Modified Scharer Phophatase Test for Milk and Soft Cheeses. Jour. Assoc. Official Agr. Chem., 29(2): 129–165. 1946.

BACTERICIDAL PROPERTIES OF SOME SURFACE-ACTIVE AGENTS*

W. S. MUELLER, EMMETT BENNETT AND JAMES E. FULLER
Departments of Dairy Industry, Chemistry and Bacteriology, Massachusetts Agricultural Experiment Station

INTRODUCTION

During the last twenty years there has been a gradual increase in the use of chemical sterilizing solutions in dairy plants and on dairy farms. Until recently, materials containing chlorine were the only recognized chemical sterilizing agents for dairy use. Although chlorine has served the dairy industry fairly well in the past, it has some well-known limitations which are of sufficient importance to have stimulated the search for better sterilizing agents. Shortly before World War II the literature called attention to the germicidal properties of some surface-active agents and their possible usefulness in the whole field of sanitation. In general the mode of action of surface-active materials on bacteria is believed to be by a disorganization of the cell membrane, and also by the denaturation of certain proteins essential to metabolism and growth. The present investigation was undertaken primarily for the purpose of evaluating the germicidal potency for dairy use of many surface-active materials and also to evaluate the germicidal stability of these products.

EXPERIMENTAL

The general plan of this study was to secure from manufacturers a reasonably large number of surface-active materials (6) representing various types, and to determine their germicidal properties under conditions simulating dairy practice. The variety of these compounds is so great that the number tested had to be limited. In order to obtain an indication of any relationship that might exist between the approximate chemical structure and the germicidal properties (1, 2, 3, 7) the samples selected were classified into what were believed to be similar chemical groups. The compounds were tested for their germicidal properties and grouped, according to their effectiveness, into three groups: effective, moderately effective, and ineffective. The germicidal properties of the compounds were then correlated with their chemical structure (4). Those materials found to be effective or moderately effective as germicides after two and one-half years storage were used for other studies such as their corrosiveness to metals, while the materials in the ineffective group were discarded.

The investigation was interrupted by the war; hence there was some delay in the first examination of some of the products. However, the inter-

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ruption was advantageous in determining the stability of some of the products.

METHODS

Germicidal properties. The germicidal properties of the surface-active agents were determined by adding 1 ml. of raw milk, containing many bacteria, to 99 ml. of the solution being tested. Immediately after the inoculum was added, each bottle was shaken rapidly 25 times. After the milk had been in contact with the test solution for 5 minutes at room temperature, proper dilutions were made and 1-ml. quantities were plated according to the Standard Method for Milk Analysis (5) procedure. The method used for evaluating the germicidal properties of the surface-active agents was such that the test was carried out in an environment where bacteria can live. The bacteria in the milk used for the inoculum were increased by adding manure, stable bedding material, and soil to the milk. No doubt the flora in the various experiments varied, but they did represent those forms that the germicide must combat in actual dairy practice, which is an important consideration. In this study, where many materials about which so little is known were being tested, it was impractical to add an inactivator to prevent a bacteriostatic action in the plate. The omission of the inactivator is not serious, however, because the chief purpose of the study was to weed out those materials which are germicidally ineffective. Therefore, if a material was ineffective in the absence of an inactivator, it is reasonable to assume that it might have been more ineffective had an inactivator been used. Where the manufacturer recommended the concentration of the surface-active material to be employed, the recommendations were followed; otherwise, the material was used in 0.5 per cent concentration by weight.

Corrosive action on metals. The corrosive properties of the surface-active materials were determined on strips, 2.5 inches by 1 inch, of the following metals: 18–8 stainless steel, monel, tin (4X), and copper tinned on one side only. The metal strips were washed, polished, washed in alcohol and ether, dried, and weighed. Each metal strip was placed in a glass-stoppered bottle (125 ml. capacity) containing 100 ml. of the solution being tested. The test solutions were of the same strength as used for the germicidal tests. The bottles were agitated for three days at room temperature by the use of a revolving agitator driven at 24 r.p.m. At the end of three days the metal strips were removed from the test solutions, rinsed in water, alcohol and ether, dried and reweighed to determine any loss in weight. The appearance of the metal strips and of the solutions, after the test, was also noted.

Hydrogen-ion concentration. The pH value of solutions of the various surface-active agents was determined with the Beckman pH meter, using a glass electrode. On some samples consistent pH determinations were difficult to obtain.

PRESENTATION OF RESULTS

Chemical classification. Forty-two surface-active agents were obtained from 14 different manufacturers. From the manufacturer's descriptive literature, and from other sources in the literature, these surface-active agents were classified in 12 groups as shown in table 1. It may be pointed out that the greater number of the surface-active agents investigated were classed as quaternary ammonium compounds or alkyl aryl sulfonates (4). The number of agents in the other groups are more evenly distributed.

Germicidal properties. The concentration at which each material was used, the inoculum employed, and the percentage survival of bacteria are

TABLE 1

Classification of the various surface-active agents investigated

	Designation	Total for each classification
Quaternary ammonium compounds	24 25 26 27 28 29 30 31 42	9
Phosphonium compounds	38 39 40	3
Substituted phenols	20 21 41	3
Alkyl aryl sulfonates	1 4 13 15 16 17 18 19 22 23	10
Aryl alkyl polyether sulfonate	33	1
Aliphatic sulfonates	2	1
Aryl alkyl polyether alcohol	35	1
Esters:		
Polyoxyalkylene of fatty acids	9 10	2
Aliphatic sulfates	32	1
Aryl alkyl polyether sulfate	34	1
Monoesters of polyhydroxy compounds	6 7 8 11	4
Unknowns	3 5 12 14 36 37	6
Total	.....	42

given in table 2. Because of the well-known inherent limitations of the agar plate method, the percentage survival data should be considered as trends and not as absolute values. While the test for each material was repeated at least three times, the data reported are not averages but are the data of single trials which conformed most closely to the general trends. The 42 agents investigated are also segregated in table 2 into three arbitrary groups according to their effectiveness as sterilizing agents. Group I are the agents which had a bacterial survival of zero to 0.5 per cent inclusive, and this is considered as being very effective. Group II are the agents which had a bacterial survival greater than 0.5 per cent and up to 3 per cent inclusive. This is considered as being only moderately effective. Group III are the agents which had a bacterial survival greater than 3 per cent, and this is considered to be ineffective from a practical viewpoint.

From table 2 it is apparent that out of 42 surface-active agents investigated, 9 were effective sterilizing agents, 9 were moderately effective, and

TABLE 2

Germicidal properties of various surface-active agents

Designation	Concentration used*	Inoculum	Percentage survival of bacteria
	Group I—Effective		
24	1:1000 dilution	60,000,000	0.0
25	1:1000 dilution	60,000,000	0.0
26	1:1000 dilution	60,000,000	0.0
27	1:1000 dilution	60,000,000	0.0
28	1:1000 dilution	46,000,000	0.0
38	0.5%	46,000,000	0.0
39	0.5%	46,000,000	0.0
40	0.5%	46,000,000	0.0
42	1:500 dilution	113,000,000	0.0
	Group II—Moderately effective		
2	0.5%	46,000,000	1.2
16	0.5%	1,287,000,000	0.8
17	0.5%	1,287,000,000	1.5
18	0.5%	1,287,000,000	1.4
19	0.5%	1,287,000,000	1.4
20	0.5%	46,000,000	2.6
21	0.5%	46,000,000	3.0
22	0.5%	46,000,000	2.4
41	0.5%	231,000,000	1.3
	Group III—Ineffective		
1	0.5%	231,000,000	7.8
3	0.5%	231,000,000	46.1
4	0.5%	231,000,000	71.2
5	0.5%	231,000,000	50.9
6	1:400 dilution	326,000,000	100.0
7	1:400 dilution	326,000,000	100.0
8	0.5%	326,000,000	100.0
9	0.5%	326,000,000	62.6
10	0.5%	326,000,000	78.0
11	0.5%	326,000,000	29.6
12	0.5%	231,000,000	34.1
13	0.5%	231,000,000	22.8
14	0.5%	231,000,000	14.9
15	0.5%	231,000,000	8.4
23	0.5%	231,000,000	13.5
29	1:1000 dilution	46,000,000	28.3
30	1:1000 dilution	326,000,000	55.2
31	1:1000 dilution	326,000,000	20.2
32	0.5%	113,000,000	77.9
33	0.5%	231,000,000	83.4
34	0.5%	231,000,000	36.6
35	0.5%	231,000,000	49.9
36	0.5%	46,000,000	37.0
37	0.5%	46,000,000	14.3

* Percentages and dilutions are based on the product as received and not on the basis of the active ingredient.

24 were ineffective. It is known that the germicidal property of a material may increase with an increase in concentration, and in some instances with a decrease in concentration. Therefore, the agents in Group II and III,

which were used at a concentration of 0.5 per cent, were retested at a concentration of 1.0 per cent and 0.05 per cent. In no instance was the group classification changed by the increase or decrease in concentration.

Table 3 shows the correlation between sterilizing property and chemical composition for the 42 agents investigated. It will be noted that the effective group includes only quaternary ammonium compounds and phosphonium compounds. The moderately effective group includes substituted phenols, alkyl aryl sulfonates, and aliphatic sulfonates. One or more of the agents of each class are represented in the ineffective group, with the exception of phosphonium compounds, substituted phenols, and aliphatic

TABLE 3

The effectiveness of the different classes of materials as sterilizing agents

	Number of materials in			
	Group I Effective	Group II Moderately effective	Group III Ineffective	Total
Quaternary ammonium compounds	6	0	3	9
Phosphonium compounds	3	0	0	3
Substituted phenols	0	3	0	3
Alkyl aryl sulfonates	0	5	5	10
Aryl alkyl polyether sulfonate	0	0	1	1
Aliphatic sulfonates	0	1	0	1
Aryl alkyl polyether alcohol	0	0	1	1
Esthers:				
Polyoxyalkylene of fatty acids	0	0	2	2
Aliphatic sulfates	0	0	1	1
Aryl alkyl polyether sulfate	0	0	1	1
Monoesters of polyhydroxy compounds	0	0	4	4
Unknowns	0	0	6	6
Totals	9	9	24	42

sulfonates. It is highly probable that the three ineffective quaternary ammonium compounds were partly inactivated by the manufacturers through the addition of foreign substance to the compounds.

Stability. Approximately one and one-half years after the first tests were made, the germicidal property and the hydrogen-ion concentration of the agents in groups I and II were checked. The stability of the agents in group III was not determined because they were eliminated in the first examination as unsuitable chemical sterilizers for dairy use. From the time they were received until the last tests were made, the agents were stored in the containers in which they were received, at room temperature away from direct sunlight. The results obtained are given in table 4. It will be noted that, with one exception, all of the agents found to be effective in the first test were still effective after two and one-half years. No. 26, the exception, had lost enough of its germicidal property during storage for reclassification as only moderately effective. It will also be noted, from table 4, that all of

the agents which were found to be moderately effective in the first test had lost enough of their germicidal properties during storage to be reclassified as ineffective. Since stability is one of the major requirements of a sterilizing agent for dairy use, only those agents remaining in the effective and moderately effective groups after two and one-half years storage were considered for further study.

An examination of the hydrogen-ion concentration data in table 4 reveals no correlation between pH, germicidal property, and stability of the agents.

TABLE 4

Stability of the effective and moderately effective sterilizing agents

Designation	Within 1 year after receiving samples		Two and one-half years after receiving samples	
	Percentage survival of bacteria	pH	Percentage survival of bacteria	pH
Group I—Effective sterilizing agents				
24	0.0	6.1	0.0	6.7
25	0.0	4.7	0.0	5.2
26	0.0	6.1	0.7	6.3
27	0.0	5.5	0.0	5.2
28	0.0	5.5	0.0	5.7
38	0.0	6.8	0.0	6.9
39	0.0	7.4	0.0	7.8
40	0.0	6.0	0.0	6.3
42	0.0	6.5	0.0	6.0
Group II—Moderately effective sterilizing agents				
2	1.2	3.7	24.7	3.6
16	0.8	7.3	28.9	6.6
17	1.5	6.8	100.0	7.5
18	1.4	6.8	100.0	6.6
19	1.4	6.8	100.0	6.9
20	2.6	7.2	62.5	7.7
21	3.0	8.1	56.9	8.1
22	2.4	8.0	57.5	9.1
41	1.3	11.1	14.3	11.3

Corrosive effect on metals. Only those agents remaining effective and moderately effective as sterilizing agents after two and one-half years storage were used for the corrosion of metals test. The corrosive effect of 6 quaternary ammonium compounds, 3 phosphonium compounds, and a chlorine product on four metals is shown in table 5. Since chlorine is so widely used it is included as a standard. None of the agents showed sufficient corrosion on 18–8 stainless steel or monel metal to be of any practical significance. As judged by loss in weight of metals, the quaternary ammonium compounds and phosphonium compounds were less corrosive than chlorine to tin (4X) or to copper tinned on one side only. Tin appeared to be more corroded in the presence of copper than when plated on steel. The correlation between corrosion, as judged by appearance and by loss in weight, is not very close.

TABLE 5

Corrosion of metals by various chemical sterilizers

Sterilizing agent	Amount used*	Loss (−) or gain (+) in weight per square foot area of metal				Extent of tarnishing or corrosion†			
		18–8 stainless steel	Monel	Tin (4X)	Tinned copper	18–8 stainless steel	Monel	Tin (4X)	Tinned copper‡
Quaternary ammonium compounds									
24	1 : 1000 dilution	− 0.0058	− 0.0346	− 0.0461	− 0.1901	−	−	++	C + T ++++
25	Same	− 0.0115	− 0.0518	− 0.1037	− 0.2477	−	−	++	C + T ++++
26	Same	− 0.0115	− 0.0461	− 0.0288	− 0.1843	−	−	++	C ++ T +++
27	Same	0.0000	− 0.0691	− 0.0864	− 0.0403	−	−	++	C + T ++
28	Same	− 0.0058	− 0.0058	− 0.4205	− 0.2534	−	−	++	C ++ T +++
42	1 : 500 dilution	− 0.0115	− 0.0115	− 0.1210	− 0.2074	−	−	++	C ++ T +++
Phosphonium compounds									
38	0.5%	+ 0.0576	+ 0.0230	0.0000	− 0.3728	−	−	+	C ++ T ++
39	Same	+ 0.0461	+ 0.0058	+ 0.0115	− 0.0576	−	−	+	C + T ++
40	Same	+ 0.0230	− 0.0058	− 0.0461	− 0.0230	−	−	+	C + T ++
Calcium hypochlorite	200 p.p.m. of Cl	− 0.0115	− 0.1037	− 0.4378	− 0.8410	−	−	++	C + T +++++

* Percentages and dilutions are based on the product as received and not on the basis of the active ingredient.

† Degree of tarnishing or corrosion ranges from (−), representing no change, or (+) indicating very slight dulling of luster to (+++++) indicating very badly tarnished or corroded.

‡ Copper strips tinned on one side only.

The phosphonium compounds in many instances deposited a light waxy film on the surface of the metals. This film was not easily removed with alcohol or ether, which no doubt accounts for the slight increase in weight for some of the metals. While it is difficult to give a practical interpretation of the losses in weight for tin plate and tinned copper, it appears that when the surface-active agents are compared with chlorine they would be satisfactory for dairy use from the viewpoint of corrosion.

Solubility, odor, taste, and color. Observations under this heading are reported only for those surface-active agents which were effective germicides after two and one-half years storage. All of the quaternary ammonium compounds investigated are readily soluble, have no objectionable odor or taste, and are practically colorless. However, all three of the phosphonium compounds went into solution with such difficulty that this property would probably exclude them from use as a dairy sterilizing agent. They are not objectionable from the standpoint of odor and taste. The color of their solutions is very cloudy or milky, which is not as desirable as a colorless sterilizing solution.

DISCUSSION

In this study the major considerations were germicidal properties, corrosiveness to metals, stability, solubility, odor, taste, and color. From these tests it appears that the quaternary ammonium compounds are the only group which show promise of making satisfactory sterilizing agents for dairy use. Further studies of the quaternary ammonium compounds will show how these new sterilizing agents can be used to the best advantage to the dairy industry.

The manufacturers of many of the products employed in this investigation do not make any claim that the product is a suitable dairy sterilizing agent. Therefore, it cannot be overemphasized here that the germicidal classification given as a result of this study is in no way a rating of the products for the uses they are now sold for. Since a dairy cleaner should also have considerable germicidal properties, possibly some of the surface-active agents which are not really effective sterilizing agents may have sufficient germicidal properties to be useful as ingredients for dairy cleansing purposes.

SUMMARY

1. The germicidal properties of the following 42 surface-active agents have been investigated: 9 Quaternary ammonium compounds, 3 Phosphonium compounds, 3 Substituted phenols, 10 Alkyl aryl sulfonates, 1 Aryl alkyl polyether sulfonate, 1 Aliphatic sulfonate, 1 Aryl alkyl polyether alcohol, 2 Polyoxyalkylene of fatty acids, 1 Aliphatic sulfate, 1 Aryl alkyl polyether sulfate, 4 Monoesters of polyhydroxy compounds, and 6 Unknowns.

2. In the first examination 9 were effective sterilizing agents, 9 were moderately effective, and 24 were ineffective.

3. The effective group included only quaternary ammonium and phosphonium compounds.

4. The moderately effective group included substituted phenols, alkyl aryl sulfonates, and aliphatic sulfonates.

5. One or more of the materials of each class are represented in the ineffective group, with the exception of phosphonium compounds, substituted phenols, and aliphatic sulfonates.

6. All of the 9 surface-active agents, with one exception, found to be effective by the first test, remained effective after two and one-half years storage.

7. All of the 9 surface-active agents, found to be moderately effective by the first test, had lost much of their germicidal properties after two and one-half years storage.

8. There was no correlation between pH value, germicidal property, and stability of the products.

9. The 6 quaternary ammonium compounds which remained effective or moderately effective as sterilizing agents after two and one-half years storage were satisfactory in so far as corrosiveness to metals, solubility, odor, taste, and color are concerned.

10. All three phosphonium compounds were satisfactory in so far as corrosiveness to metals, odor, and taste are concerned, but they are objectionable because they do not go into solution easily and also form cloudy solutions.

CONCLUSIONS

Of the 42 surface-active agents investigated, only the quaternary ammonium and phosphonium compounds had sufficient germicidal properties and stability for a good sterilizing agent for dairy use. The quaternary ammonium compounds go into solution readily, are practically non-corrosive to metals, odorless, tasteless, and colorless, all of which are desirable properties. The phosphonium compounds also are non-corrosive to metals and have no serious objectionable odor or taste, but they do not go into solution readily and produce cloudy solutions, which are undesirable properties.

REFERENCES

(1) BAKER, ZELMA, HARRISON, R. W., AND MILLER, B. F. Action of Synthetic Detergents on the Metabolism of Bacteria. Jour. Expt. Med., 73: 249–271. 1941.

(2) BAKER, ZELMA, HARRISON, R. W., AND MILLER, B. F. The Bactericidal Action of Synthetic Detergents. Jour. Expt. Med., 74: 611–620. 1941.

(3) JACOBS, MORRIS B. The Chemistry and Technology of Food and Food Products. Interscience Publishers, Inc., New York. 2: 394–424. 1944.

(4) SNELL, FOSTER D. Surface-Active Agents. Indus. and Engin. Chem., 35: 107–117. 1943.

(5) STANDARD METHODS FOR THE EXAMINATION OF DAIRY PRODUCTS. 8th edition. American Public Health Association, New York. 1941.

(6) VAN ANTWERPEN, F. J. Surface-Active Agents Manufactured in America and Commercially Available. Indus. and Engin. Chem., **35**: 126–130. 1943.

(7) WILSON, JOHN L. The Chemistry and Technology of Food and Food Products, Edited by Jacobs, Morris B. Interscience Publishers, Inc., New York. **2**: 211–241. 1944.

THE EFFECT OF VITAMIN SUPPLEMENTS UPON SURVIVAL OF NEW-BORN CALVES*

R. GAURTH HANSEN, PAUL H. PHILLIPS AND I. W. RUPEL[1]
Department of Biochemistry, College of Agriculture, University of Wisconsin, Madison

INTRODUCTION

Liebscher (1) reported in 1930 that calves which had previously been given colostrum, could be raised on skim milk and cod-liver oil, but they failed to gain in weight as rapidly as calves fed whole milk. Numerous reports have appeared regarding the importance of colostrum to the newborn (2, 3, 4). Phillips *et al.* (5) fed calves which had never received colostrum on a ration of skim milk and found that some of the animals would survive. It was also found that ascorbic acid and nicotinic acid would increase the survival rate of calves on their skim milk vitamin A ration.

The bovine placental membrane appears to be relatively impervious to the passage of vitamin A, as the calf is born deficient in this factor (6, 7, 8). Colostrum generally meets this deficiency for it may contain from 10–100 times the amount of vitamin A that is present in normal milk (6, 9); however, Stewart and McCallum (10) have observed a very wide variation in colostral vitamin A among cows of the same breed receiving similar rations. Under our present system of dairy calf management it is probable that the young calf may under certain conditions receive inadequate amounts of this vitamin.

This study was made to determine the amount of vitamin A needed by the calf fed from birth on a skim milk ration, and to determine the benefit to the calf of additional factors that are also known to occur in variable amounts in colostral milk.

EXPERIMENTAL

Holstein calves weighing from 90–110 lbs. were obtained from local farms before having access to colostrum. These calves were selected and alotted in such a manner that they alternately fell into their respective groups in order to eliminate as much as possible the factor of seasonal influence and variables attendant thereto. Skim milk was fed twice daily at the rate of twelve pounds of milk per hundred pounds of body weight per day. Since it has been shown (5) that under certain conditions the administration of

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[1] Now at the Texas Agricultural and Mechanical College.

ascorbic acid is beneficial, 250 mg. of this vitamin were given orally to all calves once each day. Standardized vitamin A concentrates were given in gelatin capsules at levels of 10,000 and 25,000 I.U. per calf per day as indicated.

In preliminary experiments with the skim milk ration it was found that most calves were lost during the first week of life. Little difficulty was encountered with older calves, hence fourteen days was taken as an adequate length of time to measure the effectiveness of a ration in meeting the requirements of the very young calf. For this reason the survival figures are given on the basis of a fourteen-day experimental period.

Calves fed the basal ration normally exhibited a looseness of the bowels regardless of the dietary supplements employed. If the looseness reached a severe stage, accompanied by a rise in body temperature (104–105° F.), 2–4 grams of sulfathiazole were given for one day and lighter doses thereafter as the condition of the calf indicated.

Blood was taken from the jugular vein at birth and before feeding, at 1½, 7, and 14 days. The samples were cooled immediately and plasma vitamin A determinations were made according to the method of Kimble (11).

I. *The effect of vitamin A level fed.* In agreement with previous observations (5) the blood plasma vitamin A level of the new born calf was low. Following the ingestion of colostrum there was an immediate rise from 4 micrograms per 100 ml. to a level of 10 micrograms or more per 100 ml. of blood. It is seen from the data in table 1 that the new-born calves did not develop normal blood plasma vitamin A levels within a thirty-six-hour period when only 10,000 I.U. of vitamin A were fed with the basal ration, while the administration of 25,000 I.U. of vitamin A per day produced blood plasma vitamin A levels comparable to the calves fed colostrum.

TABLE 1

The effect of vitamin supplements upon the occurrence of severe diarrhea and survival of young calves

Lot	I	II	III	IV	V	VI
Treatment	10,000 I.U. of vit. A		25,000 I.U. of vit. A			Colostrum and whole milk
	Vit. A only	+ Nicotinic acid	Vit. A only	+ Nicotinic acid	+ Nicotinic acid + biotin	
No. calves	3	6	13	16	7	4
Blood plasma vitamin A concentration μg./100 ml.						
Birth	2	5	4	4	.....	4
1–2 days	4.5	6	9	10	.....	10
6–14 days	.....	12	11	11	.....	13
No. cases diarrhea	3	5	9	11	5	.....
No. given sulfathiazole	3	5	7	9	4	.....
No. calves survived	1	3	9	12	6	4
Per cent survival	33	50	69	75	86	100

The data in table 1 further indicate that the level of vitamin A fed influenced the rate of incidence of severe diarrhea as eight out of nine calves (89 per cent) receiving 10,000 I.U. of vitamin A per day showed severe diarrhea, while only 25 out of the 36 calves (69 per cent) receiving 25,000 I.U. of vitamin A showed this symptom. The level of vitamin A administration seemed to have a direct bearing upon the capacity to survive. Only 4 out of the nine calves fed 10,000 I.U. of vitamin A per day survived the experimental period whereas 27 out of 36 (75 per cent) of the calves fed 25,000 I.U. of vitamin A survived.

II. *The effect of nicotinic acid and biotin.* The data of table 1 show that neither nicotinic acid nor its amide when given orally (50 mg. daily) increased the survival rate of calves fed the skim milk ration fortified with adequate vitamin A. There may have been a slight beneficial effect upon the survival rate when only 10,000 I.U. of vitamin A were fed but the number of calves used was too few to permit a definite conclusion on this point.

Since the biotin content of colostrum milk was found to be low and somewhat variable (12) the calves in lot V (table 1) were given biotin to determine its effect upon survival and general health of the calves. 150 micrograms of biotin were given subcutaneously as well as orally each day in addition to the basal ration fortified with vitamin A and niacin. There was no marked effect from the administration of biotin either in the prevention of diarrhea or on the survival rate of the calves.

The effect of season. The data in table 2 clearly indicate a marked seasonal variation in the occurrence of severe diarrheas and survival of young calves fed the skim milk basal ration. During the period of January 1, to June 1, 86 per cent of the calves showed symptoms of severe diarrhea. In contrast only 43 per cent of the calves developed severe diarrheas throughout the period from June 1 to January 1. This type of diarrhea usually occurred during the first week of life.

TABLE 2

The effect of season on the occurrence of diarrhea and survival of young calves

Season		25,000 I.U. of vitamin A		
		Vit. A only	Vit. A + nicotinic acid	Vit. A + nicotinic acid + biotin
Jan. 1 to June 1	No. calves	10	10	2
	No. cases diarrhea	9	8	2
	No. calves survived	6	7	1
June 1 to Dec. 31	No. calves	3	6	5
	No. cases diarrhea	0	3	3
	No. calves survived	3	5	5

The data in table 2 also demonstrate an effect of season on the survival rate of calves fed the skim milk basal with vitamin supplements as indicated. In the period from June 1st through December, 13 out of 14 calves (92 per cent) survived the experimental period, while of the calves in the January–June group that received identical treatment 14 out of 22 (64 per cent) survived.

DISCUSSION

The vitamin A requirement of the new-born calf appears to us to be considerably higher (5 to 10×) during the first few days of life than that previously established for heifers 3–6 months of age. These data support such an interpretation but they also raise the question of the efficacy of the source of vitamin A. Specifically; is one unit of vitamin A in fish-liver oil concentrate equivalent to one unit of vitamin A in colostrum milk? In these experiments it has been assumed that they were but we grant that the possibility of conjugated forms of vitamin A present in colostrum may be superior to the concentrated fish-liver oil vitamin A used. The data do indicate, however, that the vitamin A requirement of the new born calf fed a highly concentrated vitamin A fish-liver oil lies between 10,000 and 25,000 I.U. per 100-lb. calf per day. These results are in general agreement with those suggested by Lewis and Wilson in 1945 (13).

These data throw considerable light upon the specific effect of niacin in relation to blood plasma vitamin A. In 1943 Phillips *et al.* (5) presented data which indicated that B complex mixtures containing niacin favorably influenced the recovery of ingested vitamin A in the blood plasma of the calf. Subsequent observation on the administration of 5,000 I.U. of vitamin A per calf per day has usually shown this level of vitamin A ingestion to be more effective when niacin was also given. In the present study the favorable effect of niacin disappeared when higher levels of vitamin A were ingested. The niacin effect was only slightly in evidence, if at all, when 10,000 I.U. of vitamin A were given. These data point to the conclusion that in the presence of adequate vitamin A (25,000 I.U. per calf per day) supplementary amounts of niacin are unnecessary.

A seasonal effect is quite apparent from the data presented in table 2. With the fortified skim milk basal ration summer "dropped" calves showed a lower incidence of severe diarrhea and a higher rate of survival than winter calves. It does not seem likely that the trace of fat present in the skim milk could account for this difference.

Many other factors are recognized which may be involved in the summer–winter differential in survival. Factors such as crowding, ventilation, temperature, drafts, etc., are far from being alike for the two periods. Hence the difference noted in survival cannot be wholly attributed to nutrition at this time. In view of the demonstrated superiority of summer vs. winter milk for rats (14), however, it seems not unlikely that either dietary factors

present in the skim milk, or the intrauterine nutrition or fetal storage of metabolites was favorably affected by season. Storage of additional vitamin A in the fetal liver would be highly important in this regard. A few attempts to obtain blood plasma vitamin A levels of 10 + μg./100 ml. in the new-born calf by feeding moderate amounts of high vitamin A shark-liver oil to their dams did not meet with success. It is postulated as a working hypothesis, that some factor, or factors present either in "summer skim milk" or in intrauterine life or both favorably influence this seasonal survival.

SUMMARY

A study of the effect of vitamin supplements upon survival of new-born calves fed a skim milk basal ration from birth has been made. It appears that under these conditions new-born calves require vitamin A in excess of 10,000 I.U. per day when severe diarrhea and ability to survive were used as the criteria for establishing approximate requirements. Twenty-five thousand I.U. of vitamin A per day in the form of fish-liver oil concentrate greatly enhanced the chance of survival of calves fed the skim milk ration. In the presence of adequate vitamin A ingestion the favorable supplementary effect of niacin was found to be negligible. The blood-plasma vitamin A concentration followed previous observations, *i.e.,* on the average the new-born calf has a very low blood plasma vitamin A level and that normal concentrations in the neighborhood of 9–10 μg./100 ml. or more must be quickly attained (1–2 days) for a favorable chance of survival.

REFERENCES

(1) LIEBSCHER, W. Feeding Tests on Calves with Skim Milk and Cod Liver Oil Emulsion as a Substitute for Milk. Tierernähr, 2: 11. 1930.

(2) SMITH, T., AND LITTLE, R. B. The Significance of Colostrum to the New Born Calf. Jour. Expt. Med., 36: 181. 1922.

(3) DRUMMOND, J. C., COWARD, K. H., AND WATSON, A. F. Notes on the Factors Influencing the Value of Milk and Butter as Sources of Vitamin A. Biochem. Jour., 15: 540. 1921.

(4) DANN, W. J. The Transmission of Vitamin A from Parents to Young in Mammals. Biochem. Jour., 26: 1072. 1932.

(5) LUNDQUIST, N. S., AND PHILLIPS, P. H. Certain Dietary Factors Essential for the Growing Calf. JOUR. DAIRY SCI., 26: 1023. 1943.

(6) DANN, W. J. The Carotene and Vitamin A Content of Cows Colostrum. Biochem. Jour., 27: 1998. 1933.

(7) KRAUSS, W. E., MONROE, C. F., AND HAYDEN, C. C. Ohio Agr. Expt. Sta. Spec. Cir. 57. 1939.

(8) PHILLIPS, P. H., LUNDQUIST, N. S., AND BOYER, P. D. The Effect of Vitamin A and Certain Members of the B-complex upon Calf Scours. JOUR. DAIRY SCI., 24: 977. 1941.

(9) SEMB, J., BAUMANN, C. A., AND STEENBOCK, H. The Carotene and Vitamin A Content of Colostrum. Jour. Biol. Chem., 107: 697. 1934.

(10) STEWART, J., AND MCCALLUM, J. W. The Vitamin A Content of the Colostrum of Dairy Cows. Jour. Agr. Sci., 28: 428. 1938.

(11) KIMBLE, M. S. The Photocolorimetric Determination of Vitamin A and Carotene in Human Plasma. Jour. Lab. and Clin. Med., 24: 1055. 1939.

(12) POLLARD, A., HANSEN, R. G., AND PHILLIPS, P. H. Unpublished data Univ. of Wis.

(13) LEWIS, J. M., AND WILSON, L. T. Vitamin A Requirements in Calves. Jour. Nutr., 30: 467. 1945.

(14) ELVEHJEM, C. A., HART, E. B., JACKSON, H. C., AND WECKEL, K. G. The Nutritive Value of Milk—Raw vs. Pasteurized, Summer vs. Winter. JOUR. DAIRY SCI., 17: 763. 1934.

STUDIES ON MILK FEVER IN DAIRY COWS.[1] II. THE EFFECT OF VITAMIN D ON SOME OF THE BLOOD CHANGES IN NORMAL AND MILK FEVER COWS AT PARTURITION

J. W. HIBBS, W. E. KRAUSS, W. D. POUNDEN, C. F. MONROE, AND T. S. SUTTON[2]
Ohio Agricultural Experiment Station, Wooster, Ohio

Many of the usual blood changes occurring at parturition and in milk fever have been extensively investigated. Since Little and Wright (24) reported low blood calcium values in cows with milk fever much attention has been given to various mineral constituents of the blood. It appears that when an explanation is found for the changes in the mineral constituents of the blood and a way to prevent those changes can be devised, the mystery of the milk fever syndrome will be solved.

Since the report of Little and Wright (24) numerous reports have been submitted dealing with total calcium (1, 2, 10, 11, 12, 14, 15, 17, 24, 25, 27, 32, 33, 36) and diffusible calcium (15, 27, 30, 33) in the blood of normal and milk fever cows at parturition. All are in agreement that a marked decrease in both total and diffusible calcium occurs in milk fever. A sharp drop in blood inorganic phosphorus in milk fever has been repeatedly reported (2, 11, 14, 15, 27, 29, 30, 33, 36).

In contrast to serum calcium and phosphorus, serum magnesium is said to increase during milk fever (1, 2, 15, 23, 29, 30, 31, 33).

The work of Mattick and Little (26) suggests some benefit in preventing the fall of blood calcium and phosphorus at parturition as the result of feeding cod-liver oil.

The present paper is a report of studies covering the effect of irradiated yeast feeding on some of the blood changes at parturition and in milk fever, made in connection with the experiment described in the preceding paper (20) involving the effect of feeding vitamin D in the form of irradiated dry yeast on the incidence of milk fever.

EXPERIMENTAL

Method of Analysis

Calcium: Serum calcium was determined by the method of Clark and Collip (9).

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[2] N. E. Van Demark assisted in obtaining the data from the Ohio State University herd.

Phosphorus: Serum phosphorus was determined by the method of Briggs (7).

Magnesium: Serum magnesium was determined by the Briggs (6) method.

Phosphatase: Serum phosphatase was determined by the Bodansky (3) method. The results are expressed as units per 100 ml. of serum, a unit being defined as "equivalent to 1 mg. of phosphorus liberated from a sodium glycerophosphate substrate as the phosphate ion during the first hour at a pH of 8.6 and at 37° C."

Serum protein: Total serum protein was measured by the Falling Drop Method of Kagan (21) using a Kagan Proteinometer.

Vitamin D: Samples of whole blood were dried over night at 100° C. and finely ground. The appropriate amount was weighed out and mixed with 35 mg. of rachitic ration (Steenbock and Black's No. 2965) and fed to rachitic rats. After this amount of feed was consumed the rats were continued on the rachitic ration until the tenth day. The rats were then sacrificed and the ulna and radius removed. The distal end of the ulna was then split and after staining with silver nitrate the bones were photographed and the healing response was measured by the relative amount of calcification at the metaphysis. The per cent healing was determined by dividing the actual healing by 4, the value which represented complete healing. Thus an average healing response of 1 would be 25 per cent, etc.

Vitamin A and carotene: Plasma vitamin A and carotene were determined by the method of Kimble (22). In samples containing carotene in excess of 300 micrograms per 100 ml. the vitamin A was determined by the method of Boyer, Phillips, and Smith (4). The carotene and vitamin A in colostrum were determined by using essentially the same method described by Boyer, Spitzer, Jensen, and Phillips (5) except that the saponification was carried out by refluxing in a boiling water bath for ten minutes instead of the cold saponification. A few minor modifications were also made in the extraction process.

Ascorbic acid: Plasma ascorbic acid was determined by the macro-method of Mindlin and Butler (28).

An Evelyn Photo-electric Colorimeter was used for all colorimetric determinations.

Experiment Involving Type 7F Irradiated Dry Yeast—1941

In a preliminary trial, 1 million units of vitamin D were fed daily to cows in the form of type 7F irradiated dry yeast[3] for four weeks before and one week following parturition. Calcium and phosphorus determinations were made on the blood serum before yeast feeding started, within 12 hours before and within 12 hours after parturition. Samples of whole blood were collected

[3] All yeast was supplied by Standard Brands Incorporated.

TABLE 1

The effect of feeding one million units of vitamin D daily on the serum calcium and phosphorus content of cows' blood

Cow No.	4 weeks pre-fresh		12 hr. pre-fresh		12 hr. post-fresh		Milk fever	
	Ca mg. %	P mg. %	Ca mg. %	P mg. %	Ca mg. %	P mg. %	Ca mg. %	P mg. %
Control cows								
4	10.95	5.18	9.41	4.67	8.20	6.02	.....	.....
5	.....	.....	11.13	5.81	10.52	4.35	.....	.....
13*	10.93	4.65	11.23	6.06	8.92	2.60	5.46	1.51
14	10.83	4.14	10.93	5.18	8.70	3.83	.....	.....
18*	12.14	5.43	11.74	4.98	7.49	2.86	5.87	2.46
Yeast-fed cows								
2	10.73	5.71	11.33	5.56	11.74	5.75	.....	.....
7	11.74	5.65	10.63	5.15	12.14	5.78	.....	.....
10*	10.93	5.71	10.32	5.00	9.72	3.65	.....	.....
11	10.93	5.56	10.83	4.39	8.91	5.26	.....	.....
15	10.93	4.55	11.84	7.41	6.88	4.63	.....	.....
17*	11.13	5.72	11.92	7.62	8.30	4.46	.....	.....

* These cows had previous histories of milk fever.

and dried for vitamin D assay at the same times. A similar group of cows receiving no irradiated yeast supplement served as controls. The results of this experiment are presented in tables 1 and 2.

No marked change due to the irradiated yeast feeding occurred in the serum-calcium and serum-phosphorus levels of the blood although both serum calcium and serum phosphorus of the yeast-fed cows averaged slightly higher after freshening (table 1).

Table 2 shows that only one-fourth as much dried blood from the yeast-fed cows as from the control cows was required to produce the same healing response, indicating that the blood of the yeast-fed cows contained approximately 4 times the vitamin D potency of the controls.

TABLE 2

Relative vitamin D potency of dried whole blood from normal and milk fever cows

Group	No. of cows	Milligrams of dried blood	Per cent healing, 4 weeks pre-fresh	Per cent healing, 12 hours pre-fresh	Per cent healing, 12 hours post-fresh	Per cent healing, milk fever
Normal						
Control	3	1,000	10.0	5.0	5.0	.....
Yeast	6	250	5.0*	9.7	12.8	.....
Milk fever						
Control	2	1,000	10.0	5.0	9.0	6.3

* 1,000 mg. of dried blood.

TABLE 3

The effect of feeding one million units of vitamin D daily on the blood serum calcium, phosphorus, magnesium and phosphatase of normal and milk fever cows

Group	No. of cows	Blood constituent (mg. per cent)	4 wk. pre-fresh	12 hr. pre-fresh	12 hr. post-fresh	Milk fever	1 wk. post-fresh
Jerseys, normal parturitions							
Control	25	Calcium	11.10	10.15	9.40	.....	10.40
Yeast	30	Calcium	10.70	10.42	9.40	.....	10.53
Control	25	Phosphorus	5.70	4.08	3.59	.....	4.65
Yeast	30	Phosphorus	5.59	4.83	3.75	.....	5.37
Control	4	Magnesium	2.14	2.40	2.30	.....	2.13
Yeast	2	Magnesium	2.90	2.62	3.03	.....	1.78
Control	3	Phosphatase	5.07	5.03	2.55	.....	3.17
Yeast	7	Phosphatase	5.33	2.97	3.99	.....	2.58
Jerseys, milk fever parturitions							
Control	13	Calcium	10.72	10.05	7.98	4.95	10.30
Yeast	12	Calcium	10.80	9.50	7.38	4.76	10.30
Control	13	Phosphorus	5.89	4.68	2.86	1.47	4.83
Yeast	12	Phosphorus	5.38	4.90	3.32	1.73	5.33
Control	2	Magnesium	1.79	2.51	3.17	3.57	1.87
Yeast	5	Magnesium	2.11	2.82	3.08	3.57	2.18
Control	3	Phosphatase	5.35	2.69	2.00	0.83	2.02
Yeast	0	Phosphatase	.....	.....	.....	.....	.....
Holsteins, normal parturitions							
Control	23	Calcium	10.39	9.81	10.40	.....	11.30
Yeast	12	Calcium	11.40	11.30	10.69	.....	11.66
Control	23	Phosphorus	5.65	4.79	4.70	.....	5.43
Yeast	12	Phosphorus	5.30	5.57	4.38	.....	5.50
Control	5	Phosphatase	3.03	3.39	3.98	.....	4.03
Yeast	1	Phosphatase	1.70	2.02	2.25	.....	2.40
Holsteins, milk fever parturitions							
Control	0	Calcium	.....	.....	.....	.....	.....
Yeast	1	Calcium	10.40	.....	9.00	5.80	9.80
Control	0	Phosphorus	.....	.....	.....	.....	.....
Yeast	1	Phosphorus	5.48	.....	5.95	5.58	6.73
Ayrshires, normal parturitions							
Control	10	Calcium	10.28	9.59	8.21	.....	10.10
Yeast	8	Calcium	10.24	9.44	8.79	.....	10.90
Control	10	Phosphorus	5.15	4.49	4.22	.....	4.56
Yeast	8	Phosphorus	5.68	5.78	5.18	.....	6.06
Control	2	Phosphatase	4.61	4.10	4.40	.....	3.99
Yeast	1	Phosphatase	5.70	5.03	6.07	.....	2.30
Ayrshires, milk fever parturitions							
Control	1	Calcium	10.58	.....	8.26	.....	9.27
Yeast	2	Calcium	10.23	10.60	6.20	5.42	10.43
Control	1	Phosphorus	3.41	.....	2.97	.....	3.08
Yeast	2	Phosphorus	5.73	4.61	3.62	2.34	4.47
Control	0	Phosphatase	.....	.....	.....	.....	.....
Yeast	1	Phosphatase	4.65	4.80	.....	6.39	3.16

TABLE 3 (*Continued*)

Group	No. of cows	Blood constituent (mg. per cent)	4 wk. pre-fresh	12 hr. pre-fresh	12 hr. post-fresh	Milk fever	1 wk. post-fresh
Guernseys, normal parturitions							
Control	7	Calcium	11.05	9.79	8.71	.....	10.59
Yeast	6	Calcium	10.48	9.86	8.66	.....	10.17
Control	7	Phosphorus	4.79	4.51	4.55	.....	5.23
Yeast	6	Phosphorus	5.34	4.65	4.16	.....	4.81
Control	0	Phosphatase	.....	.....	.....	.....	.....
Yeast	1	Phosphatase	4.92	4.15	3.00	.....	4.37
Guernseys, milk fever parturitions							
Control	0	Calcium	.....	.....	.....	.....	.....
Yeast	2	Calcium	10.73	9.20	7.65	4.84	10.30
Control	0	Phosphorus	.....	.....	.....	.....	.....
Yeast	2	Phosphorus	5.13	4.30	2.72	1.21	4.94

Experiment Involving Type 9F Irradiated Dry Yeast

Based on these preliminary findings a more extensive experiment was set up as described previously (20). In both the control and yeast-fed groups blood samples were drawn for analyses at the following times: Four weeks before the expected date of parturition, within twelve hours both before and after parturition, and one week after parturition. If milk fever occurred, samples were taken during the attack before treatment, and at frequent intervals during the recovery period.

The results shown in tables 3, 4, and 5 include data obtained from September 15, 1941, to September 15, 1944. Table 3 is a comparison of the serum calcium, phosphorus, magnesium, and phosphatase of yeast-fed and control cows, that freshened normally and with milk fever. Table 4 gives a comparison of the vitamin D potency of the blood of yeast-fed and control cows that freshened normally and with milk fever, as measured by the relative per cent healing elicited by one gram of dried whole blood when fed to rachitic rats.

In order to study possible changes in some of the other blood constituents, determinations were made at the times previously indicated for serum protein and plasma ascorbic acid, vitamin A and carotene. These four constituents were determined in the blood of Jersey cows that freshened normally and with milk fever. The results are shown in table 5. The values found for carotene and vitamin A at parturition are in agreement with those reported by Sutton, Kaeser, and Soldner (35).

High Mineral Ration Plus Various Amounts of Vitamin D

From September 15, 1944, to September 15, 1945, the work was confined to Jersey cows in the Main and Pasture Farm Experiment Station herds and

TABLE 4

The effect of feeding one million units of vitamin D daily on the relative vitamin D content of one gram of whole dried blood. (Per cent healing of rachitic bones)

Group	No. of cows	Per cent healing				
		4 weeks pre-fresh	12 hours pre-fresh	12 hours post-fresh	Milk fever	1 week post-fresh
Jerseys, normal parturitions						
Control	12	26.2	23.8	37.5	.....	31.0
Yeast	15	30.2	56.8	42.5	.....	49.0
Jerseys, milk fever parturitions						
Control	12	19.2	11.8	22.5	18.2	20.5
Yeast	5	26.0	50.0	53.2	44.5	42.5
Holsteins, normal parturitions						
Control	5	42.5	33.2	30.0	.....	44.8
Yeast	4	53.2	56.2	60.0	.....	62.2
Holsteins, milk fever parturitions						
Control	0	.....	.....	.....	.....	.....
Yeast	1	53.2	.....	43.8	56.2	56.2
Ayrshires, normal parturitions						
Control	7	10.1	32.2	28.5	.....	29.8
Yeast	2	25.0	53.2	56.2	.....	53.2
Ayrshires, milk fever parturitions						
Control	0	.....	.....	.....	.....	.....
Yeast	1	50.0	62.5	.....	50.0	50.0
Guernseys, normal parturitions						
Control	3	40.8	47.0	40.8	.....	37.5
Yeast	1	22.0	37.5	47.0	.....	34.5
Guernseys, milk fever parturitions						
Control	1	22.0	37.5	47.0	.....	34.5
Yeast	1	50.0	58.2	66.8	50.0	.....

TABLE 5

Changes in the serum protein and plasma ascorbic acid, vitamin A and carotene content of Jersey cows' blood in normal and milk fever parturitions

Group	No. of cows	Blood constituent	4 wks. pre-fresh	12 hr. pre-fresh	12 hr. post-fresh	Milk fever	1 wk. post-fresh
Normal	7	Serum protein, mg.%	6.63	6.68	6.38	.....	6.69
Milk fever	8		6.90	6.18	6.23	6.04	6.48
Normal	7	Ascorbic acid, mg. %	0.48	0.49	0.40	.....	0.46
Milk fever	8		0.50	0.41	0.47	0.46	0.51
Normal	7	Carotene, microgm. %	757	454	459	.....	446
Milk fever	8		726	553	551	525	478
Normal	7	Vitamin A, microgm. %	18.8	16.6	12.9	.....	17.2
Milk fever	8		16.4	12.3	11.5	8.6	14.4

TABLE 6

High calcium and phosphorus ration No. 43

	Lb.
Ground corn	140
Ground oats	200
Wheat bran	400
Linseed oil meal	100
Molasses	100
Steamed bone meal	50
Iodized salt	10
Total	1,000

only those cows with at least two previous calvings were included. In order to make sure that calcium and phosphorus were not limiting factors, the ration shown in table 6 was fed during the dry period beginning eight weeks before the due date and continuing for one week after parturition.

Since previous results (tables 1 and 2) had shown no significant differ-

TABLE 7

The effect of various amounts of vitamin D on some of the blood changes in Jersey normal and milk fever cows at parturition

Group*	No. of cows	Blood constituent, mg. per cent	8 wk. pre-fresh	4 wk. pre-fresh	2 wk. pre-fresh	12 hr. pre-fresh	12 hr. post-fresh	Milk fever	1 wk. post-fresh
Normal parturitions									
I	4	Calcium	10.73	10.29	10.78	10.50	9.10	.....	10.55
II	3	Calcium	11.05	10.80	10.42	.....	9.31	.....	10.83
III	5	Calcium	10.73	10.33	10.85	11.45	9.26	.....	10.46
I	4	Phosphorus	6.13	6.95	6.00	6.50	5.25	.....	6.25
II	3	Phosphorus	6.45	7.49	7.89	.....	6.90	.....	5.05
III	5	Phosphorus	6.58	6.66	8.23	8.64	8.60	.....	6.42
I	4	Magnesium	1.89	1.93	2.09	2.12	2.43	.....	1.75
II	3	Magnesium	2.13	1.90	1.95	.....	2.28	.....	2.04
III	5	Magnesium	2.44	1.74	1.73	1.62	1.86	.....	1.34
Milk fever parturitions									
I	1	Calcium	9.98	9.24	10.62	7.10	6.40	3.50	8.50
II	3	Calcium	10.07	10.89	10.46	.....	6.86	5.49	10.22
III	1†	Calcium	11.47	11.56	11.52	.....	10.67	.....	Died
I	1	Phosphorus	5.02	8.80	7.36	3.82	2.92	1.63	4.75
II	3	Phosphorus	4.88	6.71	6.86	.....	3.99	3.49	6.61
III	1†	Phosphorus	7.64	7.35	8.40	.....	6.88	.....	Died
I	1	Magnesium	1.81	1.09	1.74	2.56	2.74	2.96	1.25
II	3	Magnesium	2.02	2.26	1.76	.....	2.18	2.55	1.82
III	1†	Magnesium	2.26	2.30	1.25	.....	1.83	.....	Died

* The following amounts of vitamin D were fed daily for four weeks before and one week following parturition: Group I—None. Group II—1 million units. Group III—2 million units.

† It is questionable whether this cow had milk fever as the blood picture is not typical and she did not respond to two calcium gluconate injections. She died later of complications.

ence in the calcium and phosphorus content of the blood between yeast-fed and control cows, it was decided to feed two million units of vitamin D daily to a few cows and observe any blood changes which might occur.

The eighteen available cows were divided into three equal groups. All the cows were fed the high mineral ration as previously described. Group I received no supplemental irradiated yeast. Group II received one million units of vitamin D daily in the form of type 9F irradiated dry yeast and Group III was given a similar amount of type 22F irradiated dry yeast which provided two million units of vitamin D daily. The yeast feeding was begun four weeks before the expected date of parturition and continued for one week after parturition. Blood samples were drawn for calcium, phosphorus, magnesium and vitamin D analyses at the same times as in previous experiments except that an additional eight-week pre-freshening and a 2-week pre-freshening sample were included.

One case of milk fever occurred in Group I and one abortion. The blood data of the abortion case are omitted from the averages. Three cases of milk fever occurred in Group II and one questionable case in Group III. The results of the blood analyses are shown in tables 7 and 8.

As a possible means of explaining the blood changes associated with milk fever, as often as possible during this experiment a sample of colostrum was secured before the calf nursed. The colostrum samples were analyzed for

TABLE 8

The effect of various levels of vitamin D intake on the relative vitamin D content of dried whole blood

Group*	No. of cows	Mg. dried blood fed	Per cent healing, 4 wks. pre-fresh	Mg. dried blood fed	Per cent healing, 2 wks. pre-fresh	Mg. dried blood fed	Per cent healing, 12 hr. post-fresh	Mg. dried blood fed	Per cent healing, milk fever	Mg. dried blood fed	Per cent healing, 1 wk. post-fresh
Normal parturitions											
I	2	1,000	46	1,000	56	1,000	48	.....	.....	1,000	48
II	1	1,000	25	500	50	500	63	.....	.....	500	58
III	4	1,000	50	250	61	250	61	.....	.....	250	61
Milk fever parturitions											
I	2	1,000	50	1,000	40	1,000	48	1,000	27	1,000	33
II	3	1,000	44	500	56	500	65	500	63	500	58
III	1	1,000	54	250	61	250	54	250	.....	250	.....

* The following amounts of vitamin D were fed daily for 4 weeks before and one week following parturition: Group I—None. Group II—1 million units. Group III—2 million units.

TABLE 9

The composition of colostrum from normal and milk fever Jersey cows fed various amounts of vitamin D

Group*	No. of cows	Units of vitamin D daily	Specific gravity	Total solids	Ash, per cent	Fat, per cent	Total protein	Carotene, γ %	Vitamin A, γ %
Normal parturitions									
I	2	None	1.067	19.0	1.27	1.78	13.5	147	68
II	4	1,000,000	1.068	22.0	1.09	2.50	14.8	208	144
III	3	2,000,000	1.061	20.3	1.03	3.20†	13.2	129	138
Average			1.065	20.8	1.11	2.40	14.0	168	126
Milk fever parturitions									
I	1	None	1.070	26.0	1.15	5.85	17.2	285	188
II	2	1,000,000	1.057	17.9	1.03	3.35	11.6†	187	146
III	0	2,000,000							
Average			1.061	20.6	1.07	4.15	14.4	220	160

* The following amounts of vitamin D were fed daily for four weeks before and one week following parturition: Group I—None. Group II—1 million units. Group III—2 million units.

† Only one cow.

the following: total solids, specific gravity, ash, total protein, fat, carotene, and vitamin A. The results are presented in table 9.

DISCUSSION

As is indicated in tables 1, 3, and 7, the feeding of 1 million units of vitamin D did not have any significant effect on the serum calcium and phosphorus levels of the blood at parturition regardless of whether or not milk fever developed. This finding probably explains why no lowering of milk fever incidence was observed at this level of vitamin D feeding, as reported in the first paper of this series (20).

When 2 million units of vitamin D daily were fed (table 6) an increase in both serum calcium and serum phosphorus occurred before parturition. It is possible, therefore, that at still higher levels of vitamin D feeding sufficient increases in blood calcium and phosphorus might be obtained to prevent the decrease of these blood constituents to the milk-fever level. Experiments are now in progress to investigate this possibility.

Of interest is the increase in serum magnesium concurrent with the fall in serum calcium and serum phosphorus in both normal and milk fever freshenings. The rise in serum magnesium is probably a compensatory phenomenon elicited by the fall in calcium and phosphorus and apparently has a marked effect on the symptoms seen during the milk fever attack. At the low level of serum calcium present in milk fever, tetanic symptoms would normally be expected rather than the flaccid, comatose condition usually observed. This is seemingly due to the high serum magnesium in relation to the low serum calcium. On the other hand when nervous symptoms

accompany milk fever the serum magnesium is nearly always normal or below normal, as reported by Barker (2).

In this connection some of our experiments, in which $MgSO_4$ was injected intravenously, are of interest. Upon injection of a solution of this salt into normal cows general anesthesia resulted when the serum magnesium reached a level of approximately 7.5 mg. per 100 ml. When the serum calcium was then raised to about 17.5 mg. per 100 ml. by injecting calcium gluconate the cows regained consciousness even though the serum magnesium remained at the same high level. The serum calcium and magnesium were both found to be back to normal levels within 5 hours after injection.

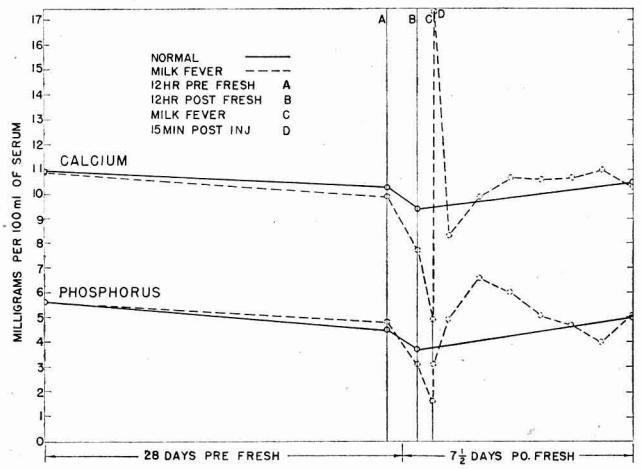

FIG. 1. Changes in the serum calcium and phosphorus of the blood before and after parturition of 55 normal Jersey cows and 25 Jersey cows that developed milk fever. (Both control and yeast fed cows are included.)

It seems, therefore, that the relationship between serum magnesium and serum calcium may be of great importance in the symptomatology of milk fever. It is also apparent that the serum calcium-magnesium relationship is more important in producing the symptoms of milk fever than is serum phosphorus.

Figure 1 shows the changes in the serum calcium and serum phosphorus of the blood of normal and milk fever Jersey cows at various times before and after parturition. Fifteen minutes after treatment with calcium gluconate (500 cc.) the serum calcium was found to be 17.1 mg. per cent which declined rapidly to near the normal level. If the fall in serum calcium continued too long a recurrent attack of milk fever ensued. Both control and yeast-fed cows are included in this figure, since, as shown in tables 1 and 2, yeast feeding at this level did not affect the blood levels of calcium and phosphorus.

The changes in the serum calcium, phosphorus, and magnesium of control and milk fever Jersey cows before and after parturition are shown in figure 2. Marked decreases in serum calcium and phosphorus occur prior to the onset of milk fever accompanied by a concurrent increase in serum magnesium.

The phosphatase content of serum was variable but it appears, as shown in table 3, that in Jersey cows especially, the phosphatase was lower at freshening than before or after freshening and that the lowest values were obtained in milk fever. This may be of significance in view of the low phosphorus content of blood serum during milk fever.

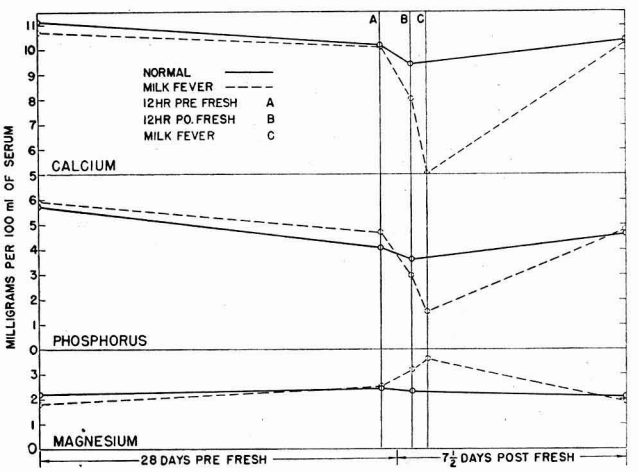

FIG. 2. Changes in the serum calcium, phosphorus, and magnesium of the blood before and after parturition of 25 control Jerseys fresh normally and 13 control Jerseys that developed milk fever.

While the serum protein values shown in table 5 were all within the normal range, a slight decrease was observed after parturition in both normal and milk fever cows. The lowest values obtained were in samples taken during the milk fever attack. It is not known whether or not this is of any importance in connection with milk fever, but it does indicate that cows in beginning lactation and in milk fever may be somewhat deficient in body protein, since low serum protein does not usually occur until after a pronounced loss of body protein. The lowering of serum protein at parturition and in milk fever is probably due, at least in part, to the formation of colostrum which is extremely high in protein, as indicated in table 9.

No marked changes at parturition were found in the plasma ascorbic acid content of the blood; however, plasma carotene and vitamin A were greatly reduced at this time. The low values obtained during milk fever indicate

that blood vitamin A and carotene values either continue to fall after the 12-hour post-freshening sample or that in milk fever the decrease is delayed. The latter concept supports the observation that in many milk fever cows the udder does not fill before parturition. Colostrum is extremely rich in these constituents, as shown in table 9. Judging from the relative decreases in the blood level of vitamin D and vitamin A it is doubtful whether or not there is relatively as high a concentration of vitamin D in colostrum as there is of vitamin A. This is also suggested by the work of Henry and Kon (18).

Table 9 shows the fat content of colostrum to be somewhat variable but on the average it contains less fat than typical Jersey milk. No difference in specific gravity, total solids, or ash content was found between colostrum from normal cows and that of milk fever cows. All three of these factors were extremely high in concentration in colostrum as compared to normal milk.

While no marked difference was observed in the composition of the colostrum of normal and milk fever cows, it is evident from the high concentration of vitamin A, carotene, protein, ash, and total solids that the formation of colostrum constitutes a sudden, heavy drain on the body reserves of the parturient cow. This is further substantiated by the corresponding fall in the blood level of these constituents.

Whether or not a cow develops milk fever seems to be determined by the success or failure of the mineral regulatory mechanism to meet the sudden demands of beginning lactation and at the same time maintain the necessary blood levels of calcium, phosphorus, and magnesium.

As indicated in tables 1, 4, and 8 the data concerning blood vitamin D were exceedingly variable. However, a few general statements can be made regarding the results. Although there was a tendency for the blood vitamin D to decrease at parturition, the decrease was not so marked as that of vitamin A and carotene. No clear-cut breed difference is noted; however, the Jerseys seemed to have a lower average blood vitamin D level than did the other three breeds. Some of the lowest values for blood vitamin D were obtained during the milk fever attack, but there was no correlation between the incidence of milk fever and the vitamin D content of the blood, since milk fever occurred in some cases even though the blood vitamin D content was twice normal. The data also show that an approximate twofold increase in the blood vitamin D, as a result of feeding 1 million units of vitamin D daily, did not affect the calcium and phosphorus level of blood serum.

When 2 million units of vitamin D were fed daily the vitamin D content of the blood was found to be approximately four times that of the controls. At this level of vitamin D feeding a slight increase in serum calcium and phosphorus was observed. It would seem, therefore, that before any marked effect on the serum calcium and phosphorus can be expected, more than 2 million units of vitamin D must be fed daily. It appears, then, that the

mere raising of blood vitamin D above the normal level will not prevent milk fever from occurring. It is possible that only when the blood vitamin D concentration reaches a level sufficient to elicit an increase in the blood calcium and phosphorus, a decrease in milk fever incidence might be expected. More work needs to be done before this question can be answered.

SUMMARY AND CONCLUSIONS

1. Determinations were made on the blood serum for calcium, phosphorus, magnesium, and phosphatase, and on whole dried blood for vitamin D content, of control cows and cows fed 1 and 2 million units of vitamin D daily for four weeks before and one week following parturition, fresh normally and with milk fever. Cows of the Jersey, Holstein, Ayrshire, and Guernsey breeds were included in the study.

2. Data are presented regarding the serum protein, plasma carotene, vitamin A, and ascorbic acid of Jersey cows that freshened normally and with milk fever.

3. A study was made of the specific gravity, total solids, ash, fat, total protein, carotene, and vitamin A in the colostrum of normal and milk fever cows fed various amounts of vitamin D.

4. No effect on the serum calcium, phosphorus, magnesium, or phosphatase was observed when 1 million units of vitamin D in the form of irradiated dry yeast were fed daily for four weeks previous to freshening and for one week thereafter. When 2 million units of vitamin D were fed, a slight increase in both serum calcium and phosphorus was noted. This was accompanied by a slight decrease in serum magnesium. It was found that both serum calcium and phosphorus decrease markedly during a few hours prior to the milk fever attack, the lowest level being reached after the symptoms appear. A concurrent increase in the serum magnesium usually occurs.

The level of the blood vitamin D was found to be approximately double the normal level when 1 million units of vitamin D were fed and about four times the normal level when 2 million units of vitamin D were fed.

Before increases in the serum calcium and phosphorus level of the blood can be obtained, sufficient to lower milk fever incidence, apparently more than 2 million units of vitamin D must be fed daily before freshening. Experiments are now in progress to determine the effect of feeding larger amounts of vitamin D on the blood picture and the incidence of milk fever.

It is of interest that some of the lowest blood vitamin D values obtained occurred in control cows during the milk fever attack; however, when vitamin D was fed milk fever occurred even though the blood level of vitamin D was relatively high. The decrease in the blood vitamin D of control cows at parturition is considered to be associated with the production of colostrum.

5. The decreases observed in serum protein and plasma vitamin A and carotene at parturition are considered to be associated with the production

of colostrum which is particularly rich in these constituents. No significant change in the level of plasma ascorbic acid was noted at parturition.

6. No significant difference was noted in the composition of colostrum between cows that freshened normally and those which developed milk fever.

7. The blood serum calcium–magnesium relationship is discussed in regard to its possible effect on the symptomatology of milk fever.

REFERENCES

(1) ALLCROFT, W. M., AND GREEN, H. H. Blood Calcium and Phosphorus of the Cow in Health and Disease. Biochem. Jour., **28**: 2220–2228. 1934.

(2) BARKER, J. R. Blood Changes and Variations in the Female Bovine Toxemias. Vet. Rec., **51**: 575–580. 1939.

(3) BODANSKY, A. The Determination of Serum Phosphatase. Jour. Biol. Chem., **101**: 93. 1933.

(4) BOYER, P. D., PHILLIPS, P. H., AND SMITH, J. K. The Separation of Carotene from Vitamin A for the Determination of Vitamin A in the Blood Plasma. Jour. Biol. Chem., **152**: 445–452. 1944.

(5) BOYER, P. D., SPITZER, R., JENSEN, C., AND PHILLIPS, P. H. Determination of Vitamin A and Carotene in Milk. A Rapid Extraction Procedure. Indus. and Engin. Chem. (Analyt. Ed.), **16**: 101–102. 1944.

(6) BRIGGS, A. P. A Colorimetric Method for the Determination of a Small Amount of Magnesium. Jour. Biol. Chem., **52**: 349. 1922.

(7) BRIGGS, A. P. A Modification of the Bell Doisy Phosphate Method. Jour. Biol. Chem., **53**: 13. 1922.

(8) CAMPBELL, I. L., AND TURNER, C. W. The Relationship of the Endocrine System to the Regulation of Calcium Metabolism. Mo. Agr. Expt. Sta. Res. Bul. No. 352. Oct. 1943.

(9) CLARK, E. P., AND COLLIP, J. B. A Study of the Tisdall Method for the Determination of Blood Serum Calcium with Suggested Modifications. Jour. Biol. Chem., **63**: 461. 1925.

(10) FISH, P. A. The Physiology of Milk Fever. II. Jour. Am. Vet. Med. Assoc., **73**: 10–37. 1928.

(11) FISH, P. A. The Physiology of Milk Fever. III. The Blood Phosphates and Calcium. Cornell Vet., **19**: 147–160. 1929.

(12) FISH, P. A. Milk Fever. 11th Internatl. Vet. Congress, 330–347. 1930.

(13) FISH, P. A. Milk Fever. Vet. Rec., **11**: 153. 1931. (Nutr. Ab. and Rev., **1**: 326. 1931–32.)

(14) GODDEN, W., AND ALLCROFT, W. M. Changes in the Composition of Cows' Blood at the Time of Calving and a Comparison of the Blood of the Calf with that of its Dam. Biochem. Jour., **26**: 1640–1646. 1932.

(15) GODDEN, W., AND DUCKWORTH, J. Variations in Serum Magnesium and the Partition of Serum Calcium in Normal Parturitions and Milk Fever. Biochem. Jour., **29**: 445–455. 1935.

(16) GREIG, J. R. Calcium Gluconate as a Specific in Milk Fever. Vet. Rec., **10**: 115–120. 1930.

(17) GREIG, J. R. Studies in the Prevention of Milk Fever. Vet. Rec., **10**: 301–305. 1930.

(18) HENRY, K. M., AND KON, S. K. A Note on the Vitamin D Content of Colostrum. Biochem. Jour., **31**: 2199. 1937.

(19) HESS, A. F., LIGHT, R. F., FREY, C. N., AND GROSS, J. A Study of the Milk, Blood and Excreta of Cows Fed Moderate and Excessive Amounts of Irradiated Yeast or Ergosterol. Jour. Biol. Chem., **97**: 369–377. 1932.

(20) HIBBS, J. W., KRAUSS, W. E., MONROE, C. F., AND SUTTON, T. S. Studies on Milk Fever in Dairy Cows. I. The Effect of Vitamin D on the Incidence of Milk Fever. JOUR. DAIRY SCI., **29**: 617–623. 1946.

(21) KAGAN, B. M. The Falling Drop Method for Determining Total Protein and Specific Gravity. Jour. Clin. Invest., **XVII**: 369–372 and 373–376. 1938.

(22) KIMBLE, M. S. The Photoelectric Determination of Vitamin A and Carotene in Human Plasma. Jour. Lab. and Clin. Med., **24**: 1055–1065. 1939.

(23) KLOUBOK, ————. Zverol Rozpravy, **6**: 13–24. Cited by Schulhof, A. (Nutr. Abs. and Rev., **4**: 699, 1934–35) and Pribyl, E. (Nutr. Abs. and Rev., **4**: 88. 1934–35.)

(24) LITTLE, W. L., AND WRIGHT, N. C. The Aetiology of Milk Fever in Cattle. British Jour. Expt. Path., **VI**: 129–132. 1925.

(25) LITTLE, W. L. Milk Fever or Hypocalcemia of Parturient Cows. Vet. Rec., **12**: 683–686. 1932.

(26) MATTICK, E. C. V., AND LITTLE, W. L. The Calcium Content of Cows' Blood. 1. The Effect of Feeding Cod Liver Oil. Vet. Rec., **13**: 238–245. 1933.

(27) MATTICK, E. C. V., AND LITTLE, W. L. The Calcium Content of Cows' Blood. 2. (a) Calcium in the Blood of Parturient Heifers. (b) Effect of Injection of Parathyroid Extract, Irradiation, etc. (c) Some Case Reports of Milk Fever. Vet. Rec., **13**: 1091–1097. 1933.

(28) MINDLIN, R. L., AND BUTLER, A. M. The Determination of Ascorbic Acid in Plasma: A Macromethod and a Micromethod. Jour. Biol. Chem., **122**: 673. 1938.

(29) Pribyl, E. The Electrolyte Content of the Blood Serum of Sheep and Goats and of Cows in Certain Diseases, Particularly in the Period of Pregnancy and in Connection with Milk Fever. Zverol. Rozpravy Suppl. Zverol. Obsor., **7**: 61–70 and 73–78. 1933. (Nutr. Abs. and Rev., **4**: 88. 1934–35.)

(30) SEEKLES, L., SJOLLEMA, B., AND VAN DER KAY, F. C. Biochem. Zeitschr., **249**: 424. 1924. (Cited by Godden, W., and Duckworth, J. Biochem. Jour., **29**: 445–455. 1934.)

(31) SCHULHOF, A. Hypoglycemia and Hypocalcemia as Causes of Milk Fever in Cows. Klin. Spisy. Skoly. Zverol. Brno., **10**: 23–45. 1933. (Nutr. Abs. and Rev., **4**: 699. 1934–35.)

(32) SJOLLEMA, B. The Biochemistry of Milk Fever of Cows. (Significance of the Electrolyte Balance of the Organism.) Biochem. Zeitschr., **200**: 300–308. 1928. (Biol. Abs., **5**: 4684. 1931.)

(33) SJOLLEMA, B., AND SEEKLES, L. Über storungen des Mineralen Regulationsmechanismus bie Krankheiten des Rindes. Biochem. Zeitschr., **229**: 358–380. 1930. (Biol. Abs., **6**: 1346. 1932.)

(34) SJOLLEMA, B. Nutritional and Metabolic Disorders in Cattle. Nutr. Abs. and Rev., **1**: 621–632. 1931–32.

(35) SUTTON, T. S., KAESER, H. E., AND SOLDNER, P. A. Changes in the Level of Vitamin A and Carotene in the Blood Plasma of Dairy Cows Associated with Parturition and Beginning Lactation. JOUR. DAIRY SCI., **28**: 933–939. 1945.

(36) WILSON, L. T., AND HART, E. B. The Chemistry of the Blood of Dairy Cows before and after Parturition and its Relation to Milk Fever. JOUR. DAIRY SCI., **15**: 116–131. 1932.

THE PANTOTHENIC ACID, NIACIN, AND BIOTIN CONTENT OF COMMERCIAL AND EXPERIMENTAL MILKS*

J. J. STEFANIAK AND W. H. PETERSON
Department of Biochemistry, College of Agriculture, University of Wisconsin, Madison

The pantothenic acid content of milk has been reported by various investigators (1, 3, 4, 11, 13) to range from 1.3 to 4.6 μg. per ml. Williams *et al.* (13) found the range to be 1.7 to 4.6 with an average value of 2.9 μg. per ml. for 30 samples. Fresh milk analyzed by Hodson (3) contained from 1.9 to 4.2 with an average of 3.1 μg. per ml.

Early methods for the determination of niacin were of a chemical nature and proved to be unreliable and figures ranging from 0.6 to 8.2 μg. per ml. have been reported (2, 7). With a reliable microbiological assay Williams *et al.* (13) obtained minimum and maximum values of 0.19 and 1.2 μg. per ml., respectively. Values given by other workers (3, 10, 12) fall within this range. Hodson (3) analyzed 31 samples collected during the month of May and found an average of 0.91 μg. per ml.

Reports on the biotin content of milk (3, 5, 8, 13) vary from 12.5 to 110 mμg. per ml. Hodson (3) reported minimum and maximum values of 32 and 84 and an average of 47 mμg. per ml.

All of these reports deal with the analysis of one sample or of samples collected for a short period of time or of milk collected at irregular intervals. It seemed desirable therefore to analyze samples of market milk collected throughout the year. At the same time an experiment was set up to determine if there is a relation between the type of silage consumed by cows and the content of these vitamins in their milk.

EXPERIMENTAL

Milks. The samples of commercial milk were either purchased in the open market or delivered by the distributor. Three distributors supply the city of Madison. These milks are classified as market and Guernsey milk and represent a daily distribution of about 110,000 lbs. of milk.

In February 1943, a number of milk samples were obtained from individual farms in the vicinity of Madison which were included in a grass silage program sponsored by the College of Agriculture. Table 2 presents the analytical results as well as the forage fed to these herds. Ordinary farm rations were being fed at the time the samples were taken.

The experimental milks were produced by animals on silage experiments at the University farm. In the 1940–41 and in the 1941–42 trials at the

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University farm, each lot contained five cows while in the 1942–43 experiments, each group consisted of nine cows. The animals were fed a ration consisting of 40 to 50 lbs. silage, 5 to 10 lbs. good hay and 3 to 12 lbs. grain per day. Variations in the ration were made according to the milk production of the animal.

Analytical methods. Aliquots of the milks were diluted with distilled water to the proper level and analyzed without further treatment. All milks were frozen after the aliquots for dilution had been taken so that repeat analyses could be run if the analytical results seemed doubtful. Recoveries of added pantothenic acid, niacin and biotin were run with all assays. If a recovery did not fall within $\pm$ 10 per cent, the determination was repeated. Repeat analyses on reference materials were run regularly as an additional check on the methods.

Pantothenic acid was determined by the method of Strong *et al.* (11). Williams *et al.* (13) reported higher values for samples of skim milk digested with takadiastase and papain than for the untreated samples. Digestion with 2 per cent of an equal mixture of takadiastase and papain was tried on our milks but no effect on the results was obtained.

The Snell and Wright method (10) for the determination of niacin with HCl in place of H_2SO_4 for hydrolysis of casein was used.

The *Lactobacillus casei* method described by Shull *et al.* (8) and modified by Shull and Peterson (9) was used for the determination of biotin. Tests in which samples were digested with a mixture of takadiastase and papain or hydrolyzed with 4.0 N HCl for 2 hours were run to determine whether biotin was present in bound form. The results obtained showed that these treatments were not necessary.

RESULTS AND DISCUSSION

Pantothenic acid. Table 1 summarizes the analyses of the commercial milks. The pantothenic acid content of the samples ranged from 2.7 to 4.5 μg. per ml. for the individual samples. Only one sample contained more than 3.9 μg. per ml. pantothenic acid. The average values for market and Guernsey were 3.4 and 3.2 μg. per ml. respectively. The pantothenic acid content did not vary greatly with seasonal changes. In 1940 and 1941, 29 other samples of commercial milk were analyzed by G. P. Bahler of this department and were found to range from 2.7 to 5.9 with an average of 4.2 μg. per ml.

Individual farm milks (table 2) were somewhat higher in pantothenic acid than commercial milks but approximately the same as the experimental milks.

The values for experimental milks in table 3 are averages of from 2 to 4 samples. The range of pantothenic acid from the low to the high samples of milk in a given feeding trial (table 3) was in most cases about 20 per

TABLE 1

B vitamin survey of commercial milks

Date	Pantothenic acid		Niacin		Biotin	
	Market*	Guernsey†	Market*	Guernsey†	Market*	Guernsey†
	μg./ml.		μg./ml.		mμg./ml.	
August 4, 1942	3.2	3.1	0.82	0.83	29	24
September 10, 1942	3.1	3.0	0.77	0.79	30	27
January 24, 1943	3.7	3.7	0.75	0.72	31	32
April 16, 1943	3.5	3.4	0.71	0.74	32	29
June 9, 1943	3.3	3.0	0.95	0.88	21	21
Total number of samples	29	15	29	15	29	15
Range of samples	2.7–4.5	2.7–3.8	0.64–1.04	0.63–1.08	18–37	17–38
Average	3.4	3.2	0.80	0.79	29	27

* The figures are the average for 6 samples at each date except the August 4, 1942, values when only 5 samples were run.

† The figures are the average for 3 samples.

cent. The commercial milks showed a variation of about 50 per cent. These variations agree with those reported by Williams *et al.* (13) and Hodson (3). The pantothenic acid values were higher for the winter and spring samples of 1940 and 1941 than for those for the following winter and spring, 1942–43. No correlation can be made between the roughage fed and the variation in the pantothenic acid content of these experimental milks.

Niacin. The niacin content of commercial milk (table 1) ranges from 0.63 to 1.08 μg. per ml. for individual samples of market and Guernsey milk. The averages for market milk and Guernsey milk were 0.80 and 0.79 μg. per ml., respectively. The niacin content increased slightly during the summer months. The values for commercial milks were generally higher than those for the individual farm milks and experimental milks. The average niacin content of experimental milks was 0.63 μg. per ml. A 10 per cent variation among samples of experimental milks and about 50 per cent among the commercial samples was found in most cases. Similar variations are reported by Williams *et al.* (13) and Hodson (3).

TABLE 2

Analyses of individual farm milks for pantothenic acid, nicotinic acid and biotin

Farm	No. of samples	Silage	Pantothenic acid	Niacin	Biotin
			μg./ml.	μg./ml.	mμg./ml.
1	1	Alfalfa and corn-cob meal silage	4.0	0.72	49
2	1	Sweet clover and corn-cob meal silage	4.6	0.75	35
3	1	Alfalfa silage—no preservative	3.2	.....	62
4	1	Corn silage	4.4	0.52	28
5	2	Alfalfa and corn-cob meal silage	6.0	0.52	38

TABLE 3

Analysis of experimental milks from the University herd

Month and year	No. of cows	No. of samples	Roughage	Pantothenic acid	Niacin	Biotin
				μg./ml.	μg./ml.	mμg./ml.
Nov.–Feb. 1940–41	5	4	Oats-peas-H_3PO_4 silage	4.4 ± 0.3	.....	40*
Nov.–Feb. 1940–41	5	4	Soybean-sudan-H_3PO_4 silage	5.0 ± 0.5	.....	59*
Nov.–Feb. 1940–41	5	4	Alfalfa-molasses silage	4.3 ± 0.5	.....	50*
Feb.–Mar. 1942	5	3	Soybean (2)–sorghum (1) silage	4.4 ± 0.4	0.68 ± 0.03	47 ± 4
April 1942	5	3	Soybean (1)–sorghum (1) silage	5.5 ± 0.5	0.61† ± 0.03	45 ± 3
Feb.–Mar. 1942	5	3	Alfalfa–10–20 lb. conc. whey/ton silage	4.3 ± 0.1	0.62 ± 0.0	53 ± 3
Feb. 1942	5	1	Alfalfa—200 lb. corn-cobmeal/ton silage	3.6	0.59	41
Mar. 1942	5	2	Alfalfa silage—no preservative	4.1 ± 0.0	0.66 ± 0.02	55 ± 3
Mar.–April 1942	6	2	Corn silage	4.1 ± 0.1	0.74*	20 ± 1
July–Sept. 1942	20	3	Pasture	3.3 ± 0.3	0.70 ± 0.09	27 ± 7
Nov. 1942	18	1	Corn silage	3.4	0.69	28
Jan. 1943	18	1	Corn silage	3.2	0.76	45
Mar.–April 1943	9	2	Alfalfa silage—200 lb. corn-cobmeal/ton	4.4 ± 0.3	0.59 ± 0.04	42 ± 4
Mar.–April 1943	9	2	Alfalfa silage—forage wilted	3.5 ± 0.9	0.37 ± 0.06	38 ± 4
June 1943	18	1	Corn silage	3.4	0.53	40

* Analysis on one sample.
† Analysis on two samples.

Biotin. The biotin content of commercial milk for the period beginning August 4, 1942, and ending June 9, 1943, varied from 17 to 38 mμg. per ml. for individual samples of market and Guernsey milk (table 1). The average values for these two classes of milk were 29 mμg. per ml. for market milk and 27 mμg. per ml. for Guernsey milk. There was a notable decline in the biotin content during the month of June when the cows were pasture fed. The milks from the individual farms (table 2) ranged from 28 to 62 mμg. per ml. of milk. This wide range may be related to the type and the quality of silage fed. High quality silage was fed on farms 1, 2, 3, and 5 and very poor quality of corn and grass silages were fed on farm 4.

The experimental milk (table 3) shows a marked variation in biotin content. A minimum of 20 mμg. per ml. and a maximum of 59 mμg. per ml. were obtained. Grass silage milks generally were higher in biotin than corn silage or pasture milk, however, high biotin values (January 1943) have been obtained when corn silage was the roughage fed. A comparison of the data from tables 1 and 3 shows that there was a decrease in the biotin content of milks produced on pasture. A part of the University herd consisting of 18 cows which was still on winter feed in June, showed no decline in the biotin content of the milk. A possible explanation of this decline may be that because of changes in the bacterial flora, the synthesis of biotin in the rumen on pasture feed does not keep pace with the increased milk flow. Lardinois *et al.* (6) have shown that an increase in the synthesis of vitamins of the B complex in the rumen of cows can be brought about by the addition of urea as a source of nitrogen in the ration. Similarly, other feeding changes might influence the biotin content of the rumen and consequently the biotin content of the milk.

SUMMARY

The pantothenic acid content of commercial market and Guernsey milks for the year 1942–43 averaged 3.4 and 3.2 μg. per ml., respectively. Analyses of 29 samples of commercial milk the preceding year averaged 4.2 μg. per ml. The niacin content of commercial milk averaged 0.80 μg. per ml. Seasonal and feeding changes did not affect the amount of these two vitamins.

Milks analyzed for biotin were found to contain from 17 to 62 mμg. biotin per ml. The average values for market and Guernsey milks were 29 and 27 mμg. per ml., respectively. The biotin content of milk showed seasonal variations which were the result of feeding changes. The type of roughage fed influenced the biotin content of milk.

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They are indebted to I. W. Rupel, Dairy Husbandry Department, and G. Bohstedt, Animal Husbandry Department, for cooperation in respect to the silages and milks of table 3. These silages were put up and fed to determine their value in maintaining the carotene and vitamin A content of winter milk. A report on this work will be given in another publication.

REFERENCES

(1) ATKIN, L., WILLIAMS, W. L., SCHULTZ, A. S., AND FREY, C. N. Yeast Microbiological Methods for the Determination of Vitamins. Pantothenic Acid. Ind. Eng. Chem., Anal. Ed., **16**: 67–71. 1944.

(2) BAILEY, E. A., JR., DANN, W. J., SATTERFIELD, G. H., AND GRINNELS, C. D. A Method for the Estimation of Nicotinic Acid in Milk. JOUR. DAIRY SCI., **24**: 1047–1053. 1941.

(3) HODSON, A. Z. The Nicotinic Acid, Pantothenic Acid, Choline and Biotin Content of Fresh, Irradiated, Evaporated and Dry Milk. Jour. Nutr., **29**: 137–142. 1945.

(4) JUKES, T. H. The Distribution of Pantothenic Acid in Certain Products of Natural Origin. Jour. Nutr., **21**: 193–200. 1941.

(5) LAMPEN, J. O., BAHLER, G. P., AND PETERSON, W. H. The Occurrence of Free and Bound Biotin. Jour. Nutr., **23**: 11–21. 1942.

(6) LARDINOIS, C. C., MILLS, R. C., ELVEHJEM, C. A., AND HART, E. B. Rumen Synthesis of the Vitamin B Complex as Influenced by Ration Composition. JOUR. DAIRY SCI., **27**: 579–583. 1944.

(7) NOLL, C. I., AND JENSEN, O. G. The Chemical Determination of Nicotinic Acid in Milk and Milk Derivatives. Jour. Biol. Chem., **140**: 755–762. 1941.

(8) SHULL, G. M., HUTCHINGS, B. L., AND PETERSON, W. H. A Microbiological Assay for Biotin. Jour. Biol. Chem., **142**: 913–920. 1942.

(9) SHULL, G. M., AND PETERSON, W. H. Improvements in the *Lactobacillus casei* Assay for Biotin. Jour. Biol. Chem., **151**: 201–202. 1943.

(10) SNELL, E. E., AND WRIGHT, L. D. A Microbiological Method for the Determination of Nicotinic Acid. Jour. Biol. Chem., **139**: 675–686. 1941.

(11) STRONG, F. M., FEENEY, R. E., AND EARLE, A. Microbiological Assay for Pantothenic Acid. Ind. Eng. Chem., Anal. Ed., **13**: 566–570. 1941.

(12) TEPLY, L. J., STRONG, F. M., AND ELVEHJEM, C. A. The Distribution of Nicotinic Acid in Foods. Jour. Nutr., **23**: 417–423. 1942.

(13) WILLIAMS, R. J., CHELDELIN, V. H., AND MITCHELL, H. K. Studies on the Vitamin Content of Tissues, II. The University of Texas Publication No. 4237, 97–104. 1942.

THE CAROTENE AND VITAMIN A CONTENTS OF WISCONSIN CHEESE*

KIYOSHI HIGUCHI AND W. H. PETERSON
Department of Biochemistry, College of Agriculture,
University of Wisconsin, Madison

INTRODUCTION

In an earlier paper from this laboratory (5) it was reported that several hundred samples of Wisconsin market milk averaged 32 I.U. of vitamin A potency per gram of butter fat for winter (December–April) milk and 55 I.U. for summer (May–November) milk. A similar survey (1) of the market butter produced in this state showed a potency of 27 I.U. per gram of butter fat for the winter product and 49 I.U. for the summer butter. A recent report (11) on the situation in the whole United States gave the average potency as 11,160 I.U. per lb. for winter and 17,955 I.U. per lb. for summer butter. Assuming 80 per cent butter fat, these figures correspond to 30 I.U. and 49 I.U. per gram of butter fat for the respective seasons. If the United States figures for butter fat are applied to cheese on the basis of 150 grams of fat per lb. of cheese and assuming no loss of potency in processing, the vitamin A potency should be about 4,500 I.U. per lb. for the winter cheese and 7,400 for the summer product. These calculations are for whole milk cheese such as cheddar, in which the butter fat is about 31 to 33 per cent. The calculated values are considerably below many of the figures found in the literature. Hathaway and Davis (6) reported about 7,000–10,900 I.U. per lb. for cheddar, 18,200 I.U. per lb. for Roquefort, and as high as 19,000 I.U. per lb. for pimento cream cheese. The first two types contained approximately 30 per cent fat while the pimento cream type contained only 18.4 per cent fat. Coward and Morgan (3) reported a sample of English cheddar cheese to have almost 25,000 I.U. per lb. Other values found in the literature are as follows: 3,400 I.U. per lb. for cheddar cheese, Davies and Moore (4), 11,000 I.U. per lb. for Roquefort cheese, Todhunter *et al.* (10), and about 9,000 I.U. per lb. for Porto Rican cheese, Cook and Axtmeyer (2). Most of these data have been obtained by the rat assay method, and in some cases undoubtedly reflect other factors than the vitamin A potency. Since the available data do not show clearly the seasonal variations in the product, it seemed desirable to make a survey of the vitamin A potency of the principal types of cheese in relation to the season of production and also with respect to the grade of the product.

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EXPERIMENTAL

Collection of samples. Cheddar and Swiss cheeses comprised the bulk of the samples, but a few other types were also examined. The collections were made monthly by the district inspectors of the State Department of Agriculture. Over five hundred samples in all were obtained during the period from the early summer of 1944 to the same season in 1945. Seven districts representing the principal cheese-producing areas were included. The samples were shipped in two-ounce screw-lid jars and kept in the cold room at below 0° C. until analyzed.

Analytical method for carotene and vitamin A. The procedure used for the determination of the carotene and vitamin A contents of cheese has been described in another paper (7). The amount of carotene was measured by its absorption of light at 440 mμ and vitamin A was determined by its absorption at 620 mμ after treatment with antimony trichloride in chloroform.

RESULTS AND DISCUSSION

Seasonal variation of the vitamin A potency of cheese. The samples were collected and analyzed monthly but the data can be adequately summarized by two- or three-month periods covering pasture and dry-feed rations. The data for cheddar cheese are given in table 1. The minimum values for carotene and vitamin A occurred in the March–April period when the cows had been off pasture for the longest time and spring pasturing had not yet begun. The values were 0.78 μg. per gram for carotene and 1.66 μg. per gram for vitamin A. The maximum values occurred in the September–October period with figures of 2.97 μg. per gram for carotene and 3.11 μg. per gram for vitamin A. Calculated as I.U. per gram on the basis that 0.6 μg. carotene and 0.25 μg. vitamin A are each equal to one I.U., the minimum and maximum values were 7.94 I.U. per gram and 17.39 I.U. per gram, respectively. This corresponds to values of about 3600 I.U. per lb. and 7900 I.U. per lb., respectively.

TABLE 1

Vitamin A potency of cheddar cheese

Period	No. samples	Carotene		Vitamin A		Total potency*
		μg./g.		*μg./g.*		*I.U./g.*
		range	ave.	range	ave.	
Jan.–Feb.	84	(0.58–1.38)	0.93	(1.35–2.03)	1.80	8.75
Mar.–April	96	(0.40–1.33)	0.78	(1.11–2.27)	1.66	7.94
May–June	75	(0.75–4.45)	2.34	(1.40–3.20)	2.50	13.90
July–Aug.	37	(1.67–3.48)	2.46	(1.90–3.42)	2.79	15.26
Sept.–Oct.	78	(2.13–3.35)	2.97	(2.80–3.72)	3.11	17.39
Nov.–Dec.	13	(1.15–1.80)	1.44	(1.80–2.60)	2.02	10.48

* Based on the assumption that 0.6 μg. carotene and 0.25 μg. vitamin A are each equal to one International Unit.

TABLE 2

Vitamin A potency of Swiss cheese

Period	No. samples	Carotene		Vitamin A		Total potency*
		μg./g.		μg./g.		I.U./g.
		range	ave.	range	ave.	
Feb.–April	18	(0.35–0.73)	0.56	(1.27–2.10)	1.70	7.73
May–July	46	(1.08–2.20)	1.69	(1.81–2.75)	2.35	12.21
Aug.–Oct.	37	(1.63–1.95)	1.75	(2.78–3.34)	2.94	14.67
Nov.–Jan.	27	(0.50–1.73)	0.99	(1.60–2.85)	1.97	9.53

* Based on the assumption that 0.6 μg. carotene and 0.25 μg. vitamin A are each equal to one International Unit.

The data for Swiss cheese are summarized by three-month periods because of a smaller number of samples (table 2). Here the same trend as with cheddar cheese was observed. The maximum values occurred in the August–October period with 14.67 I.U. per gram (6600 I.U. per lb.), and the minimum occurred in the February–April period with 7.73 I.U. per gram (3500 I.U. per lb.). The values for Swiss cheese were generally lower than in cheddar samples. The vitamin content, while not exactly proportional, roughly paralleled the fat content of the two types of cheese. Fat determinations showed about 26 per cent fat in the Swiss type and about 33 per cent fat in the cheddar type of cheese.

Grade of cheese and vitamin A potency. In order to determine whether there was any relation between grade and vitamin A potency, the data were tabulated according to the various grades reported by the inspectors for the cheddar and the Swiss samples (table 3). Though variations occurred among the different grades, there was no correspondence between grade and vitamin A potency. In the instances where there seemed to be a difference between grades the difference can probably be attributed to an insufficient

TABLE 3

Variations of the carotene and vitamin A contents of cheddar and Swiss cheese with season and grade

	Feb.–April		May–July		Aug.–Oct.		Nov.–Dec.	
	Carotene	Vit. A	Carotene	Vit. A	Carotene	Vit. A	Carotene	Vit. A
	μg./g.	μg./g.	μg./g.	μg./g.	μg./g.	μg./g.	μg./g.	μg./g.
Cheddar grades								
State	0.77	1.64	2.10	2.43	3.02	3.07	1.09	1.88
Junior	0.78	1.68	2.50	2.61	3.02	3.13	1.00	1.87
Under	0.75	1.70	2.50	2.63	2.70	3.01	0.94	1.82
Swiss grades								
A	0.51	1.47	1.77	2.38	1.65	2.84	0.76	1.80
B	0.50	1.69	1.81	2.28	1.70	3.09	1.35	2.45
C	0.53	1.70	1.88	2.43	1.80	3.01	0.97	1.86
No. 3	0.66	1.75	1.69	2.35	1.80	2.92	0.96	1.92
Grinder	0.58	1.81	1.66	2.47	1.71	2.85	1.16	2.13

number of samples. Cheese grading clearly does not distinguish differences in vitamin A potency. Because of the importance of vitamin A in nutrition, it would seem reasonable to include the vitamin A potency of the sample in establishing its grade.

Miscellaneous types of cheese. A few samples of cheese other than cheddar and Swiss were analyzed. Brick, Muenster, Limburger, and Colby all showed no significant difference from cheddar cheese of the same season in their vitamin A potency. Since about 85 per cent of the carotene and vitamin A content of the milk are found in the cheese, and curing and storage do not change the values (7), the vitamin A potency of cheese corresponds to the potency of the milk and this in turn reflects the carotene content of the ration. The vitamin A potency of milk, butter, and cheese produced during the winter months could probably be increased at least 50 per cent by making and feeding good quality grass and corn silages (8, 9).

SUMMARY

Over five hundred samples of cheese, chiefly of the cheddar and Swiss types, were collected and analyzed for their carotene and vitamin A contents.

The maximum vitamin A potency of cheddar cheese occurred in those samples made in the September–October period, 17.39 I.U. per gram (7900 I.U. per lb.); the minimum occurred in the March–April period, 7.94 I.U. per gram (3600 I.U. per lb.). The values for Swiss cheese were: August–October 14.67 I.U. per gram (6600 I.U. per lb.) and February–April 7.73 I.U. per gram (3500 I.U. per lb.). A few samples of cheese other than cheddar or Swiss were analyzed. Brick, Muenster, Limburger and Colby all showed no significant difference from cheddar cheese of the same season in vitamin A potency.

No correspondence between vitamin A potency and grade of cheese was observed among the samples.

The vitamin A potency of cheese corresponds to that of the milk from which it was made and this in turn reflects the carotene content of the ration.

REFERENCES

(1) BERL, S., AND PETERSON, W. H. Determination and Content of Carotene and Vitamin A in Wisconsin Butter. Jour. Nutr., **26**: 527–528. 1943.

(2) COOK, D. A., AND AXTMEYER, J. H. Nutrition Studies of Foodstuffs Used in Puerto Rican Dietary. VI. The Vitamin A Content of Pasteurized Milk and Native Cheese. Puerto Rico Jour. Pub. Health and Trop. Med., **9**: 90–92. 1933.

(3) COWARD, K. H., AND MORGAN, B. G. E. Quantitative Estimation of Vitamin A and D in Various Food Substances Cooked and Fresh. Brit. Med. Jour., **3908**: 1041–1044. 1935.

(4) DAVIES, A. W., AND MOORE, T. The Vitamin A Content of Cheese. Biochem. Jour., **33**: 1645–1647. 1939.

(5) DORNBUSH, A. C., PETERSON, W. H., AND OLSON, F. R. The Carotene and Vitamin A Content of Market Milks. Jour. Amer. Med. Assoc., **114**: 1748–1751. 1940.

(6) HATHAWAY, I. L., AND DAVIS, H. P. Studies on the Vitamin A Content of Cheese. Nebr. Agr. Expt. Sta. Res. Bul. **103**: 1–10. 1938.

(7) HIGUCHI, K., PRICE, W. V., AND PETERSON, W. H. Relation of Corn and Alfalfa Silage to the Quality of Cheese and Its Carotene and Vitamin A Contents. JOUR. DAIRY SCI., **29**: 157–162. 1946.

(8) JOHNSON, B. C., PETERSON, W. H., AND BOHSTEDT, G. Alflafa Silage Preservation. Ind. Eng. Chem., **32**: 1622–1625. 1940.

(9) STEFANIAK, J. J., RUPEL, I. W., AND PETERSON, W. H. Effect of Feeding Corn and Alfalfa Silages on the Fat and Water-Soluble Vitamins of Winter Milks. JOUR. DAIRY SCI., **27**: 629–630. 1944.

(10) TODHUNTER, E. N., RODERUCK, C. E., AND GOLDING, N. S. The Vitamin A Value of Roquefort Type Cheese. JOUR. DAIRY SCI., **25**: 1023–1026. 1942.

(11) U. S. DEPARTMENT OF AGRICULTURE. Vitamin A in Butter. Misc. Pub. No. 571, 1–14. 1945.

SOME OBSERVATIONS ON "QUALITY" IN HAYS*

J. G. ARCHIBALD, E. BENNETT, AND J. W. KUZMESKI
Massachusetts Agricultural Experiment Station

Much has been said and written about quality in roughage, but nobody seems to be able to define the word or to say what should be looked for in any analysis to determine quality. The ultimate verdict must of course be given by the animal which eats the roughage. If it is unpalatable, or (in the case of a milking cow) if it fails to maintain milk production satisfactorily, then we know that its quality is inferior. When that phase of the situation has been reached, however, it is often too late to do much about it. Cannot some other criterion be established which would enable the evaluation of quality before roughage is fed out, or which might explain the great variations in feeding value found in different lots of hay, especially the differences which are noted from season to season? The work here reported, although admittedly fragmentary and of a preliminary nature, constitutes a modest step toward an answer to this very important question.

The growing season of 1945 in southern New England was characterized by excessive rainfall and less than the average amount of sunshine. Although pasture and hay crops were well above average in yield, the pastures did not stimulate milk production as much as it was expected they might, and curing weather for the hay crop was so poor that much of it was stored in poor condition. Soon after the barn feeding season started in the autumn, some farmers began to complain that their cows were not holding up in production as they should, despite heavy grain feeding. By the end of the year the condition was noted in various parts of the state and some of the farmers turned to the State College for assistance. One prominent Jersey breeder submitted samples of four lots of hay grown on his farm with the information that his cows varied so in their response to them that he was curious as to what might be the cause. Questions as to his other feeding and management practice revealed the fact that the different lots of hay were the only known variable.

As a first step in the search for possible clues, a routine fodder analysis was made of all four samples. Results of this showed that in comparison with average analyses of the kinds of hay in question, protein, fat, and ash were about normal, but that fiber was invariably above average, as much as 6 to 8 per cent in some of the samples, while nitrogen-free extract was correspondingly lower. Considerable differences were noted also in the individual samples in this respect.

The question at once arose as to what might be lacking in the nitrogen-

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TABLE 1

Results of analyses of hays

Crop year	Protein	Ether extract	Fiber	N-free extract	Ash	Total sugars	Carotene	Quality[a]
Mixed grass hay—largely timothy—1st cutting								
1943	5.0	1.6	38.9	51.9	2.6	6.2	3.4	Poor*
	6.1	1.6	37.0	52.2	3.1	8.9	1.2	Fair*
	10.0	1.6	37.5	46.6	4.2	3.8	1.8	Poor*
	6.7	1.8	37.9	50.4	3.2	6.8	1.9	Fair*
Average, 1943	7.0	1.6	37.8	50.3	3.3	6.4	2.1	.....
1944	8.0	1.8	36.2	50.2	3.7	3.3	N.D.	Good*
	9.4	2.2	38.7	44.6	5.1	4.2	5.7	Fair*
Average, 1944	8.7	2.0	37.5	47.4	4.4	3.8	.....	.....
1945	8.8	1.7	40.0	43.5	6.0	1.3	3.0	Poor
	5.7	1.4	42.0	46.2	4.7	3.0	1.6	Poor
	5.7	2.0	36.3	51.1	4.8	4.2	4.0	Very good
Average, 1945	6.7	1.7	39.4	46.9	5.2	2.8	2.9	.....
For comparison	7.2	2.6	33.3	50.4	6.3	No data	3.0[b]	.....
Mixed grass hay—2nd cutting								
1944	11.1	2.0	30.2	51.3	5.5	4.4	N.D.	Good*
For comparison	13.8	3.7	27.9	46.9	7.8	No data	.....	.....
Mixed grass and legume hay —1st cutting								
1944	11.1	1.4	40.2	40.3	7.0	0.9	1.6	Fair*
1945	9.5	2.1	36.5	45.5	6.4	3.0	3.2	Good*
	13.2	1.9	37.1	41.4	6.4	5.0	6.6	Good
	8.2	1.6	35.7	48.5	6.0	5.8	3.2	Good
	11.4	2.0	37.5	43.2	5.8	3.9	4.8	Fair*
	7.5	2.0	37.8	46.9	5.8	5.8	6.4	Good
	9.2	1.9	38.6	43.7	6.6	3.1	2.9	Fair*
Average, 1945	9.8	1.9	37.2	44.9	6.2	4.4	4.5	.....
For comparison	9.8	2.3	34.2	46.6	6.0	No data	12.0	.....
Mixed grass and legume hay—2nd cutting								
1943	14.1	2.2	28.5	49.6	5.6	8.4	30.0	Excellent*
1945	15.4	3.5	36.0	36.7	8.4	1.6	15.4	Good
	19.1	2.8	32.7	36.7	8.7	2.7	20.3	Good
Average, 1945	17.3	3.2	34.4	36.7	8.6	2.2	17.9	.....
For comparison	13.3	3.3	31.3	43.4	6.5	No data	26.0	.....
Mixed grass hay—native, fine leaved grasses (cut in August)								
1945	8.2	2.5	35.1	49.5	4.7	4.6	4.4	Fair
	9.8	3.2	35.9	45.3	5.9	4.4	20.7	Good
Average	9.0	2.9	35.5	47.4	5.3	4.5	Not averaged	.....
For comparison	7.9	2.8	34.7	48.4	6.2	No data	No data	.....

TABLE 1 (*Continued*)

Red clover hay								
1943	16.0	2.6	29.8	45.3	6.4	5.2	18.3	Good*
1945	17.1	2.3	34.2	37.9	8.5	2.4	8.9	Good*
For comparison	13.4	2.9	31.0	45.5	7.3	No data	7.6	.....
Reeds canary grass								
1945	8.0	1.5	41.4	42.9	6.3	1.3	1.4	Poor
For comparison	8.3	2.6	32.0	48.9	8.1	No data	No data	.....

All values are expressed on a dry matter basis and all except carotene are percentages; carotene is expressed as thousands of International units of vitamin A equivalent per pound.

[a] Quality was determined on either or both of two bases as indicated: 1—the farmer's opinion based on milk production when the hay was fed; 2—appearance of the sample when received as judged by leafiness, color, aroma, and freedom from mold, weeds and other trash. In those cases where the farmer did not evaluate the hay, the second basis of grading had to be relied on entirely. Samples so graded are marked with an asterisk. Where the farmer expressed an opinion, his estimate was given more consideration than ours in determining quality if the two did not agree. For obvious reasons, the results of analysis were not a factor in the decisions.

[b] Average carotene values are not available in Morrison's tables. Those reported for comparison have been compiled from several sources of rather meager data. They may not be strictly comparable with the values determined in the 1943, '44, and '45 samples, due to improvement in recent years in methods for carotene determination. The improved methods now in use generally give lower carotene values, the average being about a third less.

N.D. = not determined.

free extract, and the answer seemed obvious at once—soluble carbohydrates. It was therefore decided to determine total sugars not only in these four samples but in as many additional samples of the 1945 hay crop as might be readily obtainable. Twenty-one other hay samples were secured, eleven of them from the 1945 crop, making a total of fifteen for 1945, four from the 1944 crop, and six from the 1943 crop. The samples came from five different localities well scattered over the state.

Total sugars were determined by the procedure outlined in Methods of Analysis of the A.O.A.C. (1), using the method of Lane and Eynon. Carotene was also determined using the method of Wall and Kelley (4), and routine fodder analyses were made on all samples. Table 1 shows the results classified by seasons according to kind of hay, together with average analyses as given by Morrison (2) for comparison.

Comparing the 1945 samples with those for 1943 and 1944, it is seen that protein alone in most cases was average or above. Ether extract, total ash and carotene were generally below average. With one exception fiber was higher in the 1945 samples, and wherever it was higher, nitrogen-free extract was lower. Wherever nitrogen-free extract was relatively low, total sugars were also relatively low. Some of the differences in content of total sugar from year to year are marked.

There is a rather close correlation between quality as determined by feed-

ing value and appearance, and relative amounts of desirable and undesirable components as shown by analysis. In other words, the better quality hays in general were higher in such constituents as protein, carotene, and sugar, and lower in crude fiber than those hays which were graded as of fair or

TABLE 2

Records of rainfall and sunshine at Amherst, Massachusetts, for the growing season of 1945 in comparison with 1943 and 1944, and the 50-year average

Month	1943	1944	1945	50-year average (1889–1938 incl.)	Percentage which 1945 record is of 50-year average
			Rainfall in inches		
April	3.66	3.66	5.43	3.35	162.1
May	5.62	1.35	6.45	3.60	179.2
June	2.38	4.70	7.67	3.75	204.5
July	6.18	3.88	7.36	4.10	179.5
August	2.49	4.33	2.79	4.08	68.4
September	2.40	5.31	3.57	4.24	84.2
Total for the growing season	22.73	23.23	33.27	23.12	.....
Percentage of the 50-year average	98.3	100.5	143.9	.....	.....
			Sunshine—per cent of possible hours of bright sunshine		
April	61.4	54.7	64.2	54.7	117.4
May	60.5	77.3	41.9	55.6	75.4
June	77.9	61.1	53.0	54.0	98.1
July	74.0	72.5	59.5	58.0	102.6
August	71.5	78.7	61.3	55.2	111.1
September	61.1	55.5	46.6	54.9	84.9
Average	67.7	66.6	54.4	55.4	.....
Percentage of the 50-year average	122.2	120.2	98.2	.....	.....
			Average monthly temperatures—°F.		
April	41.0	42.9	51.8	45.7	113.3
May	57.8	63.4	54.1	57.1	94.7
June	71.1	66.3	66.1	65.7	100.6
July	71.8	72.9	70.6	70.8	99.7
August	68.9	72.4	68.3	68.6	99.6
September	61.1	63.0	64.6	61.7	104.7
Average	62.0	63.5	62.6	61.6	.....
Percentage of the 50-year average	100.6	103.1	101.6	.....	.....

poor quality, and this holds true regardless of the kind of hay. It seems therefore that the complaints by many farmers as to poor feeding value of the 1945 hay crop, are corroborated to a considerable degree by these results.[1]

[1] In the absence of actual feeding trials, certain assumptions as to feeding value of these hays have been made. These assumptions are believed to be valid in the light of our general knowledge of the relationship between feeding value and composition; also they are confirmed somewhat by the opinions of the men who fed some of the lots of hay which the samples represent.

In searching for a reason for the relatively poor quality of the 1945 crop, weather conditions come to mind at once. As already noted, 1945 was an unusaully rainy season in this region, especially during the early summer. How it compared with 1943 and 1944, and how far it departed from the 50-year average at Amherst, Massachusetts, are shown in table 2.

These data, although for only one locality, are reasonably representative of conditions throughout Massachusetts in 1945. The records which stand out as unusual are the rainfall in May, June, and July and the relative lack of sunshine in May. May is the month when our first crop of hay makes most of its growth; June and July are the months when normally most of it is harvested. Since direct sunshine and photosynthetic activity are closely correlated, under definite conditions some relationship might be expected between unusually cloudy weather in May and the relatively low levels of total sugar in the 1945 samples. Probably of greater importance, however, than lack of sunshine in lowering the sugar level in the hay was the extremely unfavorable curing weather in June and July. This is evidenced by an amount of rainfall more than double the 50-year average for June, and nearly double for July. As a result, much hay was stored improperly cured, with consequent excessive "sweating," a biochemical process known to proceed at the expense of soluble organic compounds.

Good illustrations of this are the two samples of 2nd cutting mixed grass and legume hay (1945 crop). Although of good quality in other respects, these samples were low in sugar and quite high in fiber for 2nd crop hay. Examination of the samples when received revealed considerable mold and musty odor, conditions which develop when hay is stored too damp, either from natural moisture or from rain.

Average seasonal temperatures in 1945 did not deviate markedly from the 50-year average. In the month of May, however, the average temperature was considerably below the 50-year figure (3° F.), and rainfall was abundant; May is the month when the first crop of hay in this region makes most of its growth. In general, temperature and water content of the plant

TABLE 3

Composition of hays cured by different methods

Method of curing	Protein	Ether extract	Crude fiber	N-free extract	Total ash	Total sugars	Carotene	Quality
Mixed hay—largely timothy—1st cutting								
Field-cured:								
Loose	5.7	2.0	36.3	51.1	4.8	4.2	4.0	Very good
Baled	8.8	1.7	40.0	43.5	6.0	1.3	3.0	Poor
Mixed hay—grass and legumes—1st cutting								
Field-cured:								
Loose	9.0	1.9	36.9	45.9	6.2	4.0	3.1	Good
Baled	13.2	1.9	37.1	41.4	6.4	5.0	6.6	Good
Blower-dried:								
Loose	7.5	2.0	37.8	46.9	5.8	5.8	6.4	Good
Baled	11.4	2.0	37.5	43.3	5.8	3.9	4.8	Fair

are important factors in the transformation of carbohydrates. Low content of water and high temperature tend to increase the polysaccharides and to decrease the monosaccharides; the reverse conditions tend to produce opposite trends (3). That the sugar levels in the 1945 samples did not follow this generally accepted pattern, seems added evidence that, as already suggested, unfavorable haying weather was a greater influence than adverse weather while the hay crop was growing.

Some evidence is available regarding the effect of various methods of curing on composition of the hay. Most of the samples had been field cured in the usual fashion but a few of them had been field baled and some had been cured by completing the drying with forced ventilation in the barn. Results of this phase of the study appear in table 3. All of the samples so cured are from the 1945 crop. They are compared in the table with similar kinds of hay field cured in ordinary fashion, and of the same year's crop.

The results here do not uniformly favor any method of curing; although the data are rather meager, it seems probable that factors other than the curing method *per se* were of more importance in affecting composition of the hays.

SUMMARY

In an attempt to determine the reason for lowered feeding value of much of the 1945 hay crop grown in this region, twenty-five samples of hay from five localities scattered over this state (Massachusetts) were analyzed. In addition to the usual proximate fodder analysis, total sugars and carotene were determined.

In comparison with samples of the 1943 and 1944 crop, hay samples from the 1945 crop were, in general, lower in sugar, carotene, ether extract, and total ash, and higher in fiber. Protein in most cases was average or above.

The results are discussed in the light of the weather records for 1945, an exceptionally wet season, and it is tentatively concluded that insufficient sunshine while the hay was growing, and unfavorable harvesting weather, were in large measure responsible for its relatively low content of such desirable entities as sugar, carotene, and minerals.

A rather close correlation was noted between quality of the hay as judged by appearance of the samples and farmers' opinions of its feeding value on the one hand, and its apparent desirability as revealed by chemical studies on the other hand.

REFERENCES

(1) ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS. Official and Tentative Methods of Analysis. 5th edition. 757 pages. Washington, D. C. 1940.

(2) MORRISON, F. B. Feeds and Feeding. 20th edition. 1050 pages. Ithaca, New York. 1936.

(3) SPOEHR, H. A. Photosynthesis. The Chemical Catalog Co., Inc. 393 pages. New York, N. Y. 1926.

(4) WALL, M. E., AND KELLEY, E. G. Determination of Pure Carotene in Plant Tissue. Indus. and Engin. Chem., Analyt. Ed., **15**: 18–20. 1943.

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ABSTRACTS OF LITERATURE

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ABSTRACTS OF LITERATURE

BOOK REVIEWS

365. Advances in Enzymology. Volume 6. EDITED BY F. F. NORD. Published by Interscience Publishers, Inc., New York, 562 pages including cumulative index of Volumes 1–6. $6.50.

This sixth of an excellent series of reviews and discussions on enzymology prepared on invitation by authoritative workers includes sections on the following: Bacterial amino acid decarboxylases; Enzyme problems in relation to chemotherapy; Biological antagonisms between structurally related compounds; Adenosine triphosphatase properties of myosin, States of altered metabolism in diseases of muscle; Acetyl phosphate; Microbial assimilations; Chemical changes in harvested tobacco leaf; Actions of Amylases; Amylases of wheat and their relation to baking and milling technology; Tocopherol inter-relationships. The section on amylases of wheat contains an excellent review on the biochemistry of bread-making that will be of interest to those in dairy research. This and the previous five volumes on enzymology are specific tools for those in dairy research. K.G.W.

366. The Refrigerating Data Book. Refrigeration Applications Volume. Second Edition. THE AMERICAN SOCIETY OF REFRIGERATING ENGINEERS, New York, N. Y. 1946.

This volume has been prepared under the leadership of Donald K. Tressler, Chairman of the Board of Editors, made up of eight associate editors, each responsible for one of its eight parts. Eighty-nine specialists in the various branches of refrigeration applications contributed to the seventy-six chapters of the various parts. The eight parts are: I. Frozen Foods; II. Cold Storage Practice; III. Refrigeration in Food Manufacture; IV. Refrigerated Food Distribution; V. Low Temperature Applications; VI. Industrial Applications of Refrigeration; VII. Comfort Air Conditioning; VIII. Industrial Air Conditioning. At the head of each part is a list of chapter headings contained therein together with their authors. At the end of each chapter is found a reference list of detailed information sources. The 666 pages of subject matter contain many figures, graphs and tables. Chapters of paramount interest to the dairy industry are: 4. Ice Cream; 23. Milk Plants; 24. Butter Manufacture; 25. Cheese Manufacture, while others such as, 8. Bacteriology of Frozen Foods; 9. Nutritive Value of Frozen Foods; 10. Storage of Frozen Foods; 11. Cold Storage Plants; 12. Food Storage Conditions; 15. Egg Storage; 18. Ozone and Light; 20. Refrigerator Cars; 30. Refrigerated Storage of Dehydrated Foods; 32. Refrigerated Trucks; 33. Locker Plants; 34. Commerical Refrigerators; 35. Home and

Farm Freezers; 40. Dry Ice in Food Distribution; 41. Controlled Atmospheres; 46. Ice Making Plants; and 48. Dry Ice Manufacture may be of secondary interest. The publication contains a classified section of manufacturers of refrigeration equipment and supplies extending through 190 pages which should prove to be very useful as the publishers have endeavored to make the index accurate and authentic. L.M.D.

367. **Introduction to Emulsions.** GEORGE M. SUTHEIM. Chemical Publishing Co., Inc., Brooklyn, N. Y. 1946.

A book upon the fundamentals of emulsion written by a practical man for practical men. The first five chapters are concerned with the fundamentals of emulsions and are: 1. Theoretical Foundation; 2. The Physical Chemistry of Emulsifying Agents; 3. The Chemistry of the Emulsifying Agents; 4. The Formation of Emulsions; 5. The Properties of Emulsions. Chapter 6 is a brief exposition of Applications of Emulsions. In addition to the frontispiece there are twenty-two figures, largely diagrammatic, illustrating various properties of emulsions. Each chapter is followed by a number of questions designed to bring out applications of its textual matter. Appendix I, Glossary. Terms peculiar to surface activity; Appendix 2, List of Emulsifying Agents. The information conveyed in this list consists of the commercial name, chemical name or formula, group classification, type, specific reference to bibliography, and producer's name in abbreviated form keyed to their full names in an accompanying list; Appendix 3, Bibliography. Contains a list of 159 titles extending from 1910 to 1945 but largely including references in the later years of 1935 through 1944. L.M.D.

368. **Process Equipment Design.** HERMAN C. HESSE, Professor of Engineering Drawing and Design, University of Virginia, AND J. HENRY RUSHTON, Professor of Chemical Engineers, University of Virginia. O. Van Nostrand Company, Inc., New York. 1945.

A book of particular interest to equipment and plant designers. Subjects covered as indicated by the eighteen chapters are: Materials of Construction; Mechanical Properties and Strength of Materials; Riveted Pressure Vessels; Welded Pressure Vessels; Mechanics; Threaded Fasteners and Combined Stresses; Structural Analysis; Trusses and Truss Adaptation; Piping; Attachments and Closures; Non-Ferrous Construction; Concrete Construction; Wood and Other Non-Metallic Construction; Belt and Chain Drives; Toothed Gearing; Shafting and Bearings; Handling Equipment and Mechanical Frames; Special Stress Application. References are grouped at the back of the book. Numerous illustrative figures, graphs, and tables and included. Wherever desirable sample calculations involving formulas

and special applications are brought into the text to illustrate development of basic design. Each chapter has appended to it problems which in their solutions bring to bear information set forth in the chapter text. L.M.D.

369. **Surface Active Agents.** C. B. F. YOUNG, Director of Research, Clark Babbitt Industries, AND K. W. COONS, Head of Department of Chemical Engineering, University of Alabama. Chemical Publishing Co., Inc., Brooklyn, N. Y. 1945.

The authors, recognizing that the success or failure of many industrial processes depends upon the effects of surface tension, have undertaken to present information as to the origin, effects, and utilization of surface-tension phenomena in a diversity of industrial fields. It is their hope, "that the transfer of knowledge from one field to another may bear fruit in easing the problems of some workers, or in providing the germ of an idea which may improve some process or solve some problem in another field."

A unique presentation in the first part of the book is a table of illustrations set up by chapters, carrying the figure number, title of illustration and page number.

Part I contains basic information embodied in three chapters. Chapter I, Theory of Surface Tension; Chapter II, Determination of Surface Tension; Chapter III, The Structure of Wetting Agents and Specific Surface-Tension Agents.

Part II consists of applications. Chapter IV, Emulsions; Chapter V, Plating, Metal Cleaning, Pickling, and Etching; Chapter VI, Cosmetics; Chapter VII, Leather; Chapter VIII, Flotation; Chapter IX, Inks; Chapter X, Textiles; Chapter XI, Cutting Oils; Chapter XII, Adhesives; Chapter XIII, Foods; Chapter XIV, Lubrication; Chapter XV, Soldering, Brazing, and Welding.

The latter part of Chapter III, extending from page 117 through page 152, consists of an alphabetical listing of Wetting Agents and Surface-Tension Agents, giving the name, type or chemical composition, use in industry, and the manufacturer. At the end of the table is a list of the manufacturers and their addresses.

Formulas for industrial application compounds are grouped at chapter endings preceding the references. L.M.D.

370. **Physical Methods of Organic Chemistry. Volume I.** EDITED BY ARNOLD WEISSBERGER, Research Laboratories, Eastman Kodak Company. Interscience Publishers, Inc., New York. 1945.

Present Volume I comprises sixteen chapters while Volume II, to follow, will contain ten additional chapters. The subjects are handled by twenty-nine contributor specialists in the various fields of physical methods applied

to organic chemistry. The book is designed to furnish the specialist worker with information about physical methods of organic chemical problem solution with which he may not be at all familiar but that because of necessity in research work he must be forced to use. For the student it will serve to give him an insight into the many physical methods applied to organic chemistry. With the items listed in Volume II the complete coverage of physical aspects will be made. Chapter titles follow: I. Determination of Melting and Freezing Temperatures; II. Determination of Boiling and Condensation Temperatures; III. Determination of Density; IV. Determination of Solubility; V. Determination of Viscosity; VI. Determination of Surface and Interfacial Tension; VII. Determination of Properties of Monolayers and Duplex Films; VIII. Determination of Osmotic Pressure; IX. Determination of Diffusivity; X. Colorimetry; XI. Microscopy; XII. Determination of Crystal Form; XIII. Crystallochemical Analysis; XIV. X-Ray Diffraction; XV. Electron Diffraction; XVI. Refractometry. Volume II continues the chapter order with: XVII. Spectroscopy and Spectrophotometry; XVIII. Colorimetry, Photometric Analysis, and Fluorimetry; XIX. Polarimetry; XX. Determination of Dipole Moments; XXI Conductometry; XXII. Potentiometry; XXIII. Polarography; XXIV. Determination of Magnetic Susceptibility; XXV. Determination of Radioactivity; XXVI. Mass Spectrometry. Each chapter embracing a special subject has a detailed outline at its beginning, is liberally supplied with specific foot note references, and at its end a general reference list. In the 736 pages of the book there are in addition to numerous mathematical formulae 273 illustrative figures and 56 tables. L.M.D.

371. **Into the Freezer—and Out.** DONALD K. TRESSLER, Food Consultant; CLIFFORD F. EVERS, Research Director, Birds Eye-Snider Division, General Foods Corporation; LUCY LONG, General Electric Consumers Institute. Avi Publishing Co., Inc., New York. 1946.

Here at last is a book prepared by experts in the field of frozen foods for the layman, whom having had considerable experience with locker plant freezing and very limited operation of home freezers is about to become an important factor in the field of home freezer sales, once their production becomes free from restrictive shackles. Heretofore there has been a great amount of what could be called unorganized, and, even in part, unauthoritative information covering the various phases of selection, preparation and packaging, freezing and storage, and finally preparation for the table of vegetables, fruits, meats, poultry, and fish in the home. Recently there has been added to the list, partially prepared and wholly cooked bakery products, together with many cooked foods of other sorts. This book presents, in well-organized fashion and in readily comprehended language, the necessary information to insure success in the operation of a home freezer. The real answer to the home freezer operators' (both actual and contemplative) need

for helpful and expert instruction for all kinds of foods freezing preservation. Chapter headings are indicative of what the reader will find: I. The Food of the Future Has a Past; II. Freezers are Revolutionary and Democratic; III. Your Locker Man and You; IV. A 4-Point Program for Freezer Space; V. The Hidden Merit of Frozen Foods: Better Nutrition; VI. "Big Five" for Fruits and Vegetables—Variety—Maturity—Speed—Packaging—Storage Temperatures: How These Affect Success of Freezing—Planting and Harvesting Guide; VII. Step-By-Step Preparation Procedure; VIII. A Delicatessen at Your Fingertips—Cooked Foods—Baked Goods—Leftovers; IX. When Foods Come Out of the Freezer—How to Thaw, Cook, Use; X. Ice Cream in the Freezer Too!—How to Make and Package Ice Cream for the Freezer—How to Make "Ribbon" and Fruit Ice Cream—Velva Fruit; XI. Freezing Wild Game, Meat, and Fish. A number of photographic illustrations of procedure are used to supplement the instructional text. L.M.D.

BACTERIOLOGY

372. **The Activity of Penicillin in Relation to Bacterial Spores and the Preservation of Milk.** HAROLD R. CURRAN AND FRED R. EVANS, Bureau of Dairy Industry, Agricultural Research Administration, U. S. Dept. of Agr., Washington, D. C. Jour. Bact., *52,* No. 1: 89. July, 1946.

This is a study of the preserving action of penicillin in milk containing viable bacterial spores. Fifteen aerobic and two anaerobic species of bacteria were examined.

Four species of the genus *Bacillus* were relatively resistant; penicillin was not an effective preserving agent except in an impracticable concentration. Spores of the remaining 13 species were either killed or inhibited by penicillin concentrations of 5 units per ml. This concentration sterilized many cultures. Five units delayed but did not prevent spoilage by *Clostridium botulinum* and another unidentified anaerobic species. Apparently all spore cultures contain some spores susceptible to penicillin; the species differ in the proportion of resistant and sensitive cells.

It was concluded that penicillin has no application in the preservation of food. Accompanied by mild heating it might serve as a preservative in certain nonfood materials. D.P.G.

373. **The Activity of Streptomycin in Relation to Bacterial Spores and the Preservation of Milk.** HAROLD R. CURRAN AND FRED R. EVANS, Bureau of Dairy Industry, Agricultural Research Administration, U. S. Dept. of Agr., Washington, D. C. Jour. Bact., *52,* No. 1: 142. July, 1946.

This is a note dealing with a study similar to that reported on penicillin by the same authors. Many spore-forming bacteria grew in the presence of

5 units of streptomycin per ml. of milk. One hundred units per ml. were not sufficient to prevent spoilage of milk containing as few as 100 per ml. viable spores of *Clostridium botulinum* and of another anaerobe. It is concluded that streptomycin in ordinary concentrations has limited activity against bacterial spores. D.P.G.

374. The Action of *Leuconostoc dextranicum* and *Leuconostoc citrovorum* During the Ripening Process of American Cheddar Cheese. CHARLES C. PROUTY, Department of Bacteriology and Public Health, State College of Washington, Pullman, Wash. Jour. Bact., *52*, No. 1: 153. July, 1946. Proc. of Local Branches. D.P.G.

CHEESE

375. The Centrifugal Milk Clarifier in Cheddar Cheese Manufacture. E. G. HOOD AND I. HLYNKA. Canadian Dairy and Ice Cream Journal, *24*, 5: 27. May, 1945.

The three main points brought out in favor of clarification of cheese milk are: (1) removal of sediment; (2) improvement in flavor and texture; (3) reduction of fat loss in the whey. H.P.

376. Keeping Quality of Cheese With Rancid and Unclean Flavours. I. HLYNKA AND E. G. HOOD. Canadian Dairy and Ice Cream Journal, *23*, 11: 35. November, 1944.

A comparison of data on the flavor scores of three different lots of cheddar cheese at 2, 4, 6 weeks and at 6 months show that cheese made from unagitated milk maintained its flavor score throughout the ripening period. Cheese made from milk which was subjected to agitation so as to provide varying intensities of rancid and unclean flavors on the whole maintained its flavor scores up to 6 months though the results were variable. It is concluded that milk lipase is not active in cheese after it is made, and variations in flavor score are attributed to other factors. H.P.

377. Testing Cheese for Extraneous Matter. ARTHUR B. EREKSON, Plymouth, Wisconsin. Natl. Butter and Cheese Jour., *37*, No. 8: 46. August, 1946.

A special device with a V-shaped blade is used to cut an 8-ounce piece of rindless cheese from a flat surface of a Cheddar. The cut surface of the cheese is sealed by the usual operation of paraffining. Damage by mold growth during curing is eliminated by this system. The sample is shredded rather than ground; it is dissolved in a 10% sodium citrate solution at 155° F.; finally, it is filtered in an Erekson vacuum sediment tester. Sediment discs are dried, placed in cellophane envelopes and examined for amount and types of extraneous material. Discs are graded according to standards

adopted by the National Cheese Institute. Routine testing is the only basis for control of extraneous matter. W.V.P.

CHEMISTRY

378. pH and Its Many Applications in the Dairy Industry. L. R. BRYANT. Canadian Dairy and Ice Cream Journal, *24*, 4: 27. April, 1945.

There are many applications of pH in the dairy industry. The most common pH measurements are used in: (1) milk (normal pH values between 6.5–6.7); (2) cream and butter; (3) cheesemaking; (4) casein manufacture; (5) refrigeration brines; (6) boiler waters and (7) washing solutions. As the influence of pH on many dairy problems becomes more generally recognized and understood by dairy plant operators, the measurement of pH will be more widely used in dairy plant practice. H.P.

379. Use of an Ion-Exchange Resin in Determination of Traces of Copper With Special Reference to Powdered and Fluid Milk. H. A. CRANSTON, Lab. of Polarographic Analysis, Chicago, Ill., and J. B. THOMPSON, Quartermaster Food and Container Institute for the Armed Forces, Chicago 9, Ill. Jour. Ind. Eng. Chem., Analyt. Ed., *18*, No. 5: 323–326. May, 1946.

The copper content of a good dry whole milk is generally less than 1 part per million and this small concentration of metal makes the determination of copper difficult. The official army specification method of analysis lacks precision particularly at the critical range below 2.0 p.p.m. In current methods of analysis all the organic matter must be destroyed in large samples by acid digestion or ignition. This is avoided in the new method, the copper being made available by reducing the reaction to less than pH 3.0 with perchloric acid. The precipitated milk protein, carrying the fat with it, is removed by filtration. The filtrate is corrected for added acid, solids, and fat loss. The copper from this filtrate is concentrated on a synthetic ion-exchange resin in the hydrogen cycle. Copper is stripped from the ion exchanger with dilute hydrochloric acid and determined polarographically using an additive standard technique. If polarographic equipment is not available, spectrophotometric methods may be employed. Data showing the precision of the proposed method and a comparison of it with other methods for determining copper are given. B.H.W.

CONCENTRATED AND DRY MILK: BY-PRODUCTS

380. Freezing and Storing Condensed and Skim Milk. D. V. JOSEPHSON, Department of Dairy Technology, Ohio State University. Ice Cream Field, *48*, No. 1: 37. July, 1946.

Results of experimental work are reported showing that condensed whole

milk and condensed skim milk can be frozen and stored for several months without seriously affecting their quality.

The following procedure is recommended:

Select high quality whole milk and forewarm it to 190° F. for 15 minutes, then concentrate it to 39 per cent total solids (3 to 1 ratio). Cool to 40° or less and run into tinned or paper cans. Freeze as rapidly as possible and store at 10° F. or lower.

Several test cartons (quart or gallon size) should be frozen and stored under the same conditions. At intervals from three months on remove one of these cartons and test its dispersability in hot water (180° F.) in a malted milk mixer. If curd resists dispersion, the lot should be removed and used immediately.

Skim milk should be concentrated to 36 per cent total solids (4 to 1 ratio) otherwise it is processed essentially as the condensed whole milk.

The "cooked" flavor present in these products when first processed serves to prevent "oxidized" flavor from developing. It also tends to disappear during storage. W.C.C.

DISEASE

381. Control of Brucellosis in New York State Dairy Herds and Its Relation to Milk Supply. Asa Winter, N. Y. State Dept. Agr. and Mkts., Albany, N. Y. 19th Ann. Rpt. N. Y. State Assoc. Milk Sanit., p. 79. 1945.

Of all herds in New York State under the test and slaughter plan that were approved as being free from the disease on January 1, 1944, more than one-half revealed infection during the 15-month period ending April 1, 1945. These outbreaks were probably greater than normal.

In research reported by the New York State Veterinary College vaccinated calves were exposed to infection and field strains of *Brucella abortus* (not strain 19) were isolated from the milk of some heifers that remained as persistent reactors.

The program of controlling *Brucella* in New York State herds follows two plans. Plan A provides for vaccination of calves and annual blood tests of the herd. Plan B provides for heavily infected herds by vaccination of calves and also adult vaccination to reduce abortions. When a herd under Plan B becomes sufficiently negative it may be blood tested, reactors removed, and transferred to Plan A. A.C.D.

382. The Diagnosis of Chronic Brucellosis. Current Comment, Jour. Amer. Med. Assoc., *131*, No. 8: 670. June 22, 1946.

This is a thumbnail summary of present day knowledge of chronic brucellosis. Brucellosis should receive consideration early in the diagnosis of obscure illness. There is not yet available any easy adequate specific means

of diagnosis of chronic brucellosis. The agglutination test is significant only if positive. A positive intradermal test indicates increased sensitivity, but does not reveal whether the infection is still active. Negative skin tests have been observed in active brucellosis infection. The opsonophagocytic test requires perfect technic with a virulent brucellosis strain. Increased phagocytosis with a positive skin test, and symptoms suggesting brucellosis, point to a still active infection. The isolation of brucellosis by culture from the patient is the only definite diagnostic procedure. Cultural methods now in use are hardly practicable for daily routine work in the clinical laboratory. A high incidence of arthritis in brucellosis is believed to exist. In one study about half of 157 arthritis patients were believed to have brucellosis. Another observer has reported that of 427 patients with brucellosis, 74 had arthritis. Systematic study of the relation of brucellosis to chronic arthritis should be promoted. Among localizations of chronic brucellosis are those in the female genitourinary tract. More adequate cultural study with special reference to the detection of brucellosis is essential to a better understanding of various chronic infections. Cooperative investigation of the problems of diagnosis and treatment are needed. D.P.G.

383. The Incidence of Staphylococcal Mastitis in the Northwest. ERNEST C. MCCULLOCH, State College of Washington, Pullman, Wash. Jour. Bact., *52*, No. 1: 153. July, 1946. Abs. Proc. of Local Branches.

"A survey of over 3,000 cows in Washington revealed 34 per cent to have one or more quarters showing some degree of abnormality. Of the incubated milk smears examined and quarter samples cultured, 64 per cent contained staphylococci and 28 per cent streptococci, the remainder being contaminants or miscellaneous types of infection.

"The pathogenicity of staphylococci was checked by streaking on 5 per cent bovine blood agar, and after 24 hours' incubation at 37° C. by streaking the hemolytic colonies in Difco phenol red mannitol agar to which was added an additional 70 grams of NaCl per liter. Staphylococci capable of hemolyzing bovine blood, growing on 7.5 per cent salt agar, and utilizing mannitol have been considered as pathogenic.

"The infusion, immediately following milking, of 25,000 to 50,000 units of penicillin into the teat canal of quarters shedding staphylococci was followed by a temporary inability to culture staphylococci from the milk, but even the infusion of 100,000 units, repeated four times after four successive milkings, failed in several quarters to prevent the reappearance, after 7 to 14 days, of staphylococci in the milk.

"The plate counts of penicillin-treated quarters show a tendency to approach gradually the levels of staphylococci found previous to treatment." D.P.G.

384. ***Salmonella typhimurium* Food Infection From Colby Cheese.** C. B. TUCKER, M.D., GEORGE M. CAMERON, PH.D., MATTIE P. HENDERSON, M.S., AND M. R. BEYER, M.D., Tennessee Department of Public Health, Nashville, Tenn. Jour. Amer. Med. Assoc., *131*, No. 14: 1119. August, 1946.

Two hundred and fifty human cases of food-borne infection occurred in an epidemic involving six towns. History of the food eaten revealed that all patients had eaten Colby cheese 24 to 48 hours prior to the onset of the illness. Family members who did not eat cheese remained well. *Salmonella typhimurium* was isolated easily. Subsequent investigation revealed that a mouse had been removed from a ten thousand pound vat of milk which was made into cheese.

The cheese moved in interstate commerce and thus attracted the attention of the U. S. Food and Drug Administration. Six pounds of the cheese was acquired from a grocery store for bacteriologic study. It had been kept at 50° F. by the groceryman. In the laboratory the cheese was held at 43 to 48° F. Bacteriological isolations were made at monthly intervals. A small surface area was removed with a sterile scapel. A portion of cheese taken aseptically from beneath this area was macerated in a bottle containing selenite F liquid media and incubated at 37° C. for 24 hours. Several loopfuls of selenite F broth culture were then streaked on S. S. agar plates. After 24 hours at 37° C. typical colonies were transferred to Krumwiede's triple sugar agar slants and incubated 24 hours at 37° C. Further identification was obtained by incubation of the appropriate differential culture media. Final verification was secured by the assistance of the Salmonella Center, University of Kentucky. *S. typhimurium* remained viable in this cheese for a period of 302 days at 43 to 48° F.

It is suggested that pasteurization of milk and cheese curd in the process of cheese manufacture should be carried out. It is believed that other sanitary measures should be strictly enforced in the manufacture of cheese. Pasteurization of the milk alone would not have prevented the occurrence of this outbreak. D.P.G.

FOOD VALUE OF DAIRY PRODUCTS

385. **Vitamin C in Milk Products.** Current Comment, Jour. Amer. Med. Assoc., *131*, No. 10: 828. July 6, 1946.

A considerable part of the daily requirement of ascorbic acid is contributed by fresh milk. A quart of milk may contain 22 mg. of reduced vitamin C, almost a third of the recommended allowance. Commercial handling and processing drastically reduces this value. One study indicates that pasteurized milk from consumers' homes and retail stores averaged 5.8 mg. per liter, while reconstituted evaporated milk averaged 2 mg. Fresh pow-

dered whole milk after being reconstituted averaged 12.5 mg. per liter, and the vitamin C in the dried milk was retained after 12 months at room temperature to the extent of 80%. D.P.G.

ICE CREAM

386. A Roadside Ice Cream Business. Ice Cream Field, *48*, No. 1: 18. July, 1946.

The success of roadside stands depends upon many things. Mention is made of the importance of location, the advisability of adequate parking to encourage profitable curb service as well as proper layouts for attractive and efficient stands.

The balance is from an article by Charles W. Alexander which appeared in *Printers Ink*. Alexander states that millions of people travel the "open road" and this will affect many phases of manufacturing, mediums of advertising as well as sales methods. He warns against doing the wrong thing when starting a roadside business and stresses the following:

1. Pick spot carefully. Traffic count alone is not enough, ratio of customer cars to total traffic is important. It is stated that "Roadside business is much more a creature of the weather than metropolitan outlets."
2. It is vitally important to know the type of road. It is claimed that traffic lights are an asset and a corner location of important intersecting highways is desirable.
3. The road may change, hence it is important to contact the State Highway authorities, especially the road inspector under whose jurisdiction your prospective site will come. He can be of considerable help.
4. Pick the outside of a curve but not a winding section of the road likely to be replaced by a straight one.
5. The apex of a forked road is a good location.
6. Check zoning restrictions.
7. Check land characteristics and drainage as well as availability of electrictiy, gas and telephone.
8. Suitable sized plot with ample frontage as well as good landscaping are important.
9. Building should be designed by experienced architect and be air-conditioned.
10. Operating costs should be considered in deciding whether to lease or buy. All agreements should be in writing.
11. Radio and mail advertising can be effective in promoting sales. Names of car owners who frequent your stand can be secured from the State Registrar of Automobiles. W.C.C.

387. Nectar Research Progressing. W. V. CRUESS AND I. G. A. GLAZEWSKI, Division of Food Technology, University of California, Berkeley, California. Ice Cream Field, *48,* No. 1: 42. July, 1946.

Results of experiments on fruit purees and nectars are briefly described.

Apricot nectar, consisting of 50 per cent apricot puree and 50 per cent light sugar sirup, was originated about fifteen years ago. It has been produced commercially in sterilized form, but the frozen nectar is also an excellent product with many uses, the authors claim.

Several blends were found to be very pleasing. Thus apricot nectar 2 parts, apple juice 1 part and 6.6 per cent lemon juice was very palatable. Likewise a blend of Valencia orange juice and apricot juice was pleasing. Pineapple juice blended with apricot nectar required acidification for best results. Blends of pineapple juice and apricot puree were too viscous.

Good peach nectars were also made from the following varieties: J. H. Hale, Elberta and Rio Oso Gem. Acidification to 0.3 per cent to 0.4 per cent citric acid was beneficial.

Pear-peach nectar blends, plum nectar and several other blends were found pleasing in flavor. W.C.C.

388. Preparation and Pretreatment of Fruits for Freezing. LEONORA A. HOHL, Division of Food Technology, University of California, Berkeley, California. Food Freezing, *1,* No. 8: 287. June, 1946.

A summarization of research to date on the control of changes in color, flavor, and texture in fresh frozen fruits. Much progress has been made in the direction of retaining natural color and flavor but it appears that knowledge of the complex physico-chemical or colloidal changes which take place during the processes of freezing and thawing is insufficient to make specific recommendations concerning the most effective way of preserving the natural texture of fruits. In general, it is probably true that the importance of this reason for quick freezing has been overemphasized. Minimization of chemical and microbiological changes are still good arguments in favor of rapid freezing. Pretreatments of fruits for freezing to minimize or control oxidation fall into four general categories: (1) selection of suitable varieties, (2) exclusion of air, (3) anti-oxidants, (4) blanching. Two tables are included, I. Recommended Quantities of Ascorbic Acid for Inhibition of Browning in Frozen Fruits, II.Condensed Directions for Preparation of Important California Fruits for Freezing. L.M.D.

MILK

389. Effect of Cooling on Extent of Fat Dispersion in Agitated Milk. E. G. HOOD. Canadian Dairy and Ice Cream Journal, *24,* 4: 35. April, 1945.

Morning's milk was cooled at 85°, 75°, 65°, 55°, and 45° F. Sixteen

gallon lots of milk taken at each temperature were agitated by churning for some 5 minutes, warmed to 85° F., and separated. A sample of each lot of skim milk was analyzed for butterfat. In a second set of experiments evening's milk which had been cooled to below 45° F. was warmed up to each of the above temperatures and the same procedure followed. As the temperature at which milk was agitated became progressively lower, the butterfat content of the skim was also lower. On the basis of previous work the results are interpreted to mean that vigorous agitation of milk causes larger fat globules to break up but that this effect is minimized by cooling the milk to below 55° F. H.P.

390. Transfer of Unsatisfactory Dairies Between Health Jurisdictions. CARSON H. OUTWATER, Dept. of Health, New York, N. Y. 19th Ann. Rpt. N. Y. State Assoc. Milk Sanit., p. 159. 1945.

As no recognized health agency desires unsatisfactory dairies under their jurisdiction it is suggested that dairies desiring transfer should be carefully inspected. It is necessary to (1) keep control records, (2) require application for transfer with pertinent prior record, (3) require investigation and inspection before permitting transfer, and (4) in questionable cases require letter of authorization from health agency which the dairyman wishes to leave. A.C.D.

391. Postwar Milk Bottle. V. L. HALL, Glass Container Mfg. Inst., New York, N. Y. 19th Ann. Rpt. N. Y. State Assoc. Milk Sanit., p. 155. 1945.

The square milk bottle is being strongly recommended to the dairy trade in all sizes. The quart size weighs 17¾ ounces and has an opening of 51 mm. or less. Satisfactory washing and breakage are no problems as compared with the round bottle. A case of round bottles occupies 47½% more space than a case of square bottles. In a refrigerator 12 square bottles occupy the space of 8 round bottles. A.C.D.

392. The Future of Fiber Milk Containers. FRED C. BASLET, American Can Co., New York, N. Y. 19th Ann. Rpt. N. Y. State Assoc. Milk Sanit., p. 145. 1945.

In 1944 there were 375 dairies in the U.S.A. using 2,000,000,000 fiber milk containers. They were used chiefly in stores. The percentages of all store accounts sold in paper were Chicago 80, Los Angeles 75, San Francisco 60, and New York 45. About 60% of all housewives prefer paper. The trend toward homogenized milk has tended to minimize the objection to paper containers that the cream layer does not show.

Three types of paper containers are used, namely, (1) those formed

and paraffined in the dairy as needed, (2) those which are pre-fabricated and require special machines for filling, and (3) those which are pre-fabricated and may be filled in ordinary glass milk bottle machines.

Cost is vital. At one cent each, an increase of one mill per container means an increased cost to consumers of $3,000,000 per year. If costs double, few paper containers will be used, but if costs could be cut in half then nearly all milk would be sold in paper. A.C.D.

393. Problems in Design, Installation, and Operation of H.T.S.T. Pasteurizers. C. W. WEBER, N. Y. State Dept. of Health, Albany, N. Y. 19th Ann. Rpt. N. Y. State Assoc. Milk Sanit., p. 121. 1945.

There is need to standardize the design and installation of H.T.S.T. pasteurizers to assure most perfect results. From a public health standpoint the H.T.S.T. pasteurizer equals or exceeds any other type of pasteurizer. Attention needs to be given to certain phases of the process as (1) heating every particle of milk to 160° F. or higher, (2) holding every particle for 15 seconds or longer, (3) prevention of contamination of properly pasteurized milk with pre-pasteurized milk, (4) installing homogenizer in the system, (5) cleaning and sterilizing, and (6) handling breakdowns during operation. A.C.D.

394. Preliminary Report on Coliform Studies of Pasteurized Milk and Milk Products. LEON BUCHBINDER AND JOHN W. FERTIZ, N. Y. City Dept. of Health, and School of Public Health, Columbia University, New York City. 19th Ann. Rpt. N. Y. State Assoc. Milk Sanit., p. 103. 1945.

This study was conducted on some 2,250 samples of pasteurized milk collected at the bottle fillers or in the refrigerators of 46 plants in New York City.

It was found that the coliform counts were much higher in the summer than in the winter. There were 400 quarts of milk collected at the pasteurizers and only one sample was coliform positive, thus proving that coliform bacteria in pasteurized milk represent recontamination. Very little difference was obtained by using sodium desoxycholate agar and brilliant green bile broth.

All milk samples were plated in 1-ml. quantities, also 20 ml. milk were divided in 4-ml. portions and poured into 5 plates, and finally the remaining quart of milk (900 ml.) was incubated overnight at 37° C. and then plated in 1-ml. quantities. Tests showed that 2 to 5 coliform bacteria per quart of milk would give positive for coliform after incubation overnight at 37° C. The data showed that most milk was coliform positive if the samples were large enough, and the number of coliform bacteria increased with an increase in the mean outdoor temperature.

Analysis of the data lead the authors to think that two standards for coliform counts should be used, one for May through November, and a lower count for November through April. Trials are being made to try the application of 10 or more colonies per 5 ml. of milk in summer and 6 or more colonies per 20 ml. of milk in winter as representing unsatisfactory milk.

A.C.D.

MISCELLANEOUS

395. Tomorrow's Packages. An open letter to materials manufacturers. C. I. SAYLES, Associate Professor, Institution Engineering, School of Nutrition, Cornell University. Food Freezing, *1*, No. 7: 246. May, 1946.

A plea is made for standardization of the moisture-vapor test for wrapping materials used in frozen foods packaging in order that the customer whether locker plant operator or processor will be in a position to exercise selection of protective wrappers on a comparative basis. The recommendation made contains four points for test standardization: (1) Moisture-vapor results be reported in the same units of weight and area; (2) That a common relative humidity test condition be chosen as a standard; (3) That a standard 0° F. test be sought; (4) That eventually all these results shall be brought to the public in a simple form related to the length of storage time so that a particular wrapping material may be designated as a "six months" or a "year paper." L.M.D.

396. Sanitation in the Processing Plant and Its Relation to the Microbial Quality of the Finished Product. REESE H. VAUGHN AND THRESSA C. STADTMAN, Division of Food Technology, University of California. Food Freezing, *1*, No. 7: 334. July, 1946.

Proper blanching of vegetables will reduce the number of microorganisms at least 99.9 per cent. Post-blancher contamination is the vital factor that the processor must keep in mind. Contaminated cooking water, conveying belts, and miscellaneous handling equipment should be guarded against. Even the human element should be considered. Continuous sanitizing of equipment such as belt conveyors and elevators is recommended. Sanitizing agents that may be used are: (1) chlorine in residual strength above breakpoint to be great enough to destroy microbial growth and prevent slime formation; (2) sulfiting by means of SO_2 gas or sulfite salts; (3) surface-active agents possessing disinfecting powers as well as detergency. Diagrams are included showing methods for applying sanitizing solutions to conveyor belts.

L.M.D.

397. Handling of Long-Distance Shipments of Frozen Foods. EDWIN SMITH, Senior Horticulturist, Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, Soils and Agricultural Engineering, ARA, U.S.D.A. Food Freezing, *1,* No. 7: 331. July, 1946.

A discussion of results observed in long-distance shipment of frozen foods in various types of refrigerator cars. Many details are touched upon which indicate that the refrigeration protection of frozen foods in transit present new problems compared to the older standard practices in vogue for many years for handling frozen meat carcasses and cold pack fruits. In general the best results in temperature control were obtained with the "Preco" fan car and the overhead brine tank car. Emphasis is given to the need for improvement in handling at loading and receiving ends to keep down the temperature rise of car on the one hand and the temperature rise of the goods on the other. One important fact was brought out that goods thought to be "zero or lower" often were in the twenties. This condition puts undue load upon the car's refrigeration facilities. Car design must be such that packages are not stowed in direct contact with car walls. Heavy insulation is a must for long-distance transport because cars spend a large percentage of the overall haulage time actually standing idle, which in the case of the fan car means air circulation ceases. In this case the overhead brine tank car offers an advantage in that the refrigeration effect is obtained through convection. L.M.D.

Abstractor's comment: Forced convection with the overhead brine tank would seem to offer some advantage where close storage of cartoned goods is practiced.

398. Studies of Frozen Food Samples Bought in Open Market. Part I. Observations on color, texture, appearance and odor of frozen vegetables. W. V. CRUESS, Professor of Food Technology, University of California. Food Freezing, *1,* No. 7: 243. May, 1946.

The quality of frozen vegetables that the consumer is receiving is an important factor in their merchandising, a phase of the retailing of frozen foods which should be given increased attention. The author used in his investigations a score card containing the items, Color and Appearance—20, Texture—20, Flavor—45, and Odor—15, Total 100. Under the heading "Comments," are included a description of the odor and flavor, state of maturity of the vegetable, character of color, and the degree of catalase and peroxidase activity. Also a check of actual weight found against declared net weight. The procedure was first to thaw at room temperature and then test for catalase and peroxidase. The vegetables were then cooked either in steam or in a pot with a very small amount of water; then were tasted.

Ten tables of score results on different vegetables are included together with comments. In general the results indicate considerable chance for improvement in quality under all items of scoring and a need for control of stage of maturity in selection of vegetables for freezing. L.M.D.

399. The Rôle of Water in Freezing Foods. MILO R. DAUGHTERS AND DAWRENCE S. GLENN, Western Research Laboratories, Frosted Foods, Inc. Refrigerating Engineering, *52*, No. 2: 137. August, 1946.

As a result of the beneficial results observed when figs that have begun to shrivel are frozen (they withstand freezing better and have better texture when defrosted than do those that are in a plump and succulent stage of maturity) the authors have advanced the theory that bound water is the responsible factor. The assumption that all moisture in foods is changed to ice crystals at sub-zero temperatures may not be correct. According to the theory only free water is changed to ice and the bound water is held in some form which either does not freeze at the temperatures employed in quick freezing, or, if it does, freezes to crystals of molecular size which do not rupture the tissue. Once the methods of reducing the percentage of free water in foods, or the means for transforming it into bound water are known, there should be a decided improvement in the quality of many of the frozen foods now coming into the market.

The authors, employing the calorimetric method, determined the proportional distribution of free and bound water in several fruits and vegetables. It is a possibility that partial dehydration may become a processing step in commercial food freezing which could be combined with deactivation of enzymes. L.M.D.

400. Evacuation and Dehydration of Field Installations. HOWARD A. BLAIR AND JOEL N. CALHOUN, B. F. Sturtevant Company, Division of Westinghouse Electric Corporation, Boston, Mass. Refrigerating Engineering, *52*, No. 2: 125. August, 1946.

The authors review the means for removal of moisture from Freon refrigeration systems, the undersirable effects of excessive moisture and recommend a combination of vacuum evacuation and dehydration of installations in the field as the most efficient means of moisture removel where Freon-12 is concerned. L.M.D.

401. Refrigerated Transport. PAUL B. REED, Perfex Corporation, Milwaukee, Wis. Refrigerating Engineering, *52*, No. 2: 115. August, 1946.

A comprehensive survey of the field covering rail, motor, and air refrig-

erated transport. Most recent developments in attempts to develop efficient and economical refrigeration applications in the transportation of perishable foodstuffs are described. The author believes that the present state of refrigeration equipment of cars, trucks, and trailers, much of which is antiquated and badly worn, offers great opportunities for exploitation, development, and expansion. L.M.D.

402. The Control of Microorganisms in Food Storage Rooms. W. L. MALLMAN AND E. S. CHURCHILL, Section of Bacteriology and Public Health, Michigan Agricultural Experiment Station, East Lansing, Mich. Refrigerating Engineering, *51,* No. 6: 523. June, 1946.

Ultraviolet rays were found to produce reduction of bacteria on surfaces directly irradiated, followed by a gradual increase after prolonged irradiation. In closed rooms, surfaces shielded from direct irradiation showed some reduction in surface populations. In closed rooms ozone atmospheres gave results comparable to those obtained with shielded ultraviolet rays.

Carbon dioxide in laboratory tests in concentrations of 2.5 per cent suppressed the rate of growth of bacteria on surfaces, while those of 5 and 10 per cent caused reductions in populations of 45–90 per cent depending upon the type of bacteria.

Glycols when vaporized into the air caused reductions of 40–90 per cent, but had no lethal action upon spores.

Ultraviolet rays, ozone, carbon dioxide and glycols may be classified as marginal sanitizing agents which have specialized use in refrigerated and food handling rooms. In all instances they should be tested out fully for each specific application before permanent installations are made.

Both hypochlorites and quaternary ammonium chlorides are applicable to surface disinfection in food preparation and storage rooms, but due to the fact that surface sanitizers have only a limited action on bacterial spores, gross contamination of spores must be removed by thorough washing and flushing of surfaces in order to achieve successful results in maximum reduction of surface contamination. L.M.D.

403. Technical Phases of Home Freezer Development. C. E. LUND, Director of Research, Seeger Sunbeam Corp., St. Paul, Minn. Refrigerating Engineering, *51,* No. 6: 513. June, 1946.

Emphasis is placed upon the design of freezer cabinets from the standpoint of economics of operation. It is brought out that four inches, five at the most, is the limit in insulation thickness. Beyond this, additional thicknesses result in no economy of operation or in prevention of surface condensation ("sweating"), nor was insulation beyond four inches of thickness

warranted when considering maximum storage space against external dimensions. Top thickness and that of lids can be set at about two and one-half inches. Vitally important is vapor sealing. Whether double gaskets or single gaskets are used on lids there must not be any appreciable vapor leakage. Care should be exercised to use materials for obtaining structural strength and rigidity between the inner and outer walls of a freezer cabinet. A table (3) of performance characteristics for four capacities of home freezer cabinets operating at 0° F. cabinet temperature is included. The cabinets are of 6, 9, 12, and 18 ft. sizes. At two and one-half cents per kwh at 70° F. the costs were per month, 0.84, 1.27, 1.50, 1.76 dollars, respectively, while at 100° F. they stepped up to 1.86, 2.24, 2.65, 3.10 dollars. L.M.D.

404. Tomorrow's Packages—and Their Merchandizing Value in Display. J. ALFRED ANGLADA, Sylvania Industrial Corp. Food Freezing, *1*, No. 6: 220. April, 1946.

Stress is placed upon the fact that a frozen food package must possess sales appeal in addition to affording protection against deterioration of its contents. In providing an attractive package, cellophane not only allows visual appraisal of contents through the media of window sections, lift tops, window tabs, etc., but, if properly sealed, insures the food product against dehydration, oxidation and under some conditions added moisture. The multiple unit package is advocated as a merchandising unit for complete frozen meals, or a number of units of the same food. Cellophane as an overwrap lends itself to printing, particularly on the reverse side which enhances the beauty of the print job by lending gloss and depth of color to the ink. Sealing cellophane by heat calls for one second or less at 250° F. to 300° F. L.M.D.

405. Studies of Frozen Food Samples Bought in Open Market. Part II. Observations on the color, texture, appearance and odor of frozen fruits. W. V. CRUESS, Professor of Food Technology, University of California. Food Freezing, *1*, No. 8: 306. June, 1946.

Part I, on frozen vegetables, appeared in Food Freezing, May, 1946. Samples of frozen fruit purchased in the open market were subjected to scoring upon a rating basis of—color and appearance 20, texture 20, flavor 45, and odor 15 for perfect score. Three tables showing results for apricots, apple sauce, and sliced peaches are included. Orange juice and other fruits are commented upon. In general it is indicated that much improvement remains to be made if frozen products are to compete with fresh and canned one. The author recommends flash pasteurization of citrus juices following vacuumizing before packing and freezing. Fruits should be covered with syrup. In the case of vegetables more thorough blanching should be prac-

ticed. Fluming may be responsible for a large part of the loss of flavor of vegetables and apples. Net weights of fruits were generally over declaration because of added syrup but practically all vegetables were below declared net weight, shortages of 1 oz. being common. This indicates a need for protective packaging that is more efficient in preventing moisture vapor passage. The loss is probably through sublimation of ice. L.M.D.

406. Illinois Co-op Locker Associations "Come of Age." THEODORE H. KIMBLE. Food Freezing, *1,* No. 8: 292, 314. June, 1946.

An exposition of the results of a field survey of 25 cooperative locker plant associations in 25 Illinois counties made by Paul C. Wilkins of the research division of the U. S. Dept. of Agriculture's Farm Credit Administration in cooperation with the St. Louis Bank of Cooperatives. The 25 associations constituted 48 complete plants and 12 branch rooms. The complete plants had an average capacity of 510 lockers; the 12 branch rooms (without processing equipment) averaged 203 apiece. A total of 26,975 lockers were being rented at the close of the fiscal year last July 31. Tables, Comparative Analysis of Expenses Per Locker Rented and of Income Per Locker Rented are given showing the high, low and average figures itemized. The average total expense was $21.03, average total income $23.09, resulting in a net saving of $2.06. It is evident from the average income from product items that the bulk of services is in connection with meat products, relatively small returns being received from vegetable and fruit processing and poultry. Two major difficulties encountered by most associations in operating their processing rooms at a profit were: (1) Insufficient volume of products in one or more of the plants. (2) Wide fluctuations in volume from month to month. Many associations are considering consolidation of processing plants in one centralized location and converting processing and high temperature rooms to additional low temperature storage space. Also the increase of poultry processing in the summer months together with freezing fruits and vegetables for re-sale out of season to locker patrons and other local sales outlets should offer a means of fuller utilization of centralized processing plant personnel and equipment. L.M.D.

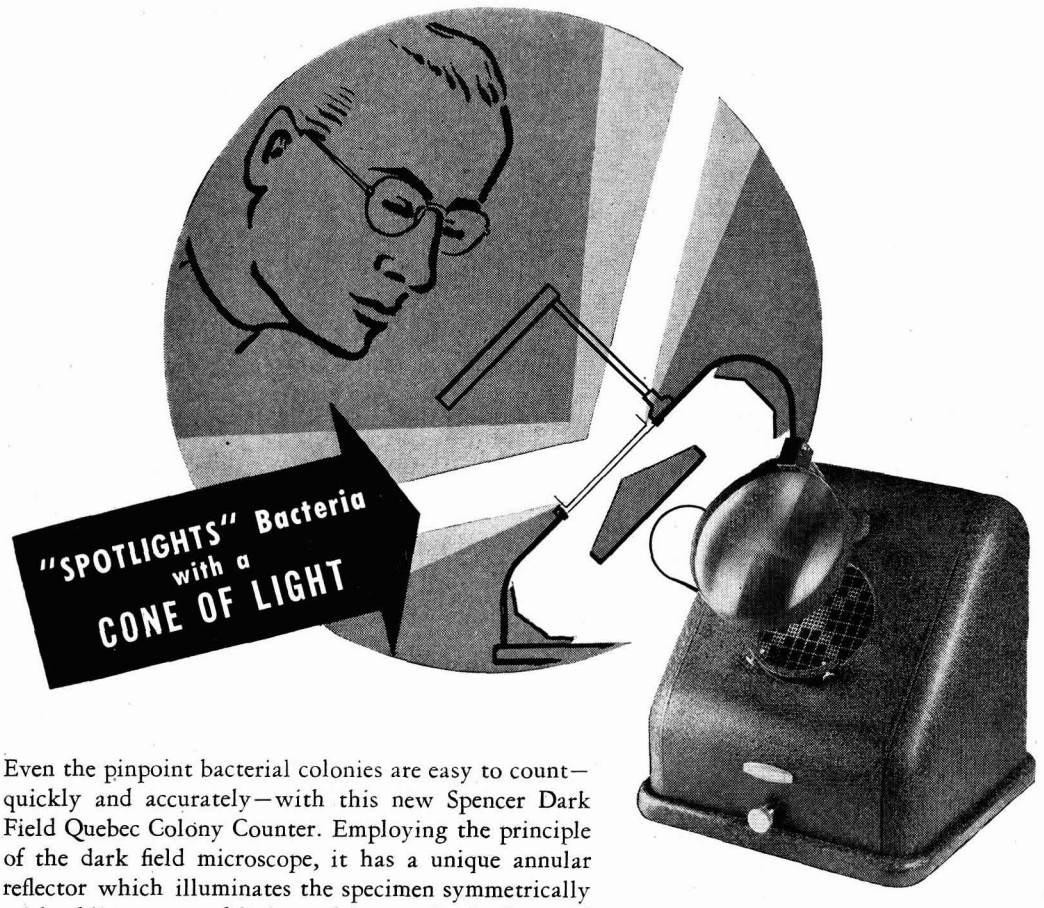
407. Tomorrow's Packages—and the Use of Petroleum Waxes. J. F. BUTTERWORTH, Staff Engineer, Process Products, Socony Vacuum Oil Co., Inc. Food Freezing, *1,* No. 8: 296. June, 1946.

The most universally used packaging material—be it in frozen food or in other food requirements—is *paper treated with petroleum wax.* There are two broad classifications of waxes, namely, paraffin-type waxes and microcrystalline type waxes. The former are hard, brittle, white, and crystalline; while the latter are generally darker in color, have great ductility at all tem-

peratures, are tacky, have smaller crystals and higher melting points. For low temperature use in frozen food packaging refined paraffin waxes are blended with microcrystalline waxes to obtain low temperature flexibility. Converting paper with waxes can be grouped into three classifications: 1. "Dry-Waxed" or impregnated papers; 2. "Wet-Waxed" or surface-coated papers; 3. Laminated papers.

While dry-waxed papers are waterproof for practical purposes they are not moisture-vapor barriers. Wet-waxed papers possess moisture-vapor-proofness and if the paraffin wax has a small quantity of microcrystalline wax blended with it the coating will have the necessary ductility for low temperature use. Laminated papers making use of microcrystalline wax combine the desirable characteristics of two materials. L.M.D.

NEW SPENCER COLONY COUNTER



Even the pinpoint bacterial colonies are easy to count—quickly and accurately—with this new Spencer Dark Field Quebec Colony Counter. Employing the principle of the dark field microscope, it has a unique annular reflector which illuminates the specimen symmetrically with oblique rays of light and leaves the background subdued. As a result it provides:

1. **Remarkably uniform illumination**—the colonies are lighted from all sides.

2. **Greater brilliance**—reflector collects and concentrates the light rays.

3. **Freedom from glare**—only light reflected from the specimen reaches the eyes of the observer.

4. **Enclosed illuminating system**—protected from dust, yet permitting easy replacement of the household 40 watt lamp.

Equipped with a standard 1.5x lens, this compact, efficient instrument will be welcomed by health departments, hospitals, dairies, breweries, canneries—wherever colony counts are made. Write dept. L28 for details.

American Optical
COMPANY
Scientific Instrument Division
Buffalo 15, New York

Manufacturers of the **SPENCER** *Scientific Instruments*

If We Missed You at ATLANTIC CITY—

The first post-war Dairy Industries Exposition is past history. One of its highlights was the combination of old and new faces... men who kept the dairy industry "clicking" during the difficult war years... returned service men ... men just starting out in the industry.

If you didn't get to Atlantic City, we're sorry we missed seeing you. If you are a newcomer to the dairy field, we want to welcome you into the industry which one of our old publisher friends aptly described in a recent editorial as *"a good business to be in."*

Building time-saving and product-improving processing equipment for the Dairy Industry has been CP's sole business for nearly sixty years. From CP's research and engineering have come some of the Industry's most important developments.

Creamery Package offers you a complete line of modern equipment for processing milk, butter, cheese and ice cream; complete refrigerating machinery; an extensive line of dairy plant supplies; and coast to coast service through 21 company owned and operated branches.

To both old and new friends we offer this reminder: Look to CP for the best in Dairy Plant Equipment, Supplies and Service.

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