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Contents

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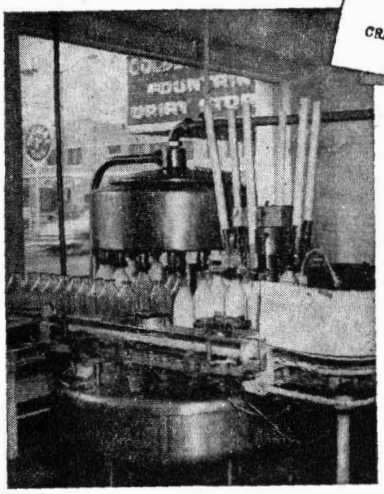

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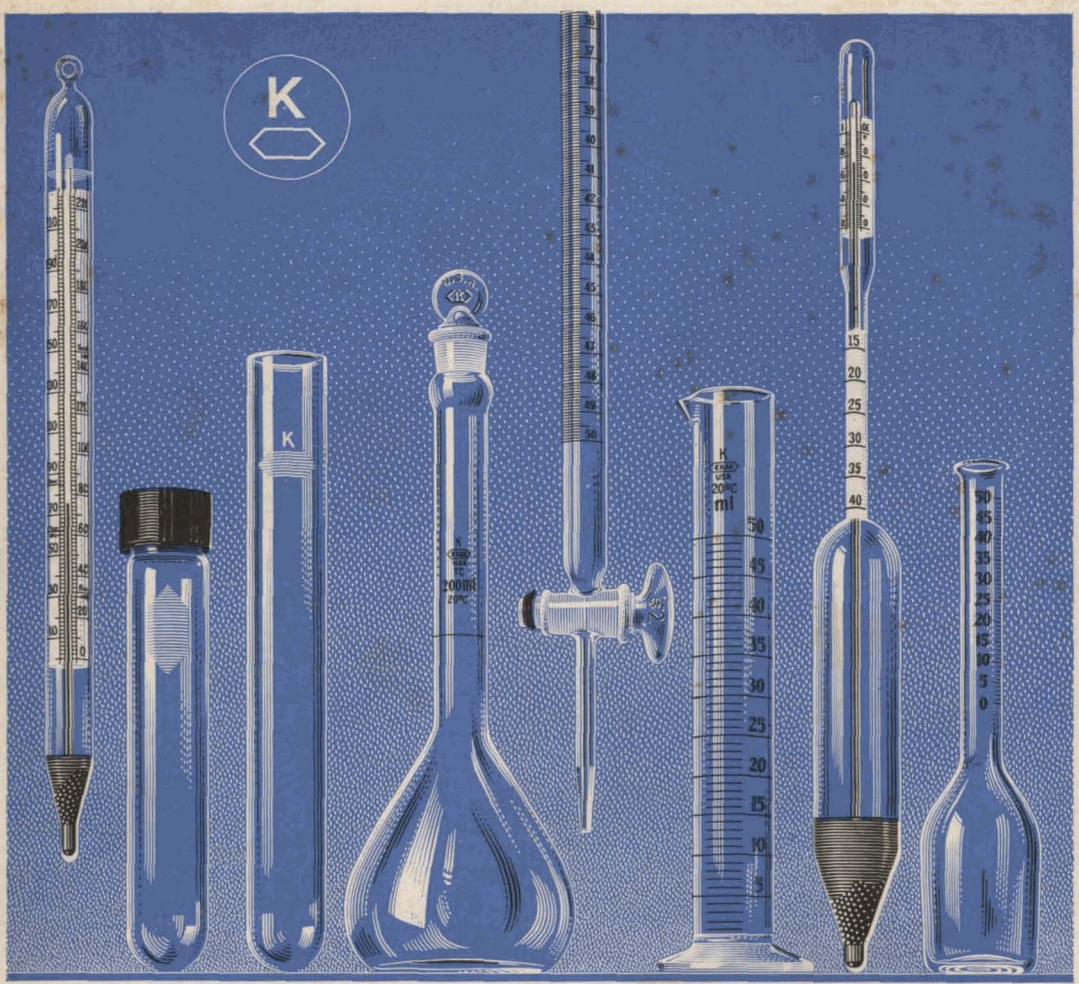

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LIPASE: A REVIEW

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These pages were written with the hope that they might prove useful to those who know little about lipase, and to those who know much. The references cited may not be the first paper on a given topic nor the last. The first publication may be indecisive, incomplete. The last may be less informative than an earlier publication. Some may feel that too much has been omitted; an equal number may feel that too much has been included. So be it.

If these pages help some to understand what is already known; if they point the way for further research; if they stimulate some to start new investigations, then the time spent in writing this has been well spent.

I. THE RANCIDITY[1] PROBLEM

At one time, some chemists doubted that normal milk contained lipase. It is now accepted that the great majority of cows secrete milk containing fat-splitting enzymes but, in most cases, these enzymes are inactive. Under certain conditions they may become active and cause serious loss. In view of this fact, it is more important to know the activity of lipase in raw milk than to know the quantity; and handlers of milk must pay particular attention to those factors which increase or reduce the activity of the enzyme.[2]

Several times in the history of the dairy industry, new methods of handling or processing milk have activated the lipase system and produced rancid flavors. Homogenization is the best known example of this. Raw milk becomes rancid so quickly after homogenization that the sale of homogenized milk would be impossible if lipase could not be inactivated by simple means.

As a second example of changes in practice which led to trouble with lipase, we may cite the change in temperature of separation which occurred in the market cream industry some years ago. At one time, milk was warmed to a fairly

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[1] In this paper, rancidity refers only to hydrolysis with liberation of fatty acids. Oxidation is not included.

[2] "Activation of lipase" should not be interpreted in a narrow sense. The phrase is used in place of the more accurate statement: "acceleration of the process of enzymatic hydrolysis."

high temperature (110-120° F.) before separation to reduce fat losses in the skimmilk. When it was realized that a more viscous product could be produced by separating at a lower temperature (80-90° F.) and improvements in separators made this possible, the temperature of separation was gradually lowered. At the same time, there was an increase in the incidence of rancidity. The relation between these two events was not understood until the phenomenon of "temperature activation"[3] was reported in 1939 *(51)*.

Recently, there has been an increase in the number of reports reaching this department concerning rancid milk. This time, the trouble seems associated with the introduction of pipeline milkers and farm tanks into the New York area. Because the use of such equipment offers many advantages and is likely to be adopted widely, rancidity may become an important problem once more.

It is known that several investigations of the effect of pipeline milkers and bulk handling upon the quality of milk are now under way, but little or nothing has yet been published. Until the results of others appear, comment must be based largely upon our own investigations. Full details of these will be published later.

According to reports reaching us, the milk handled by the newer methods frequently contains butter particles and in some cases it is rancid. Churning is evidence of agitation, and the agitation of raw milk is known to accelerate lipolysis *(52)*. It seemed probable that the two defects could be traced to the same source.

In a number of cases, the trouble was traced to "risers" in the pipe lines, vertical sections connecting one pipe line to another at a higher level. The milk is carried up by a stream of air which produces considerable agitation and foam. Any given portion of milk may be thrown up and fall back a number of times before it passes over.

In New York State, where milk is collected each day, the average acid degree[4] of milk reaching pasteurizing plants is approximately 0.5 unless it has been handled in a pipeline milker *(32)*. In that case, the acid degree may be much higher. To show clearly the effect of a pipeline milker upon lipolysis, a sample of the entire night milking was taken on three successive days at a farm where there were five risers in the pipe line. On the second day of the sequence, pail milkers were used and the milk was carried by hand to the milkhouse; on the first and third days, the milk passed through the pipe line. The acid degree values after holding for 36 hours were 2.87, 0.92, and 2.59, respectively.

If risers could be eliminated, much of the trouble would disappear. Ultimately, it may be possible to design risers which cause less damage to the milk. The mechanism by which agitation activates lipolysis is not understood in detail. It is difficult to understand why agitating warm raw milk with air in a vertical

[3] The rate of lipolysis in cold raw milk is increased greatly if cold milk is warmed momentarily to 85° F. and recooled. Warming to higher or lower temperatures produces less "activation."

[4] Acid degree is the number of milliliters of normal alkali required to neutralize the free acid in 100 g. of fat.

pipe should produce much more activation than agitation for the same time in a Waring Blendor. That is the case if the Waring Blendor is filled completely so that no space is left for air.

The effectiveness of agitation is not determined by its violence alone. Other factors must be considered. The role of temperature is important. As fresh milk cools, it passes through a critical temperature zone where it is most susceptible to churning *(43)*. If pipe lines in barns were heated to prevent cooling of the milk, churning in the pipe could be prevented, but this would not reduce trouble from rancidity. The sensitivity of milk to activation by agitation is related to the fluidity of the fat, and it increases rapidly as the temperature is increased up to 75 or 85° F.

With the introduction of farm tanks and tank truck transportation, there is a growing interest in the possibility of alternate day pick-up from the farms. If this practice is adopted, an increased effort will be needed to control lipase. Milk supplies which do not develop organoleptic rancidity under present methods of handling may do so if the milk is held raw for an additional 24 hours. Under such conditions, any factor which may accelerate or delay the appearance of rancidity must be given careful consideration.

The effect of agitating fresh warm milk has been mentioned. Fortunately, the agitation of cold milk has much less effect upon lipase activity. The most critical periods are when the warm milk is in the pipeline and when warm milk enters an empty tank and does not completely cover a propeller-type agitator. Crowe *(12)* compared milks cooled in cans with milks cooled in a vat. The vat-cooled milk contained more water-insoluble acid, and the value increased with increasing amounts of agitation. The agitation of milk in tank trucks during shipment is of lesser consequence because the temperature is usually very low and the subsequent holding period is relatively short.

The possibility of temperature activation when fresh warm milk is added to cold milk in the tank should not be overlooked. In the worst possible case, all of the second milking would be added at once to the cold milk in the tank. Under such conditions, the temperature might exceed 68° F. This is high enough to produce a definite increase in the rate of lipolysis *(51)*. In practice, however, the warm milk is added over a period of time while the refrigeration unit is in continuous operation, and the cold milk is not warmed enough to produce such a great effect. In some cases, however, this small effect might be just enough to carry the milk across the threshold of organoleptic rancidity, especially if alternate day pick-up is practiced.

On the positive side, only one means of controlling rancidity has been discovered that might be put into practice on the dairy farm. It is instantaneous cooling. If the milk is cooled in a few seconds by means of a tubular or surface cooler, lipolysis during subsequent storage is reduced greatly *(34)*. Furthermore, this avoids all risk of temperature activation and reduces activation by agitation in the vat. Instant cooling may become an important step in a program of alternate day hauling.

It should be emphasized that low temperature alone does not check lipolysis.

In natural milk, the reverse is true; lipolysis is accelerated as the temperature is reduced *(53)*. If the holding period is prolonged, microorganisms may attack the fat at higher temperatures and, under these conditions, investigators may observe less lipolysis at intermediate temperatures (60° F.) than at higher or lower temperatures *(12, 70)*.

It seems probable that there will be increased interest in the study of lipase in the near future. Those making such studies, or reading about them, should understand some of the differences between the methods used to study lipase. Failure to do this has been the cause of much confusion.

Some methods measure the deterioration of milk fat itself, but they do not measure the same kind of deterioration. For that reason, their results may not agree closely with each other. Other methods do not measure the deterioration of milk fat. They yield results which may or may not be significant in the actual handling of milk. Such data must be interpreted with caution. The most widely used methods which measure changes in milk fat are:

1. *Organoleptic examination.* This is primarily a measure of free, short-chain, water-soluble, fatty acids. Liberation of long-chain acids has little effect on the flavor or odor of the milk. This method yields results of most significance in terms of consumer response but it does not yield numerical data, and the method is of low sensitivity. It can not detect changes before they become commercially significant.
2. *Surface tension measurements.* If the change in surface tension is due to the liberated fatty acids, as some suppose, then it should be most sensitive to the longer water-soluble acids (capric, caprylic). Butyric acid, the most important factor in the organoleptic measurement, has little effect on the surface tension of milk *(10)*. On the other hand, the possible role of monoglycerides and of diglycerides in reducing surface tension must not be overlooked. If they are the major factors causing surface tension depression, then the depression of surface tension should be nearly independent of the nature of the acid liberated.

   Unfortunately, some of the published measurements of surface tension are difficult to interpret for several reasons. First, merely cooling fresh milk and immediately rewarming it to room temperature will produce a change in surface tension of several dynes which is not due to lipase action *(50)*. If workers using surface tension measurements have taken this into account in planning their experiments, they have not always made the fact clear, nor explained what provision was made to eliminate it. Second, there is some doubt about the correctness of the values reported in the literature. Few details have been published concerning the techniques that have been employed so that others might duplicate the work. For example, the surface tension of a fresh surface decreases rapidly at first, then more slowly. The first change is so rapid that it can scarcely be observed unless the milk is diluted *(83)*. Subsequently, the surface tension falls more slowly, probably because fat globules are accumulating in the surface. (The surface tension of cream is lower than that of whole milk;

the surface tension of skimmilk is higher.) Because of this slower change, one who works rapidly will obtain higher readings than one who works more slowly. More important still, measurements made with a du Noüy tensiometer (the instrument most widely used today) must be corrected as explained by the makers of the instrument. Personal correspondence has revealed that some workers have not made this correction. There is a possibility that others, also, have failed to correct their data. This does not necessarily invalidate the conclusions of an individual investigator, but it does make it difficult to compare the values of one worker with those of another unless there is assurance that both have applied proper corrections to their measurements. (With our own instrument, the correction is approximately −9%.)

3. *Measurement of fat soluble acids (acid degree values).* Most of these acids have little effect on surface tension or upon the flavor of milk. Several procedures are in use for their measurement. Some prefer to recover the fat by extraction, some by churning *(46)*. (It may be possible to use some of the emulsion breakers investigated by Stine and Patton, *78.)* Some have titrated the acids in alcohol, and some have used ether and other solvents *(80)*. These modifications all yield essentially the same results though there are small differences.[5] Measurements of fat soluble acids have the advantage that they yield numerical results which are reproducible and which reveal very small changes in the fat. Before any change can be detected organoleptically, the titration value will usually increase from an initial value of 0.20 to 0.30 ml. [the normal range for fresh milk fat *(32, 33)*] up to 1.5 to 2.0 ml. *(17)*. Consequently, the effect of minor changes in handling milk can be detected easily by this method.

It should be emphasized that each of these three methods measures deterioration of fat by hydrolysis. However, it is a common observation that any one of the three measures of deterioration (organoleptic rancidity, surface tension depression, or water-insoluble acids) may change independently of the others. For that reason, there are some who believe that several different enzymes are present in milk and that they do not always occur in the same proportions.

Other methods have been used to study lipase which do not measure the breakdown of milk fat directly. For example, some have measured the breakdown of substrates foreign to milk, tributyrin *(18, 55)*, p-nitrophenol stearate *(45)*, naphthol acetate *(62, 74, 75)*, etc. With these methods, it is possible to show differences in milks, but the significance of such data is not clear. The limitation of these methods is apparent when you consider that the effects of homogenization and of temperature activation upon lipolytic activity in milk can not be shown by such techniques. These methods may be useful in estimating the quantities of enzyme but, in practice, the activity of the enzyme system is of much more importance than the quantity when handling raw milk.

[5] Hillig's method for water-insoluble acids is relatively specific for acids of 16 carbons or more. WIA values are not strictly comparable with measurements of total fat soluble acid (acid degree).

II. SOME RECENT WORK ON LIPASE

Several reviews dealing with lipase have appeared in recent years. That of Herrington *(31)* dealt primarily with lipase in milk and its products. Ammon *(1)* did not restrict his review to any particular field. Bradshaw *(5)* was concerned with lipase in cereal products, and Desneulle *(14)* with lipase in digestive processes. Frisell's *(23)* review of nonoxidative, nonproteolytic enzymes lists only 12 references to lipase. In contrast, Dunkley's paper *(17)* was not intended primarily as a review, but it does cite many references to the literature of the subject. Those interested in extensive bibliographies should consult these papers for early references. This paper is intended to supplement, not duplicate, these earlier reviews.

LIPASE IN MILK

Dunkley and his associates published a series of articles of much interest. In the first of these *(17)*, Dunkley compared the acid degree values of 92 samples of cream with organoleptic ratings. Samples classified as slightly rancid ranged in acid degree from 1.47 to 4.88, with an average of 2.59. Since fresh milk fat has an acid degree of approximately 0.3 *(32, 33)*, it is evident that extensive hydrolysis may ocur in some samples before it can be detected organoleptically. He found that surface tension values were a better index of organoleptic rancidity than acid degrees. In general, samples having a surface tension below 45 dynes at 20° C. were rancid, and those above 46 were seldom rancid. However, the fat content of the milk should be taken into consideration when making predictions. Some samples containing only 2% of fat showed no organoleptic rancidity even though the surface tension fell below 42 dynes. He made the curious observation that samples stored in test tubes differed in surface tension from those held in ¼-pint bottles by approximately 1.4 dynes. He also measured the surface tension of milk from individual quarters of the same udder and occasionally found large differences.

In a second paper, Fredeen *et al. (22)* reported that lipolytic activity, as revealed by surface tension measurements, varied with season and stage of lactation but not with gestation or estrous cycle. This last observation should be contrasted with the results of another study at Maryland on the relation beween lipolytic activity and both natural and induced estrus *(2)*. In the Maryland investigation, they measured changes in the acidity of the fat and found a maximum concentration of lipase on the day of estrus, and a maximum acidity in the fat on the day following estrus. When estrus was induced by injections of diethyl stilbestrol, the milk became very rancid. Perhaps the difference between these conflicting reports is due to differences in the cows, perhaps to differences in the methods used to detect lipolysis.

Fredeen *et al.* reported that injections of pituitrin and stilbestrol sometimes caused a decrease of almost 15 dynes in the surface tension of the milk secreted, but five cows did not show this response to the injection. No information about organoleptic rancidity was given.

In the third paper of the series, Dunkley and Smith *(18)* compared the

activity of individual samples of skimmilk upon tributyrin and upon a special homogenized milk fat substrate. In both cases, they observed the same pH optimum, 9.5 at 10° C. At 37° C., the optimum fell to pH 8.8 with tributyrin; no value was given for milk fat. The results with different milks on the two substrates showed very high correlation: 0.984, 0.923, and 0.941 in three sets of samples. They concluded that measurements of tributyrinase are a useful measure of lipase. It should be noted that they did not compare either measurement with changes occurring in the original milk.

In the fourth paper, tributyrinase activity was compared with changes in surface tension in natural and in temperature-activated milk. During the first 3-4 months of lactation, the two showed considerable correlation but there was none during the latter half of the lactation period. They make the curious statement that the amount of tributyrinase present is not a limiting factor in producing rancidity in the latter part of the lactation period (when the amount of tributyrinase is low and lipase activity as measured by surface tension changes is high), but it is a limiting factor in the latter part of the lactation cycle when the tributyrinase values are high and lipolysis (measured by surface tension) is low. This can be interpreted in several ways. What they meant is not clear. In their experiments, they found that the tributyrinase activity of skimmilk was independent of the separation temperature and concluded that cooling milk did not cause an increase in adsorption of lipase upon the fat globules. This is in agreement with the conclusions of Krukovsky *(50)*, who found no difference between skimmilks prepared at 10° C. and at 45° C. when tested against tributyrin and against heated homogenized cream. Krukovsky went further and found that there was no difference between the creams if the lipase was activated by homogenization. Without activation, the 45° cream showed no lipolysis but the 10° cream became rancid very quickly.

These findings are in conflict with the view of Tarassuk and Jack *(79)* that milk lipase is adsorbed upon the fat when it is cooled. It is true that their experimental conditions were very different. Their conclusion was based upon the observation that some samples of milk will become rancid if cooled but do not become rancid if kept warm. It is possible to explain their observations by the fact that lipolysis in natural milk is more rapid at low temperature, a phenomenon which can not be explained by adsorption alone.

Weinstein and Trout *(82)* compared the susceptibility of milk of different breeds to activation by homogenization. No definite conclusions can be drawn since they studied only 18 cows distributed among five breeds.

LIPASE IN MILK PRODUCTS

Shotwell *et al. (77)* made a study of rancidity in ice cream. They employed a solvent extraction method to recover the fat for measurement of acid degree values. Samples of vanilla ice cream collected from ten manufacturers at four periods during the year had a mean acid degree value of 2.91 and a range of 1.33 to 6.24. In a separate study of organoleptic rancidity of ice cream, they prepared rancid samples by using homogenized raw cream. Samples having acid

degree values of 4-5 were judged slightly rancid. Those above five were judged rancid, or worse. They made similar studies of chocolate ice cream. The mean values for acid degree were higher but the organoleptic threshold was higher also.

Hood *(44)* has published statistics on the incidence of rancidity in 718,096 Canadian Cheddar cheeses, and others *(47)* have reported on rancidity in the butter of Quebec.

Christensen *et al. (8)* found that spray powders made from milks which had not been preheated were rancid. They attributed this to the agitation in the vacuum pan during condensing. The acidity of the fat continued to increase slowly during storage even though the moisture content was less than 3%. Preheating for 20 minutes at 140° F., or higher, was sufficient to prevent rancidity.

ANALYTICAL METHODS

Papers dealing with analytical procedures may be divided into two groups: those measuring changes produced by lipolysis and those attempting to measure the enzyme itself. Obviously, the two are closely related.

Hillig's *(35)* method for the determination of water-insoluble acids (WIA) in butter has been adopted by the A.O.A.C. It enables the analyst to judge the quality of the original cream, since the WIA are not lost during churning *(39)*. Most of the butyric acid is lost when butter is made in conventional churns *(42)*, though one-third may remain in the butter made by the continuous process *(40)*. This fact has been turned to advantage in judging whether deterioration in quality has occurred before or after churning. Microbial activity in butter usually results in a sharp increase in the butyric acid/WIA ratio *(49)*.

Because of the importance of such measurements, the Hillig test has been the subject of many papers. Several have presented evidence concerning its reliability *(4, 28, 36, 39, 41)*. It has been found to correlate reasonably well with the alpha naphtholphthalein (ANP) test when the WIA value did not exceed 400 mg/100 g. *(3, 30)*. When compared with organoleptic scores, there were many inconsistencies *(4)*. This is to be expected since the water-insoluble acids, themselves, are practically tasteless and odorless. It has been reported *(69)* that the results of the Hillig test were sometimes much higher when 2.0 ml. of excess alkali (beyond that required for neutralization) was used. Hillig *(37)*, however, has explained this by showing that excess alkali is needed to insure complete extraction of the free fatty acids. The official method recognizes this by prescribing 0.5 ml. of excess alkali.

The original Hillig test is time-consuming. A quicker procedure has been described *(38)*, which measures the WIA by volumetric titration (instead of isolation and weighing) and the assumption that the mean equivalent weight of the acids is 270. By actual test, the value fell between 260 and 280 in 71 out of 73 samples. Ramsey and Hess *(72)* reported good results on a collaborative study of the butyric acid test, but they used aqueous solutions of short chain acids, not milk fat. It should be noted that lipolysis in butter and in sour cream is due chiefly to enzymes of microorganisms, not of the milk *(42)*.

Armstrong and Harper *(3)* described an improved form of the ANP test

and adapted it for the examination of cream *(30)*. This test can not measure small differences in WIA values but they recommend it as a sorting test. Goiffon *(28)* described another colorimetric test based upon the fact that Nile blue, which ordinarily turns red in the presence of excess sodium carbonate, does not do so in the presence of free unsaturated fatty acid. He describes both colorimetric and titrimetric procedures based upon this fact. Such a test might permit the rapid estimation of oleic acid in the WIA of butter. The value of such information is not yet known.

Tucker and Bird *(80)* substituted methanol for ethanol in preparing their standard alkali solutions because it was cheaper and easier to obtain. This produced turbidity during the titration, but that trouble was eliminated by dissolving the fat in a mixture of Skellysolve B and propanol, 4 + 1.

The chemical methods which have been used to study the breakdown of milk fat are all measures of the acid products of hydrolysis. Desnuelle *(14, 15)* and Mattson *et al. (57)* have measured the other products (diglyceride, monoglyceride, and glycerine) formed during the digestion of fats. The first step in the hydrolysis seemed most easy; the last, most difficult. The yield of glycerine was increased by the addition of calcium and bile salts *(15)*. Perhaps some one will be able to make similar studies on rancid milk. Because of the great surface activity of the mono- and diglycerides, such information might be useful.

ESTIMATION OF LIPASE

Interest in methods of measuring lipase is not limited to the dairy industry. Copenhauer *(9)* has described a Warburg method by which he could measure the esterase activity of liver at pH 8.4, using as little as 0.5 mg. of fresh tissue. Lubert *et al. (55)* developed an extraction-titration method which could be used over a wide range of pH values. Fiore and Nord *(20)* recommended polyvinyl alcohol as an emulsifying agent in lipase assays. Para-nitrophenol esters have been used by some investigators to measure lipase activity *(45)*. This colorless substrate yields a yellow color upon hydrolysis. However, Dirks and Boyer *(16)* found that many substances which were not enzymes would catalyze this reaction. Glutathione was particularly active, and crystalline bovine serum albumin, crystalline egg albumin and β-lactoglobulin possessed activity, even after heat treatment. Similar studies should be made to determine whether other nonfat substrates such as the beta naphthol esters are specific reagents for enzymes.

ISOLATION OF LIPASES

A few have attempted to concentrate and purify lipase. Zechmeister *(85)* and Giri *(27)* have described chromatographic techniques, and Wallenfels *(81)* used electrophoresis in a paper sheet to separate the enzymes of a fungus. He was able to identify zones of amylase, proteinase, lipase, and phosphatase. However, lipase was identified by the hydrolysis of para-nitrophenyl stearate and, in view of Dirks' results, the identification of lipase is not conclusive.

Morton *(59)* reported that many enzymes could not be extracted from natural materials because they were bound to solid particles of lipid material. In some

cases they could be released by extracting the lipids with butanol. This may be helpful in the study of lipase. [It is of interest to dairymen that he was able to concentrate milk phosphatase 5,600-fold by this technique *(60)*.] Nashif and Nelson *(63)* were able to precipitate the lipase of *Pseudomonas fragi* from solution with $(NH_4)_2SO_4$, but the temperature must be kept very low to avoid inactivation. Shipe *(76)*, also, used ammonium sulfate to precipitate the enzyme from cultures of *Penicillium roqueforti* and *Aspergillus niger*, but the broth was concentrated first by removing approximately three-fourths of the water as ice.

SOME EFFECTS OF LIPOLYSIS

The growth of *S. lactis* is inhibited in strongly rancid milk. Costilow and Speck *(10)* studied this phenomenon by adding small amounts of purified fatty acids to milk inoculated with test organisms. Butyric acid, caproic, oleic, linoleic, linolenic, arachidonic, and palmitic acids had no appreciable effects upon growth. Capric acid was most toxic, followed by caprylic and lauric. Toxicity did not correlate with the depression of surface tension. In a second paper *(11)* they reported that the growth of *S. zymogenes*, of *S. bovis*, and of *E. coli* was retarded in rancid milk, but less than that of *S. lactis*. They confirmed the fact that the surface tension of rancid milk tends to rise during incubation with *S. lactis*. This parallels the observations of Cherrington and Hammer *(7)*, who found that rancidity in young Cheddar cheese was removed by the action of an organism thought to be a variant of *S. lactis*.

As little as 0.1% rancid milk fat proved to be a very effective foam breaker during the condensing of skimmilk and whey *(6)*. The effect was attributed to the mono- and diglycerides present. Sagar *(73)* found that rancid milk fat was assimilated by young children even more rapidly than homogenized milk. This, also, was attributed to the surface activity of the mono- and diglycerides. He measured the interfacial tension between a 1% solution of olive oil in mineral oil and aqueous solutions of alpha mono-stearyl glyceride, alpha, beta di-stearyl glyceride, and bile. The interfacial tensions were 2, 4, and 12 dynes, respectively. With no addition, the interfacial tension was 22 dynes.

Mukherjee *(61)* has repeated the observation of Greenbank and Holm *(29)* that fat containing free fatty acid is more susceptible to oxidation. Mukherjee held milk fat under various conditions at 37° C. and examined it periodically for 85 days. The peroxide value and the Kreis test increased more rapidly in the fats having higher acid values. The possible relation between lipase activity and oxidation in market milk deserves more attention than it has received.

THE NATURE OF LIPASE

Relatively little is actually known about the lipases. There is evidence that some can be split into a thermostable coenzyme and a thermolabile apoenzyme *(84)*. Perhaps this is true of all lipases. When lipases from different sources are compared with respect to pH range, temperature of inactivation, activity against different substrates, and response to different activating or inactivating agents, it seems that scarcely any two enzymes are exactly alike. Do we have as many

lipases as we have sources, or are we dealing with mixtures of a much smaller number of enzymes? If we are dealing with mixtures, how can we separate them? How specific are individual components in their action? These questions need answers, and some investigators are attempting to find them.

Paul J. Fodor has published many papers on the properties of enzymes from insects and other sources.[6] Others *(21, 58, 63, 64, 65, 66, 67, 68, 71, 76)* have studied the lipases of organisms important in the dairy industry. From the published data, it is clear that these enzymes are different and not interchangeable.

Shipe *(76)* described a new technique for the study of relative specificity. He used an equimolecular mixture of tributyrin and tricaprylin as a substrate and determined the relative amounts of butyric and caprylic acids set free using a chromatographic separation. The lipase from *Aspergillus niger* liberated 4 mols of caprylic acid per mol of butyric, that from *Penicillium roqueforti* liberated only ⅓ mol per mol of butyric acid. With equal activity on tributyrin, there is a twelvefold difference in activity on tricaprylin. Clearly, we must dismiss the idea that "lipases are of low specificity."

An even more striking case of specificity was reported by Martin and Peers *(56)*, who found that oat lipase would split only one molecule of butyric acid from tributyrin. It had no action on either alpha, alpha dibutyrin or alpha, beta dibutyrin, nor would it attack either alpha or beta monobutyrin. It did attack triacetin (at one-third the rate on tributyrin). During purification, the relative activities against tributyrin and triolein remained constant. This may mean that the same enzyme is involved in both cases, but the evidence is not conclusive.

Fujimura and Hamaguchi *(24, 25, 26)* have published several papers which seem of unusual interest though the original manuscripts are not yet available to the reviewer. They found that a complex of casein and ascorbic acid possessed esterase activity which was lost by destruction of the ascorbic acid. It was restored by the subsequent addition of ascorbic acid. This should open a new field of inquiry regarding the possible relation of ascorbic acid to lipase activity in milk. It may be significant that both lipase and ascorbic acid are destroyed by exposure of milk to sunlight *(48)*, and both are sensitive to oxygen *(54)*.

From the practical viewpoint, an understanding of the phenomena of activation may be of greater value to the dairy industry than information regarding the enzyme itself. In the original report *(32)* describing temperature activation it was stated that: "It seems probable that activation is more dependent upon changes in the state of the fat than upon changes in the lipase." This view has been expressed more recently by Dunkley and Smith *(19)*, who wrote: "Activation treatments such as temperature-fluctuation, agitation and homogenization generally are considered to depend on changes in the substrate for their effectiveness."

The writer would go one step further and postulate that activation by

[6] These papers are not listed here but may be located through the author index to Chemical Abstracts.

temperature changes, by agitation, or by homogenization is nonselective with respect to the kind of acids subsequently liberated. The relative amounts of the different acids set free in any given sample will depend upon the nature of the enzymes present. If this view is correct, then the choice of method for studying the effect of processing upon lipolytic activity in milk need be based only on sensitivity and convenience.

The exact nature of the changes in the fat surface during activation needs further study. It seems likely to the writer that at least two kinds of activation must exist. The original surface material may be removed irreversibly by mechanical forces, or the adsorptive and reactive properties of the fat may be changed by the phase transformations which follow cooling. The completion of a thesis dealing with the relation between lipolysis and the properties of the surface of the fat globule has been announced *(13)*. No further information is available at present, but the title suggests that the results may be of unusual interest.

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AMINO ACIDS AND PEPTIDES IN THE PROTEIN-FREE FRACTION OF MILK BEFORE AND AFTER INCUBATION WITH *STREPTOCOCCUS LACTIS*[1]

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Several investigators have shown that *Streptococcus lactis* grown in milk can cause breakdown of milk protein. Little is known, however, about the changes that take place in milk protein as the result of the action of microbial proteinases. Morgan and Nelson *(3)* reported marked increases in leucine, isoleucine, valine, threonine, arginine, methionine, histidine, tryptophan, tyrosine and phenylalanine in tungstic and lactic acid filtrates of milk after incubation with *S. lactis* for 15 days at 21° C. It was shown by van der Zant and Nelson *(4)* that *S. lactis* growing in milk caused a rapid increase in soluble nitrogen and also in tyrosine and tryptophan during the first 24 hours, followed by a smaller but gradual increase during the next 2 to 3 days.

In this study, protein-free fractions prepared from skimmilk after incubation with *S. lactis* for different lengths of time were investigated for their content of amino acids and peptides by paper chromatography.

EXPERIMENTAL METHODS

The culture of *S. lactis* was strain 26 used in a previous study *(4)*; it was handled as described in that publication.

For these trials fresh skimmilk was heated for 20 minutes at 85° C. to destroy as many undesirable organisms as possible, without subjecting the milk constituents to changes that might occur during sterilization. One-liter quantities of heated milk were inoculated with 0.1% of a 24-hour culture of *S. lactis* grown in milk and incubated for 0, 24, and 96 hours at 32° C. After incubation, 100 g. of trichloroacetic acid was added to each culture, and the precipitate was allowed to settle for 1 hour at 2° C. The precipitate was removed by centrifugation and filtration through paper. The excess trichloroacetic acid was removed by extraction with ether. The amino acids and peptides in the protein-free filtrate were removed by adsorption on and elution from a column of the ion exchange resin Duolite C-3, as described by Block *(1)*. An aliquot of the solution containing amino acids and peptides was hydrolyzed by boiling under reflux for 24 hours in 20 ml. of 6 *N* HCl per milliliter solution. The excess acid was removed by concentration in vacuo, and the residue was diluted with a 10% solution of isopropanol to give the same concentration of nitrogen as in the unhydrolyzed solution.

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Two dimensional chromatograms of the unhydrolyzed and hydrolyzed fractions were carried out, using Whatman No. 1 filter paper (7.5 × 7.5 in.). The solution (0.01 ml.) was applied with a micropipet in one of the corners 1 in. from the edges of the paper. After drying, the paper was rolled into a cylinder and stapled so that the edges would not touch. After the run in the first dimension with phenol (80 parts of phenol and 20 parts of water), the paper was dried and restapled for the run in the second dimension. A mixture of butyl alcohol, butyric acid, and water (BABW 2:2:1) was used as developing medium in the second dimension; this run was repeated once. Development was carried out at 21° C. in wide-mouth reagent bottles with tight-fitting lids. Each paper was immersed 2 cm. in the solvent; developing was continued until the solvent reached the top of the paper. All chromatograms were run in duplicate; one was used for color development with ninhydrin; the other was viewed under ultraviolet light after heating at 100° C. for 15 minutes. Color development was carried out by spraying the paper with a solution of 1% acetic acid in 0.1% ninhydrin in butyl alcohol. The paper then was dried in a hood and heated at 85° C. for 10 minutes. Known amino acids were run under the same conditions.

The solution containing amino acids and peptides was fractionated by placing 20 0.01-ml. aliquots equi-distant from each other and 1 in. from the bottom of a 12 × 16 in. sheet of Whatman No. 1 filter paper. Five sheets were used. Phenol was used as developing medium. After development, the sheets were dried in a hood, washed twice with redistilled ether, dried, heated at 100° C. for 15 minutes and viewed under ultraviolet light to locate the amino acids and peptides. The bands were cut out and thoroughly extracted with hot water and concentrated to dryness in vacuo, and each residue was dissolved in 1 ml. of 10% aqueous isopropanol. Aliquots of these solutions were hydrolized as described earlier. Two dimensional chromatograms then were carried out on the unhydrolyzed and hydrolyzed solutions as described above. The spots on the paper viewed under ultraviolet light were cut out and put in test tubes. The color which developed after addition of ninhydrin was measured as described by Fowden *(2)*. Differences in color extracted from spots representing identical amino acids then were used to compare semi-quantitatively the amounts present.

EXPERIMENTAL RESULTS

In Table 1 are presented data showing the amino acids present in unhydrolyzed and hydrolyzed protein-free filtrates of cultures of *S. lactis* incubated for 0, 24, and 96 hours. Free alanine, glutamic acid, glycine, leucines, and valine were present in the protein-free filtrate at 0 hour incubation. Incubation with *S. lactis* for 24 hours resulted in the appearance in the protein-free filtrate of free lysine, phenylalanine, proline, serine, threonine, and tyrosine; an increase was observed in free alanine, glutamic acid, glycine, leucines, and valine which already were present at 0 hour incubation. As the period of incubation was extended to 96 hours, free aspartic acid appeared in the protein-free filtrate; increases also were detected in free alanine, glutamic acid, leucines, lysine,

TABLE 1

Amino acids in unhydrolyzed and hydrolyzed protein-free fractions of milk incubated with S. lactis for 0, 24, and 96 hours

	0 hr.		24 hr.		96 hr.	
Amino acid	Unhydr.	Hydr.	Unhydr.	Hydr.	Unhydr.	Hydr.
Alanine	+[a]	++[a]	++	+++[a]	+++	+++
Arginine						+
Aspartic acid				+(?)	+	+
Cystine						+
Glutamic acid	+	++	++	+++	+++	++++[a]
Glycine	+	++	++	++	++	++
Leucines	+	++	++	+++	+++	++++
Lysine			+	++	+++	+++
Phenylalanine			+	++	+++	+++
Proline			++	+++	+++	++++
Serine		+	+	++	++	++
Threonine		+	+	++	++	+++
Tyrosine			++	++	+++	+++
Valine	+	++	++	+++	++	+++

[a] + weak, ++ medium, +++ strong, ++++ very strong (on paper).

phenylalanine, proline, serine, threonine, and tyrosine over the amounts found after incubation for 24 hours.

Hydrolysis of the protein-free filtrate caused in some instances the appearance of amino acids which were not present in the free form before hydrolysis and caused increases of some of the amino acids present in the free form before hydrolysis. A two-dimensional chromatogram representing the amino acids found in a hydrolyzed protein-free filtrate of a culture of *S. lactis* incubated for 24 hours is presented in Figure 1.

Fractionation showed evidence of a number of peptides. In the filtrate from the culture at 0 hour incubation were present peptides containing glutamic acid and glycine; alanine, glutamic acid, glycine and leucines; glutamic acid, leucines, proline, serine and valine.

In the filtrate from the culture incubated for 24 hours were present peptides containing alanine, glutamic acid and glycine; glutamic acid, leucines, and valine; aspartic acid (?), glycine, leucines, tyrosine, and valine; alanine, leucines, lysine, proline, threonine, and tyrosine; alanine, glycine, leucines, phenylalanine, and serine.

In the filtrate from the culture incubated for 96 hours were present peptides containing glycine and tyrosine; glycine, leucines and phenylalanine; alanine, lysine, tyrosine and valine; leucines, lysine, proline and threonine; glutamic acid and glycine; glutamic acid, glycine, leucines, proline and valine.

The order in which the amino acids in the peptides are written is arbitrary and does not imply their amounts or structural arrangement in the peptide. Threonine was present in the hydrolyzed aliquot of the protein-free filtrate (0 hour) but not in the peptides, whereas proline was found in one of the peptides and not in the hydrolyzed filtrate. Arginine, aspartic acid, cystine, and serine were found in the hydrolyzed filtrate of a culture that was incubated for 96 hours, but these amino acids could not be traced in the peptides of this filtrate.

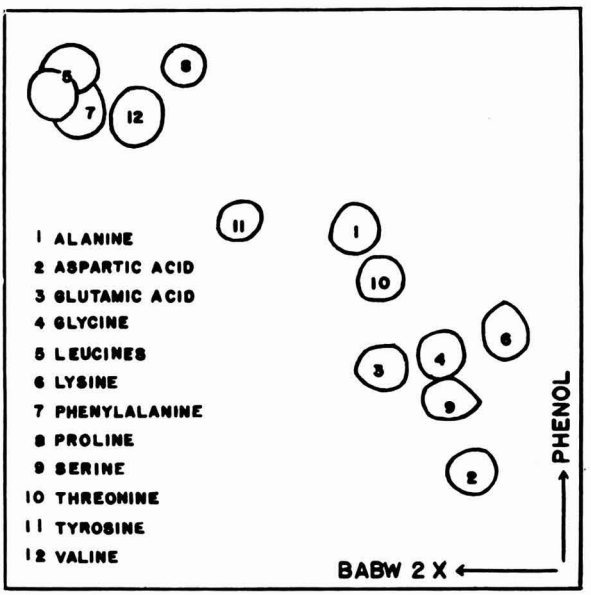

FIG. 1. Chromatogram of hydrolyzed protein-free filtrate of a culture of *S. lactis* incubated at 32° C. for 24 hours.

DISCUSSION

It is too much to hope that a single study would reveal the pattern followed in the breakdown of milk protein by the proteolytic enzyme system of *S. lactis*. The free amino acids (alanine, glutamic acid, glycine, leucines and valine) found in the protein-free filtrate of a milk culture of *S. lactis* at 0 hour incubation also were reported by Block *(1)* in a protein-free fraction of skimmilk. Aspartic acid and serine were reported by Block to be present in very small quantities but were not found in this study at 0 hour incubation. Methionine, which is required for growth of *S. lactis*, was not detected in any of the protein-free filtrates. This amino acid is present in large amounts in the milk proteins and it can be expected that the enzyme system of *S. lactis* makes methionine available. Failure to detect methionine in the filtrates after organism growth may be due to *(a)* methionine being present but in such a small amount that the methods employed did not reveal its presence or to *(b)* destruction of methionine during preparation of the samples, which may be true especially for the hydrolyzed filtrates.

Block found four peptides in the protein-free fraction of skimmilk; in this study three peptides were found at 0 hour incubation, of which one contained the same amino acids as one reported by Block. The peptides found by Block contained the same amino acids as the peptides found in this study at 0 hour incubation, with the exception of aspartic acid, which was present in one of the peptides reported by Block. Determination of the order in which the

amino acids occur in the peptides found in the various protein-free filtrates undoubtedly would contribute to the knowledge of the mode of action of the proteolytic enzyme system of *S. lactis*.

Concerning the peptides found in the protein-free filtrates, it is interesting to note that a cell-free extract of *S. lactis* was able to hydrolyze various peptides such as glycylglycine, glycyl-L-leucine, glycyl-L-tyrosine, DL-alanyl-glycine, DL-leucylglycine, glycylglycylglycine and DL-leucylglycylglycine *(5)*. Quite possibly the reason other peptides were not found in these studies is that the bacteria have destroyed some peptides. The increases in free amino acids which were found as incubation progressed might be interpreted to indicate the probability that some of the several bacterial peptidases might have attacked peptides formed by action of bacterial proteinases. A peptide containing the amino acids glycine and tyrosine was present after incubation for 96 hours.

It seems advisable in future experiments of this type to use a less complex substrate than milk; the complexity of this substrate increases the difficulties in the interpretation of the results. A similar study employing pure fractions of casein as substrate and purified enzyme extracts probably would eliminate some of the difficulties encountered with milk.

SUMMARY

The protein-free fraction of skimmilk incubated with *S. lactis* for 0, 24, and 96 hours was investigated for the presence of amino acids and peptides by paper chromatography. Evidence was presented for the presence in the protein-free fraction of three peptides at 0 hour, five peptides after 24 hours, and six peptides after 96 hours of incubation.

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CHARACTERISTICS OF SOME ENDOCELLULAR PEPTIDASES OF *STREPTOCOCCUS LACTIS*[1]

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In a previous paper *(12)* it was reported that the proteolytic activity of a cell-free extract of *Streptococcus lactis* against skimmilk was destroyed at pH 7.0 by heating at 61.7° C. for only 2 minutes. Subsequent experiments showed that these heated extracts were active against various di- and tripeptides. Numerous reports have appeared in the literature on bacterial peptidases, but in most cases only a few data illustrating the fact that peptides were hydrolyzed are presented. Berger *et al. (1, 5)* reported in some detail on the peptidases of a great number of microorganisms. In most cases optimum hydrolysis occurred at pH 8.0 to 9.0. Metal activation of various microbial peptidases was shown by Berger *et al. (3, 4)*. Dudani *(7)* determined some of the characteristics of peptidases present in a cell-free extract of *S. liquefaciens*. Zimmerman *(14)* reported that a cell-free extract of *S. cremoris* showed optimum activity at pH 8.0 against both glycyl-L-leucine and DL-alanylglycine; the effect of several cations on the rate of hydrolysis also was studied.

In the present study some of the characteristics of the endocellular peptidases of *S. lactis* were determined.

EXPERIMENTAL

The two cultures of *S. lactis* were strains 18 and 26 used in the previous studies *(11, 12)*; they were handled as described in these publications. Cultures were transferred daily for three transfers prior to each trial, using the culture medium employed in the experiment. Cell-free extracts of cells of *S. lactis* grown in either skimmilk or broth media were prepared by sonic vibration, as described in a previous study *(12)*.

M/30 solutions of glycyl-L-leucine, glycyl-L-tyrosine, glycylglycine, glycylglycylglycine and *M*/15 solutions of DL-alanylglycine, DL-leucylglycine and DL-leucylglycylglycine were used as substrates. These substrates, in 25-ml. quantities, were prepared by dissolving the required amount of peptide in approximately 15 ml. of distilled water and 7.5 ml. of a 0.05 *M* composite buffer of the desired pH, containing equimolar concentrations of acetate, borate, and phosphate. The solution then was brought to the desired pH with dilute sodium hydroxide or sulfuric acid and made up to volume with distilled water. Merthiolate was added to all substrates at the rate of 0.6 mg. per 25 ml. substrate as a preservative; this concentration had no adverse effect on the activity of the

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peptidases. All substrates were stored at 2° C. Unless stated otherwise, 0.2 ml. of cell-free extract was added to 3 ml. of substrate and incubated at pH 8.0 and 37.5° C. for 1 hour. Hydrolysis of the peptides was determined by titration of the carboxyl groups with ethanolic KOH, by using thymolphthalein as indicator *(8)*. In the course of purification of the cell-free extract, protein determinations were carried out by using the biuret test as proposed by Weichselbaum *(13)*. A standard curve was constructed with the protein of a cell-free extract of *S. lactis;* protein nitrogen was determined by the Kjeldahl method. The amount of protein nitrogen was read from the standard curve after a blank determination had been subtracted. The color was measured in a Klett-Summerson photoelectric colorimeter with a filter of 555 mμ wave-length.

Bacterial counts were made according to *Standard Methods for the Examination of Dairy Products (1),* and plates were incubated at 32° C.

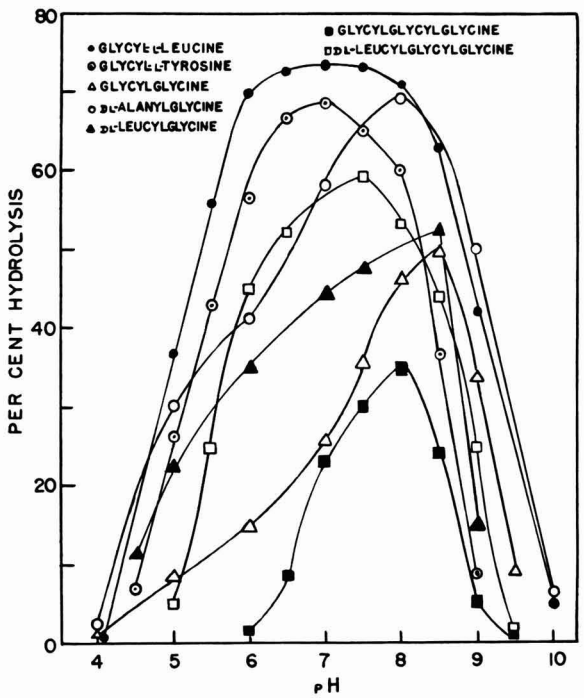

FIG. 1. pH optima for enzyme preparation from *S. lactis* 26 against different peptides (0.2 ml. enzyme preparation per 3 ml. substrate incubated at 37.5° C. for 1 hour).

RESULTS

In Figure 1 are presented representative data showing the effect of pH of substrate on the hydrolysis of five dipeptides and two tripeptides by a cell-free extract of *S. lactis* 26 grown in milk. In all tests maximum activity was found

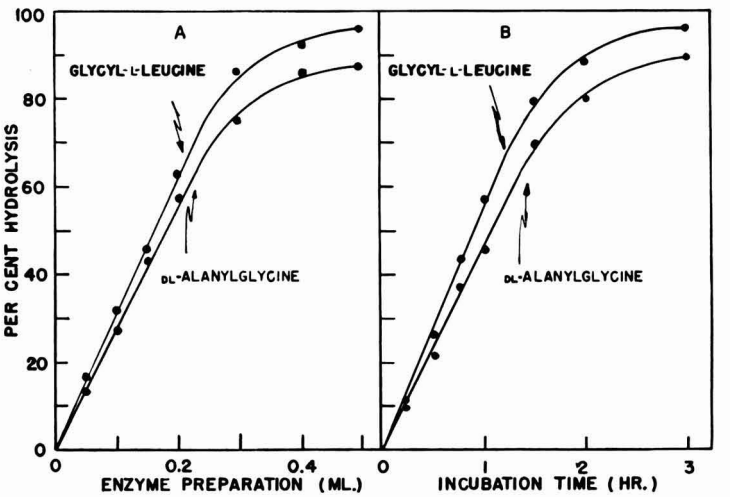

FIG. 2. Effect of increasing quantities and time of incubation on hydrolysis of peptides by preparation from *S. lactis* 26. A: incubated at 37.5° C. and pH 8.0 for 1 hour, B: 0.2 ml. preparation per 3 ml. substrate, incubated at 37.5° C. and pH 8.0.

between pH 7.0 and 8.5. Usually a rather sharp decline in activity was noticed on either side of optimum pH. As shown in Figure 2A, there is a direct relationship between the quantity of cell-free extract used and hydrolysis of glycyl-L-leucine and DL-alanylglycine, at least up to 0.2 ml. of cell-free extract. Up to at least 1 hour of incubation (Figure 2B), a direct relationship between time of incubation and amount of hydrolysis of glycyl-L-leucine and DL-alanylglycine existed. Optimum activity against these two dipeptides, within the temperature limits studied, was found at 45° C. (Figure 3); however, the increase in rate with increasing temperature was somewhat less above 21° C. The results found with a cell-free extract prepared from cells of *S. lactis* 18 grown in milk were similar to those reported for strain 26.

Influence of growth medium on enzyme production. A study, employing media and growth conditions such as were used in studies on proteinase pro-

TABLE 1

Peptidase activity of a cell-free extract[a] *of S. lactis 26 grown in different media*[b]

Medium	Standard plate count	Per cent hydrolysis	
		Glycyl-L-leucine	DL-alanylglycine
	(millions per ml.)		
Niven and Sherman *(9)*	570	56	42
Amundstad *(2)*	1,100	56	40
Vitamin-test casein A	1,200	58	42
Vitamin-test casein B	2,000	62	46
Tryptone medium	1,800	58	48
Casein medium	1,900	58	42
T. G. E. broth	900	56	50

[a] 1 ml. extract represents 3×10^{10} cells.
[b] 0.2 ml. enzyme preparation per 3 ml. substrate incubated at 37.5° C. and pH 8.0 for 1 hour.

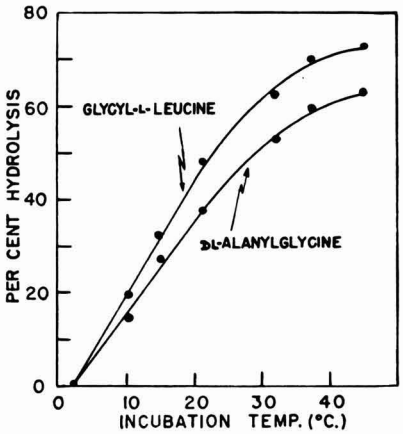

FIG. 3. Effect of temperature of incubation on hydrolysis of peptides by preparation from *S. lactis* 26 (0.2 ml. preparation per 3 ml. substrate incubated at pH 8.0 for 1 hour).

duction reported in a previous publication *(12)*, showed (Table 1) that cell-free extracts prepared from cells of *S. lactis* 26 grown in seven different media possessed peptidase activities against glycyl-L-leucine and DL-alanylglycine comparable to those of an extract prepared from cells grown in milk (Figure 1). The possible effect of tryptic digestion of casein in the vitamin-test casein medium A was investigated. The digestion of casein was carried out as described previously *(12)*. There was no indication that the presence of whole protein (casein) in the growth medium stimulated the peptidase activities of the cell-free extract. A study of the effect of omission of individual vitamins from vitamin-test casein medium A *(12)* on the activity of a cell-free extract of *S. Lactis* 26 against glycyl-L-leucine and DL-alanylglycine showed (Table 2) that omission of biotin, nicotinic acid, and pyridoxine decreased the activity against glycyl-L-leucine

TABLE 2

Effect of omission of vitamins from vitamin-test casein medium A on the activity of cell-free extract[a] against glycyl-L-leucine and DL-alanylglycine[b]

Vitamin omitted	Standard plate count	Per cent hydrolysis	
		Glycyl-L-leucine	DL-alanylglycine
	(millions per ml.)		
All	0.1	—	—
Riboflavin	720	60	46
Ca-pantothenate	110	56	42
Nicotinic acid	170	50	40
Pyridoxine	200	52	52
Thiamine	710	70	46
Biotin	540	50	43
Folic acid	790	60	52
Vitamin B_{12}	870	62	46
None	1,260	62	50

[a] 1 ml. extract represents 4×10^{10} cells.
[b] 0.2 ml. enzyme preparation per 3 ml. substrate incubated at 37.5° C. and pH 8.0 for 1 hour.

somewhat; the same was true for the omission of biotin, nicotinic acid, and calcium pantothenate when DL-alanylglycine was used as substrate, although some of these may be borderline cases. In all subsequent experiments vitamin-test casein medium B was used to grow *S. lactis*. Data on the effect of pH, increasing quantities of cell-free extract, and time and temperature of incubation on the hydrolysis of peptides indicated that the results on cells (both strain 18 and 26) grown in vitamin-test casein medium B parallel closely those of cells grown in milk.

Effect of metallic ions and cysteine on peptidase activities. One-ml. quantities of the following solutions in 0.01, 0.001 and 0.0001 *M* concentrations were used: $MnSO_4 \cdot 4H_2O$, $CuSO_4 \cdot 5H_2O$, $ZnSO_4 \cdot 7H_2O$, $MgSO_4 \cdot 7H_2O$, $NiCl_2$, and $CoSO_4 \cdot 7H_2O$. The cell-free extracts (0.2 ml.) were incubated with 1 ml. of the metal solution for 1 hour at 37.5° C., followed by incubation with peptide substrate for 1 hour at 37.5° C. and pH 8.0. As Table 3 shows, when glycyl-L-leucine was used as substrate there was increased activity at all three concentrations of Mn^{++} tested; Cu^{++}, Zn^{++}, and Ni^{++} were inhibitory in all three concentrations, whereas Mg^{++}, Co^{++} and cysteine did not show any great effect. When DL-alanylglycine was used as substrate, Co^{++} in all three concentrations used increased the activity; Mg^{++} gave some activation but only in the highest concentration tested. Cu^{++}, Zn^{++}, and Ni^{++} showed inhibitory effect in the 0.01 *M* concentration; Mn^{++} and cysteine did not show much effect on peptidase activity.

TABLE 3

Effect of metallic ions and cysteine on the peptidase activity of a cell-free extract of S. lactis 26[a]

Metal	Per cent hydrolysis when ion was added in concentration of:		
	10^{-2} *M*	10^{-3} *M*	10^{-4} *M*
		Glycyl-L-*leucine*	
None	52		
Mn^{++}	75	69	61
Cu^{++}	25	32	39
Zn^{++}	10	20	35
Mg^{++}	50	48	50
Ni^{++}	16	31	43
Co^{++}	45	50	55
Cysteine	47	50	52
		DL-*alanylglycine*	
None	57		
Mn^{++}	55	50	52
Cu^{++}	46	51	56
Zn^{++}	32	50	52
Mg^{++}	67	60	60
Ni^{++}	21	45	50
Co^{++}	71	70	65
Cysteine	50	50	55

[a] 0.2 ml. enzyme preparation per 3 ml. substrate incubated at 37.5° C. and pH 8.0 for 1 hour.

Stability of peptidase activities. Preliminary experiments showed that a cell-free extract of *S. lactis* could be heated at 55° C. for 30 minutes at pH 7.0 without loss of activity against the different peptides used in this study when

tested at pH 7.0, whereas the proteinase activity, as determined by release of tyrosine and tryptophan from casein, was destroyed almost completely by heating at 55° C. for 15 minutes *(12)*. Portions of a cell-free extract of *S. lactis* 26 were adjusted to pH values ranging from 4.0 to 9.0 and heated at 61.7° C. for different lengths of time, as described in a previous paper *(12)*. These heated extracts then were tested against glycyl-L-leucine and DL-alanylglycine with incubation at pH 7.0. Maximum stability of the peptidase activity (Table 4) occurred

TABLE 4

Effect of heating at 61.7° C. at different pH levels on the peptidase activity of a cell-free extract of S. lactis 26[a]

pH of cell-free extract	Per cent hydrolysis in control	Per cent hydrolysis after heating for:			
		1 min.	3 min.	5 min.	10 min.
		Glycyl-L-leucine			
4		0	0	0	0
5		62	48	36	30
6		70	68	62	56
7	74	74	70	70	68
8		70	65	52	48
9		67	51	32	28
		DL-*alanylglycine*			
4		0	0	0	0
5		52	42	25	20
6		56	45	40	43
7	60	62	62	60	58
8		66	51	40	39
9		56	42	35	20

[a] 0.2 ml. enzyme preparation per 3 ml. substrate incubated at 37.5° C. and pH 7.0 for 1 hour.

at pH 7.0. Heating at 61.7° C. at pH 4.0 for only 1 minute destroyed the peptidase activities. Considerable destruction of activities against both peptides took place when heated for more than 3 minutes at pH levels of 5.0 and 9.0.

For determination of the stability of peptidase activities when held at different pH levels, cell-free extracts adjusted to pH levels ranging from 4.0 to 9.0 were stored at 2° C. for various lengths of time. After storage, the activity was determined at pH 8.0 against glycyl-L-leucine and DL-alanylglycine. The peptidase activities (Table 5) appeared to be stable between pH 6.0 and 9.0. Considerable destruction of activities was observed when stored for 4 days at pH 5.0; holding at pH 4.0 for 12 hours destroyed the peptidase activities completely.

Purification of the cell-free extract. The results obtained in the present and in a previous study *(12)* indicate that there are at least two different proteolytic systems present in a cell-free extract of *S. lactis;* a proteinase inactivated when heated at 61.7° C. at pH 7.0 for 2 minutes and peptidases which could withstand this heating without appreciable loss of activity. It was found that both proteolytic systems could be precipitated from the cell-free extract with $(NH_4)_2SO_4$ in the range of 40 to 75% saturation. The procedure used in the separation of some of the activities is outlined in Figure 4. All steps in this procedure were

TABLE 5

Stability of peptidase activity at different pH levels when held at 2° C. for various lengths of time[a]

pH of cell-free extract (strain 26)	Per cent hydrolysis after holding for:			
	12 hr.	24 hr.	48 hr.	96 hr.
		Glycyl-L-leucine[b]		
4	0	0	0	0
5	50	50	42	30
6	60	50	46	42
7	70	60	50	45
8	72	65	65	60
9	60	62	60	50
		DL-*alanylglycine*[c]		
4	0	0	0	0
5	50	52	45	32
6	62	60	60	50
7	70	65	62	50
8	70	70	72	60
9	60	62	60	52

[a] 0.2 ml. enzyme preparation per 3 ml. substrate incubated at 37.5° C. and pH 8.0 for 1 hour.
[b] Hydrolysis without holding at 2° C. = 72%.
[c] Hydrolysis without holding at 2° C. = 74%.

performed in a room at 2° C. Either purified Kaolin or $Al(OH)_3$ Cγ *(6)* was used as an adsorbent. Either one volume of $Al(OH)_3$ Cγ (20 mg. per milliliter) was used per six volumes of dialyzed material or 25 mg. of Kaolin per 10 ml. The proteolytic activities of the original cell-free extract, precipitate III and supernatants III, IV, and IVA against casein, glycyl-L-leucine and DL-alanylglycine were expressed as activity per milligram of protein nitrogen present in the material tested. These activities were determined as proteinase activity on casein *(12)* and as per cent hydrolysis of the two peptides. Data in Table 6 show that a considerable amount of inactive material was removed from the cell-free extract by the described method. The adsorbent showed a preference for the enzyme active against DL-alanylglycine over the one against glycyl-L-leucine (compare supernatants III and IV). Supernatant IV A did not show any activity against glycyl-L-leucine; this may have been because either the adsorbent showed preference for the activity against DL-alanylglycine or for some other reasons, such as inactivation or loss during the process of adsorption and elution. The proteolytic system active against casein was not adsorbed by Kaolin. Similar results were observed when $Al(OH)_3$ Cγ was used as adsorbent. Addition of absolute ethanol to cell-free extract of *S. lactis* 26 showed that the activities against casein, glycyl-L-leucine, and DL-alanylglycine were present in a fraction obtained by increasing the ethanol concentration from 45 to 60 volume per cent. Subsequent experiments with supernatant IVA showed that the optimum pH for the activity against DL-alanylglycine was at pH 8.0. The supernatant could be heated for 10 minutes at 61.7° C. (pH 7.0) without loss of activity against DL-alanylglycine; Co^{++} increased the activity. Supernatant III showed optimum activity against glycyl-L-leucine at pH 7.0; heating the extract for 10 minutes at 61.7° C. (pH 7.0) did not affect its activity. Mn^{++} increased the activity against glycyl-L-

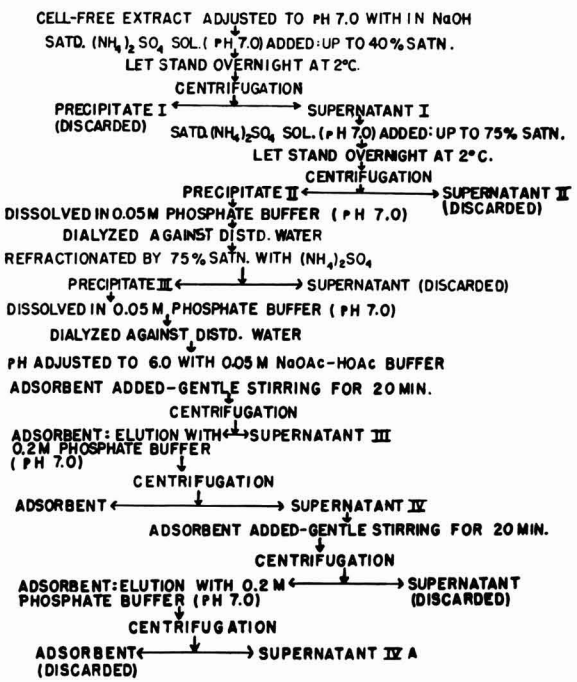

FIG. 4. Fractional precipitation and adsorption of proteolytic activities.

leucine. These characteristics agree well with those found for the crude extracts. Attempts to obtain an active fraction from the supernatants III, IV, and IVA in crystalline form by various adjustments in pH and salt concentration were not successful.

DISCUSSION

Production of the endocellular peptidases of *S. lactis* does not seem to be affected by the absence of whole protein (casein) in the growth medium in contrast to production of endocellular proteinase activity *(12)*, which was stimulated by the presence of casein in the growth medium. Unless growth was affected adversely, omission of one vitamin at a time from the growth medium failed to influence to a great extent activity of either of the two peptidases tested for; this may be attributed to the same reasons as described for continued production of proteinase activity after omission of vitamins *(12)*. The activity of a cell-free extract of *S. lactis* against glycyl-L-leucine was somewhat greater than against DL-alanylglycine, in contrast to the findings of Zimmerman *(14)*. It should be mentioned, however, that the extract used by Zimmerman was prepared from cells of *S. cremoris* which were grown in a medium different from that used in the present study. The effect of metals on the activity of the peptidases active against glycyl-L-leucine and DL-alanylglycine was similar to that reported by Zimmerman. The differences observed in the effect of pH and metal-

TABLE 6

Results of fractional precipitation with ammonium sulfate and adsorption with Kaolin

Material tested	Substrate	Proteolytic activity	Protein nitrogen in enzyme preparation tested
			(mg.)
Cell-free extract	GL[a]	81[c]	1.37
	AG[b]	55[c]	1.37
	casein	65[d]	6.86
Precipitate III	GL	90	0.228
	AG	77	0.228
	casein	46	1.140
Supernatant III	GL	92.5	0.196
	AG	15	0.196
	casein	40	0.392
Supernatant IV	GL	15	0.045
	AG	87.5	0.045
	casein	0	0.225
Supernatant IVA	GL	0	0.04
	AG	75	0.04
	casein	0	0.40

[a] GL = glycyl-L-leucine.
[b] AG = DL-alanylglycine.
[c] Per cent hydrolysis (incubation at 37.5° C. and pH 8.0 for 1 hour).
[d] Increase in tyrosine and tryptophan per ml. trichloroacetic acid filtrate above control values (incubation at 37.5° C. and pH 7.0 for 18 hours).

lic ions on the hydrolysis of two or more peptides seem to indicate the presence of different peptidases; this also would be supported by the results of the adsorption experiments which effected a separation of the activities against glycyl-L-leucine and DL-alanylglycine. Although fractional precipitation with $(NH_4)_2SO_4$ or ethanol and subsequent adsorption with Kaolin or $Al(OH)_3$ Cγ resulted in some separation of the activities against glycyl-L-leucine and DL-alanylglycine, it should be kept in mind that other peptidases still may be present in both fractions. In an impure system such as a cell-free extract of *S. lactis*, a multiplicity of peptidases probably exists and the action observed against a certain peptide may be the result of the action of several enzymes. Some of the peptidases present in a cell-free extract of *S. lactis* proved to be still active at pH values that prevail in cheese of most common types and could contribute to the hydrolysis of smaller protein fragments. The inhibitory effect of copper on the hydrolysis of glycyl-L-leucine and DL-alanylglycine is interesting, since very small amounts of copper are known to be effective in retarding normal flavor development in Cheddar cheese *(10)*.

SUMMARY

Cell-free extracts prepared from *S. lactis* grown in either milk or vitamin-test casein medium B showed the presence of several peptidases which had their optimum activity at pH values ranging from 7.0 to 8.5. Some of the characteristics of these peptidases were studied. There was no indication that the production of these peptidases was stimulated by the presence of whole protein

in the growth medium. Some of the peptidases were found active at pH values that usually prevail in cheese of the most common types. Fractional precipitation with $(NH_4)_2SO_4$ or absolute ethanol and adsorption with Kaolin or $Al(OH)_3$ $C\gamma$ were helpful in removing inactive material from the cell-free extract and separation of the peptidases.

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A STUDY OF HEMOGLOBIN LEVELS IN THE BLOOD OF YOUNG DAIRY CALVES AND THE ALLEVIATION OF ANEMIA BY IRON

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It has been well established that young animals fed on an exclusive milk diet often become anemic. Hart *et al. (9)* observed that an anemia developed when young chicks were fed milk and yellow corn. The condition gradually disappeared as the chicks became older. Dogs *(8, 19)* and piglets *(10, 17, 22)* restricted to an all-milk ration and not allowed access to soil developed an anemic condition and in the latter species it was often fatal. Low hemoglobin concentrations in rats *(15, 23)* and rabbits *(5, 24)* were observed when they were fed only whole milk. Pijoan and Elkin *(18)* encountered low blood hemoglobin values in Shoshone Indian infants due to the continued and exclusive use of milk in their diet. Several investigators *(7, 14, 24)* have produced a nutritional anemia in calves by feeding milk exclusively.

It has been shown that milk alone does not contain sufficient quantities of various metallic elements to support the formation of normal amounts of hemoglobin in growing animals. Supplementing milk diets with iron alone or in combination with other trace elements prevented the occurrence of anemia in young animals *(8, 9, 10, 11, 14, 15, 17, 19, 22, 23, 24)*.

In some areas in the world forages grown on certain types of soil may not contain a sufficient amount of trace elements for proper hemoglobin formation. Becker and Henderson *(3)* observed that in certain parts of Florida the dairymen had ceased trying to raise their own replacements because of large losses and the constant development of an anemic condition. They found that iron, or iron and copper, or iron, copper, and cobalt prevented this anemia. Archibald *et al. (2)* described a condition of young dairy animals in Massachusetts that was characterized by emaciation, anorexia, and a diminution in number of red blood cells and the hemoglobin concentration of the affected animals. A spectacular recovery was reported to follow the administration of iron compounds.

The hemoglobin values of animals receiving normal rations reported in the literature have been obtained from older calves and dairy animals *(1, 4, 6, 16)*. Wise *et al. (26)* reported values for 11 young calves. The small number of young calves used in the previous studies has given no reliable evidence on possible breed and sex differences. The following experiment was undertaken to help establish average hemoglobin values for young dairy calves of different breeds and sexes and to study the incidence and explore the causes of anemia under normal conditions.

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EXPERIMENTAL

Three hundred fifty-six calves in the nutrition and breeding herds at the Bureau of Dairy Industry, Beltsville, Maryland, were used in this study. Data on the calves from the two herds are shown separately since there were some differences in the feeding regime and management of the two herds. The calves in the breeding herd were given a liberal allowance of milk and grain, and alfalfa hay was supplied ad libitum. Calves from the nutrition herd that were used in this study were raised on a limited milk, limited grain regime plus unlimited but weighed amount of alfalfa. Most Jersey and Holstein cows were entirely barn-fed. All Red Dane and crossbred cows were on pasture in season.

Data on the nutrition herd calves extend from late 1946 to early 1950. The data on the breeding herd calves were obtained from late 1946 to early 1948, and again in 1952-53. During this interval the use of a trace mineralized salt mixture was initiated in the grain ration of calves and cows in the breeding herd. A comparison of the hemoglobin values in this herd for the two periods should indicate whether these values are influenced by feeding a trace mineralized salt.

Calves in the breeding herd were bled by venipuncture at approximately 14-day intervals (occasionally 21-day intervals), and most calves in the nutrition herd were bled at 7-day intervals. The results have been grouped according to breed groups into age intervals of 15 days up to the 105th day. Where there were sufficient data the values for each sex within the one breed have been tabulated separately.

Citrated or oxalated blood was slowly measured into aqueous 0.1% sodium carbonate solution, and the transmission of the resulting solution was determined at 540 mμ on a Cenco-Sheard spectrophotolometer. Concentration was calculated with the specific absorption coefficient of 15, which was determined by Horecker *(12)*.

In the study on the effect of iron, copper, cobalt, and manganese salts on the hemoglobin values, these salts were fed in combination to two calves and singly to seven, four, three, and four calves, respectively. Calves selected for studying these effects were all under 30 days of age, averaging 20 days, and their hemoglobin values for a previous 7 days were well below 9.0 g. %. The amounts fed were: 100 mg. iron daily as ferric sulfate; 25 mg. copper daily as cupric sulfate; 5 mg. cobalt daily as cobalt sulfate, and 155 mg. manganese daily as manganese chloride. The salts were fed by capsule each day for 31 days.

RESULTS AND DISCUSSION

The average values and their standard errors are shown in Table 1 according to age, breed, and sex, where the number of individuals warranted a partition of the sexes. An analysis of variance showed that the differences between age groups and between breeds were significant at the 1% level. A definite trend in hemoglobin levels according to age of the young calf was found. This is emphasized by the data shown in the next to bottom line of Table 1, where the values of all calves were averaged according to age. Hemoglobin levels started

TABLE 1

Average hemoglobin values with their standard error for young dairy calves of the ages, sexes, and breeds studied

Breed and sex[a]	Year	Age in days							Av. No. of calves
		1-15	16-30	31-45	46-60	61-75	76-90	91-105	
Breeding herd		*(grams hemoglobin per 100 ml. blood)*							
H ♀	1947	11.76 ±0.3	10.90 ±0.3	9.71 ±0.2	9.44 ±0.2	9.18 ±0.2	9.31 ±0.2	9.30 ±0.2	27
H ♂	1947	10.19 ±0.4	9.66 ±0.4	8.94 ±0.4	8.80 ±0.3	8.45 ±0.2	8.69 ±0.2	9.47 ±0.2	20
H ♀	1952-53	10.56 ±0.3	10.08 ±0.3	9.15 ±0.2	8.94 ±0.2	9.03 ±0.2	8.84 ±0.1	9.34 ±0.2	25
H ♂	1952-53	9.88 ±0.3	9.10 ±0.3	8.58 ±0.3	8.20 ±0.2	8.31 ±0.2	8.94 ±0.1	9.10 ±0.2	22
H ♀	47 & 52	11.19 ±0.2	10.48 ±0.2	9.5 ±0.2	9.15 ±0.2	9.14 ±0.1	9.06 ±0.1	9.32 ±0.1	52
H ♂	47 & 52	10.02 ±0.2	9.39 ±0.3	8.76 ±0.2	8.54 ±0.2	8.38 ±0.1	8.85 ±0.1	9.25 ±0.1	42
J ♀+♂	47 & 52	10.99 ±0.4	10.26 ±0.4	8.88 ±0.3	9.34 ±0.3	9.25 ±0.2	9.97 ±0.3	10.40 ±0.2	24
X ♂	1947	9.95 ±0.4	8.88 ±0.8	9.09 ±0.6	9.82 ±0.5	10.03 ±0.7	10.05 ±0.3	10.24 ±0.3	10
X ♀	1947	11.25 ±0.4	10.27 ±0.4	9.7 ±0.4	9.79 ±0.4	9.78 ±0.1	10.69 ±0.3	11.02 ±0.3	21
RD ♀+♂	1947	11.47 ±0.6	10.35 ±0.6	10.43 ±0.6	9.94 ±0.4	10.73 ±0.5	10.16 ±0.4	10.46 ±0.4	13
SXJ ♀+♂	1947	11.99 ±0.4	11.85 ±0.5	11.57 ±0.4	10.98 ±0.6	10.86 ±0.4	10.33 ±0.5	10.68 ±0.9	9
Nutrition herd									
H ♀	1946-50	10.84 ±0.6	10.20 ±0.5	9.55 ±0.4	8.85 ±0.3	8.61 ±0.3	8.62 ±0.3	8.94 ±0.4	13
H ♂	1946-50	10.64 ±0.6	9.87 ±0.4	9.09 ±0.4	8.99 ±0.2	8.46 ±0.3	8.30 ±0.2	8.77 ±0.3	18
J ♀	1946-50	10.38 ±0.3	9.63 ±0.3	9.43 ±0.3	9.39 ±0.2	9.79 ±0.2	9.76 ±0.2	9.91 ±0.1	36
J ♂	1946-50	9.77 ±0.3	8.83 ±0.2	8.13 ±0.2	8.51 ±0.2	8.93 ±0.2	9.22 ±0.2	9.36 ±0.2	46
All animals		10.66	9.86	9.21	9.14	9.24	9.37	9.63	284
Calves fed synthetic milk		.....	9.9	10.0	9.7	9.9	10.2	10.0	22

[a] H denotes Holstein; J, Jersey; X, crossbred; RD, Red Dane, and SXJ, Sindhi-Jersey crossbred.

to decline shortly after birth and reached their minimum value at 40 to 60 days of age. After this time there was a tendency for the hemoglobin concentration to increase. This occurred in practically all calves examined. The time that the levels increased coincides with the time that calves had started to consume alfalfa in large quantities. A similar trend is evident in the data presented by other investigators *(1, 26)*. The average values found for calves under 15 days of age was somewhat lower than the value of 12.12 reported by Reid *et al. (29)* for newborn calves after colostrum feeding.

The distribution of calves into various groups based on minimum hemoglobin levels is presented in Table 2. The calf's minimum hemoglobin level represented

TABLE 2

Distribution of lowest average hemoglobin values over a 14-day period for calves of different breeds and herds

Breed	Year of observation	No. of calves	Hemoglobin (g/100 ml. blood) 5-6	6-7	7-8	8-9	9-10	10-11	> 11
Beltsville breeding herd			*(%)*	*(%)*	*(%)*	*(%)*	*(%)*	*(%)*	*(%)*
Holstein	1947-8	57	3.5	6.9	8.6	48.3	27.6	5.2	0
Holstein	1952-3	49	0	6.1	20.4	61.2	12.2	0	0
Jersey	1947 & 52	30	0	16.7	6.7	20.0	43.3	13.3	0
Cross-bred	1947-8	31	0	6.5	9.7	35.5	16.1	16.1	16.1
Red Dane	1947-8	17	0	0	17.6	17.6	29.4	5.9	29.4
Sindhi × Jersey	1947-8	10	0	0	0	10.0	40.0	10.0	40.0
Beltsville nutrition herd									
Holstein	1946-50	31	0	15.2	21.2	48.5	12.1	3.0	0
Jersey	1946-50	85	8.1	7.0	22.1	33.7	26.7	1.2	1.2
♂ Calves as % of each group			78	80	61	46	36	6	47

the lowest average for two or three consecutive hemoglobin values which occurred during any 14-day period in the first 3 months of life.

The data in Tables 1 and 2 show that the Jersey and Holstein calves have lower hemoglobin values than crossbred, Red Dane, or Sindhi-Jersey crossbred calves. The Jersey and Holstein breeds also had a greater percentage of their population in the low hemoglobin groups than did the three other breeds. Previous reports *(13, 21)* have indicated that Sindhi-Jersey crossbred animals had higher hemoglobin values than some other breeds. The bottom line in Table 2 shows that the male calves are in the majority in the low hemoglobin groups and that they form a minority in the higher hemoglobin groups.

Female Holstein calves in the breeding herd were higher in hemoglobin concentrations than male calves up to the 75th day of age. After this time there were no significant differences between the sexes. This occurred in the data for 1947 and for 1952. At each of the five age periods, between 0 and 75 days of age, the differences were highly significant ($P < 0.01$) when the data for the 2 years were combined. In these age periods for each of the two individual years the significance of the differences ranged from $P = 0.1$ to < 0.01.

The sex difference in the nutrition herd Jersey animals was also significant. The females were higher than the males for each age group and the significance for each age group ranged from $P = 0.10$ to < 0.01.

The data for nutrition herd Holstein calves and breeding herd crossbred calves showed no significant difference between sexes, although the females had higher hemoglobin values than the males for most age groups. The number of Jersey male calves in the breeding herd was only six, which was insufficient for an adequate comparison between sexes. Similarly there were only five Jersey calves in 1952-53 so that no comparison between years in this breed was possible.

This observed effect of age, breed, and sex on the hemoglobin concentration in young calves has not been reported by previous investigators *(1, 4, 6, 15, 26)*. Anderson *et al. (1)* found higher values in female animals, but McCay *(16)*

found higher values in males. However, most of these animals were older than those in the present study.

A comparison between the 1947 and 1952 values for the same sex, age, and breed showed no differences. Thus, the use of a trace mineralized salt to replace iodized salt in the grain ration of cows and calves in the breeding herd had no influence on the hemoglobin concentrations of the young calves up to 105 days of age. The trace mineralized salt when used at the rate of 1 lb. per 100 lb. grain mix furnished only 5.6 mg. iron, 1.5 mg. copper, 0.5 mg. cobalt, and 6.7 mg. manganese per pound of grain mix. It is obvious that this amount of iron added to the concentrate ration was insufficient to influence hemoglobin concentrations under conditions existing in this herd.

An attempt was made to increase the hemoglobin values of calves which showed low values and to prevent the decrease described previously. Two anemic young calves were given a mixture of iron, copper, cobalt, and manganese by capsule for 30 days. This treatment brought about a rapid increase in hemoglobin concentration. The response of these two calves, presented in Column 2 of Table 3, shows a marked increase in hemoglobin level by the 20th day after initiation of the mineral feeding. The maximal effect was after 30 to 40 days, which coincides with the approximate life of the red cell.

With this technique of using a somewhat anemic calf under 30 days of age any increase in hemoglobin value before 60-70 days of age should not be confused with the "spontaneous" increase that was found to occur in calves after this age. Additional trials were carried out to determine which of the four salts produced the increase in hemoglobin concentrations.

The hemoglobin values before and after supplementation are shown in Table 3 along with the average age at initiation of the supplemental feeding of the metallic salt under investigation. The hemoglobin values for the 11 calves

TABLE 3

Average hemoglobin levels of calves before and after supplementation with the minerals under investigation

	2 calves	5 calves	4 calves	4 calves	3 calves	2 calves
	Supplements given					
Time interval	All 4 minerals	Iron	Manganese	Copper	Cobalt	Folic acid
	(grams hemoglobin per 100 ml. blood)					
8 days before	8.8	7.3	7.3	7.7	9.0	8.2
1 day before	7.8	7.1	7.2	7.0	8.0	6.5
6 days after	7.1	7.2	7.9	6.5	7.7	7.9
13 days after	8.4	8.0	6.5	7.0	7.0	7.4
20 days after	9.2[a]	8.7[a]	6.6	7.1	7.0	7.9
27 days after	9.8[a b]	9.3[a b]	6.9	7.0	7.3	8.3
34 days after	10.4[a b]	10.2[a b]	6.7	7.2	8.2	8.5
41 days after	10.5[a b]	10.3[a b]	6.9	8.0	7.8	8.2
Age at initiation supplementation	22.	18.	18.	24.	24.	26.

[a] Increased above other groups receiving only trace minerals $P = 0.02$ to < 0.01.
[b] Increased above presupplemental value $P = 0.02$ to < 0.01.

given copper, cobalt, or manganese salts show no increase up to 40 days after starting supplementation. The five calves receiving only iron salt responded with an increase in hemoglobin concentration, which was noticeable 20 days after the beginning of supplementation. Two other calves fed only iron gave a similar response but were bled too infrequently for their data to be used in Table 3. These seven calves showed the same type and degree of response as did the two calves given all four minerals.

The hemoglobin values between the six groups in Table 3 before supplementation showed no significant differences. The hemoglobin values for the calves receiving iron were significantly ($P = 0.05$ to < 0.01) greater than for any of the groups receiving copper, cobalt, or manganese at either 20, 27, or 34 days after the feeding of the salt was initiated. The value for the iron-fed calves after receiving the supplement for 34 days was significantly ($P < 0.01$) increased above their presupplemental value, whereas the values for the calves receiving copper, cobalt, and manganese showed no significant change during this time.

A hematocrit determination and red cell counts were made on some of the calves. The calves receiving iron showed an increase in hematocrit and in number of red blood cells at the time their hemoglobin values increased. The calves not receiving iron showed no change until the spontaneous increase occurred, which was after they were 70 days of age. The administration of iron salts appears to have stimulated the hemoglobin and the red cell production in these calves, whereas the three other metals had no effect.

Folic acid at 9 mg. per day had no curative effect on this type of anemia in two calves. Their hemoglobin values at 20, 27, and 34 days were not significantly different from the 11 calves receiving copper, cobalt, and manganese but were significantly lower ($P < 0.05$) than the seven calves receiving iron salts.

Others *(26)* have called the temporary decrease in hemoglobin levels of calves a "physiological anemia." No such temporary decrease was noted in calves fed a synthetic milk. This milk provided 140 mg. iron per 100 lb. body weight per day; its composition was as given previously *(24, 25)*. The data on these calves is presented in the bottom line of Table 1. This information, together with the alleviation of moderate anemia in calves fed iron (Table 3), indicates that such treatment might prevent the temporary decrease noted in other calves. If this is so, it would not be valid to use the term "physiological anemia" to describe this temporary decrease in normally fed calves.

The importance of this temporary decrease of hemoglobin level in the raising of calves is not known. Similarly, the relation of hemoglobin level to the calf's performance has not been determined. The relation between hemoglobin level and body weight gains from birth to 90 days of age were estimated in this study for the Holstein and Jersey calves in the breeding herd. All body weight gains were expressed as per cent of normal for that respective breed and sex. When these values were averaged for the hemoglobin level groups in Table 2 the gains were 88, 103, 106, and 107% for hemoglobin groups of 7.0, 7-8, 8-9, and 9.0, respectively. The gain of calves with minimum hemoglobin values averaging

less than 8 g. per 100 ml. was 96%. The gains of calves whose similarly averaged minimum levels were more than 8 averaged 106%. This difference was significant ($P < 0.01$).

The correlation coefficient between minimum hemoglobin level (as determined for Table 2) and body weight gain from birth to 90 days of age expressed as per cent of normal for 129 calves was 0.238. This relation was significant ($P < 0.01$). For 112 calves in the nutrition herd a similar relation was significant at $P = 0.07$. The correlation between average hemoglobin level and relative gain for the 90-day period was significant at $P = 0.05$. The correlation between average lowest hemoglobin level and relative gain during the period of low hemoglobin values was significant ($P < 0.05$). The practical significance or application of this relationship needs further study.

SUMMARY

The hemoglobin concentration of calves was determined at weekly or biweekly intervals for periods ranging from 1 to 105 days of age. The values were highest during the first 15 days of life and gradually decreased until the 30th to 70th day of life. After this time there was a gradual increase in hemoglobin level. A large variation in hemoglobin levels between individual calves was evident. In breeds of which a large number of calves of each sex were studied the females had higher hemoglobin levels from birth to 75 days of age than did the males. Calves of the Red Dane breed and Sindhi-Jersey crossbreds and usually mixed crossbreds had higher hemoglobin values than calves of the Jersey and Holstein breeds.

Feeding 100 mg. of iron per day to calves that were moderately anemic caused an increase in the number of their red blood cells and hemoglobin levels and thus prevented a further decrease, which normally would have been expected. Iron alone or iron in combination with the copper, cobalt, and manganese cured the anemic condition in these young calves, whereas copper, cobalt, and manganese fed alone had no curative properties. A significant correlation between hemoglobin level and body weight gains was observed in the calves of one herd. Further studies are necessary on the practical importance of these findings.

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STUDIES ON SULFUR TO NITROGEN RATIOS IN FEEDS FOR DAIRY COWS

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It has been demonstrated that when urea furnishes the only nitrogen in the ration of ruminants the utilization of nitrogen depends upon a dietary source of sulfur *(12)*. On a sulfur-free diet the animals lost weight and died. The addition of inorganic sulfates permitted retention of sulfur and of the nitrogen from urea.

Other workers *(11)* have demonstrated increased retention of both nitrogen and sulfur when supplemental sulfur was supplied to lambs being fed on purified rations.

With radioactive sodium sulfate *(4, 5, 6)* it was shown that the tagged sulfur was rapidly incorporated into the cystine and methionine of bacterial protein in rumen contents, into the casein of milk, and into the proteins of wool, thus showing directly that synthesis of sulfur-containing amino acids from nonprotein nitrogen and inorganic sulfur depended upon bacterial activity, as earlier suggested *(8)*. On the basis of these findings it has been proposed that an inorganic sulfur source should be added to the rations of dairy cattle and other ruminants when urea or ammonia furnishes part of the nitrogen so that the elements provide a ratio of approximately 15 parts of nitrogen to one part of sulfur, as found in the average mixed proteins of body tissues.

No attempts have been made to determine the quantitative sulfur requirements of ruminants or to study the utilization of sulfur from different sources. Many of the sulfur values of feeds summarized by Morrison *(9)* were determined years ago and may not be applicable to present day feeds. Beeson *(2)* tabulated the published sulfur analyses for many crops and found wide variations in the same crop grown on different soils. Under some conditions the yield and the sulfur content of crops can be increased by sulfur fertilization *(3, 14)*.

From these considerations it is evident that more complete information on the sulfur content of feeds and the requirements of ruminants is needed. Studies were undertaken to determine the sulfur content of currently available concentrate feed ingredients and by an experiment with dairy cows to find whether the addition of sulfates to a concentrate mixture containing urea would improve lactation performances.

EXPERIMENTAL

Sulfur analyses. Representative samples of various feeds used in formulating concentrate mixtures for dairy cows were obtained from two feed manufacturers. The feeds were analyzed by A.O.A.C. *(1)* methods for the usual constituents. Total sulfur was determined by oxidation with sodium peroxide in the

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TABLE 1
Chemical composition of feeds analyzed for total sulfur

	Moisture	Protein	Ether extract	Crude fiber	Nitrogen-free extract	Ash	Total sulfur	Ratio N:S	Total sulfur from Morrison
	(%)	(%)	(%)	(%)	(%)	(%)	(%)		(%)
Alfalfa meal[a]	9.5	13.7	1.8	20.7	45.3	9.0	0.31	7:1	
Brewers' dried grains[a]	7.6	26.1	7.4	13.7	41.5	3.7	0.35	12:1	
Brewers' dried grains	5.6	30.9	6.6	12.6	41.0	3.3	0.48	10:1	
Citrus pulp[a]	11.4	5.8	4.4	10.1	63.3	5.0	0.23	4:1	
Cocoanut oil meal	5.6	20.7	6.6	13.7	46.2	7.2	0.36	9:1	
Corn meal	7.9	8.3	4.9	2.5	74.9	1.5	0.11	12:1	0.12
Corn gluten feed	4.5	24.1	2.9	7.4	51.4	9.7	1.30	3:1	0.09
Corn gluten feed	6.7	25.7	2.1	9.4	49.1	7.0	0.73	6:1	
Corn gluten feed	6.5	26.1	2.4	9.9	49.4	5.7	0.64	6:1	
Corn gluten feed	5.9	25.6	2.5	8.1	50.2	7.7	0.63	6:1	
Cottonseed meal[a]	7.8	42.3	6.2	6.8	30.3	6.6	0.46	15:1	0.46
Cottonseed meal	6.9	43.5	5.0	14.2	24.6	5.8	0.46	15:1	
Distillers' dried grains	4.2	26.3	11.4	11.9	40.7	5.5	0.56	7:1	
Distillers' dried solubles[a]	13.3	23.3	9.1	0.8	47.1	6.4	0.58	6:1	
Hominy feed	7.4	10.3	5.7	4.9	69.2	2.5	0.18	9:1	0.02
Linseed meal[a]	8.7	39.3	4.3	4.8	37.3	5.6	0.42	15:1	0.40
Linseed meal	5.7	34.3	3.6	9.5	41.2	5.7	0.40	14:1	
Milo, ground[a]	9.9	11.0	2.5	1.6	73.5	1.5	0.12	15:1	
Molasses, Cuban cane[a]	24.7	2.4	0.0	0.0	67.3	5.6	0.15	2:1	0.44
Molasses, Pacific cane	34.7	3.9	0.0	0.0	56.3	5.1	0.29	2:1	
Oats, ground	7.1	12.2	4.3	12.2	60.9	3.3	0.19	10:1	0.21
Rice bran[a]	7.7	13.0	7.9	10.2	47.2	14.0	0.18	11:1	
Rice bran solvent-extracted[a]	9.3	14.3	4.6	10.4	47.1	14.3	0.20	11:1	
Rice bran	6.1	12.3	14.4	9.3	45.4	12.5	0.18	11:1	
Soybean oil meal	6.7	48.7	1.2	6.1	31.8	5.5	0.45	17:1	
Wheat, ground	8.7	15.3	2.1	3.8	68.3	1.8	0.18	13:1	0.20
Wheat bran[a]	10.0	16.7	3.2	8.6	55.2	6.3	0.21	13:1	0.21
Wheat bran	8.1	19.3	3.9	10.7	52.6	5.4	0.30	10:1	
Wheat gray shorts[a]	10.1	16.8	3.3	5.0	60.2	4.6	0.25	11:1	

[a] Proximate analyses performed by Nutrition Laboratory, Uncle Johnny Mills, Houston, Tex.

Parr Bomb *(10)* and precipitation as barium sulfate by the A.O.A.C. method *(1)*. The results are summarized in Table 1.

Certain values for total sulfur in Table 1 differ decidedly from those listed by Morrison *(9)*. A striking difference was seen in hominy feed which was reported to contain only 0.02% of sulfur as compared with a value of 0.18% found in the sample analyzed. The value found is somewhat higher than for corn meal. Morrison reports a value of 0.09% sulfur for corn gluten feed. Four different samples of corn gluten feed from different processors contained from 0.63 to 1.31% total sulfur. The wide variation in the sulfur content of corn gluten feed from different sources is probably due to different amounts of residue remaining from the manufacturing process. The values for most feeds listed in Table 1 agreed more closely with published figures. Several feeds were analyzed for which no published values were found.

Lactation experiment. A lactation experiment was conducted with 19 purebred Holstein cows assigned at random to the three concentrate mixtures described in Table 2. The mixtures were formulated on the basis of the sulfur values reported by Morrison *(9)* since the analyses shown in Table 1 were not

TABLE 2
Ingredients used in the concentrate mixtures

Ingredients	I	II	III
	(lb.)	*(lb.)*	*(lb.)*
Hominy feed	1200	1582	1574
Ground oats	300	300	300
Wheat bran	150	150	150
Corn gluten feed	600	600	600
Molasses	240	240	240
Soybean oil meal	450	—	—
Urea	—	68	68
Sodium sulfate	—	—	8
Dicalcium phosphate	30	30	30
Iodized salt	30	30	30
Totals	3000	3000	3000

[a] Plus 3 g. cobalt sulfate added to each mixture.

available at that time. Ingredients were selected to give a basal mixture as low as possible in sulfur content, and urea or soybean meal was added to give mixtures judged adequate in crude protein based on accepted feeding standards *(7)*. Sodium sulfate was added to Mixture III to equal the estimated sulfur content of Mixture I. The chemical composition of the concentrate mixtures and of the roughages fed is shown in Table 3.

The lactation study was a continuous feeding trial 8 weeks in duration with six cows per group. The average daily yield of fat corrected milk (FCM) by three groups of cows is shown in Fig. 1-A. The highest yield was produced by the cows receiving soybean meal with the urea groups being similar in production. None of the differences were statistically significant, possibly because of the small numbers and rather large differences in level of milk production among the cows in each group. If one plots the average FCM for each group during the 3 week preliminary period before the test was started and the average for the last 3 weeks of the test (Fig. 1-B), an idea is obtained of the relative rates of decline of the three groups. Expressing the yield during the last 3 weeks as a percentage of the preliminary yields, values of 88, 79, and 77% of preliminary FCM production are obtained for the mixtures containing soy-

TABLE 3
Chemical composition of the concentrate mixtures, corn silage, and hay fed

Feed	Percentage composition							
	Moisture	Protein	Ether extract	Crude fiber	Nitrogen-free extract	Ash	Total sulfur	Ratio N : S
Concentrate mixture containing:	*(%)*	*(%)*	*(%)*	*(%)*	*(%)*	*(%)*	*(%)*	
I—Soybean meal	6.9	17.2	4.9	5.3	59.9	5.8	0.35	8 : 1
II—Urea	6.5	19.0	5.4	5.3	58.6	5.2	0.30	10 : 1
III—Urea + SO_4	6.5	18.4	5.6	4.8	58.8	5.9	0.36	8 : 1
Corn silage	77.0	1.8	0.5	7.0	12.9	0.8	0.06	5 : 1
Timothy hay	10.0	6.2	1.5	35.4	43.7	4.9	0.20	5 : 1

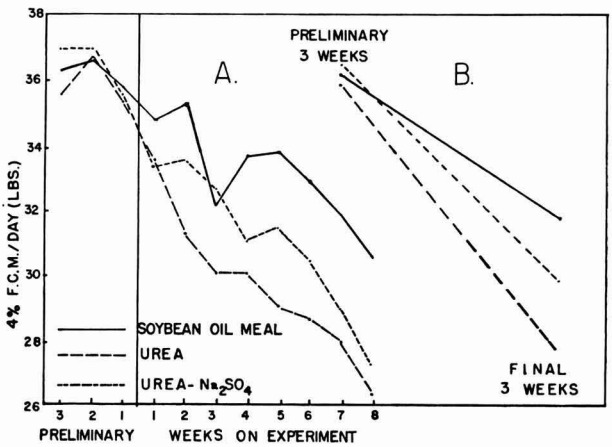

FIG. 1. Average daily production per cow of FCM.

bean meal, urea plus sulfate, and urea, respectively. This evidence might be interpreted as suggesting that the soybean oil meal was superior to urea as a nitrogen source and that added sulfate was of questionable value; however, the data are too limited to justify definite conclusions.

Feed intakes for the three groups were very similar and showed no marked differences in the utilization of feed for milk production. The data are not reported but will be made available by the authors upon request. There were no differences in the fat percentages of the milk produced on the different feeds.

DISCUSSION

Inorganic sulfur, if it is to be of benefit to ruminants, must be included in a ration which is deficient in sulfur and must be in a form which can be utilized by the animal. The ability of ruminants to use inorganic sulfur has been established by several workers *(4, 5, 6, 8, 11, 12)*. There has been no attempt to estimate the sulfur requirement of ruminants; therefore, it is not known at what level rations might become deficient in sulfur for these animals.

The analyses reported in Table 1 indicate that few feeds are lacking in sulfur, according to the only standard which we now have, which is the nitrogen to sulfur ratio in ruminant proteins of approximately 15:1. There is some evidence *(15)* to indicate that soils low in sulfur produce roughages which are deficient in this element. However, there is no evidence that feeds grown on ordinary soils show this condition. More work is needed to determine the normal sulfur content of roughages in order to be able to evaluate their contribution of this element to most rations. Hay usually contains appreciable quantities of inorganic sulfur, which may be as fully available to the rumen bacteria as sulfur in the proteins of feeds.

In evaluating the results of the lactation experiments, one would hardly expect Concentrate Mixture II to be low enough in sulfur to obtain a response

from added sulfate. The feeds used to make up this ration were shown by data obtained earlier to be deficient or relatively low in sulfur. Current analyses indicate that these feeds are among the better sources of sulfur. It may be that analyses made on these feeds several years ago do not accurately reflect the sulfur content of material available today.

From these preliminary data, it appears that unless ruminant rations are composed of feeds grown on sulfur-deficient soils there is little chance of a sulfur deficiency occurring when nonprotein nitrogen is fed at levels up to 3% of the concentrate ration. Further information needs to be obtained on the quantitative sulfur requirement of the ruminant and on the content and availability of sulfur in feed stuffs before recommendations can be made that sulfur should be added to dairy feeds.

SUMMARY

Data are presented on the total sulfur content of common feeds used in concentrate mixtures for dairy cows. The samples of hominy feed and corn gluten feed analyzed contained approximately 10 times as much sulfur as earlier values indicate.

In a lactation experiment, no advantage was apparent for the addition of sulfate to a concentrate mixture containing urea.

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INHIBITION OF THE OXIDIZED FLAVOR OF MILK WITH CHELATING COMPOUNDS[1]

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The relationship of the oxidized flavor of milk to the presence of copper is well established, and considerable data are available that demonstrate the role of copper in its development. The experimental work relating to oxidized flavor was reviewed in 1948 by Greenbank *(6)*. References cited in this review report the use of certain materials, which, when added to milk, act to retard or inhibit development of the oxidized flavor. Among the antioxidants used were: a dried culture of bacteria, a pancreatic enzyme, concentrated or dried milk, hydroquinone, ascorbic acid, and oat flour. In 1944 Lundberg *(7)* first reported the antioxidant property of nordihydroguaiaretic acid (NDGA) in fats, and in 1948 Stull *et al. (8)* observed that this material retarded the development of oxidized flavor in milk and other dairy products. Additional data on the use of certain hydrolytic enzymes have been reported recently by Forster *et al. (3, 4)*, and an inhibitory effect of manganese was reported by Garrett *(5)*.

The research reported in this paper deals with the inhibition of the oxidized flavor in fresh milk by certain chelating compounds which have the property of complexing or tying up metal ions when in solution.

EXPERIMENTAL

Pasteurized homogenized milk processed in commercial plant equipment was used for the experimental samples. No effort was made to isolate milk from individual cows nor from different sources which might be highly susceptible to development of oxidized flavor. The flavor defect was induced by additions of copper in the form of $CuSO_4 \cdot 5H_2O$ in aqueous solution.

The chelating agents used were three of the salts of ethylene diamine tetra acetic acid (EDTA). These were: the disodium salt, the disodium calcium salt and the tetrasodium salt. Sodium diethyl dithiocarbamate was used in a few trials. Although not generally considered to be a chelating agent, this compound was included because of its property of precipitating copper from solutions. These materials were dissolved in Pyrex-distilled water and prepared in concentrations such that the additions did not appreciably dilute the milk.

In the preparation of some of the samples, copper was added first, followed by addition of the chelating agent, and to others it was added after the chelate

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TABLE 1

Effect of varying levels of chelating agents upon the copper induced oxidized flavor of milk

Chelating agent	Added copper	Oxidized flavor after storage for 48-96 hours[a]							
		M/l of chelating agent							
		None	1.5×10^{-5}	6.5×10^{-5}	1.25×10^{-4}	2.5×10^{-4}	5.0×10^{-4}	10^{-3}	1.5×10^{-3}
	(M/l)								
Disodium salt of EDTA	5.0×10^{-5}	+++		(3) ++	(3) ++	(5) 0	(2) 0		
	8.0×10^{-5}	++++			(3) +++	(4) ++	(6) 0		
	10^{-4}	++++						(5) 0	(1) 0
Disodium calcium salt of EDTA	5.0×10^{-5}	+++		(1) ++	(1) +	(10) 0	(7) 0	(1) 0	
	8.0×10^{-5}	++++			(1) +++	(1) +			
	10^{-4}	++++				(1) 0	(8) 0	(7) 0	(4) 0
Tetrasodium salt of EDTA	5.0×10^{-5}	+++				(5) 0	(1) 0		
	8.0×10^{-5}	++++							
	10^{-4}	++++					(5) 0	(4) 0	(1) 0
Sodium diethyl dithiocarbamate	5.0×10^{-5}	+++	(1)[b] 0	(1) 0					
	8.0×10^{-5}	++++	(2) 0	(1) 0	(2) 0	(1) 0			
	10^{-4}	++++							

[a] ++++ badly oxidized, +++ oxidized, ++ slightly oxidized, + questionable to slightly oxidized, 0 not oxidized

[b] Figures in parentheses represent numbers of samples.

had been added. After storage at 36 to 40° F. for 48 to 96 hours, the samples were examined organoleptically by three experienced judges. No attempt was made to score the samples, but the judges reported their findings with regard to the presence or absence of the flavor defect and its relative intensity. An arbitrary rating as follows was established: ++++ badly oxidized, +++ oxidized, ++ slightly oxidized, + questionable to very slightly oxidized, *0* not oxidized.

It was anticipated that the milk would vary in the degree of susceptibility to induction of oxidized flavor; therefore, each series of samples was prepared to include control samples to which copper only was added. The chelate-treated samples were then rated with reference to the controls in each trial.

In trials designed to observe the effect of pasteurization, both the copper and the chelates were added to samples of raw milk and the samples were laboratory pasteurized at 143° F. for 30 minutes.

RESULTS

Data representing the inhibitory effect of the different chelates at the various concentrations are summarized in Table 1. The major portion of the study was conducted with levels of chelating agents at 2.5×10^{-4} to 1.0×10^{-3} *M* per liter, but lower concentrations were used in some trials in order to observe effects when the amount was insufficient for complete protection.

The three salts of EDTA were approximately equally effective in their inhibitory action. The quantity required for complete protection was of the order of five times the amount of added copper on a molar basis. Samples to which 5.0×10^{-5} *M* per liter of copper were added required 2.5×10^{-4} *M* per liter of the chelate. In other samples, containing a similar concentration of copper, 1.25×10^{-4} *M* per liter of the chelate appreciably reduced the susceptibility but did not give complete protection against development of the defect. The reduction in intensity was proportional to the amount of the chelate added.

Although the results reported in Table 1 represent examinations made at 48 to 96 hours, the majority of the samples did not change in flavor rating when storage was extended to 120 or 144 hours. In a few of the samples which contained small quantities of the chelates, and in which the flavor defect developed slightly, there was a slight increase in intensity of the flavor after prolonged storage.

Sodium diethyl dithiocarbamate appeared to be more effective than the salts of EDTA since lower concentrations, on a molar basis, prevented development of the oxidized flavor. This compound may, however, impart a slight off-flavor to the milk.

Pasteurization did not alter the effectiveness of the chelates in preventing development of the oxidized flavor (Table 2). When samples of raw milk were treated with copper and with a chelating agent, and the milk subsequently pasteurized, no change in the relative inhibitory action was observed.

TABLE 2

The effect of pasteurization upon the action of chelates in preventing oxidized flavor

Chelating agent	Cu added	Oxidized flavor rating[a]					
		M/l of chelating agent					
		None	6.5×10^{-5}	1.25×10^{-4}	2.5×10^{-4}	5.0×10^{-4}	10^{-3}
	(M/l)						
Disodium salt of EDTA	5.0×10^{-5}	+++	++	++	0		
	8.0×10^{-5}	++++		+++	++	0	
	10^{-4}	++++				0	0
Disodium calcium salt of EDTA	5.0×10^{-5}	+++	++	+	0		
	8.0×10^{-5}	++++		++	+	0	
	10^{-4}	++++				0	0
Tetrasodium salt of EDTA	5.0×10^{-5}	+++			0		
	10^{-4}	++++				0	0

[a] ++++ badly oxidized, +++ oxidized, ++ slightly oxidized, + questionable to slightly oxidized, 0 not oxidized

TABLE 3

The effect of adding chelates prior to the addition of copper upon the action of the chelates in preventing oxidized flavor

Chelating agent	Cu added	Oxidized flavor rating[a]			
		M/l of chelating agent			
		None	2.5×10^{-4}	5.0×10^{-4}	10^{-3}
	(M/l)				
Disodium salt of EDTA	10^{-4}	++++	0	0	0
Disodium calcium salt of EDTA	5.0×10^{-5}	+++	0		
	10^{-4}	++++	0	0	0
Tetrasodium salt of EDTA	5.0×10^{-5}	+++	0		
	10^{-4}	++++		0	0

[a] ++++ badly oxidized, +++ oxidized, ++ slightly oxidized, + questionable to slightly oxidized, 0 not oxidized

The data in Table 3 were obtained on samples to which the chelating materials were added prior to the additions of copper. The chelates were equally effective when added before or after the addition of copper. The results demonstrate that the order of addition is without effect.

DISCUSSION

The results of this experiment demonstrate the inhibitory action of certain chelates against the copper-induced oxidized flavor of milk. The inhibition is attributed to complexing or tying up of the copper ions and probably other ions, rendering them ineffective as catalysts or as activators for certain enzyme systems. By using the compounds in the manner described, it appears that the antioxidant property can be attributed only to the mechanism of chelation of the copper.

Any consideration of the use of these compounds in market milk or other dairy products must be based upon information to clearly establish their freedom from any harmful effects. Although no long-term feeding experiments with various species have been reported, there appears to be no evidence from other studies that EDTA is harmful to laboratory animals when consumed orally in quantities many times greater than would be present in milk if added to prevent the oxidized flavor. Child *(2)* fed rats up to 5% of the sodium salt of EDTA for 4 weeks, and the animals failed to develop abnormalities. Unpublished results *(1)* from an experiment accompanying this study showed no detrimental effects when the disodium salt of EDTA was fed to rats. Weanling rats in this experiment were fed 300 and 900 p.p.m. of the salt in a mineralized milk sucrose diet. Over a 10-week period, no adverse effects on the rats were evident.

SUMMARY

Samples of normal milk were treated with copper to induce oxidized flavor and at the same time were treated with certain chelating compounds. The chelates, which were the disodium salt, the disodium calcium salt, and the tetra-

sodium salt of ethylenediamine tetraacetic acid (EDTA), were effective in preventing development of the oxidized flavor. Quantities of the chelates which were five times the amount of added copper on a molar basis provided complete protection, and smaller quantities of the chelates gave some inhibitory action. The chelates were equally effective when added either before or after pasteurization or when added prior to or after the addition of copper.

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EFFECTS OF FORMALDEHYDE AND COPPER SALTS ON THE HEAT STABILITY OF EVAPORATED MILK

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The milk industry has long felt the need for an improved forewarming process to regulate the heat stability of evaporated milk. Such a process depends on control of protein denaturation and is complicated by the fact that so little is known of the chemical and physical changes involved. The Sommer-Hart "salt balance theory" *(3)* has proved useful, but additions of trace elements to raw whole milk and variations in processing affect the heat stability of evaporated milk more than can be accounted for by this theory.

Salts of cobalt, copper, gold, iron, lead, mercury, and platinum, as well as formaldehyde and hydrogen sulfide, have been added to raw milk at various times, but the available literature has very little information relating directly to the effects of these substances on the heat stability of evaporated milk in the cooker process. With the possible exception of the formaldehyde data of Townley and Gould *(5)*, no results have been found in the literature bearing directly on the effect of these substances on the heat stability of evaporated milk, although the substances have been discussed to some extent in industry meetings.

This paper presents results of pilot plant experiments, made in August and September, 1953, showing effects of small amounts of formaldehyde and copper salts on the heat stability of evaporated milk.

EXPERIMENTAL METHODS

These experiments were performed with grade A milk from a local dairy. All equipment used in processing was fabricated of stainless steel.

The procedure used in the preparation of evaporated milk was as follows: a 25-lb. lot of milk was heated in a jacketed kettle to 205° F. and held 10 minutes at this temperature. Approximately 10 minutes was required to attain 205° F. This was followed by evaporation, cooling to 40° F., and standardization to 27% total solids and 7.9% fat. The milk was not homogenized.

The standardized evaporated milk was filled into 211 × 400 type cans — 300-ml. capacity. The heat stability was determined by using a cooker process which would not destabilize the least stable milk beyond a satisfactory point of viscosity measurement. In general, cans of evaporated milk were heated in a pilot sterilizer, described in an earlier paper *(1)*, for 6 minutes at 210° F., followed by a cooker process which varied for the several lots from 5 to 14 minutes at 250° F.

The relative amount of volatile sulfur was determined by a method essentially the same as the one used by Townley and Gould *(4)*. The amount of sulfur evolved from 770 ml. of whole milk during a heating period of 20 minutes at

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203° F., followed by an aspiration period of 20 minutes, was used to estimate the relative effect of the various amounts of formaldehyde added. A fresh sample of milk was used for each addition of formaldehyde.

The destabilizing action of hydrogen sulfide was measured by the addition of a freshly prepared, saturated solution of hydrogen sulfide in water to evaporated milk prior to sterilization.

RESULTS

Seven lots of milk were prepared for estimating the effects of formaldehyde and copper on the heat stability of milk. These lots were divided into three groups: Lots 1, 2, and 3 contained 5 ml., 10 ml., and 15 ml. respectively, of copper solution added in the form of a 1.6% solution of $CuSO_4 \cdot 5H_2O$ to milk before forewarming; Lots 4, 5, and 6 contained the corresponding amounts of copper added to the milk after forewarming; Lot 7 did not contain copper. (Five ml. of the copper solution in 25 lb. of milk is equivalent to 1.7 p.p.m. of copper.) Formaldehyde (35%) was added to each can of the various lots in amounts varying from 0 to 12 drops. A can of milk in each lot was used as a control sample to estimate the heat stability of the lot without formaldehyde. Owing to the strong destabilizing action of copper in Lots 1, 2, and 3, it was necessary to add 2 g. of anhydrous disodium phosphate to Lot 1, 4 g. to Lot 2, and 8 g. to Lot 3, in order that a cooker process of 5 minutes at 250° F. could be used. All lots contained 25 lb. of whole milk. On the other hand, the copper in Lots 4, 5, and 6 increased the heat stability of the milk so that a sterilizing process of 12 minutes at 250° F. was required for Lot 4, 14 minutes at 250° F. for Lot 5, and 12 minutes at 250° F. for Lot 6. Heating for 10 minutes at 250° F. was required for Lot 7. The relative stability of the milk in each lot, after noting the phosphate addition in Lots 1, 2, and 3, was estimated from the viscosity data of the milk after sterilizing. These data are given in Table 1.

The maximum increase in heat stability of evaporated milk was produced by approximately 35 drops of 35% formaldehyde per 300 ml. of milk. At this point it required approximately 3 ml. of 10% calcium chloride solution and a heat treatment of 14 minutes at 260° F. (approximately 20 minutes at 250° F.) to produce a moderate degree of heat coagulation. Formol titrations indicated that approximately 30 drops of 35% formaldehyde were required for combination with the free amino groups. These results were obtained on additional lots of milk.

The repressing effects of small amounts of formaldehyde on the evolution of volatile sulfur are shown by the data in Table 2. Although the data are only relative, it is evident that a minimum evolution of sulfur was reached at approximately 6 drops of formaldehyde per 770 ml. of whole milk. Further addition of formaldehyde did not decrease the rate of evolution of sulfur.

The data on the effect of hydrogen sulfide on the heat stability of evaporated milk are incomplete. However, it can be stated that 1 ml. of a freshly prepared, saturated solution of hydrogen sulfide in water — about 3 mg. added to 14.5 oz.

TABLE 1

Effect of formaldehyde and copper on the heat stability of evaporated milk viscosity in Mojonnier-Doolittle units at 75° F.[d]

Sample	Formaldehyde drops[a]	Copper added before forewarming, p.p.m.[b] 1.7	5.1	8.5	Copper added after forewarming, p.p.m. 1.7	5.1	8.5	Control samples, no copper
		Lot 1	Lot 2	Lot 3	Lot 4	Lot 5	Lot 6	Lot 7
Sterilization time *(Min. at 250° F.)*		5	5	5	12	14	12	10
(Grams per sample) Na_2HPO_4		0.15	0.30	0.40	0	0	0	0
1	0	24	250	115	14	16	16	15
2	1	36	gel	180	35	48	23	40
3	2	48	gel+[c]	240	178	170	40	gel
4	3	55	290	235	325	230	65	gel+
5	4	54	200	190	325	218	78	gel++
6	5	42	119	110	280	135	68	gel+++
7	6	26	55	77	205	95	50	gel+++
8	7	20	32	39	55	50	33	gel+
9	8	16	25	28	40	30	25	gel
10	9	14	20	25	18	20	20	250
11	10	12	18	20	14	16	16	35
12	11	12	16	16	14	14	14	20
13	12	11	16	16	14	14	14	13

[a] One drop is equivalent to 32 mg. of 35% formaldehyde.

[b] Equivalent parts per million of copper in raw milk.

[c] Plus (+) denotes increase in firmness of gel.

[d] The viscosity of unsterilized evaporated milk at 75° F. in terms of Mojonnier-Doolittle units is approximately 12.

of evaporated milk — immediately prior to sterilization will generally reduce the maximum holding time possible for evaporated milk by approximately 3 minutes at 250° F. As a comparison, it would require approximately 25-50 mg. of calcium chloride to produce a comparable destabilization.

Salts of cobalt, iron, lead, mercury, and platinum in amounts of 10 p.p.m. of evaporated milk were found to be without marked effect. However, gold salts on a molar basis are approximately equivalent to copper salts in their effect on the heat stability of evaporated milk.

TABLE 2

Effect of formaldehyde on the evolution of volatile sulfur from whole milk

Drops of formaldehyde[a]	Volatile sulfur in mg. evolved from 770 ml. of milk Lot 1	Lot 2	Lot 3
0	0.260	0.225	0.255
1	0.070		
3	0.035		
4	0.020		
6	0.015	0.015	0.045
9	0.055	0.035	0.035
12	0.045		0.035
15	0.050		
24	0.030		

[a] One drop is equivalent to 32 mg. of 35% formaldehyde.

DISCUSSION

The data in Table 1 offer ample opportunity for speculation on the role played by amino and labile sulfur groups in the heat stability problem. The fact that formaldehyde can be both a stabilizer and a destabilizer appears to be due to its ability to condense with both sulfhydryl and amino groups. Schubert *(2)* found that formaldehyde forms stable compounds with sulfhydryl-containing proteins which, however, dissociate readily in solution and yield a nitroprusside test. That a similar compound is formed in milk is indicated by the data in Table 2, in which the volatile sulfur, although reduced greatly, did not disappear with increased amounts of formaldehyde. These data, together with the fact that maximum destabilization of milk occurred with approximately minimum evolution of sulfur, suggest that the presence of the labile sulfur group is important in increasing the heat stability of the protein.

The destabilizing effect of formaldehyde was reduced by copper salts. This is more evident in Lots 1, 2, and 3 (Table 1), in which the copper salts were added prior to forewarming, than in Lots 4, 5, and 6. It is also noticeable that in both groups the point of maximum destabilization occurred with less formaldehyde than was required by the milk in Lot 7.

The striking increase in heat stability resulting from the addition of increased amounts of formaldehyde has been known in the evaporated milk industry for some years. It is only recently that a correlation between the formol titration and the point of maximum heat stability has been suggested. However, in these experiments, the maximum heat stability was not defined sharply, so that it is not known whether the amount of formaldehyde required for the formol titration and the amount required for maximum heat stability are in close agreement.

It is difficult to account for the effects of copper salts on the heat stability of milk. Speculation suggests that copper added prior to forewarming inhibits the appearance of the sulfhydryl group. Thus the effect of formaldehyde in this case is additive to the effect of copper. When copper salts are added after the appearance of the sulfhydryl group, as, for example, in Lots 4, 5, and 6, formaldehyde has a destabilizing effect. The copper may act now to reduce the number of sulfhydryl groups by increasing the redox potential so that part of the sulfhydryl groups is converted to sulfide groups. This may contribute to an increased stability by blocking the reaction with formaldehyde. The fact that hydrogen sulfide is a reducing and destabilizing agent lends some support to this idea, although it is recognized that hydrogen sulfide may act to cross-link protein groups.

It is possible that in the usual forewarming operation a stabilizing action results from a reduction in the calcium and magnesium ion concentration and the formation of sulfhydryl groups. The holding period in the forewarming process is both stabilizing and destabilizing. It is stabilizing to the extent that the calcium and magnesium ion concentration is reduced and destabilizing to the extent that labile sulfur is lost. It is well known that a prolonged holding

period, for example, 30 minutes at 210° F., usually results in a loss of heat stability, which cannot be restored either by disodium phosphate or copper salts. A trace of copper salts added early in the forewarming process may prevent loss of these labile sulfur groups.

CONCLUSIONS

1. A possible correlation exists between the amount of formaldehyde required for maximum heat stability of evaporated milk and that required for combination with the free amino groups as determined by a formol titration.

2. Copper salts in traces reduce the destabilizing action of formaldehyde but apparently do not interfere with its stabilizing action.

3. A trace of copper salts in whole milk prior to forewarming results in an evaporated milk of below normal heat stability. The entry of a trace of copper salts into milk after normal forewarming process results in an evaporated milk of above normal heat stability.

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THE EFFECT OF LACTOSE CRYSTALLIZATION ON PROTEIN STABILITY IN FROZEN CONCENTRATED MILK

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The coagulation of the colloidal protein in milk during frozen storage has limited the development of a marketable frozen concentrated milk. Webb *(13)* patented a process for the production of frozen concentrated milk but observed *(14)* that protein coagulation restricted the storage period to several weeks in the temperature range 0 to 10° F. The rate of coagulation of the protein, identified as calcium caseinate by Doan *et al. (5, 15)*, has been directly related to the temperature of frozen storage *(3, 4, 5)*.

Attempts to improve the stability of frozen concentrated milk by manipulation of such prefreezing process variables as forewarming temperature and homogenization pressure have not been particularly successful. Significant improvements have been attributed to rapid freezing of milk subsequent to concentration and to the use of sugars and calcium sequestrants as stabilizing additives *(5, 15)*. Reduction of the calcium content of milk by ion exchange treatment was reported to exert a retarding effect on protein flocculation in frozen milk *(6, 15)*. Wildasin and Doan *(15)* speculated that lactose nuclei in concentrated milk might "accelerate the formation of casein structures" based on the observation that the addition of fine mesh sand to concentrated milk lowered the stability of the frozen product.

Research in these laboratories by Stimpson *(11)* revealed that a stable frozen concentrated milk could be produced by treatment of the milk with a lactase enzyme prior to freezing. The improvement derived from the application of a lactose hydrolyzing enzyme implied that lactose has a significant influence on the stability of the proteins in frozen concentrated milk.

EXPERIMENTAL METHODS AND RESULTS

The milk processed for this study was concentrated to 35% total solids with a minimum of 25% serum solids and 10% butterfat. The milk was clarified, forewarmed to 145° F. for 15 minutes, vacuum condensed at 120-130° F., adjusted to the proper fat to solids-nonfat ratio, homogenized at 2,000 p.s.i., and pasteurized at 155° F. for 30 minutes. The product was then cooled to 40° F., packaged, frozen statically at −20° F. for 24 hours, and stored at 15° F.

The extent of protein flocculation in the stored samples was measured as the total volume of insoluble material and expressed as a solubility index, according to a method described for nonfat milk solids *(1, 2)*. The frozen concentrate was thawed in a 77° F. water bath and reconstituted to 12% solids. A 50-ml. aliquot of the reconstituted milk was centrifuged in a calibrated tube for 5 minutes at

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800 r.p.m. in an 18-in. diameter centrifuge. The supernatant was decanted and replaced with distilled water, and the precipitate was recentrifuged. The volume of precipitated protein, in milliliters, was recorded as the solubility index. The values obtained with freshly frozen concentrated milk were invariably under 0.2 ml., whereas values as high as 18.0 ml. were observed with milk that had totally coagulated in storage. A stored sample with an index value greater than 1.0 was considered unacceptable for fluid consumption.

Stability of lactase-hydrolyzed frozen milk. The quantitative relationship between lactose hydrolysis and protein stability was determined in a series of samples in which 5, 10, 15, 20, 30, 50, and 85% of the lactose was hydrolyzed. Skimmilk containing 31% solids was treated with a lactase enzyme prepared from the yeast *Saccharomyces fragilis*, using one part by weight of the dry enzyme preparation to 40 parts lactose in the milk. Within 4 hours at 123° F., 85% of the lactose was hydrolyzed. The extent of hydrolysis was determined by measurement of the monose concentration in the milk according to a method reported by Tauber and Kleiner *(12)*. The lactase-treated concentrated skimmilk was then adjusted to contain 35% total solids and 10% fat by the addition of 40% cream. This product was then mixed with untreated concentrated whole milk to obtain the desired level of lactose hydrolysis in the final product. The samples were then pasteurized and processed as described for control milk. The direct correlation between the degree of lactose conversion and frozen storage stability is shown in Figure 1.

Lactose crystallization in frozen milk. Careful visual and microscopic examination of control and lactase-treated milk samples at intervals during frozen storage consistently revealed the appearance of crystalline lactose immediately preceding protein flocculation. Significantly, it was observed that the rate of crystallization of lactose and the coagulation of casein, in frozen milk, were

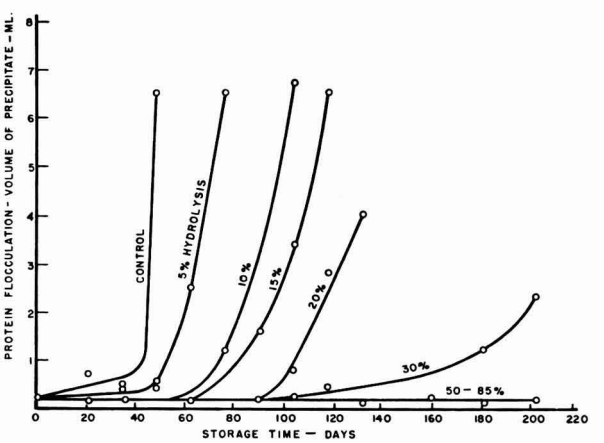

FIG. 1. The influence of enzymatic lactose hydrolysis on the protein stability of concentrated whole milk (35% solids — stored at 15° F.).

suppressed in direct relation to the extent of hydrolysis of the lactose, by the lactase enzyme.

A polarimetric method devised by Sharp and Doob *(10)* for the quantitative determination of crystalline lactose in dried milk and whey was adapted, with slight modification, to the measurement of the rate of crystallization of lactose in frozen milk. This method utilizes the mutarotation of deproteinated solutions of milk powder or whey as a basis for evaluating the quantity of crystalline lactose in the dried product. The expression,

$$\% \text{ anhydrous alpha lactose} = \left(\frac{\text{initial rotation}}{\text{final rotation}} - 0.635\right) \times 101.1,$$

was derived, by Sharp and Doob, to correlate the extent of mutarotation with the alpha lactose content in the dried milk. Any increase in the alpha lactose content above the 37% equilibrium level represents crystallized alpha lactose.

Procedure: Solution A — 0.9 g. oxalic acid/1,000 ml. water

Solution B — 264 g. mercuric chloride dissolved in 1,000 ml. of 95% ethyl alcohol.

A 10-g. sample of frozen milk was weighed rapidly in a 100-ml. volumetric flask. The protein was precipitated by the addition of 40 ml. of the oxalic acid solution, 25 ml. of distilled water, and 10 ml. of the alcoholic mercuric chloride solution, in succession. The solution was shaken vigorously for 2 minutes after the addition of each reagent to ensure complete solution of the lactose crystals. The volume was finally adjusted to 100 ml. with distilled water, and the solution was transferred to a filter paper. The filtrate was run directly into a polarimeter tube to facilitate immediate polarization. The entire procedure was accurately timed from the moment of contact of milk sample and oxalic acid reagent. The first optical rotation measurement of the deproteinated lactose solution was obtained in approximately 10 minutes, and rotation values were recorded every few minutes for the first hour. The final rotation value was obtained at 8 hours. Optical rotations were measured at 20° C., with sodium light through a 4-decimeter tube in a polarimeter accurate to 0.01°. Extrapolation of the rotation-time curve to zero time (Figure 2) yielded the initial rotation value used to calculate the percentage of crystalline lactose in the frozen milk sample. Freshly frozen milk concentrates were invariably nonmutarotating, evidence that lactose crystallization does not occur during the freezing process. When protein flocculation was measured simultaneously with lactose crystallization at successive time intervals during frozen storage of concentrated milk, the lactose crystallization invariably preceded the flocculation of the casein (Table 1).

To demonstrate that acceleration in the rate of lactose crystallization in concentrated milk would cause a corresponding increase in the rate of protein coagulation, a sample of concentrated milk was seeded with crystalline lactose prior to freezing. This milk showed both extensive lactose crystallization and protein coagulation within 5 days of storage at 15° F. (Table 1 and Figure 2).

It was anticipated, from these results, that resolubilization of the lactose crystals formed during storage of frozen milk prior to the onset of protein

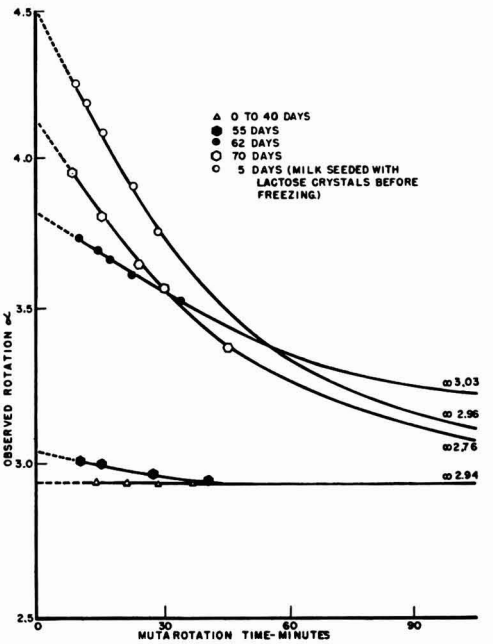

FIG. 2. The crystallization of alpha lactose in concentrated whole milk (35% solids) measured by the increase in mutarotation of samples withdrawn at successive intervals of storage at 15° F.

flocculation would afford renewed stability to the milk proteins. A series of samples of 35% solids whole milk concentrate was stored at 15° F. At the end of 4 weeks of storage, traces of lactose crystals were evident in the frozen milk. Several samples in the series were rapidly thawed and heated at 155° F. for 1 hour to resolubilize the lactose crystals. This reheated milk was immediately

TABLE 1

The rate of lactose crystallization and protein flocculation in 35% solids whole milk stored at 15° F.

Storage time	Optical rotation[a] – Initial – (extrapolated)	Optical rotation – Final – (observed)	Alpha lactose crystallized (Calc.)	Protein flocculation solubility index
(Days)	*(Degrees)*	*(Degrees)*	*(%)*	*(ml.)*
6	2.94	2.94	0.0	0.1
9	2.92	2.92	0.0	0.2
13	2.93	2.91	0.5	0.1
40	2.94	2.87	2.2	0.2
55	3.03	2.96	2.2	0.2
62	3.82	3.03	40.6	6.0
70	4.10	2.76	77.8	11.0
5 (seeded with lactose crystals before freezing)	4.50	2.96	82.5	18.0

[a] Optical rotation and solubility index measurements were made simultaneously on each sample.

refrozen and stored at 15° F. The samples not receiving the heat treatment coagulated within several days of additional frozen storage and showed extensive lactose crystallization. The reheated samples, however, were stable for an additional 4 weeks, at which time lactose crystals started to re-form. Several of these samples were reheated, as before, and the others were left in storage at 15° F. The reheated samples again did not coagulate on subsequent frozen storage for an additional month, whereas the unheated controls showed heavy lactose crystallization and extensive protein flocculation within a few days.

The stability of lactose depleted milk in frozen storage. The protection afforded frozen milk by partial lactose hydrolysis has been directly correlated with the decreased rate of lactose crystallization resulting from such conversion. The retardation of lactose crystallization in frozen milk was duplicated by another method. Lactose crystallization was induced in 45% solids skimmilk by seeding with fine lactose crystals and refrigerating the milk at 40° F. for 24 hours. The crystallized lactose was then centrifuged from the milk in a conventional milk clarifier. A reduction of 25% of the lactose content was readily attained by this procedure. The partially delactosed skimmilk concentrate was then combined with cream and water to a 35% total solids whole milk concentration. The lactose deficit was restored with either sucrose, glucose, or an equal mixture of glucose and galactose. The milk was heated at 155° F. for 1 hour to ensure resolubilization of the residual lactose crystals, as such crystals were found to nucleate the frozen milk and markedly accelerate protein flocculation. Milks partially depleted of lactose by this method were stable for over 3 months at 15° F., whereas controls crystallized and coagulated within 4 weeks.

Influence of temperature on the rate of lactose crystallization in frozen concentrated milk. The importance of avoiding temperature fluctuations during the storage of frozen concentrated milk is implicit in the influence of lactose crystallization on the stability of the casein. Successive thawing and freezing of the milk will permit rapid nucleation of the lactose with its concomitant effect on the protein stability. The evaluation of the influence of storage temperature on the rate of lactose crystallization and the stability of the milk must, therefore, be performed under carefully controlled storage temperatures.

A series of constant temperature baths, each equipped with an immersion heater and a bimetallic thermoregulator, was assembled in a −20° F. cold room. The baths were filled with water and sufficient ethylene glycol to prevent freezing. Milk samples for frozen storage evaluation were sealed in small cans and submerged in the baths, which, with constant stirring, were capable of maintaining the samples within 1° F. of the desired temperature. The 35% solids whole milk used in this experiment was processed as previously described. All possible precautions were taken to avoid the formation of lactose crystal nuclei in the processing of the milk. The samples were then stored immediately at the selected temperatures. Polarimetric examination of the milk at intervals during storage revealed that all samples were free of lactose crystals for definite periods following the freezing process. Only after a prolonged induction period in each case did lactose crystallization become detectable. The ensuing mass crystallization

of the lactose in the frozen milk was invariably accompanied by coagulation of the casein. The influence of temperature on frozen milk stability is shown by the data in Table 2.

TABLE 2

The influence of temperature on the stability of frozen concentrated whole milk (35% solids)

Storage time	−20° F.[a]		−10° F.		−5° F.		0° F.		5° F.		15° F.	
	A[b]	B[b]	A	B	A	B	A	B	A	B	A	B
(Days)	*(%)*	*(ml.)*	*(%)*	*(ml.)*	*(%)*	*(ml.)*	*(%)*	*(ml.)*	*(%)*	*(ml.)*	*(%)*	*(ml.)*
15	0.0	0.1	0.0	0.1	0.0	0.1	0.0	0.1	0.0	0.1	0.0	0.1
36	0.0	0.1	0.0	0.1	0.0	0.1	0.0	0.1	0.0	0.1	0.0	0.1
55	0.0	0.2	0.0	0.1	0.0	0.1	54.0	1.0	54.0	5.5	40.0	6.0
65	0.0	0.1	0.0	0.1	0.0	0.1	72.0	6.5	60.0	7.0	80.5	11.5
112	0.0	0.1	0.0	0.1	20.0	1.0	73.0	12.0	79.0	14.0	71.0	16.0

[a] Samples continued in storage for over 1 year at −20° F. showed no lactose crystallization or protein flocculation.

[b] A = % lactose crystallized. B = volume flocculated protein (solubility index ml.).

The freezing curve of milk. The crystallization of lactose in milk stored at freezing temperatures was taken as evidence that only part of the water in the "frozen milk" was frozen. As freezing point data on high solids milk concentrates were not available in the scientific literature, the ice-water equilibrium in milk frozen at various temperatures was evaluated by calorimetric measurements. The high heat of fusion of ice provides a practical basis for the calorimetric determination of the ice content of partially frozen milk.

A low temperature bath was prepared from an insulated glass tank filled with a nonfreezing ethylene glycol-water mixture. A cold finger containing dry ice and equal parts of chloroform and carbon tetrachloride was used to refrigerate the bath. A mercury thermoregulator was set to control the temperature by actuating an infrared heater focused on the wall of the bath. With vigorous agitation, the temperature could be maintained within ±0.1° C. at temperatures as low as −28° C. Skimmilk, concentrated to 27% solids, was used in all the calorimetric measurements to eliminate the influence of the fusion of butterfat on the heat balance. It was determined experimentally that the ice-water equilibrium in milk held at constant freezing temperatures could be established with small samples in less than 1 hour. The concentrated skimmilk samples were weighed in 50-ml. glass centrifuge tubes, stoppered, and immersed in the freezing bath controlled at the specified temperature. If ice crystallization was not evident after 30 minutes, the milk was seeded with an ice crystal, and freezing equilibration was continued for a minimum of 1 hour. The sample was then quickly transferred to a calorimeter containing distilled water at a known temperature. The calorimeter was prepared from a 1½-pint silvered vacuum bottle fitted with a thick cork stopper, a standardized thermometer calibrated in 0.1° C., and a glass stirrer. The total observed temperature change was recorded when the calorimeter contents reached equilibrium. Correction for the heat capacity of the calorimeter and sample tube was applied to the calculation of the ice content of the frozen milk. At each temperature a parallel control determination was

made with a weighed sample of distilled water. The total quantity of ice recovered in each distilled water control was within 2% of the theoretical. The determination of the freezing point of 27% solids skimmilk was made with a conventional Beckmann cryoscope. The results of this study are summarized in Table 3 and Figure 3.

TABLE 3

Calorimetric measurement of ice in concentrated skimmilk equilibrated at freezing temperatures

Freezing temperature		Sample weight of 27% solids skimmilk	Weight of water in calorimeter	Calorimeter temperature decrease	Weight of ice in sample (calc.)	Water frozen in sample
(° C.)	(° F.)	(g.)	(g.)	(° C.)	(g.)	(%)
−2.8	26.9	40.0	400	3.1	6.5	22.2
−4.8	23.4	40.0	400	4.9	16.9	57.9
−10.0	14.0	40.0	400	6.0	21.6	73.8
−18.2	−0.76	40.0	400	6.9	25.0	85.6
−28.0	−18.4	40.0	400	7.9	27.6	94.5

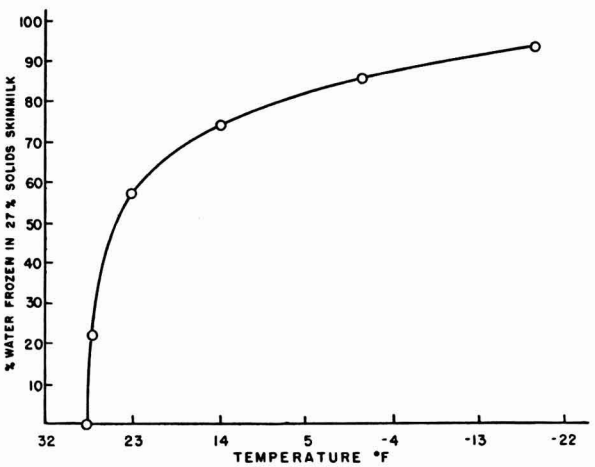

FIG. 3. Calorimetric measurement of ice in 27% solids skimmilk equilibrated at freezing temperatures.

DISCUSSION

The freezing point of fluid whole milk is approximately −0.55° C. This value reflects the depressing influence of the lactose and salts on the freezing point of the water. Lowering the temperature of milk below its freezing point concentrates the milk solids by withdrawal of water from the system as ice. This increase in solute concentration further depresses the freezing point of the residual unfrozen milk. It would be anticipated that a considerable quantity of water remains unfrozen in milk at all but very low storage temperatures. Measured calorimetrically, approximately 75% of the water in 27% solids skimmilk is frozen at 15° F. The unfrozen portion is highly supersaturated with respect to

lactose, which is slow to crystallize because of the high viscosity and unusual stability of supersaturated lactose solutions. Mass crystallization in this labile concentrated phase does not occur for approximately 50 days at 15° F. if all possible precautions are taken to eliminate the development of lactose crystal nuclei during the processing of the milk. Temperatures which afford complete protection against protein destabilization (approximately −20° F.) coincide with almost total freezing of the water in the milk. At such temperatures the lactose is in a stable amorphous glass state, unable to crystallize without dilution. At temperatures above this stable zone and below 0° F. the crystallization of lactose is significantly retarded and frozen concentrated milk is stable for periods longer than 100 days. Temperatures in the range of 0 to 15° F. (15-25% unfrozen water in 27% solids skimmilk) permit fairly rapid lactose crystallization and colloid flocculation, generally within a 50- to 70-day storage period. The partial hydrolysis of lactose to monoses, by the lactase process, suppresses the rate of crystallization of the lactose by lowering its level of supersaturation and thereby stabilizes the casein colloid in the frozen concentrated milk.

The large discrepancies in storage stability data reported in the literature probably reflect the variations in the extent of lactose nucleation permitted during the processing of the milk. Identical milk samples, stored simultaneously at controlled freezing temperatures, often showed variations of several weeks in the lactose crystallization induction period. Storage stability evaluation studies should not rely on the analysis of single samples in view of this chance variation in the rate of spontaneous lactose crystallization in frozen milk.

It was observed in the course of this work that flocculation of the casein in frozen milk, seeded with lactose, can be partially suppressed if the milk is fortified with sucrose. Wildasin and Doan *(15)* reported that frozen concentrated milk was stabilized by sucrose and noted the presence of crystallized lactose in the frozen product. The stability of the colloidal casein in sucrose fortified frozen milk in the presence of crystallized lactose indicates that coagulation of frozen milk is not a direct consequence of the physical intervention of the lactose crystals in the micellar structure of the casein. The protection afforded milk by partial removal of calcium by ion exchange *(6)* and the stability of oxalated milk in frozen storage *(15)* suggest that the high salt concentration developed in the unfrozen milk phase promotes the destabilization of the casein. However, the mechanism by which soluble lactose protects the stability of the casein in frozen concentrated milk has not been resolved. Hawke and Lea *(7)* recently reviewed the subject of the stabilization of protein systems by carbohydrates and demonstrated that lactose and sucrose suppress the insolubilization of freeze-dried lipovitellin stored at high humidities. Herrington *(9)* reported on the formation of molecular compounds of lactose and calcium salts and speculated on the possibility that such compounds, if formed in ice cream and other concentrated milk products, may account for the unusual stability of their supersaturated lactose solutions.

The results obtained in this study emphasize the importance of lactose in maintaining the stability of the casein in frozen concentrated milk. The lactose

may stabilize the casein by "sequestration" of the highly concentrated milk salts in the unfrozen phase of the milk, thereby suppressing the salting-out of the casein. Additionally, the lactose may intervene in the molecular organization of the protein so as to suppress structural changes that would lower the stability of the colloid during prolonged periods of frozen storage. The withdrawal of the moderating influence of soluble lactose, as a consequence of its crystallization in frozen concentrated milk, is suggested as the primary cause of coagulation of the casein colloid. Whether lactose crystallization is a factor in the coagulation of casein in unconcentrated frozen milk is the subject of a current investigation.

An interesting parallel can be drawn between the "insolubilization" of casein in frozen milk and in high moisture milk powder, as follows:

1. Lactose crystallization precedes the development of protein insolubility in high moisture ($> 7\%$) milk powder and in frozen concentrated milk.

2. The casein insolubility developed in both systems is "reversible" with heat initially but gradually becomes irreversible on continued storage.

3. Depletion of the calcium content of either system stabilizes the casein under storage conditions that induce extensive lactose crystallization.

4. The insolubilization of the casein in both systems is suppressed by the presence of sucrose.

Henry *et al. (8)* related the protein insolubilization in high moisture milk powder to the interaction of the amino groups of the casein with lactose. However, the low temperatures at which casein is rapidly insolubilized in frozen milk is in direct contrast to the high temperature coefficient characteristic of sugar-protein interactions. In addition, the hydrolysis of lactose stabilizes the casein in frozen milk, atlhough such conversion renders the more reactive glucose available to the casein. Although sugar-protein interaction does not appear to be a factor in the coagulation of casein in frozen milk, the physical similarity of this system to that of high moisture milk powder suggests that certain aspects of the protein insolubilization may be common to both.

SUMMARY AND CONCLUSIONS

1. The coagulation of casein during the storage of frozen concentrated milk is a direct consequence of the crystallization of the lactose in the milk. The mechanism by which lactose crystallization initiates the flocculation of the colloidal casein in frozen milk remains obscure. It is suggested that the soluble lactose in the metacryotic fluid of partially frozen milk may sequester the concentrated calcium salts and thereby moderate their destabilizing influence on the colloidal casein. Withdrawal of soluble lactose from the unfrozen phase by crystallization would eliminate the sugar as a stabilizing factor and permit the casein to flocculate.

2. The rate of crystallization of lactose in milk frozen at 15° F. to −20° F. has been determined by polarimetric measurement of the increase of alpha lactose above the equilibrium level. Lactose crystallization and flocculation of the casein colloid in frozen milk occur in close sequence after well-defined induction periods that are related to the freezing temperature.

3. The quantity of unfrozen water in concentrated skimmilk, equilibrated at various freezing temperatures, has been evaluated calorimetrically. Approximately 75% of the water is frozen in 27% solids skimmilk at 15° F. The residual unfrozen phase is highly saturated with lactose which crystallizes rapidly after a characteristic induction period during which crystal centers are formed. At temperatures sufficiently low to freeze over 90% of the water, the amorphous lactose phase is stable against crystallization. Under these conditions, flocculation of the casein is suppressed.

4. Enzymatic hydrolysis of lactose in concentrated milk markedly stabilizes the frozen product by retarding the rate of crystallization of the lactose in proportion to the degree of hydrolysis.

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ACTION OF AEROSOLS OF CERTAIN VIRICIDAL AGENTS ON LACTIC STREPTOCOCCUS BACTERIOPHAGE[1]

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Since the announcement in 1935 of the discovery of bacteriophage as an important cause of lactic acid culture failure, much research has been devoted to control methods. Carrying cultures in sealed cans, rotation of a series of cultures, and the use of multiple strain cultures have been advantageous but not entirely satisfactory methods. More research on the subject of control of air-borne phage through the use of chemical agents seemed desirable.

Whitehead and Hunter *(12)* recovered lactic streptococcus bacteriophage from the air by three different techniques. Anderson and Meanwell *(1)* reported that bulk starter was protected successfully from phage infection by the use of a special air-tight can with a small opening plugged with cotton. Whitehead and Hunter *(13)* considered droplet infection of the air from whey separators or other equipment to be an important channel of infection of cheese milk and recommended the use of sealed vessels for starter, filtering of air into the building and keeping the inside pressure slightly greater than that on the outside.

Henle and Zellat *(5)*, Robertson *et al. (8, 9)*, Stokes and Henle *(11)*, Biggs *et al. (3)*, Rosebury *et al. (10)*, and others have reported the inactivation to some degree of certain air-borne viruses by vapors of one or more of the glycols. Wolf *et al. (15)* reported inconclusive but apparently not satisfactory results from the use of propylene glycol mist in attempts to destroy air-borne lactic streptococcus bacteriophage. Fine mists containing available chlorine were said to give protection from this phage for one-half hour when the humidity was well in excess of 50%. Apparently no tests have been made of the effect of quaternary ammonium compounds in aerosols upon bacteriophage. Prouty *(7)* reported the inactivation of lactic streptococcus bacteriophage in liquids by each of six quaternary ammonium compounds under certain conditions.

EXPERIMENTAL METHODS

Three strains of bacteriophage, F10, F67, and F69, which are specific in their actions against the *Streptococcus cremoris* strains 122-1, 144F, and ML1, respectively, were selected for use in this investigation. The phage strains were immunologically distinct, according to Wilkowske *(14)*. The phages and their host bacteria were obtained from the collection of the Dairy Industry Section of the Iowa Agricultural Experiment Station. Ethylene glycol, propylene glycol, triethylene glycol, calcium hypochlorite, and alkyldimethylbenzylammonium chloride were selected as viricidal agents. Glycols of technical grade were used.

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The strengths of solutions of hypochlorite and chloramine-T were tested immediately before use by titrating with 0.1 *N* sodium thiosulfate, using a 2% solution of soluble starch as an indicator. One ml. of 0.1 *N* thiosulfate was regarded as being equivalent to 0.003546 g. of available chlorine. The solutions of alkyldimethylbenzylammonium chloride were prepared volumetrically on the basis of the manufacturer's stated strength of an industrial solution (10%).

The phages were prepared in milk, acid whey from which was filtered through a sterile filter paper and then through a sterile #03 Selas microporous filter. Filtrates containing the respective phages were mixed for simultaneous testing and the pH was adjusted when necessary by the addition of 1 *N* NaOH. The most probable numbers of active bacteriophage particles in test liquids were determined by the three-tube limiting dilution technique described by Krueger *(6)*. Litmus milk fortified with 1% nonfat dry milk solids and 10% V-8 juice was used as the medium. Since simultaneous tests were made on the three phages, triplicate sets of tubes were prepared from each test sample and its serial dilutions. One set was inoculated with one drop per tube of culture 122-1; the two other sets were inoculated similarly with cultures ML1 and 144F, respectively. Tubes failing to show normal coagulation and reduction of litmus after 12 to 16 hours incubation at 32° C., the same as occurred in the phage-free control tubes, were considered as containing active bacteriophage. The most probable numbers of particles per milliliter were obtained by referring to the tables of Buchanan and Fulmer *(4)*.

DeVilbiss No. 127 atomizers were used for atomizing whey filtrates for the infection of a closed room having a volume of 1,370 cu. ft. Phage was recovered by aspiration of 6.7 cu. ft. of air through 25 ml. of distilled or buffered distilled water in 35 × 200 mm. test tubes and by settling on stainless steel discs 90 mm. in diameter or in petri dishes which usually contained 20 ml. of buffered distilled water (pH 7.2). A sufficient quantity of glass beads 3-5 mm. in diameter was placed in each tube so that they came about to the surface of the water.

Aerosols of the viricidal agents were dispersed into the infected room by means of DeVilbiss No. 127 atomizers. Tubes and dishes for the collection of samples after the treatment of the room with calcium hypochlorite or chloramine-T were charged with distilled water containing 3.2 mg. of sodium thiosulfate per liter and 1/3200 *M* phosphate buffer adjusted to pH 7.2. Buffered water without inhibitors was used in testing the action of the glycols and of alkyldimethylbenzylammonium chloride.

All apparatus and test materials which came into contact with the cultures and phage samples were sterilized before use, with the exception of the stock viricidal agents.

RESULTS

Results representative of the nine test series made on recovering the bacteriophage from the air by aspiration and by deposition on stainless steel discs when the room was infected with an atomized whey filtrate are presented in Table 1. The high results with bacteriophage strains F67 and F69 on discs exposed during

TABLE 1

Bacteriophage recovery from room infected with atomized whey filtrate at pH 4.45 containing lactic streptococcus bacteriophages

Period sample taken after infection began	Mpn/ft³ of phage particles recovered from the air by aspiration through buffered distilled water		
(min.)	F10	F67	F69
10- 17	17,000	9,400	940,000
73- 80	110	110	2,800
131-138	0	1.1	36
259-266	0	0	0
490-497	0	0	0
Period disc exposed after infection began	**Mpn/ft² of phage particles recovered by rinsing stainless steel discs with buffered distilled water**		
(min.)	F10	F67	F69
0- 7	30,000	30,000	300,000
0- 17	11,000	30,000	53,000
0- 80	3,000	11,000	300,000
0-138	30,000	11,000	30,000
0-266	30,000	130,000	30,000
0-497	30,000	530,000	110,000
0-1220	5,300	480	11,000
0- 7	30,000	30,000	300,000
15- 22	350	11,000	110,000
78- 85	11	0	180
136-143	4.8	0	4.8
264-271	4.8	0	0
495-502	0	0	0
497-1220	0	300+	300+

60 ml. of whey filtrate containing 250,000,000, 25,000,000, and 110,000,000 particles/ml of F10, F67, and F69 phages, respectively, were atomized into the room. Time required for atomizing was 5 min.

the interval 497-1,220 minutes were atypical. The filtrates in the other tests ranged from pH 4.85 to pH 6.85. Petri dishes containing or not containing distilled water or buffered distilled water were used in some tests for sampling for the phages deposited from the air. No significant differences resulting from variations in pH or in receptacles were observed. Neither did a range from 54 to 91% in relative humidity seem to affect the results.

Most of the phage carried in whey filtrates atomized into the air settled or otherwise disappeared from the air in 1 to 2 hours, but some phage was recovered in certain trials after a considerably longer period. In one test, 2.6 phage particles per cu. ft. were recovered from still air and 940 particles per cu. ft. were recovered after vigorously using a dry mop on the floor 3 days after infection of the room. No phage was recovered in two other tests at the end of 1 and 2 days, respectively, after vigorous mopping. Phage was recovered from the surfaces in the room 2 days after dispersal into the air.

The results of treatment of the infected room with an aerosol of 1 ml. of triethylene glycol in 650,000 cc. of air (44 ml. per 1,000 cu. ft.) are shown in Table 2. Two similar tests of ethylene glycol and one test of propylene glycol likewise failed to indicate any destructive effect of these agents.

TABLE 2

Effect of triethylene glycol aerosol on numbers of phage particles recovered from room infected with lactic streptococcus bacteriophages

Period sample taken after infection began	Mpn/ft³ of phage particles recovered from the air by aspiration through buffered distilled water		
(min.)	F10	F67	F69
10-17	750,000	280,000	940,000
20-27	940,000	170,000	940,000
40-47	9,400	56,000	17,000
Period dish open after infection began	**Mpn/ft² of phage particles recovered from buffered distilled water standing in petri dishes**		
(min.)	F10	F67	F69
0- 7	1,100,000	530,000	3,000,000
0-10	3,000,000	1,100,000	3,000,000
0-17	5,300,000	3,000,000	3,000,000
0-27	3,000,000	1,800,000	3,000,000
0-47	530,000	1,100,000	3,000,000
0- 7	1,100,000	530,000	3,000,000
10-17	300,000	30,000	530,000
20-27	30,000	11,000	110,000
40-47	8,900	5,300	3,000

55 ml. of whey filtrate with a pH of 6.6 containing 150,000,000, 45,000,000, and 150,000,000 particles/ml of F10, F67, and F69 phages, respectively, were atomized into the room. 60 ml. of triethylene glycol were dispersed simultaneously with the filtrate.

An aerosol of calcium hypochlorite which provided 0.61 g. of available chlorine per 1,000 cu. ft. of air space, applied simultaneously with the infection of the room, resulted in no active phage particles being recovered at any time during the test. Table 3 presents the results of a similar test except that the aerosol

TABLE 3

Effect of calcium hypochlorite aerosol dispersed between infections on numbers of phage particles recovered from room infected with lactic streptococcus bacteriophages

Period sample taken after infection began	Mpn/ft³ of phage particles recovered from the air by aspiration through buffered thiosulphate solution		
(min.)	F10	F67	F69
10-17	0	0	0
46-53	750	17,000	360,000
77-84	170	17,000	75,000
Period dish open after infection began	**Mpn/ft² of phage particles recovered from buffered thiosulphate solution standing in petri dishes**		
(min.)	F10	F67	F69
0-37	0	0	0
37-47	30	30,000	110,000
10-17	0	0	0
46-53	0	24	1,100
77-84	180	890	53,000

60 ml. of whey filtrate with a pH of 6.55, containing 1,500,000, 150,000,000, and 150,000,000 particles/ml of F10, F67, and F69 phages, respectively, were atomized into the room. Time required for atomizing was 6 min. Ten ml. of calcium hypochlorite solution containing 8.3% available Cl were dispersed into the room 7-9 min. after infection began. A second infection of 60 ml. of the filtrate was made 37-45 min. after the first began, and a third of 20 ml. was made 71-73 min. after the first.

was applied after infection of the room and the room was infected again 28-36 minutes after disinfection and again 26-28 minutes later. The phage was completely inactivated immediately after disinfection, but not enough available chlorine remained in the air to inactivate phage dispersed into the room 28 minutes or more after disinfection. Table 4 shows the effects of a calcium hypochlorite

TABLE 4

Effect of calcium hypochlorite aerosol dispersed before infection on numbers of phage particles recovered from room infected with lactic streptococcus bacteriophages

Period sample taken after infection began	Mpn/ft^3 of phage particles recovered from the air by aspiration through buffered thiosulphate solution		
(min.)	F10	F67	F69
7-14	1.1	1.1	3.4
30-37	360	17,000	75,000
54-61	940	56,000	170,000
Period dish open after infection began	**Mpn/ft^2 of phage particles recovered from buffered thiosulphate solution standing in petri dishes**		
(min.)	F10	F67	F69
0-22	0	30	24
22-50	540	30,000	180,000
7-14	0	8.0	0
30-37	0	2,400	3,500
54-61	300	11,000	30,000

60 ml. of whey filtrate with a pH of 6.5, containing 4,500,000, 4,500,000, and 75,000,000 particles/ml of F10, F67, and F69 phages, respectively, were atomized into the room. Time required for atomizing was 7 min. Twelve ml. of calcium hypochlorite solution containing 8.5% available Cl were dispersed into the room 5-2 min. before infection began. A second infection of 60 ml. was made 22-29 min. after the first and a third of 20 ml. was made 50-54 min. after the first.

aerosol providing the same amount of available chlorine applied to the room during the period of 5 to 2 minutes before infection. Almost all of the phage dispersed into the room at first was inactivated, but much more survived from later dispersals. Treatment of the infected room with calcium hypochlorite aerosols providing 0.18 g. of available chlorine per 1,000 cu. ft. of air space in three other trials resulted in the recovery of fewer phage particles than from the untreated room, but not all of the phage was inactivated in any of these trials.

The effects of an aerosol of alkyldimethylbenzylammonium chloride (0.73 g. per 1,000 cu. ft.) on numbers of phage particles recovered in the infected room are shown in Table 5. The phage was not completely inactivated and the aerosol had a highly astringent effect upon the respiratory tract of a person in the room, which alone would make its use impracticable in rooms where workers are present.

DISCUSSION

The present data agree in general with those of Wolf *et al. (15)* in demonstrating a rapid decrease in numbers of active phage particles recovered from the air of a room infected with phage. The rapidity with which the phage settled out of the air or became inactivated seems to limit greatly the dissemination of lactic streptococcus bacteriophage through the air.

TABLE 5

Effect of alkyldimethylbenzylammonium chloride aerosol on numbers of phage particles recovered in room infected with lactic streptococcus bacteriophages

Period sample taken after infection began	Mpn/ft³ of phage particles recovered from the air by aspiration through buffered distilled water		
(min.)	F10	F67	F69
10-17	0	1.1	940
38-45	56,000	17,000	17,000
70-77	75,000	940,000	94,000
Period dish open after infection began	**Mpn/ft² of phage particles recovered from buffered distilled water standing in petri dishes**		
(min.)	F10	F67	F69
0-28	5,400	11,000	30,000
28-58	540,000	1,400,000	240,000
10-17	3,000	11,000	890
38-45	54,000	110,000	110,000
70-77	5,400	30,000	11,000

60 ml. of whey filtrate with a pH of 6.5, containing 25,000,000, 200,000,000, and 25,000,000 particles/ml of F10, F67, and F69 phages, respectively, were atomized into the room. Time required for atomizing was 6 min. Ten ml. of 10% alkyldimethylbenzylammonium chloride solution were dispersed into the room 5-2 min. before infection began. A second infection of 50 ml. of filtrate was made 30-35 min. after the first began and a third 60-62 min. after the first.

The present results also agree with those of Wolf *et al.* in indicating ineffectiveness of the glycols as aerosols against this type of phage. The effectiveness of glycols dispersed by other means than by the DeVilbiss atomizer was not tested in the present investigation.

The dispersal of approximately 0.61 g. of available chlorine from a hypochlorite per 1,000 cu. ft. of air space apparently represents the minimum effective treatment. This treatment is effective for a very short time. Relatively fine mists are essential. The corrosive action of chlorine upon metals should be kept in mind in its use.

Aerosols of alkyldimethylbenzylammonium chloride appear not to be adaptable to the control of air-borne lactic streptococcus bacteriophage.

Although 0.61 g. of available chlorine per 1,000 cu. ft. completely inactivated the phage in the air at the time of dispersal in these trials, the use of 1 g. per 1,000 cu. ft. in a fine mist is recommended to allow a margin of safety.

SUMMARY AND CONCLUSIONS

The persistence of air-borne infection with three lactic streptococcus bacteriophages and the action of certain viricidal agents against these phages suspended in air have been studied in this investigation.

Most of the *Streptococcus cremoris* phage carried in whey filtrates atomized into the air settled or otherwise disappeared in 1 to 2 hours, but some phage was recovered in certain of the trials after a considerably longer period.

It was possible in some cases to resuspend the phage in the air after settling. Phage was recovered on the surfaces in the room 2 days after dispersal into the air.

Glycols were not found sufficiently effective to recommend their use as aerosols in control of lactic streptococcus bacteriophage.

Aerosols of calcium hypochlorite supplying a minimum of 0.61 g. of available chlorine per 1,000 cu. ft. of air completely inactivated air-borne phage under the conditions of this investigation.

Alkyldimethylbenzylammonium chloride aerosol was ineffective.

The use of 1 g. of available chlorine per 1,000 cu. ft. from hypochlorite in a fine aerosol is recommended for the inactivation of air-borne lactic streptococcus bacteriophage.

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ACTION OF CERTAIN VIRICIDAL AGENTS ON LACTIC STREPTOCOCCUS BACTERIOPHAGE IN LIQUIDS[1]

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Following the investigation of the action of aerosols of certain viricidal agents upon lactic streptococcus bacteriophage by these authors *(2)*, it was decided to study the action of the same agents when applied to liquids containing the phage.

Dunham and MacNeal *(4)* inactivated vaccinia virus by exposure to 70% propylene glycol for 3 minutes in watery suspension. On the other hand, Tilley and Anderson *(12)* found 100% ethylene glycol ineffective against the virus of Newcastle disease.

Beckwith and Rose *(1)* and Diénert *(3)* found most strains of bacteriophage from sewage or water contaminated with fecal matter resisted treatment with chlorine. Hunter and Whitehead *(5)* inactivated lactic streptococcus phage in 1 minute in a 1:2 dilution of whey with a solution containing 0.05% available chlorine. Whitehead and Hunter *(11)* found that more than 200 p.p.m. of available chlorine were required to destroy low titer phages and over 600 p.p.m. of available chlorine were needed to eliminate phage from high titer whey.

Maier *(9)* found that dilution of 1:50,000 of a quaternary ammonium compound did not interfere with the reproduction of staphylococcal phage. Klein *et al. (7)* proved three quaternary ammonium compounds were lethal for *Shigella dysenteriae* and *Staph. albus* phages but not for *E. coli* phage. Kalter *et al. (6)* described a method for the isolation of *E. coli* phage from sewage by means of cationic detergents. Prouty *(11)* reported inactivation of lactic streptococcus bacteriophage by 100 p.p.m. of each of six quaternary ammonium compounds in 16 out of 17 trials which he conducted.

EXPERIMENTAL METHODS

Three strains of bacteriophage, F10, F67, and F69, and the respective host strains, 122-1, 144F, and ML1, of *Streptococcus cremoris* which were used previously *(2)* in testing aerosols were used in this investigation also. Technical grades of ethylene glycol, propylene glycol, and triethylene glycol and commercial preparations of calcium hypochlorite, chloramine-T, alkyldimethylbenzylammonium chloride, N-(acylcolaminoformylmethyl) pyridinium chloride, 9-octadecenyldimethylethylammonium bromide, methyldodecylbenzyltrimethylammonium chloride and diisobutyl (or *p* – tertiaryoctyl) – phenoxyethoxyethyldimethylbenzylammonium chloride were selected for test as viricidal agents.

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Solutions of the viricidal agents were made by the same procedures as in the previous study *(2)* except they were prepared in double strengths.

Whey filtrates containing each of the three phages were prepared and the most probable numbers of phage particles per milliliter were determined by the techniques described for testing aerosols *(2)*.

The action of the viricidal agents was determined by a modification of the procedure proposed by Weber and Black *(13)* for evaluating practical performance of germicides proposed for sanitizing food utensils. Six to 10 ml. of the test solution of the viricidal agent in double strength were placed in a 25 × 150 mm. test tube and covered with a 30 × 50 mm. glass or aluminum cap. Distilled water for preparing test solutions, the metal caps, and all glassware used in these trials were autoclaved at 15 lb. pressure for 30 minutes. Five-ml. quantities of phage filtrate or diluted phage filtrate were carefully transferred to another 25 × 150 mm. tube, avoiding touching the sides of the tube in transfer. This tube also was covered with a 30 × 50 mm. glass or aluminum cap. Both capped tubes were placed in a 400-ml. beaker of water for obtaining and maintaining the test temperature.

The tempered tube containing the phage was set in a 2-oz. glass jar in a vertical position out of the water bath. With a 5-ml. tip-delivery bacteriological pipet 5 ml. of the viricidal solution were transferred to the tube containing the phage. The contents of the pipet were discharged quickly by blowing them into the tube. As the transfer was begun, a stop watch with a sweep second hand was started. The pipet was discarded quickly and the contents of the tube were swirled vigorously. With an APHA 1.1 ml. bacteriological pipet, 1 ml. of the treated filtrate was removed from the tube and quickly discharged into a 9-ml. inhibitor blank prepared as described below at 15 seconds. The inhibitor tube was swirled. Additional transfers of the treated filtrate into separate inhibitor blanks were made in a similar manner after 30, 60, 120, and 300 seconds, respectively.

The 9-ml. inhibitor blanks were prepared in 25 × 150 mm. Pyrex tubes which were plugged with cotton. The solution for preparing the 9-ml. inhibitor blanks to be used in testing 50 p.p.m. of available chlorine in hypochlorite or chloramine-T contained 80 mg. of sodium thiosulfate ($Na_2S_2O_3 \cdot 5\ H_2O$) and 1.25 ml. of stock *M*/4 phosphate buffer made up to 1 l. and adjusted to pH 7.2. For higher concentrations of available chlorine, the concentration of sodium thiosulfate was increased proportionately.

The solution for preparing the 9-ml. inhibitor blanks as used for testing 200 p.p.m. of quaternary ammonium solutions contained 2.222 g. of asolectin, 15.6 ml. of Tween 80, and 1.25 ml. of stock *M*/4 phosphate buffer per liter and was adjusted to pH 7.2, as recommended by Weber and Black *(13)*. For testing higher concentrations of quaternary ammonium compounds the asolectin and Tween 80 were increased proportionately. Both types of inhibitors were sterilized in the autoclave at 15 lb. pressure for 20 minutes.

In order to have sufficient volume of material for assay, 10 ml. of sterile distilled water were pipetted into each 9-ml. inhibitor tube to which 1 ml. of

treated filtrate had been added. The contents of each tube then were assayed for each strain of bacteriophage, and the results were doubled to compensate for the final water dilution. The most probable numbers of phage particles per milliliter were calculated from the titers of the stock phage preparations.

The pH of the original and treated filtrates was determined with a Beckman Model G pH meter with glass electrodes or a Leeds and Northrup hydrogen-ion potentiometer with calomel and quinhydrone electrodes.

RESULTS

Glycols. The results of treatment of the bacteriophages in 10^{-1} and 10^{-2} dilutions of whey filtrate with ethylene glycol are presented in Table 1. Little, if any, destructive action in concentrations as high as 1,000 p.p.m. in a filtrate

TABLE 1

Effect of ethylene glycol on lactic streptococcus bacteriophages in dilutions of whey filtrates at 25° C.

Bacteriophage	pH of filtrate	P.p.m. of glycol in treated filtrate	pH of treated filtrate	Calc. mpn/ml in control	Mpn[a]/ml after exposure time (in sec.) of:				
					15	30	60	120	300
				Whey filtrate diluted 10^{-1}					
F10	6.5	500	7.05	1,100					500
F69	6.5	500	7.05	25,000					5,000
F10	6.5	1,000	7.1	25,000					50,000
F69	6.5	1,000	7.1	95,000					50,000
F10	6.65	1,000	7.15	11,000					9,000
F67	6.65	1,000	7.15	1,100,000					500,000
F69	6.65	1,000	7.15	2,500					15,000
	ca.			Whey filtrate diluted 10^{-2}					
F69	4.7	100	—	45,000	19,000	19,000	5,000	19,000	2,300

[a] Assay of treated filtrate diluted 10^{-1} in sterile milk.

diluted 10^{-1} occurred in 300 seconds. Some destruction in a 10^{-2} dilution may have occurred. No more effect was evident when propylene glycol and triethylene glycol were tested separately.

Calcium hypochlorite and chloramine-T. Representative results of tests to determine the action of calcium hypochlorite upon the phages in whey filtrates diluted 1:2 are shown in Table 2. A concentration of 100 p.p.m. of available chlorine reduced the numbers of active particles in filtrates with a pH of 5.0 from 25,000,000-55,000,000 per milliliter to 1.4-5,000 per milliliter in 300 seconds, with most of the destructive action occurring within 15 seconds of exposure time. A concentration of 400 p.p.m. of available chlorine inactivated all three phages in filtrates with a pH of 5.0 within 15 seconds, but when the filtrate had 10%

TABLE 2

Effect of calcium hypochlorite on lactic streptococcus bacteriophages in 1:2 dilutions of whey filtrates at 25° C.

Bacteriophage	pH of filtrate	P.p.m. of av. Cl in treated filtrate	pH of treated filtrate	Calc. mpn/ml in control	Mpn[a]/ml after exposure time (in sec.) of:				
					15	30	60	120	300
F10	5.0	100		25,500,000	500	500	500	90	1.4
F67	5.0	100		55,000,000	5,000	5,000	5,000	5,000	5,000
F69	5.0	100		55,000,000	500	500	90	90	30
F10	5.0	200		225,000	0	0	0	0	0
F67	5.0	200		2,250,000	900	900	900	300	150
F69	5.0	200		10,000,000	900	5,000	900	90	90
F10	5.0	300		1,250,000	3		0	0	0
F67	5.0	300		2,250,000	150		50	90	50
F69	5.0	300		3,750,000	50		50	50	50
F10	5.0	400		1,250,000	0		0	0	0
F67	5.0	400		2,250,000	0		0.6	0.8	0
F69	5.0	400		3,750,000	0		0	0	0
F10[b]	4.6	500	4.8	1,250,000	500	90	50	0.6	0
F67[b]	4.6	500	4.8	2,250,000	50	50	50	50	50
F69[b]	4.6	500	4.8	1,250,000	9	50	9	1.4	0
F10	6.5	400	6.6	1,250,000	50		9	5	0
F67	6.5	400	6.6	1,250,000	50		50	50	50
F69	6.5	400	6.6	2,250,000	220		90	50	5
F10	6.5	500	6.6	1,250,000	0		0	0	0
F67	6.5	500	6.6	1,250,000	50		90	0	0
F69	6.5	500	6.6	2,250,000	9		0	0	0

[a] Assay of treated filtrate diluted 10^{-1} in thiosulphate inhibitor.

[b] 10% susceptible culture of lactic streptococcus added just before treatment with viricide solution.

of a milk culture of the homologous organisms added, 500 p.p.m. was hardly sufficient for complete inactivation within 300 seconds. In filtrates with a pH of 6.5, a concentration of 500 p.p.m. of available chlorine inactivated all three phages within 120 seconds in one trial but 1.4 F67 particles per milliliter and 19 F69 particles survived an exposure of 300 seconds in another trial. Larger numbers of particles survived treatment with 400 p.p.m. of available chlorine.

Representative results of tests of the action of calcium hypochlorite upon the phages in 10^{-1} dilutions of whey filtrates are given in Table 3. The phages suspended in a dilution of whey with a pH of 5.0 were inactivated in 60 seconds by 100 p.p.m. of available chlorine but survived in small numbers an exposure of 300 seconds at pH 6.9-6.95. The effect of calcium hypochlorite upon the phages in 10^{-2} dilutions of filtrates is shown in Table 4. The phages suspended in dilutions of whey at pH 5.4-6.0 were inactivated in 15 seconds by 25 p.p.m. of available chlorine but were not inactivated in 300 seconds by the same concentration of chlorine at pH 7.2-7.4.

TABLE 3

Effect of calcium hypochlorite on lactic streptococcus bacteriophages in 10^{-1} dilutions of whey filtrates at 25° C.

Bacteriophage	pH of filtrate	P.p.m. of av. Cl in treated filtrate	pH of treated filtrate	Calc. mpn/ml in control	Mpn[a]/ml after exposure time (in sec.) of:				
					15	30	60	120	300
F10	4.6	50		4,500,000	50	50	19	4	1.4
F67	4.6	50		11,000,000	90	220	90	90	6
F69	4.6	50		1,100,000	50	50	50	19	9
F10[b]	4.6	100	4.8	450,000	0	9	19	5	1.8
F67[b]	4.6	100	4.8	9,500,000	300	150	80	90	19
F69[b]	4.6	100	4.8	400,000	50	50	90	150	19
F10	5.0	100		4,500,000	0	50	0	0	0
F67	5.0	100		11,000,000	0	50	0	0	0
F69	5.0	100		11,000,000	0	50	0	0	0
F10	6.5	100	6.9	2,000,000	5	1.4	0	0	0
F67	6.5	100	6.9	1,500,000	1.4	3	5	0.8	0
F69	6.5	100	6.9	1,500,000	19	6	2.2	0.6	0

[a] Assay of treated filtrate diluted 10^{-1} in thiosulphate inhibitor.
[b] 10% culture of susceptible organism added to filtrate immediately before treatment with hypochlorite solution.

TABLE 4

Effect of calcium hypochlorite on lactic streptococcus bacteriophages in 10^{-2} dilutions of whey filtrates at 25° C.

Bacteriophage	pH of filtrate	P.p.m. of av. Cl in treated filtrate	pH of treated filtrate	Calc. mpn/ml in control	Mpn[a]/ml after exposure time (in sec.) of:				
					15	30	60	120	300
F10	4.6	15	6.0	25,000	19	1.8	1.4	3.2	9
F67	4.6	15	6.0	45,000	50	3	3	3	1.4
F69	4.6	15	6.0	25,000	1.8	9	2.2	1.8	1.2
F10	4.6	25	6.0	25,000	0	0	0	0	0
F67	4.6	25	6.0	45,000	0.8	0	0	0	0
F69	4.6	25	6.0	25,000	0	0	0.6	0	0
F10	4.6	50	6.4	2,500	0	0	0	0	0
F67	4.6	50	6.4	450,000	0	0	0	0	0
F69	4.6	50	6.4	150,000	0	0	0	0	0
F10	6.5	25	7.2	200,000	3	50	0	1.8	0.8
F67	6.5	25	7.2	150,000	50	50	50	50	50
F69	6.5	25	7.2	150,000	50	50	50	50	500

[a] Assay of treated filtrate diluted 10^{-1} in thiosulphate inhibitor.

Less action was obtained from available chlorine supplied by chloramine-T than when calcium hypochlorite was the source. The numbers of phage particles per milliliter which survived 300 seconds exposure when the former was used were as follows: 8-5,000 in 1:2 dilutions of whey at pH 7.7 when treated with 500 p.p.m. of available chlorine; 400-220,000 in 10^{-1} dilutions of whey at pH 7.6-8.1 when treated with 100 p.p.m. of available chlorine and 80-5,000 in 10^{-2} dilutions of whey when treated with 25 p.p.m. of available chlorine at pH 7.7-8.1.

Quaternary ammonium compounds. Representative results of treating the phages in 1:2 dilutions of whey filtrates with alkyldimethylbenzylammonium chloride are given in Table 5. A concentration of 1,000 p.p.m. of the compound inactivated the phages in 30 seconds when they were suspended in dilutions of whey at pH 6.6-6.65 but failed to inactivate them at pH 4.6.

Representative results of treating the phages in 10^{-1} dilutions of whey filtrates with alkyldimethylbenzylammonium chloride are presented in Table 6. Six hundred p.p.m. of the compound inactivated the phages in 120 seconds in dilutions of whey at pH 4.8 and 300 p.p.m. inactivated the phages in 15 seconds at pH 6.9. Table 7 contains representative results of treating the phages in 10^{-2} dilutions of whey filtrates with alkyldimethylbenzylammonium chloride. Fifty p.p.m. inactivated the phages in 15 seconds at pH 5.1, but 25 p.p.m. was insufficient for inactivation in 300 seconds at pH 7.0-7.05.

Results of similar tests with diisobutyl (or *p*-tertiaryoctyl) – phenoxyethoxyethyldimethylbenzylammonium chloride, methyldodecylbenzyltrimethylammoni-

TABLE 5

Effect of alkyldimethylbenzylammonium chloride on lactic streptococcus bacteriophages in 1:2 dilutions of whey filtrates at 25° C.

Bacteriophage	pH of filtrate	P.p.m. of viricide in treated filtrate	pH of treated filtrate	Calc. mpn/ml in control	Mpn[a]/ml after exposure time (in sec.) of:				
					15	30	60	120	300
F10	5.0	400		1,250,000	5,000,000	3,000,000	5,000,000	1,900,000	190,000
F67	5.0	400		2,250,000	5,000,000	4,000,000	900,000	5,000,000	500,000
F69	5.0	400		3,750,000	90,000	150,000	190,000	40,000	500,000
F10	4.6	1,000	4.6	1,250,000	90	50	9	0.6	0
F67	4.6	1,000	4.6	7,500,000	22,000	22,000	90	50	0.8
F69	4.6	1,000	4.6	2,250,000	1,300,000	500,000	22,000	1,900	50
F10	6.5	600	6.6	5,500,000	90,000	50,000	5,000	50	0.6
F67	6.5	600	6.6	2,250,000	500,000	220,000	5,000	90	0.6
F69	6.5	600	6.6	1,250,000	5,000	5,000	900	90	1.4
F10	6.5	800	6.6	12,500,000	5	0.6	0	0.6	0
F67	6.5	800	6.6	1,000,000	80	1.4	0	0	0
F69	6.5	800	6.6	2,250,000	5,000	90	5	0.6	0
F10	6.5	1,000	6.65	750,000	0.6	0	0	0	0
F67	6.5	1,000	6.65	1,250,000	0	0	0	0	0
F69	6.5	1,000	6.65	75,000	9	0	0	0	0

[a] Assay of treated filtrate diluted 10^{-1} in asolectin-Tween 80 inhibitor.

TABLE 6

Effect of alkyldimethylbenzylammonium chloride on lactic streptococcus bacteriophages in 10^{-1} dilutions of whey filtrates at 25° C.

Bacteriophage	pH of filtrate	P.p.m. of viricide in treated filtrate	pH of treated filtrate	Calc. mpn/ml in control	Mpn[a]/ml after exposure time (in sec.) of:				
					15	30	60	120	300
F10	4.6	300	5.0	4,500,000	150	50	50	19	0
F67	4.6	300	5.0	11,000,000	2,200	900	900	300	1.8
F69	4.6	300	5.0	1,100,000	900	90	50	80	0.8
F10	4.6	600	4.8	110,000	0	0.8	0.8	0	0
F67	4.6	600	4.8	450,000	19	1.8	0	0	0
F69	4.6	600	4.8	1,500,000	9	0.8	0.8	0	0
F10	6.5	200	7.05	2,500,000	5,000	5	0	0	0
F67	6.5	200	7.05	200,000	9,000	300	1.8	0.6	0
F69	6.5	200	7.05	450,000	22,000	5,000	19	9	0.6
F10	6.5	300	6.9	1,100,000	0	0	0	0	0
F67	6.5	300	6.9	450,000	0	0	0	0	0
F69	6.5	300	6.9	250,000	0	0	0	0	0

[a] Assay of treated filtrate diluted 10^{-1} in asolectin-Tween 80 inhibitor.

TABLE 7

Effect of alkyldimethylbenzylammonium chloride on lactic streptococcus bacteriophages in 10^{-2} dilutions of whey filtrates at 25° C.

Bacteriophage	pH of filtrate	P.p.m. of viricide in treated filtrate	pH of treated filtrate	Calc. mpn/ml in control	Mpn[a]/ml after exposure time (in sec.) of:				
					15	30	60	120	300
F10	4.6	50	5.1	4,500	0	0	0	0	0
F67	4.6	50	5.1	25,000	0	0	0	0	0
F69	4.6	50	5.1	45,000	0	0	0	0	0
F10	4.6	100	5.3	2,500	0	0	0	0	0
F67	4.6	100	5.3	25,000	0.6	0	0	0	0
F69	4.6	100	5.3	3,500	0	0	0	0	0
F10	4.6	200	5.3	25,000	0	0	0	0	0
F67	4.6	200	5.3	45,000	0	1.8	0	0	0
F69	4.6	200	5.3	25,000	0.6	0	0	0	0
F10	4.6	200	5.1	25,000	0	0	0	0	0
F67	4.6	200	5.1	45,000	1.8	0	0	0	0
F69	4.6	200	5.1	25,000	0	0	0	0	0
F10	4.6	300	5.2	25,000	0	0	0	0	0
F67	4.6	300	5.2	1,100,000	4	0.6	0	0	0
F69	4.6	300	5.2	9,500	0	0.8	0	0	0
F10	6.5	25	7.0	15,000	900	800	500	300	90
F67	6.5	25	7.0	25,000	5,000	5,000	500	50	5
F69	6.5	25	7.0	1,500	1,900	500	400	500	9

[a] Assay of treated filtrate diluted 10^{-1} in asolectin-Tween 80 inhibitor.

um chloride, N-(acylcolaminoformylmethyl) – pyridinium chloride and 9-octadecenyldimethylethylammonium bromide did not reveal any significant differences between actions of these compounds and the action of alkyldimethylbenzylammonium chloride.

DISCUSSION

The presence of organic matter very definitely reduced the viricidal action of solutions of either calcium hypochlorite (Tables 2-4) or alkyldimethylbenzylammonium chloride (Tables 5-7) of a given concentration. Because of the filtration methods employed, the filtrates probably contained a lower percentage of organic matter than the usual whey from cheese. The few tests made on filtrates containing 10% added culture (Table 2) indicate that ordinary cheese whey may require heavier dosages of chlorine than are indicated in the results of this investigation.

Since lower concentrations of the viricides inactivated the phages when the filtrates were diluted 10^{-1} or 10^{-2}, it is important that rinse solutions be kept as nearly free as possible of milk and whey or other organic matter. Thorough washing of utensils, followed by a preliminary rinse before the use of viricidal solutions, is highly recommended.

Decreases in pH increased the viricidal efficiency of calcium hypochlorite but had the opposite effect upon the action of alkyldimethylbenzylammonium chloride. This factor would be important in practical applications.

Distilled water was used as a diluent for the viricides in this investigation. McCulloch *(10)* and Lawrence *(8)* present information indicating that impurities in some water supplies may affect the action of viricides. Especially is this true in hard water used in diluting quaternary ammonium compounds.

Chloramine-T was found to be much less effective than calcium hypochlorite when the concentrations of available chlorine were approximately equal. The higher alkalinity of the chloramine-T product may have been a disadvantage. Tests at the same pH may have given more nearly comparable results.

The few tests which were made on the different kinds of quaternary ammonium compounds did not reveal any outstanding differences in their actions. More differences might be observed upon a more extended investigation.

The results of the action of quaternary ammonium compounds on lactic streptococcus bacteriophage obtained in this investigation are far from agreement with those reported by Prouty *(11)*. Since Prouty did not use an inhibitor to stop the action of the compounds at the end of certain periods of exposure, the action observed by him probably was partly static, rather than entirely viricidal. Lawrence *(8)* has reviewed several reports on this point.

A rinse containing 100 p.p.m. of available chlorine from hypochlorite or 200 p.p.m. of an approved quaternary ammonium compound equal in effectiveness to alkyldimethylbenzylammonium chloride is recommended for the treatment of all utensils, equipment, walls, and floors. These amounts make reasonable allowances for variations in pH and other factors. This treatment should always be preceded by thorough cleansing and by rinsing with clear water, because of the

deleterious effect of organic matter and the possibility that the pH might be influenced adversely by a residue of washing powder and/or milk solids. Clear water rinsing of utensils and surfaces of equipment coming in contact with milk following treatment with quaternary ammonium compounds may be advisable to avoid possible inhibitory action of residues upon culture organisms.

SUMMARY AND CONCLUSIONS

The action of certain viricidal agents against three lactic streptococcus bacteriophages in whey and in diluted whey have been studied in this investigation.

Glycols were not found sufficiently effective to recommend their use in control of lactic streptococcus bacteriophage.

Available chlorine from calcium hypochlorite, ranging in concentration from 400 p.p.m. in 1:2 dilutions to 25 p.p.m. in 10^{-2} dilutions of the whey, inactivated the phages therein in 15 seconds at pH 5 to 6. The greater the dilution of the whey, the lesser was the concentration of chlorine which was required for inactivation.

Available chlorine from chloramine-T was much slower in action and was very noticeably less effective even after 300-second exposures than available chlorine from calcium hypochlorite. A higher pH level in the former may have been a factor.

Alkyldimethylbenzylammonium chloride, ranging in concentration from 1,000 p.p.m. in 1:2 dilutions to 50 p.p.m. in 10^{-2} dilutions of the whey, inactivated the phages therein in 15-30 seconds at certain pH levels. Lesser concentrations of this compound were required for inactivation as the dilution of the whey was increased.

Presence of organic matter decreased the effectiveness of either available chlorine or the quaternary ammonium compounds studied in the destruction of the phage. Available chlorine was more effective in the destruction of the phages at the lower pH levels than at the higher levels within the range of pH 4.6 to 7.4. The opposite was true of alkyldimethylbenzylammonium chloride.

No outstanding differences in the actions of the five quaternary ammonium compounds on lactic streptococcus bacteriophages were observed.

A final rinse containing 100 p.p.m. of available chlorine from hypochlorite or 200 p.p.m. of an approved quaternary ammonium compound equal in effectiveness to alkyldimethylbenzylammonium chloride is suggested for the destruction of the phage on cleaned surfaces.

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EFFECT OF TWO LEVELS OF INTAKE OF A VITAMIN A DEPLETION RATION ON SOME BLOOD CONSTITUENTS AND DEFICIENCY CRITERIA IN THE DAIRY CALF[1]

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The level of feed intake must be considered when studying specific nutrient deficiency symptoms, because biochemical, physiological, and histological changes have been demonstrated to be dependent in many instances upon this factor *(8)*. With respect to the onset of vitamin A deficiency symptoms in the dairy calf, no data were found in the literature on the effect of level of feed intake. However in the ovine, Sapsford *(16)* reported more rapid rates of decrease of blood plasma vitamin A levels and earlier appearance of morphologically abnormal spermatozoa in rams on a high level of intake of a carotene deficient ration than those for rams on a low intake. In possible contrast, withholding the feed from 25-day-old rats has been reported *(12)* to result in more rapid rates of decrease of blood plasma vitamin A and vitamin A stores than controls fed a vitamin A-free diet.

The present study was concerned with the effect of two levels of intake of a vitamin A depletion ration, one adequate for normal growth and another inadequate, on rates of change of some blood constituents and on terminal physiological and histological alterations in the dairy calf.

EXPERIMENTAL

Animals and rations. Twenty one-day old calves (two Ayrshire, two Guernsey and 16 Holstein) were on experiment from February through June, 1952. These calves were either allowed to nurse their dams for a 24-hour period after birth or fed colostrum at the rates of 7.0 lb. for Guernseys and 8.0 lb. for Ayrshires and Holsteins. Thereafter, they were placed in individual tie-stalls and raised to 35 days of age on a standard limited whole milk, limited dry calf starter and ad libitum hay system *(4)*. Each calf received a 250 mg. oblet of aureomycin for the first 3 days of age, and 14 of the Holstein calves received 100,000 U.S.P. units of vitamin A from fish liver oil[2] for the first and second days of age.

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[2] The fish liver oil contained 25% by weight of crude soybean lecithin and 25,000 U.S.P. units of vitamin A per gram.

On the 36th day of age, calves were paired in chronological order of birth with restrictions as to breed and assigned according to a previously randomized allotment to one of two levels of intake of the same vitamin A depletion ration. With the 35th day weight as the basis, the "low" level of intake was fed to give a 7-day rate of increase of 5 lb. and the "high" level to give a 7-day increase of 10 lb. The depletion ration allowance was fed *(4)* according to the following formula: $Y = 0.0562W^{0.87}$ (where Y = the pounds of feed required daily and W = anticipated weight of the calf in pounds). Feeding of the vitamin A depletion ration was continued for each calf until the blood plasma vitamin A level had decreased to less than 4.0γ% for two consecutive 7-day periods. The calves were then slaughtered.

The milk was from Holstein cows, and the starter was similar to that previously described *(4)*. Either U.S. No. 1 mixed hay or ¼ in. artificially dehydrated alfalfa pellets were fed each pair of calves. The depletion ration, a mixture by weight of one-third of dried beet pulp and two-thirds of a grain mixture composed of the following: 419.5 lb. ground barley, 500 lb. crimped oats, 500 lb. wheat bran, 150 lb. linseed oil meal (expeller process), 150 lb. soybean oil meal (expeller process), 200 lb. cane molasses, 40 lb. 500-potency B-Y dried fermentation solubles, 20 lb. steamed bone meal, 20 lb. iodized salt, and 0.5 lb. irradiated yeast (Standard Brands type 9-F) per ton of mixture, contained less than 0.10 mg. of carotenoids per pound.

Observations and analyses. Daily feed fed and refused was weighed to the nearest 0.1 lb. Between 1:00 and 2:00 P.M. on the 35th day of age and at successive 7-day intervals thereafter, body weights to the nearest pound were recorded. Similarly, blood samples were obtained by puncture of the jugular vein for hemoglobin *(6)* and plasma carotenoids and vitamin A *(1, 11)* determinations. In addition, for seven pairs of the Holstein calves whole blood and plasma ascorbic acid *(15)* also were determined. Samples of the depletion ration were obtained at successive 28-day intervals during the experiment for carotenoid analysis *(14)*.

Two observations on heart rate by auscultation of the left chest wall and one observation of rectal temperature were taken for three successive days on each of seven pairs of Holstein calves at approximately the middle of the experimental period, at which time their average age was 73.8 ± 4 days. In the morning immediately prior to slaughter, spinal fluid pressure *(13)* was taken. At slaughter each calf's liver was removed for carotenoid and vitamin A analysis *(2)*, and various tissues *(5)* were taken for histological examination *(9, 10)*.

The statistical analysis *(3)* was concerned with the variability between level of intake of the depletion ration and among pairs of calves. Rates of change of the various criteria on time fed the depletion ration were explored by separate analyses of the first and second order orthogonal polynomial coefficients *(3, 7, 18)*. In the case of muscular incoordination or histological alterations in which only the frequency of its occurrence or nonoccurrence was observed, contingency tables were formed and appropriate significance tests were applied *(3)*.

RESULTS AND DISCUSSION

Feed consumed and body weight. Calves refused some of the feed fed during the first 5 to 10 days on the vitamin A depletion ration. However, by the 45th day of age, all consumed the depletion ration allowance. This continued until each calf's blood plasma vitamin A level had decreased to less than 4.0γ% for two consecutive weeks, at which time the animals were slaughtered.

The average body weight and its standard error at the start of the experimental period was 121 ± 5 lb. for the low level intake group and 120 ± 5 lb. for the high level group. The rate of gain in body weight per 7-day period during the vitamin A depletion period was 7.1 ± 1.5 and 11.0 ± 0.4 lb., respectively, for the low and high level intake groups.

Blood constituents. Average values on the 35th day of age, the day prior to the feeding of the depletion ration, and average rates of change per 7-day period during the feeding of the depletion ration for hemoglobin, plasma carotenoids, and plasma vitamin A and whole blood and plasma ascorbic acid are contained in Table 1. Only plasma carotenoids and plasma vitamin A exhibited significant over-all trends with consecutive 7-day periods during which the vitamin A depletion ration was fed. During the initial stages of feeding the vitamin A depletion ration, plasma carotenoids decreased at a more rapid rate (as evidenced by positive orthogonal polynomial coefficients $P < 0.001$) than plasma vitamin A. The latter's change was essentially a uniform linear rate of decrease ($P < 0.001$). Neither plasma carotenoids nor vitamin A values were significantly affected by the level of intake of the vitamin A depletion ration.

Hemoglobin showed a decrease in the calves on the low level of intake of the depletion ration whereas no apparent change was evident in the high level group ($P < 0.05$ for differences in level of intake). Whole blood and plasma ascorbic acid failed to exhibit over-all trends or to be affected by level of intake of the depletion ration.

The lack of an apparent effect of level of intake of a depletion ration on blood plasma carotenoids and vitamin A, as well as ascorbic acid, during vitamin A depletion suggests that other mechanisms may regulate these blood constituents. The difference in these results and those for sheep *(16)* with respect to plasma vitamin A might possibly have been due to species, relative age, or relative body weight increase.

Physiological and histological observations. Heart rates (Table 2) were significantly higher in calves fed the high level of intake of the depletion ration than those of calves on the low level intake ($P < 0.01$). This was probably due to the greater intake of total digestible nutrients by the former group *(17)*. Neither rectal temperatures nor magnitude of spinal fluid pressures were materially affected by level of intake of the depletion ration.

Although the occurrence of muscular incoordination, papillary edema, and squamous metaplasia of the interlobular ducts and Stenson's duct of the parotid gland (Table 2) was greater in the high level group, only the frequency of occurrence of squamous metaplasia of the interlobular ducts was significantly dif-

TABLE 1

Effect of feeding two levels of a vitamin A depletion ration on rate of change of some blood constituents in the calf

	Level of feeding		Standard error[d]
	"Low"	"High"	
Number of calves	10	10	.....
Average number of 7-day periods per calf	10.0	10.3	0.6
Hemoglobin *(g.%)*			
Initial value[a]	12.21	11.10	0.62
Rate of change per 7-day period[b]			
Linear	−0.136	0.032	0.029
Quadratic	0.041	0.013	0.007
Plasma carotenoids *(γ%)*			
Initial value	47	71	7
Rate of change per 7-day period			
Linear	−2.104	−2.944	0.312
Quadratic	0.633	0.740	0.123
Plasma vitamin A *(γ%)*			
Initial value	16.1	14.0	0.6
Rate of change per 7-day period			
Linear	−1.443	−1.445	0.060
Quadratic	−0.018	−0.041	0.020
Whole blood ascorbic acid *(mg.%)*[c]			
Initial value	0.69	0.70	0.05
Rate of change per 7-day period			
Linear	0.000	−0.011	0.004
Quadratic	0.000	−0.001	0.000
Plasma ascorbic acid *(mg.%)*[c]			
Initial value	0.52	0.49	0.05
Rate of change per 7-day period			
Linear	0.006	−0.006	0.004
Quadratic	−0.002	−0.002	0.000

[a] Value for the 35th day of age, the day prior to being placed on the assigned level of the depletion ration.

[b] Linear and quadratic rates of change represent 1st and 2nd order orthogonal polynominal coefficients *(2, 17)*.

[c] Values for 7 pairs of calves.

[d] These standard errors apply to the values of either of the preceding columns.

ferent ($P < 0.05$). The concentration of carotenoids and vitamin A in the livers of the high level intake group (Table 2) was greater than that of the low, but this difference was not statistically significant. The weights of the livers of the high group were greater ($P < 0.01$), as would be expected because of their greater body weight.

It was recognized that if the calves had been slaughtered at different intervals during the depletion period rather than terminally when the blood plasma level of vitamin A for each calf had decreased to less than 4.0γ% for 2 consecutive weeks, the occurrence of the histological change might have been more pronounced between levels of intake of the depletion ration. The difference in frequency of histological changes between the main duct of the parotid gland (Stenson's duct) and the interlobular ducts indicated that the former should be examined in ascertaining vitamin A deficiency in the calf.

TABLE 2

Effect of feeding two levels of a vitamin A depletion ration on some physiological and histological changes in the dairy calf

	Level of feeding		Standard error[c]
	"Low"	"High"	
No. of calves	10	10	.....
Heart rate *(beats/min)*[a]	105	125	11
Rectal temperature *(° F.)*[a]	101.9	102.1	0.2
Spinal fluid pressure *(mm. H_2O)*	162	164	23
Occurrence of muscular incoordination *(No. of calves)*	2	4	.....
Histological alterations			
Eye (papillary edema)			
No lesion	1[b]	1	.....
Lesion	7	9	.....
Parotid gland (squamous metaplasia)			
Interlobular ducts			
No lesion	8	2	.....
Lesion	2	8	.....
Stenson's duct			
No lesion	1	0	.....
Lesion	9	10	.....
Liver			
Weight *(g.)*	1388	1799	64
Carotenoids *(γ/g)*	0.37	0.32	0.05
Vitamin A *(γ/g)*	0.21	0.15	0.04

[a] Values for 7 pairs of calves.
[b] Values for 8 calves.
[c] These standard errors apply to the values of either of the preceding columns.

SUMMARY

Ten pairs of 36-day-old calves were fed a vitamin A depletion ration at two levels of intake, low and high, until the blood plasma vitamin A values decreased to less than 4.0γ% for two consecutive 7-day periods. This resulted in a 7-day increase in body weight of 7.1 ± 1.5 and 11.0 ± 0.4 lb. for the low and high levels of intake, respectively. Over-all, plasma carotenoids and vitamin A were found to decrease while whole blood and plasma ascorbic acid exhibited no change. None of these constituents were affected by the level of intake of the depletion ration. Hemoglobin was observed to decrease in the calves on the low level of intake of the depletion ration but no change was found for the calves on the high level. Heart rates of animals on the high level of intake averaged 20 beats per minute higher than those on the low level of intake, whereas rectal temperatures and terminal spinal fluid pressures were unaffected. Among the histological changes, only the frequency of occurrence of squamous metaplasia in the interlobular ducts of the parotid gland was significantly greater in the high level group.

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A WETTABILITY METHOD FOR POWDERED MILK[1]

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When dried milk is to be used for beverage purposes the dispersibility of the product is of primary importance. It is generally recognized that spray-dried milks are almost completely soluble when dispersed in small amounts by rapid agitation. Most solubility methods demonstrate this. However, under practical conditions with limited agitation the powders are less completely reconstituted. Even a slight amount of undispersed powder gives a grainy appearance to the sides of the container, making the final product unpleasant as a beverage.

Many different methods have been proposed for measuring the solubility of milk powders. These methods have been discussed by Waite and White *(5)*. All of the methods call for rather vigorous agitation for reconstituting the milk; they differ in their means of measuring the insoluble fraction. For spray-dried milk powder, however, this fraction remains a very small percentage of the total.

Few methods have been proposed in the literature for measuring the rate of dispersion of milk powder. One suggested by Ashworth and Bendixen *(1)* uses a standardized shaking procedure for 7 seconds with a mixture of one part milk powder to seven parts water. After rapid straining thru 0.5-mm. openings (Gooch type crucible), the undispersed residue is completely dispersed by means of warm water and agitation. An aliquot is then dried for total solids. The method is shown to be more sensitive than the regular solubility tests for freshly made spray-dried milk powders. Individual variations in the shaking technique may limit the reproducibility. A method for measuring static dispersion or wettability has been described by Kleinert *(4)*. He dusted 1 g. of milk powder through a 0.5-mm. mesh screen onto the surface of 500 ml. of water in a graduated cylinder and after a definite interval of time removed 200 ml. of the solution for turbidity measurements. The turbidity was compared with that found for 1 g. of the same milk powder completely dispersed in 500 ml. of water. Considerable variations were observed, which may be attributed, at least in part, to the time required for the dispersed powder to diffuse uniformly through the large volume of water used.

The first step in the solution or dispersion of any powdered material is the wetting of the solid particles by the solvent. In spray-dried milk powder this first step may well be the limiting factor governing the entire dispersion process. It is a common observation that without agitation milk powders vary considerably in their rate of dispersion. Some samples sink into solution readily but others remain floating on the surface indefinitely. This paper reports an attempt to find a method for measuring this tendency to wet. The method proposed is empirical but has been found to be a sensitive criterion of dispersibility.

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EXPERIMENTAL PROCEDURE

A dry 250-ml. or 400-ml. beaker may be used for the test. The bottom of the beaker is covered with a filter paper to keep the dry powder from adhering to the glass and to allow uniform distribution of the water. A sample consisting of 2.5 g. of milk powder is worked through a coarse "tea strainer" (16-20 mesh) to form a uniform layer of milk powder on the filter pad below. By means of a Babcock pipette 17.6 ml. of water at 30° C. are allowed to flow gently down the side of the beaker and form a layer under the filter paper. The powder and water are allowed to stand 5 minutes at room temperature without disturbance. At the end of this time the beaker is carefully picked up without agitating the contents and the dispersed milk is strained through an empty Gooch crucible to remove the unwetted powder. Ten seconds are allowed for the beaker to drain and approximately another 30 seconds for the crucible to drain. Plastic centrifuge tubes of 50 ml. capacity may be used to receive the milk. A 5-ml. aliquot of the strained milk is measured into aluminum foil dishes for total solids determination. This value, multiplied by a factor, gives the percentage of the original powder wet by the water in 5 minutes. The factor is found by dissolving a known amount of powder in 17.6 ml. and determining the total solids on a 5-ml. aliquot as before.

TABLE 1

Average confidence intervals for wettabilities

Wettability range	45° F. storage		85° F. storage	
(%)	*(No.)*[a]	*(C.I.)*[b]	*(No.)*[a]	*(C.I.)*[b]
20-29	4	±3.3	100	±3.5
30-39	15	4.4	119	4.3
40-49	13	5.8	79	5.6
50-59	18	6.5	72	5.7
60-69	42	6.1	60	7.0
70-79	46	5.1	72	6.5
80-89	204	4.5	106	5.2
90-99	242	3.2	62	3.4
Over-all weighted aver.		±4.20		±5.01
Total	584		670	

[a] Number of observations.
[b] Confidence interval calculated from range (see ref. 3).

Reproducibility. The nature of the wetting process is such that several replications are necessary to insure accuracy. Generally three or four replications are sufficient to give a confidence interval below ± 5% wettable *(3)*. A few powders, however, seem to show more variation in their wettability characteristics; this is particularly true of those falling in the middle range of wettability, as shown in Table 1. Some of this variation has been traced to slow straining through the Gooch crucible, as is shown later on in this report. Better reproducibility is secured when the layer of powder above the filter paper is not over 4 mm. thick. The packing density of the powder may be a factor in this respect. Table 1 shows the parallel trends between powders stored at 45° compared with those stored at 85° F.

Effect of surface area exposed. It might be assumed that the area of the bottom of the beaker over which the powder sample is distributed is an important factor influencing the amount of powder dispersed in the 5 minutes allowed. This would be true, no doubt, if the time interval were short enough; but during the 5 minutes allowed for the powder to disperse, fresh surface is continually being exposed because of the sinking of the heavier suspension of powder in the liquid phase. Consequently, the size of the beaker used is not critical, as may

TABLE 2

Influence of area of powder exposed to water on amount dispersed during 5 minutes of contact

Size of beaker *(ml.)*	250		400		600	
Diameter *(mm.)*	63		72		83	
Area exposed *(cm.²)*	31.2		40.7		54.1	
Sample No.	Amount dispersed[a]		Amount dispersed		Amount dispersed	
	in 5 ml.	per sq. cm.	in 5 ml.	per sq. cm.	in 5 ml.	per sq. cm.
	(mg.)	*(mg.)*	*(mg.)*	*(mg.)*	*(mg.)*	*(mg.)*
846	329	10.5	344	8.4	365	6.7
847	457	14.6	477	11.7	533	9.8
849	187	6.0	197	4.8	231	4.3
846	293	9.4	335	8.2	384	7.1
847	508	16.3	524	12.9	529	9.8
849	176	5.6	183	4.5	198	3.7
726	187	6.0	201	4.9	211	3.9
Average	305	9.77	323	7.91	350	6.47

[a] Each value in the table represents the average of 4 replications when 2.5 g. of powder were weighed into each beaker and 17.6 ml. of water added.

be observed from Table 2. The same amount of powder and water was used in all beakers, and each value represents four replications. The areas of the bottoms of the beakers varied from 31 to 54 sq. cm. The absolute amount of powder dispersed when 600-ml. beakers were used instead of the 250-ml. size increased on the average about 15%. However, when these amounts were divided by the area of the beakers used, the larger beakers gave lower amounts dispersed per unit area by about 34%.

It is important for comparative purposes, of course, to use the same sized beaker. For the amount of water and powder given in the procedure a 400-ml. beaker was found to give slightly more consistent values for wettability than either of the other two sized beakers used. Another important consideration was to have the layer of powder shallow enough to prevent any packing of the powder. Otherwise gel formation will lower wettability greatly.

Effect of amount of powder used and time of standing after water addition. To determine whether the sample size of 2.5 g. was optimum and whether 5 minutes of standing was adequate, several experiments were performed. The results of one trial are shown in Table 3. The amounts of sample used varied from 1 through 5 g. and the time allowed for dispersion varied between 1 and 5 minutes. The whole experiment was repeated several times. Some variations are shown, but the trends are definite except for the largest amount used. The time allowed seems to be adequate for all amounts up to 3 and possibly 4 g. The

TABLE 3

Comparison of amounts of powder used with time of standing in contact with water on the amounts dispersed

	Amounts of powder weighed out in grams				
	1	2	3	4	5
Time	Amounts dispersed				
(Min.)	*(g.)*	*(g.)*	*(g.)*	*(g.)*	*(g.)*
1	0.92	1.57	1.69	1.98	2.44
2	0.96	1.54	2.49	2.20	2.96
3	0.96	1.62	2.50	3.18	3.93
4	0.96	1.59	2.51	3.23	4.33
5	0.96	1.61	2.48	3.31	3.41
% dispersed during 5 min.	96	81	83	83	68

percentage of each amount dispersed decreased as the amount of powder used was increased.

There was some indication that either a larger sample size or a shorter standing time might be better for the highly wettable powders because the 3-g. samples were almost completely dispersed after 2 minutes of standing and were not benefited by the remaining 3 minutes of time. Consequently, a large number of comparisons were made between the use of 2.5-g. and 4-g. samples. Table 4 shows a summary of some direct comparisons. Sixteen replications on each of eight powders and each amount used were run. The powders are arranged according to their wettability, using 2.5-g. samples. The table shows that the standard deviations were for the most part greater when the 4-g. amount was used. It also shows that the greatest variability is found in the middle wettability range. Both sample sizes ranked the powders roughly in the same order of wettability. Arc sine transformations were used since the extreme values expressed as percentages tend to show minimum variation, whereas values close to 50% show maximum variation, because of the relationship between mean and variance in such data. Analysis of variance assumes that every treatment is subject to the same variance, a condition which is approximately achieved by arc sine transformation *(2)*.

TABLE 4

A comparison of the reproducibility of 4-g. compared with 2.5-g. samples (Each value is the mean of 16 replications)

Mean wettabilities		Arc sine transformations			
4 g.	2.5 g.	4-g. samples		2.5-g. samples	
(%)	*(%)*	*(Mean)*	*(s)*	*(Mean)*	*(s)*
10.3	16.3	18.7	2.3	23.8	1.1
43.7	46.6	41.4	7.3	43.0	6.3
63.2	59.0	52.6	10.9	50.2	4.6
63.5	63.1	52.8	6.4	52.6	4.3
75.8	65.1	60.5	6.6	53.8	7.8
80.9	75.2	64.1	8.8	60.1	6.2
91.3	89.8	72.8	2.4	71.4	2.8
93.9	91.2	75.7	2.7	72.7	3.6

Effect of water temperature. In the range between 20° and 30° C. the wettability increased on the average about 2% per 1° C. rise in temperature of the water. This value was calculated in parts per 100 of the wettability value, which was given in per cent. Poorly wettable powders were not affected greatly by water temperature in the absolute amount dissolved. The use of 25° C. water gave slightly better reproducibility than either the 20° or 30° C. water.

In general the powders which had been stored at 85° F. were benefited more by the higher temperature water than were those which had been stored at 45° F. Most powders showed considerable increases in wettability when the temperature of the water was increased to 40° C.

Variations in the method for separating undispersed powder. A critical step in the procedure seemed to be that of separating the undispersed from the dispersed powder. This straining procedure must be done rapidly and with a minimum of agitation. One possible reason for poor reproducibility of the method in the middle wettability range (50-70%) is that powders falling in this range are slow to strain through the Gooch crucibles. As a result of this, an attempt was made to improve the method of separation.

Straining through plugs of cotton or sintered stainless steel crucibles failed to show any improvement. Then it was found that when a piece of 16-mesh screen (window screen) was slipped down the pouring edge of the beaker, practically all the undispersed powder remained in the beaker. This modification was found to greatly improve the reproducibility in the middle range of wettability.

TABLE 5

Comparison of the variance calculated from 4 replications for each of 3 modifications of the wettability method

Wettability average	Variance found		
	Plain method	Screens added	Second filter
(%)			
57	599	637	139
63	4,015	725	2,055
73	2,250	2,955	481
97	797	821	27
Av.	1,916	1,284	676

A second modification which has proved to be even better is that of dropping a second filter paper over the partially dispersed powder just before straining the liquid through the Gooch crucible. It is necessary that the edges of this filter be wetted by the liquid underneath. It will then gently hold back all traces of undispersed powder. For this modification, 400-ml. beakers were used together with 7-cm. filter paper. Table 5 shows some of the comparisons made.

SUMMARY

A method for measuring the first step in the dispersion of milk powder, that of wetting the powder with water, has been discussed. The standard procedure described is simple to use and has a reproducibility within approximately

5%. The method shows great sensitivity as a test for determining the manufacturing and storage conditions of whole milk powders. It has been used over a period of more than 4 years, during which time several thousands of determinations have been made.

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METHODS OF DETERMINING THE PER CENT TOTAL SOLIDS IN MILK BY MEANS OF THE LACTOMETER[1]

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There are a number of sources of errors involved in the estimation of total solids from the lactometer reading and fat test. Generally, these may consist of errors in making the lactometer reading, errors in determining the fat content, the particular formula used to calculate the results, and possibly the composition of the milk. If the calculated total solids are to be compared with the observed total solids, then the errors involved in the determination of total solids must also be considered.

The purpose of this investigation was to establish suitable methods whereby such errors may be held to a minimum and to test the formula derived by Sharp and Hart *(7)* and later modified by Herrington *(4)* used in estimating the percentage of total solids by lactometry. Because of its general use, the Babcock short formula was also included in this study.

METHODS

Lactometer.[7] The preferred lactometer is 31.0 cm. long; the stem is 14.5 cm. long and 5 mm. in diameter. The bulb is 35 mm. in diameter. The lactometer is made of Pyrex glass, calibrated to read in 0.1° Quevenne at 60° F/60° F. The scale ranges from 13 to 28.

Lactometers of this type were used by all collaborators except collaborator B, whose lactometer was broken in shipment.

Each hydrometer was standardized as follows: 61.31 g. of sucrose and 10 mg. methylene blue were dissolved in freshly distilled water in a liter flask and the contents made to 1 liter with freshly distilled water. This solution was warmed to exactly 30° C. and a portion was transferred to a cylinder in a water bath at 30.0° C. The lactometer was allowed to stand in the water bath for 5 minutes, quickly dried, and slowly immersed in the sucrose solution. Corrections were made on the basis of a specific gravity of 1.0202, which was the specific gravity of the sucrose solution determined at 30.0/15.5° C. in a pycnometer.

A similar procedure was used for calibrating the lactometer to be used at 15.5° C. except that a solution of sucrose containing 82.40 g. sucrose per liter

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was used. This solution as determined at 15.5/15.5° C. in a pycnometer had a specific gravity of 1.0318.

The thermometer was checked in a melting ice bath or against an N.B.S. thermometer. The well-mixed sample of milk was brought to a temperature of 15.5° C. and gently poured down the side of a glass graduate (no smaller than 50 mm. in diameter) which was supported in a water bath maintained at 15.5° C. The lactometer and cylinder were thoroughly tempered at 15.5° C. The cylinder was filled with milk so that any foam present could be blown off. The lactometer was quickly removed from the water bath, dried, and slowly submersed in the milk so that very little milk was on the exposed stem of the lactometer at the time of reading. The lactometer was read to the top of the meniscus as soon as it came to rest, recording to the nearest 0.1°.

The milk was warmed to 45° C., held for 2 minutes, and cooled to 30° C. The lactometer and the cylinder were tempered and maintained at 30.0° C. Duplicate determinations were made.

Babcock test. The A.D.S.A. modification *(3)* was used in this investigation. The test was made after completing both lactometer readings.

Presence of total solids. This determination also was made after completing the lactometer readings by the Mojonnier method *(6)*.

Several cans of the same lot of evaporated milk were supplied to each collaborator to be run with each group of tests. One gram of this was weighed and 1 ml. of water was added prior to heating on the hot plate.

General. Approximately 1 qt. of milk was required for analysis. It represented herd milk rather than milk from individual cows or milk from many herds. The importance of thoroughly mixing samples of milk before removing aliquots for individual tests was emphasized.

All results were reported in duplicate and when data were accumulated from at least 12 samples of milk, the following formulas were used for calculating total solids on the basis of the fat test and lactometer readings:

Short Babcock:

$$\% \text{ T.S.} = 0.25 \times (\text{Quevenne } 15.5^\circ \text{ C.}) + 1.2 \times (\% \text{ fat})$$

Sharp and Hart (Herrington modification):

$$\% \text{ T.S.} = 0.2594 \times (\text{Quevenne } 30^\circ \text{ C.} + 3.0) + 1.2648 \times (\% \text{ fat})$$

This equation may also be written as:

$$\% \text{ T.S.} = 0.7782 + 1.2648 \times (\% \text{ fat}) + 0.2594 \times (Q_{30})$$

RESULTS

Table 1 presents the specific gravity of the 6% and 8% sucrose solutions obtained with a pycnometer and a lactometer at various temperatures. These results indicate that the lactometer should read 20.2 on a 6% sucrose solution at 30° C. Lactometer readings made on milk at 30° C. were corrected by the amount of the difference between 20.2 and the reading obtained with the lactometer on 6% sucrose solution at 30° C. Similarly, corrections were applied to the readings made at 15.5° C.

TABLE 1

Specific gravity of 6% and 8% sucrose solutions (average of three trials at Laboratory A)

Method	Sucrose	15.5°/15.5° C.	20°/15.5° C.	30°/15.5° C.	20°/20° C.	30°/30° C.
Pycnometer	6%	1.0237	1.0228	1.0202	1.0230	1.0239
	8%	1.0318	1.0310	1.0281	1.0312	1.0318
Lactometer ‡0 R & B	6%	1.0238	1.0231	1.0208	.....	.....
	8%	1.0320	1.0311	1.0286	.....	.....

Obtained from Bureau of Standards Circular 44 (1918)
6% solution 20°/20° C.—1.02366 (61.31 g. per liter)
8% solution 20°/20° C.—1.03176 (82.40 g. per liter)

Table 2 shows the results obtained by Laboratories A and C, A and D, and A and E, each using the same lactometer but preparing its own sucrose solution. The results obtained by Laboratory B on a different lactometer are presented also. The correction applied by Laboratory B was therefore −0.5; by Laboratory C, −0.1; by Laboratory D, −0.2; and by Laboratory E, −0.1° Q.

Table 3 presents results obtained by five laboratories on different samples of herd milk using methods outlined above. Although the use of evaporated milk as a control in testing whole milk is open to criticism, on 68 trials the results ranged from 25.85 to 26.78%, which would indicate that good precision in determining total solids by the Mojonnier test is lacking. Laboratory D has the greatest standard deviation from the mean on the evaporated milk sample and also the greatest standard error of estimate for total solids calculated by the Sharp and Hart equation.

This may also account for the large value of the constant in the regression equation obtained from the data of Laboratory D. The total solids of the evaporated milk sample reported by Laboratory E were low, and this may account for the low value of the constant in the regression equation obtained from the data of Laboratory E.

When the regression equation calculated from the data of each laboratory is applied to the data from which it was derived, there is a decrease in the value

TABLE 2

Standardization of lactometer at 30° C. with 6% sucrose solution

Lactometer No.	Lab. A	Lab. B	Lab. C	Lab. D	Lab. E
R & B ‡638	20.3	.....	20.3	.....	.....
	20.3	.....	20.3	.....	.....
	20.3	.....	20.3	.....	.....
R & B ‡641	20.4	.....	.....	20.4	.....
	20.4	.....	.....	20.4	.....
	20.4	.....	.....	20.4	.....
Kimble ‡559	.....	20.7	.....	.....	.....
	.....	20.7	.....	.....	.....
	.....	20.7	.....	.....	.....
R & B ‡636	20.3	.....	.....	.....	20.3
	20.3	.....	.....	.....	20.2
	20.4	.....	.....	.....	20.3

TABLE 3
Summary of results

Laboratory		A	B	C	D	E	Mean (All data)
Number of Samples		19	19	12	16	12	78*
Mean Mojonnier total solids		13.183	12.207	12.930	12.828	12.351	12.706
Mean Babcock fat		4.380	3.636	3.918	4.106	3.500	3.936
Mean lactometer 30° C.		26.900	26.526	27.242	26.150	27.033	26.729
Mean total solids calculated by formula of	Babcock	12.938	12.168	12.794	12.678	.....	12.627
	Sharp & Hart	13.297	12.259	12.800	12.755	12.217	12.690
	Regression equation	13.185	12.206	12.930	12.829	12.351	12.706
Std. error from Moj. T. S. based on formula of	Babcock	±0.224	±0.137	±0.158	±0.277	.....	±0.206
	Sharp & Hart	±0.186	±0.130	±0.149	±0.225	±0.167	±0.176
	Regression equation	±0.145	±0.101	±0.069	±0.206	±0.095	±0.174
Evaporated milk control sample	No. trials	10	20	8	28	2	68
	Mean T. S.	26.264	26.124	26.239	26.153	26.005	26.164
	Std. dev. from mean	±0.047	±0.060	±0.072	±0.215	±0.161	±0.154

* Number of samples for Babcock calculated total solids = 66

of the standard error of estimate. However, when the regression equation derived from all the data is applied, the standard error of estimate is not significantly different from the value obtained by the Sharp and Hart formula.

Table 4 presents regression equations for each laboratory for estimating total solids based on the Babcock fat test. The standard error of estimate for the data is ± 0.327% total solids.

Table 5 presents regression equations for each laboratory for estimating total solids based on the Babcock fat test and the lactometer reading at 30° C. The standard error of estimate for the data is ± 0.174% total solids.

TABLE 4
Regression equations (calculated Mojonnier total solids based on Babcock fat test)

Laboratory	No. samples	Regression equations
A	19	T.S. = 5.5605 + 1.7483 × (fat)
B	19	T.S. = 8.1573 + 1.1242 × (fat)
C	12	T.S. = 7.8153 + 1.3056 × (fat)
D	16	T.S. = 7.2257 + 1.3468 × (fat)
E	12	T.S. = 7.6240 + 1.3595 × (fat)
All data	78	T.S. = 7.3073 + 1.3717 × (fat)

Table 6 presents data obtained at Laboratory A on the effect of increasing lactose concentration in milk. These data are limited but indicate that the addition of lactose to the milk influences the calculation of total solids by the Sharp and Hart equation.

The data in Table 7, obtained by Laboratory A, include the protein content, along with the fat, total solids, and lactometer reading, on 14 samples of milk. Protein was determined by the Kjeldahl Arnold Gunning method *(1)* with a

standard error of ± 0.098%. The regression equation for total solids using fat and lactometer reading was found to be:

$$\%\ \text{T.S.} = 0.6688 + 1.2358\ (\%\ \text{fat}) + 0.2645\ (Q_{30})$$

The multiple correlation coefficient was +0.995 with a standard error of estimate of ± 0.138% total solids.

When the per cent protein was included, the regression equation was found to be:

$$\%\ \text{T.S.} = 0.7070 + 1.1514\ (\%\ \text{fat}) + 0.1993\ (Q_{30}) + 0.6585\ (\text{protein})$$

The multiple correlation coefficient was +0.998 with a standard error of estimate of ±0.080.

TABLE 5

Regression equations (calculated Mojonnier total solids based on Babcock fat test and lactometer [30° C.] reading)

Laboratory	No. samples	Regression equations
A	19	T.S. = 1.1526 + 1.2638 (fat) + 0.2426 (lact. 30°)
B	19	T.S. = 0.8462 + 1.3685 (fat) + 0.2406 (lact. 30°)
C	12	T.S. = 1.0532 + 1.2732 (fat) + 0.2529 (lact. 30°)
D	16	T.S. = 3.8246 + 1.2508 (fat) + 0.1479 (lact. 30°)
E	12	T.S. = 0.3238 + 1.3365 (fat) + 0.2718 (lact. 30°)
All data	78	T.S. = 1.0467 + 1.2566 (fat) + 0.2512 (lact. 30°)

TABLE 6

Effect of addition of lactose in milk on determination of T.S. by lactometer (obtained by Laboratory A)

Sample	% added lactose	Lactometer 15.5°C.	Lactometer 30.0°C.	Fat	Moj. T.S.	Calc. T. S. Bab.	Calc. T. S. S & H	Calc. T. S. Est.	Moj.–Bab.	Moj.–S&H	Moj.–Est.
328	0	30.2	26.1	3.63	12.04	11.91	12.14	12.17	.13	–.10	–.13
	.5	31.8	27.9	3.65	12.48	12.32	12.62	12.64	.12	–.14	–.16
	1.0	33.7	29.6	3.67	12.87	12.85	13.11	13.12	.02	–.24	–.25
	1.5	35.4	31.3	3.64	13.30	13.22	13.50	13.50	.08	–.20	–.20
329	0	29.0	24.9	3.41	11.74	11.34	11.55	11.60	.40	.19	.14
	.5	30.8	26.4	3.42	12.10	11.80	11.94	11.99	.30	.16	.11
	1.0	32.7	28.2	3.41	12.52	12.21	12.40	12.44	.31	.12	.08
	1.5	34.3	30.0	3.40	12.85	12.66	12.73	12.88	.19	.12	–.03
330	0	28.1	23.5	3.51	11.48	11.24	11.29	11.34	.24	.19	.14
	.5	29.9	25.4	3.50	11.93	11.68	11.79	11.83	.25	.14	.10
	1.0	31.7	27.1	3.50	12.36	12.13	12.24	12.26	.23	.12	.10
	1.5	33.6	29.0	3.50	12.80	12.60	12.73	12.75	.20	.07	.05

(In 1st and 2nd trials, lactose added directly to milk — in 3rd trial lactose dissolved in 100 ml. water and added to 600 ml. milk — water added to milk for blank) Results obtained in duplicate and averaged.

DISCUSSION

On the 78 samples tested by five laboratories, the regression equation obtained by the method of Fisher described in Brownlee *(2)* was found to be:

$$\%\ \text{T.S.} = 1.0467 + 1.2566 \pm 0.0133\ (\%\ \text{fat}) + 0.2512 \pm 0.0182\ (Q_{30})$$

TABLE 7

Effect of protein on lactometric determination of total solids (obtained by Laboratory A)

Sample No.	% fat	Moj. T. S.	Lactometer		% protein	Ratio SNF to protein	Calculated T.S.		Moj.– Bab.	Moj.– S&H
			15.5°C.	30°C.			Bab.	S&H		
11755	4.41	11.88	26.1	21.8	2.74	2.74	11.81	12.01	0.07	–0.13
11757	4.71	13.96	32.8	28.4	3.34	2.77	13.85	14.10	0.11	–0.14
7506	4.81	13.47	30.3	26.6	3.11	2.78	13.35	13.76	0.12	–0.29
8815	4.16	12.05	27.7	22.7	3.02	2.61	11.96	11.93	0.09	0.12
8816	3.33	9.29	20.4	17.6	1.92	3.10	9.10	9.56	0.19	–0.27
8818	5.69	14.79	31.6	26.8	3.10	2.83	14.73	14.93	0.06	–0.14
10773	4.51	12.16	26.7	21.6	2.80	2.73	12.09	12.09	0.07	0.07
3	5.09	14.43	33.3	28.4	3.56	2.62	14.45	14.58	–0.02	–0.15
6153	5.25	14.46	32.4	27.3	3.47	2.65	14.40	14.50	0.06	–0.04
6154	5.01	14.47	33.0	27.9	3.53	2.68	14.26	14.35	0.21	0.12
5	4.86	14.24	33.4	29.0	3.20	2.93	14.18	14.45	0.06	–0.21
4422	5.13	13.34	28.6	24.6	2.90	2.83	13.31	13.65	0.03	–0.31
4426	4.66	12.48	27.6	23.2	2.77	2.82	12.49	12.43	–0.01	0.05
4444	3.43	11.81	30.4	26.0	2.90	2.89	11.72	11.86	0.09	–0.05

At the 95% level of probability, the partial regression coefficients for the fat would range from 1.2300 to 1.2832, and for the lactometer would range from 0.2144 to 0.2880. It is obvious, then, that the above equation is not significantly different from the Sharp and Hart equation as modified by Herrington. These results based on direct analysis, therefore, substantiate the equation derived from the specific gravity of the milk constituents by Sharp and Hart and the calculations of Herrington of the effect of using a lactometer instead of a Westphal balance.

It should be noted that the above results were obtained with the lactometer reading made at 30.0° C. No attempt has been made to establish temperature correction factors, since it is felt that for practical purposes the temperature of the sample many be thermostatically controlled. Since the data contain readings at both 15.5° C. (60° F.) and 30.0° C. (86° F.), it is interesting to note that the decrease in degree Quevenne per increase in degree F. varied from a minimum of 0.138° Q. per 1° F. to a maximum of 0.219° Q. per 1° F. with an average of 0.175° Q. per 1° F. applied at 60° F. in the Babcock formula.

It should also be noted that while the mean per cent of total solids calculated by the Babcock formula was found to be 12.627, it does not differ significantly at the 95% level from the value of 12.690% for the Sharp and Hart formula, based on the Student T test. The slightly better agreement calculated by the Sharp and Hart equation is due either to the more accurate partial regression coefficients or to the physical state of the fat in the Sharp and Hart method. In this connection and although the results reported in this study were made by heating the milk to 45° C. and cooling to 30° C., the results reported by Sharp and Hart would indicate that the heating to 45° C. may be unnecessary. They reported that the average of 30 experiments in which milk was held at 20° C. and warmed to 30° C. resulted in a specific gravity of 1.03008. The same milk held ½ minute at 45° C., then cooled to 30° C., resulted in a specific gravity of 1.02998. This point should be investigated further, since the elimination of the step of heating to 45° C. would simplify the method.

Another point of interest is the regression equation obtained from estimating the per cent of total solids from the butterfat content. This equation is:

$$\% \text{ T.S.} = 7.3073 + 1.3717 \ (\% \text{ fat})$$

The standard error estimate in using this equation on the 78 samples tested was $\pm 0.327\%$ and indicates that a significant increase in accuracy is obtained when the lactometer reading is included in the calculations. When the Jacobson *(5)* formula was applied to these same data, the standard error of estimate was found to be $\pm 0.436\%$. Thus, equations for estimating per cent of total solids from the per cent of fat alone would yield rather unsatisfactory results.

The use of suitable experimental controls in a study of this nature is complicated by the necessity of dealing with unhomogenized whole milk. The shipment of samples of fluid whole milk usually results in some degree of churning. Studies of the precision of the method were therefore made by one laboratory.

On a total of 20 trials, the precision of the Modified Babcock test was found to be $\pm 0.042\%$, of the lactometer reading was found to be $\pm 0.077°$ Q., and of the Mojonnier total solids was found to be $\pm 0.060\%$. These values indicate that the lactometric method should yield results with a precision of $\pm 0.106\%$ total solids.

Although the data in Table 7 are limited, the standard error of estimate using fat and lactometer reading only resulted in a value of $\pm 0.138\%$. When protein was included, the standard error of estimate was found to be $\pm 0.080\%$. Since the precision of the method would account for an error of $\pm 0.106\%$, as indicated in the previous paragraph, the square root of the sums of the squares of the variations due to protein and to errors in the method is equal to $\pm 0.136\%$, or approximately the same as the value of the total error of estimate found for the data of Table 7.

CONCLUSIONS

1. Methods are presented for determining per cent of total solids by means of the lactometer and the A.D.S.A. Modified Babcock test.

2. The per cent total solids was calculated on 78 samples by means of the Babcock formula (mean = 12.627%, standard error of estimate = $\pm 0.206\%$) and the Sharp and Hart equation as modified by Herrington (mean = 12.690%, standard error of estimate = $\pm 0.176\%$) and compared with the Mojonnier total solids (mean = 12.706%).

3. The regression equation derived from 78 samples of milk was shown to substantiate the partial regression coefficients in the Sharp and Hart equation as modified by Herrington.

4. The protein content of the milk was shown by the results of one laboratory to have some influence on the accuracy of the determination of total solids by lactometry.

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EFFECTS ON ECONOMY AND EFFICIENCY OF MILK PRODUCTION WHEN THYROPROTEIN IS FED FOR A SHORT PERIOD OF TIME TO MILKING COWS

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No carefully planned experiments with milking cows have been reported which show the effects of feeding thyroprotein on the efficiency of feed utilization and monetary returns. Several articles have appeared in which information on feed utilization was reported, but this was not the primary object of the studies *(2, 13, 15, 16, 17, 18)*. Consequently, the results of these experiments have not provided an adequate basis for the proper evaluation of the economy of feeding thyroprotein under practical conditions.

The data reported show that cows given thyroprotein produced more milk, on the average, per pound of total digestible nutrients (TDN) consumed than similar cows that were not fed thyroprotein, when changes in body weight were not considered *(2, 6, 13, 15, 16, 17)*. In some of these experiments there was much more variability in efficiency of milk production among cows on the same treatment than between the groups of cows on different treatments *(2, 6, 15)*. When body weight changes were taken into account, production efficiency was somewhat reduced in some cases *(2, 6, 16, 17)* but not in others *(13, 15, 18)*. When thyroprotein was fed for successive lactations, the efficiency of production decreased in the later lactations *(17)*. These results indicate that it is undesirable to feed thyroprotein for long periods of time, especially in successive lactations *(1, 5, 9, 17)*.

Experimental evidence indicates that large doses of thyroprotein may decrease dry-matter digestibility and increase rate of passage of food through the gut *(5, 12)*. Nitrogen balance was decreased when feed intake was limited *(11, 12)*, but it was positive when feed intake was more liberal *(12)*. Basal heat production was increased in calves and cows fed large amounts of thyroidally-active materials *(11, 18)*, and these authors calculated that the extra feed intake per unit of extra milk produced was markedly above normal. This is true for any increased feed intake when it is fed in addition to a normal total requirement.

However, the dairyman is primarily interested in the ratio of total milk produced to total feed consumed. This ratio is usually termed gross efficiency and is conveniently expressed as fat corrected milk (FCM) produced per pound of TDN consumed. The experiments reported in this paper were performed in order to obtain more information on this relationship.

Thyroprotein is presently sold as a commercial product in the form of a pelleted concentrate mixture which contains 5 g. of iodinated casein per pound.

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[1] Present address: Montana Agricultural Experiment Station, Bozeman.

[2] Present address: Tennessee Agricultural Experiment Station, Lewisburg.

It is recommended by the manufacturer that the concentrate mixture be fed at the rate of 3 lb. per cow per day in addition to the usual ration. A comparison between the cost of this extra concentrate and the value of the extra milk produced is easily made, but such a comparison is an incomplete measure of true monetary return and actual efficiency of milk production. Two additional factors that should be considered when making such a comparison are: *(a)* TDN necessary for body-weight changes and *(b)* any increased consumption of roughages, pasture, or other feed that is fed without being weighed when the thyroprotein-containing concentrate also is fed. These experiments were planned to obtain information on these factors.

EXPERIMENTAL PROCEDURE

Three experiments (A, B, and C) were performed, all having a similar general plan. Experiment A was carried out at Huntley, Mont., during the winter of 1952-53; Experiment B at Bozeman, Mont., during the winter of 1953; and Experiment C at Lewisburg, Tenn., during the winter of 1951.

In all experiments daily records were kept on milk production and the amount of each feed that was fed and refused. Feed and weigh-back samples for analysis were obtained every third day for 150 days in Experiment A and every day for 120 days in Experiment B, and the samples from both experiments were composited by 30-day periods for proximate analysis. TDN values were calculated from these analyses by means of appropriate digestion coefficients *(10)*. In Experiment C, TDN values were assumed from a description of the feeds and refusals.

Butterfat tests were obtained on a 2-day composite milk sample taken on the fifth and sixth days of each 10-day period and also at monthly intervals before and after the experimental period in Experiment C.

Body weights were taken on three consecutive days at 10-day intervals in Experiments A and B and at monthly intervals, or whenever there was a change in feeding regime, in Experiment C.

The experiments were divided into pre-experimental, experimental, and post-experimental periods. This and other pertinent information on the experimental design and cows are presented in Table 1. At the end of the post-experimental period the cows in Experiments B and C were placed on pasture. Data obtained while the cows were on pasture were not used in calculating production efficiency or monetary returns, although the trends in production and body weight are shown in Figures 1 and 3 for the pasture period.

Enough hay was fed that there was a large refusal (8 to 35%, depending on its quality). In Experiments A and B the hay was alfalfa and in Experiment C it was a red clover–orchard grass. A small amount of corn silage was fed to all cows in Experiment A. In all experiments grain was usually fed at the rate of 1 lb. to 4 lb. milk to Holstein cows and at the rate of 1 to 3 to Jersey cows.

In short-term trials, as in these experiments, the effect of thyroprotein feeding on efficiency of production and monetary returns should be determined by

TABLE 1

Data on the design and cows used in three experiments

Experiment No.	A				B				C		
Group No.	1	2	3	4	1	2	3	4	1	2	4
No. of cows	4	4	4	4	4	4	4	4	5	5	5
Breed of cows	Holstein				Holstein & Jersey				Jersey		
Av. days in milk[a]	79	86	86	93	80	75	105	94	112	93	104
Av. age in months[a]	62	65	62	64	53	38	52	51	70	64	65
Extra grain *(lb.)*[b]	0	0	3[c]	3[c]	0	0	3	3	0	0	3
Thyroprotein *(g.)*[b]	0	15	0	15	0	15	0	15	0	15	15
Pre-exp't. period *(days)*	30				30				10		
Experimental period *(days)*	60				60				60		
Post-exp't. period *(days)*	60				30				5		

[a] At beginning of pre-experimental period.
[b] Fed during the experimental period per cow per day.
[c] Fed during the experimental period per cow per day plus 10 days of post-experimental period.

combining the data for the experimental thyroprotein-feeding period and the data for a reasonable post-experimental period. The post-experimental period should extend beyond the period of rapid weight increase which occurs when thyroprotein is withdrawn, since the composition of this rapid gain and also the large weight losses which occur when thyroprotein is fed cannot be assumed to be normal *(5, 6, 15, 17)*. The usual correction factors for gain or loss in weight are not, therefore, applicable for the purpose of assigning a TDN value to these immediate weight changes. On the other hand, the composition of the body probably is approximately the same at 1 or 2 months after thyroprotein was fed as it was before it was fed. Therefore, the usual TDN values for the small difference in weight at these two times were used without reservations *(8)*. Also, a lower than normal level of production occurs for 30 to 60 days after the removal of thyroprotein *(1, 4, 6, 9, 16)*. In calculating returns in these experiments, the authors considered the lowered level of production after thyroprotein feeding was discontinued and also the increased production during thyroprotein feeding. These factors (production and weight changes) were properly taken into account by combining the data for the thyroprotein-feeding period with the post-experimental period in calculating efficiency and monetary returns.

RESULTS AND DISCUSSION

Effect on production. All cows increased in milk production after thyroprotein was added to the ration. The average increase for 26 cows (Table 2) fed thyroprotein was 5.1 and 3.9 lb. FCM per day per cow for the first and second months, respectively, of thyroprotein feeding when calculated according to the method mentioned by Baily *et al. (3)*. The 23 control cows decreased 10.2%, whereas the cows fed thyroprotein increased 11.7% between the pre-experimental and the first experimental 30-day period. The net difference in level of

TABLE 2

Average daily FCM production before and during thyroprotein feeding and the relative change when it was discontinued

Group and ration fed	Period			10 to 40 days post-exp't. as % of pre-experimental period	
	Pre-exp't.	1st 30 day exp't.	2nd 30 day exp't.		
		(lb/day/cow)		Exp't. A & B	Exp't. C
1 (Control)	34.3	30.8	27.1	72.6	72.2
2 (Thyro)	35.2	39.3	32.9	54.8	76.8
3 (Extra grain)	38.6	34.7	31.9	67.9	—
4 (Extra grain and thyro)	34.0	38.0	35.3	59.9	80.6

production between control and thyroprotein groups was 21.9 and 18.1% for the first and second experimental months, respectively, when compared to the pre-experimental period.

The immediate response in milk production was not enhanced by the feeding of extra grain with thyroprotein. This is indicated by the similar responses shown in Figure 1 and in Table 2 for Groups 2 and 4. Holstein cows showed a larger response than Jersey cows ($P < 0.01$), but the higher initial production

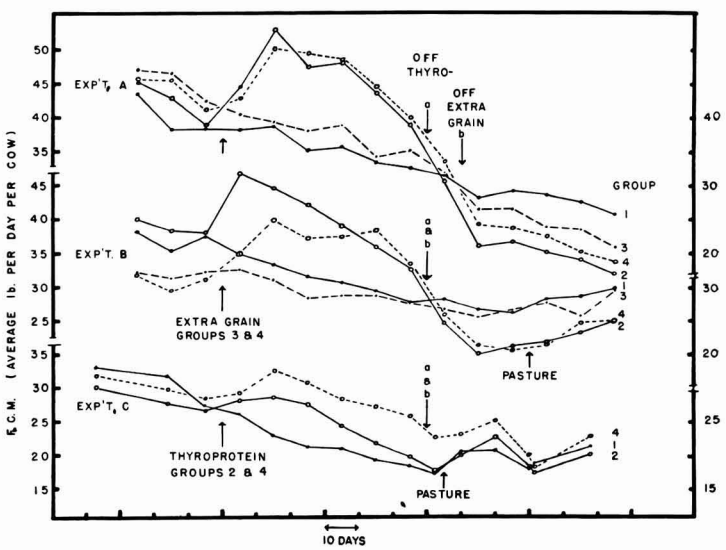

FIG. 1. Trends in average milk production for the three experiments showing the effects of feeding thyroprotein to Group 2, feeding extra grain to Group 3, and feeding both thyroprotein and extra grain to Group 4 compared to the normally fed cows in Group 1.

of the Holstein cows may have been wholly or partially responsible for the difference in breed response. The maintenance of increased levels of production varied widely between individual cows. It remained above pre-experimental level for 10 days with one cow and for 60 days with nine cows. FCM production during the last 10 days of thyroprotein feeding averaged 93% of the last 10-day pre-experimental level with a range from 38 to 130%. The large variation would seem to preclude the feeding of this material to all animals in a herd. A dairyman should discontinue feeding thyroprotein to cows whose response is unprofitable.

In Experiment A, the cows in Group 4, which were fed thyroprotein and extra grain, did not maintain the increase any better than did the cows in Group 2, which received no extra grain. However, in Experiments B and C, the cows in Group 4 showed a tendency to maintain the increased level better than the cows in Group 2. The control cows that received extra grain, Group 3, were no more persistent than were the cows in Group 1; this was especially true in Experiment A. Previously, it has been shown that the feeding of extra grain (more than in this experiment) to cows in early stages of lactation had a marked effect on increasing persistency *(16, 17)*. The feeding of extra grain (3 lb. per day) to cows fed thyroprotein in Group 4 for 2 months did not have the marked effect of increasing persistency which has been observed previously *(7, 15, 16, 17)*. The stage of lactation, length of experiment, amount of extra grain, or individual cow variation may have been responsible for these differences.

A marked reduction in level of milk production occurred when thyroprotein was withdrawn. In Experiments A and B, the production of Groups 2 and 4 was below that of the control groups at 30 and 60 days after the withdrawal of thyroprotein. This is shown in Figure 1 and in Column 5 of Table 2. Production levels of these cows in the entire post-experimental period was below the levels of control cows or the projected levels based on pre-experimental production. There was more total FCM produced for the 60-day experimental period plus a 30- or a 60-day post-experimental period by the two groups fed thyroprotein than was produced by the two control groups. However, with the continued lower level of production for the remainder of the lactation it appears doubtful that any increased amount of milk was obtained for the entire lactation for the cows fed thyroprotein in this experiment. Investigations in England showed only a slight or no increase in total milk per lactation when thyroprotein was fed under similar conditions *(1, 9)*.

There was no decrease in level of production when thyroprotein was withdrawn in Experiment C and the cows were turned to fresh spring pasture 5 days later. This is shown in Figure 1 and in Column 6 of Table 2. It is apparent that the procedure in this experiment probably increased the yield of milk for the entire lactation. Placing cows on pasture 30 days after the removal of thyroprotein, as was done with Groups 2 and 4 in Experiment B, did not increase their production up to the expected levels, as did the procedure used in Experiment C.

All cows in Experiments A and B showed an increase in butterfat test when

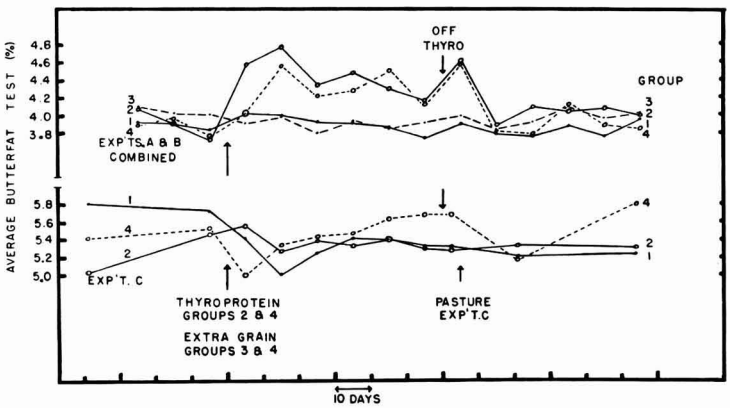

FIG. 2. Trends in butterfat tests as influenced by feeding thyroprotein alone (Group 2) and in combination with extra grain (Group 4), compared to control animals (Groups 1 and 3).

thyroprotein was added to the ration. Only a few cows in Experiment C showed a definite increase in fat test as a result of thyroprotein feeding. The average responses are shown in Figure 2. The average change was approximately 0.5% increase in actual fat test. There was a tendency, especially in Group 4 of Experiment C, for a better maintenance of the increased test when extra grain was fed. These results are similar to previously published observations *(2, 3, 4, 9, 15, 16, 17)*.

Effect on body weight. The feeding of thyroprotein caused a large and rapid decrease in body weight of each cow. Cows in the control groups showed only small changes in body weight. The average trends are shown in Figure 3.

The average body weight decrease for the 13 cows in Group 2 was 138 lb.

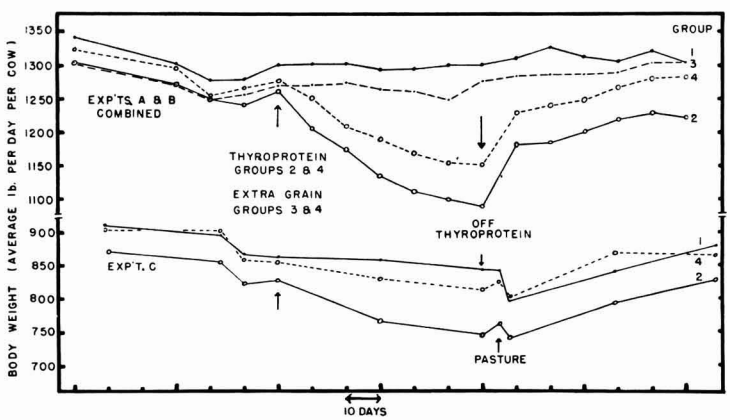

FIG. 3. Body-weight trends of cows fed thyroprotein (Group 2), extra grain (Group 3) and both thyroprotein and extra grain (Group 4) for 60 days compared to cows fed at normal rate (Group 1).

during the 60 days that thyroprotein was fed. The cows in Group 4 lost only 93 lb. during this period. The cows in Group 4 regained initial weight by 40 to 50 days after the removal of thyroprotein, but the cows in Group 2 had not regained initial weight by the end of the observation period. The cows in Groups 2 and 4 of Experiments A and B gained 8.5 lb. per day per cow during the 10 days immediately after the removal of thyroprotein. Control cows gained 0.8 lb. per day per cow during this period. In the short-term trials, the feeding of extra grain with the thyroprotein appeared to have a more pronounced influence on body weight and general appearance than on milk production.

TABLE 3

Efficiency of production as influenced by extra grain feeding and thyroprotein feeding, singly and in combination

Days	FCM per lb. TDN				FCM per lb. TDN corrected for weight change[a]			
	Group No.				Group No.			
	1	2	3	4	1	2	3	4
	Experiment A (Pre-experimental period)							
1-30	1.81	1.79	1.98	1.92	1.73	1.72	1.90	1.62
	(Thyroprotein fed to Group 2 & 4 — Experimental period)							
31-60	1.58	2.05	1.56	1.93	1.52	1.30	1.49	1.35
61-90	1.48	1.89	1.48	1.79	1.80	1.57	1.58	1.64
	(Thyroprotein removed — post-experimental period)							
91-120	1.37	1.12	1.25	1.21	1.51	3.95	1.50	2.86
121-150	1.26	.87	1.12	.95	1.32	1.08	1.45	1.24
Av. 31-120	1.48	1.70	1.46	1.66	1.52	1.56	1.52	1.60
Av. 31-150	1.42	1.50	1.38	1.49	1.47	1.46	1.50	1.52
	Experiment B (Pre-experimental period)							
1-30	1.82	1.92	1.68	1.62	1.90	1.84	1.74	1.70
	(Thyroprotein fed to Groups 2 & 4 — Experimental period)							
31-60	1.59	2.25	1.52	1.78	1.55	1.53	1.52	1.43
61-90	1.42	1.76	1.37	1.74	1.46	1.43	1.41	1.43
	(Thyroprotein removed — post-experimental period)							
91-120	1.31	1.04	1.29	1.11	1.34	2.05	1.33	2.09
Av. 31-120	1.44	1.67	1.39	1.55	1.46	1.54	1.42	1.49
	Experiment C (Pre-experimental period)							
1-10	1.66	1.64	—	1.73	1.55	1.84	—	1.60
	(Thyroprotein fed to Groups 2 & 4 — Experimental period)							
11-40	1.36	1.72	—	1.53	1.32	1.28	—	1.39
41-70	1.13	1.33	—	1.33	1.05	1.19	—	1.24
	(Thyroprotein removed — post-experimental period)							
71-75	0.95	1.15	—	1.30	0.95	4.30	—	2.34
Av. 11-75	1.22	1.49	—	1.43	1.17	1.28	—	1.34

[a] Corrected for TDN necessary for body weight changes, according to the following:

$$\frac{\text{FCM produced}}{\text{TDN consumed} + 2.73 \times \text{weight loss or} - 3.53 \times \text{weight gain.}}$$

A relation between increase in milk production and decrease in body weight was noted. The correlation coefficient was −0.67, and the regression line was found to be body weight decrease = 7.44X + 38.75. The relationship was statistically significant (P = 0.01) but there were large variations from the calculated regression line.

Effect on feed consumption. It was expected that the hay consumption of Group 2 might increase when thyroprotein was fed, but no such increase was found. The hay consumption of the normally fed control cows (Group 1) was the highest of all groups. Feeding extra grain to Group 3 caused a decrease in the hay consumption of these animals compared to that of Group 1. A similar trend was noted between Groups 2 and 4. In these experiments, roughage was fed ad libitum but in such a manner that consumption was measured. No increase in roughage consumption was found in cows fed thyroprotein as compared to cows not fed thyroprotein. This is shown by the average amounts of hay consumed, given in Table 4 for Groups 2 and 4. Herdsmen feeding the cows in two of these experiments commented that cows fed thyroprotein seemed too tired to eat.

Effect on efficiency of production. The gross efficiency of production for the three experiments is shown in Table 3. The first four columns of data were calculated without considering body weight changes and the second four columns were calculated considering body weight changes. Separate values are shown for the periods before, during, and after thyroprotein feeding. The values for the entire experimental plus the post-experimental periods are given on the lower line for each experiment.

During the pre-experimental period the average values for the groups were fairly close, and higher values for efficiency were noted for the groups that gave the most milk. As shown in Table 3, the values for each 30-day period following thyroprotein feeding and its withdrawal were erratic and overly influenced by body weight changes. However, when the experimental and post-experimental periods were considered as one unit, a more constant value was observed. This value has been used for comparative purposes because it is more reliable and reasonable. The reasons for its use have been mentioned previously.

Without consideration of body weight changes, the cows fed thyroprotein were more efficient than the control cows. However, when the TDN values of the changes in weight *(8)* were included in the calculations, there was a small increase in efficiency in Experiments A and B for the 31st to the 120th day. When a longer post-experimental period was included, as in Experiment A from the 31st to the 150th day of observation, there was no difference in the efficiency between control and thyroprotein-fed groups. There was an increase in efficiency for Groups 2 and 4 in Experiment C, where no decrease in production followed the removal of thyroprotein. The failure of cows fed thyroprotein to regain their expected levels of production within 1 to 2 months following its removal caused a large reduction in efficiency after the thyroprotein-feeding period. In practice, the extent and value of this handicap will vary with each individual situation.

TABLE 4

Average production, consumption and body weight changes, and calculated returns above feed costs, showing the probable advantage due to the feeding of thyroprotein

Group No.	Milk	Average test	Feed consumed		Body weight change	Return above feed cost[a, b]	Advantage of feeding thyroprotein[b]
			Alfalfa	Grain			
	(lb/day /cow)		*(lb/day /cow)*	*(lb/day /cow)*	*(lb/day /cow)*	*($/day/ cow)*	*($/day/ cow)*
				Experiment A[d]			
30-day pre-experimental period							
1	42.72	3.59	24.01	11.17	−0.375	1.33	—
2	44.30	3.71	27.21	11.08	−0.367	1.39	—
3	44.66	4.09	23.78	12.15	−0.342	1.58	—
4	46.46	3.65	25.10	11.52	−1.542	1.47	—
60-day experimental plus 30-day post-experimental							
1	36.16	3.54	29.31	8.65	+0.197	0.97	—
2[c]	38.60	4.04	27.22	10.10	−0.736	1.13	+.16
3	36.19	3.61	26.57	11.32	+0.258	0.95	—
4[c]	41.43	3.72	25.75	12.69	−0.325	1.15	+.20
60-day experimental plus 60-day post-experimental							
1	34.39	3.55	29.26	8.24	+0.208	0.90	—
2[c]	33.82	4.00	28.41	8.85	−0.254	0.90	00
3	33.08	3.64	26.92	10.01	+0.527	0.84	—
4[c]	36.60	3.69	27.12	10.94	+0.117	0.94	+.10
				Experiment B			
30-day pre-experimental period							
1	36.85	4.03	27.63	9.88	+0.225	1.22	—
2	38.73	4.01	26.86	10.20	−0.333	1.30	—
3	34.15	3.94	26.94	8.43	+0.183	1.11	—
4	30.90	3.99	26.85	8.15	+0.242	0.95	—
60-day experimental plus 30-day post-experimental							
1	29.48	4.12	32.48	7.89	+0.075	0.77	—
2[c]	31.33	4.62	30.00	9.17	−0.625	0.92	+.15
3	28.01	4.12	29.95	9.33	+0.111	0.69	—
4[c]	29.51	4.60	28.16	10.47	−0.297	0.80	+.11
				Experiment C			
10-day pre-experimental period							
1	21.69	5.76	21.61	7.56	−0.100	0.82	—
2	21.88	5.47	21.13	7.50	−0.125	0.80	—
4	23.16	5.51	20.59	8.02	−0.100	0.89	—
60-day experimental plus 5-day post-experimental							
1	17.86	5.27	24.74	6.15	−0.308	0.45	—
2[c]	20.16	5.40	22.10	6.94	−1.015	0.54	+.09
4[c]	23.53	5.43	23.73	10.75	−0.462	0.62	+.17

[a] Milk price used was $5.90/cwt at 4% with 8c differential per 0.1% butterfat. Costs of feed used were : Alfalfa = $42.00/ton; grain = $80.00/ton; silage = $16.00/ton; thyroprotein containing concentrate = $7.00/cwt.

[b] Corrected for body weight changes by assuming 1 lb. body weight loss = 2.73 lb. TDN; 1 lb. body weight gain = 3.53 lb. TDN and 1 lb. TDN = 0.3 lb. FCM.

[c] These groups received thyroprotein during the 60-day experimental period at 15 g/cow/day.

[d] Corn silage fed to all cows in Experiment A at 10 lb/cow/day.

If the abrupt decrease in milk production following the withdrawal of thyroprotein can be prevented, as it was in Experiment C, then there will be an increase in efficiency over the experimental plus the post-experimental period as a result of feeding thyroprotein.

In Experiments A and B the cows fed thyroprotein produced an average of 419 lb. more of FCM than did the control cows during the 90 days of experimental plus post-experimental periods. Their net TDN consumption was 185 lb. more per cow. This gave a ratio of 2.27 lb. of FCM per pound of extra TDN consumed. In Experiment C the cows in Groups 2 and 4 averaged 339 lb. more of FCM during the 65 days of experimental plus post-experimental periods than did the cows in Group 1. They consumed an average of 127 lb. more of TDN. This ratio was 2.66 lb. of extra FCM per pound of extra TDN. However, when the differences in body weight were considered (as indicated in footnote of Table 4) the values were 1.1 and 2.58, respectively. A normal value for an entire lactation is approximately 1.6 *(7, 15, 16, 17)*. These data are not in complete agreement with the calculations made by others *(11, 18)* which indicated that the extra milk produced per unit of extra feed consumed was smaller than normal.

Economic considerations. The weights of feed and milk and the prices used to calculate returns over feed costs are given in Table 4. Other values can be used in calculations where these values do not apply. The returns over feed costs during the pre-experimental period reflect level of production and were reasonably close between groups in each experiment.

There was an increased return over feed costs of approximately \$0.11 to \$0.20 per cow per day as a result of thyroprotein feeding for the experimental plus a 30-day post-experimental period in Experiments A and B. However, the returns for the experimental plus a 60-day post-experimental period in Experiment A show no gain for Group 2 and a reduced gain for Group 4. Since the rate of production decreased below its expected level and remained there after the removal of thyroprotein from the ration, this period of lowered production offset much of the value of the increased production during the period of thyroprotein feeding. If the lowered level of production continued for the remainder of the lactation there would be a decrease in profit for the entire lactation. This same result undoubtedly would have been shown in Experiment B, but these cows were placed on pasture and this value could not be estimated accurately. This method of comparing combined data of all periods which were influenced by thyroprotein feeding appears to be the only proper practical procedure that has been presented to date.

In Experiment C there was no reduction in production level after the removal of thyroprotein, and as a result there was a net increase in returns over feed costs for the experimental plus the short post-experimental periods. In this case, including the data for a longer post-experimental period would not change the net result nor the values given in Table 4.

Whether or not there was an increased efficiency or monetary return as a result of feeding thyroprotein depended on the prevention of the large decrease in production after thyroprotein feeding was discontinued. The feeding of extra

grain for a short period after discontinuing thyroprotein feeding, as in Group 4 of Experiment A, did not prevent the large decrease in production. Placing the cows on pasture 1 month after discontinuing thyroprotein feeding, as in Groups 2 and 4 of Experiment B, did not help the cows regain the expected levels of production. However, in Experiment C the cows were placed on pasture 5 days after thyroprotein feeding was discontinued, and no decrease in production occurred. In fact all cows in Experiment C, control and experimental, increased production in a similar manner at this time (Figure 1). Confirmation of this observation is needed.

Removing thyroprotein gradually from the ration over a period of 3 to 4 weeks has been reported to prevent the large decrease in production at this time *(4, 14)*. However, some of these results were confounded by comparing the rate of decrease to control cows whose rate was abnormal and changed from 12 to 24 and 24 to 11% per month coinciding with the time thyroprotein was fed and withdrawn from the experimental group and the use of too short a post-experimental period *(14)*. In another experiment *(4)*, the total milk produced during the post-experimental period was the same with gradual as with abrupt removal of thyroprotein. Other experiments where this procedure has been used also have shown large post-experimental decreases in milk production *(1, 9)*. Further experiments are needed and are in progress in this field.

SUMMARY

Three experiments involving 47 cows were performed, in which thyroprotein was fed for 60 days to determine its effect on milk production, body weight, butterfat test, roughage consumption, efficiency of production, and monetary returns. Data from the experimental feeding period were combined with data from the post-experimental period in order to make a proper evaluation of the over-all effect of thyroprotein feeding on total milk produced, efficiency of production, and monetary returns.

The average increase in milk production was about 20% for the period during which thyroprotein was fed. The variation in milk production response between individual cows was large. In two of the three experiments production decreased to a lower than normal level after thyroprotein was withdrawn from the ration, and production continued at this lowered level for the remainder of the observation period. When only a short post-experimental period of decreased production was considered, there was an advantage in total FCM, efficiency, and monetary returns for the groups of cows fed thyroprotein, but longer post-experimental periods of decreased production lessened this advantage.

In one experiment the cows were placed on pasture 5 days after thyroprotein feeding ceased and no decrease in production occurred. There was a net increase in milk produced and in monetary returns under these conditions as a result of feeding thyroprotein.

The presence or absence of the decrease in milk production after thyroprotein was withdrawn from the ration appears to be the determining factor in the desirability of its use over a short term and its effect on milk yield, efficiency

and monetary returns for the entire lactation. However, under practical conditions the increased milk produced during a particular period has other advantages, and this is especially true during the period when a fluid-milk base is being established.

In these three experiments the feeding of thyroprotein did not cause an increase in the consumption of hay when it was fed ad libitum. The extra energy necessary for the proper maintenance of body weight and increased milk yield must be furnished in the form of extra grain. When thyroprotein was fed, there was an average increase in fat test. All cows showed a large decrease in body weight, which was alleviated but not prevented by feeding extra grain.

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RELATIVE VALUE OF CAROTENE FROM ALFALFA AND VITAMIN A FROM A DRY CARRIER FED AT MINIMUM LEVELS TO HOLSTEIN CALVES[1]

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Vitamin A is a limiting dietary essential in calf nutrition *(4, 12, 19)*. For the prevention of night blindness, a daily intake between 11 and 16γ of carotene per pound of body weight or between 2.3 and 2.9γ of vitamin A per pound of body weight has been reported to be adequate *(16, 17, 18, 25)*. Similar values for growth were between 11 and 33 or 10.0 and 25.0 *(7, 13, 14, 17, 22, 24, 29)*, for maintenance of blood plasma vitamin A levels of 10.0γ per cent between 34 and 57 or 8.2 *(5)*, and for adequate liver reserves between 71 and 102 or between 5.4 and 29.0 *(13, 14, 17, 24)*. With the exception of the requirements based on blood plasma levels *(5)*, the experiments on responses to carotene feeding were not run concurrently with those on responses to vitamin A feeding and consequently cannot be compared directly. Although the carotene requirements for the prevention of papillary edema and the maintenance of normal spinal fluid pressure have been studied and these criteria found more sensitive to changes in the vitamin A status of the calf than night blindness or growth *(25, 27, 28)*, data with respect to the effect on these of intake of vitamin A per se were not reported.

The present study was undertaken to determine quantitatively the relative value of carotene from alfalfa and vitamin A from a dry carrier fed at minimum levels employing as criteria blood and liver values of vitamin A, spinal fluid pressure, occurrence of papillary edema, and tissue alterations in the Holstein calf.

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EXPERIMENTAL

Animals and rations. Forty 1-day-old Holstein male calves were placed on experiment during the period November, 1952, to March, 1953. These calves were obtained from various Connecticut state institution herds and placed in individual tie-stalls at the University research barn. On arrival at the research barn, each calf received a 500-mg. oblet of aureomycin[2] and 200,000 U.S.P. units of vitamin A in the form of fish liver oil.[3] Levels of colostrum and milk fed were as previously described *(10)*. A standard calf starter *(11)* was fed up to 3.0 lb. per day and U.S. No. 1 alfalfa hay chopped to ¼ in. in length was allowed ad libitum. This preliminary feeding period continued through each calf's 63rd day of age.

On the 64th day of age, each calf was fed an intake of a vitamin A depletion ration *(11)* to give an anticipated 7-day rate of increase in body weight of 10 lb. The depletion ration allowance was calculated from the formula, $Y = 0.0562W^{0.87}$, in which Y = the daily depletion ration allowance in pounds and W = the anticipated body weight *(11)*. These ration allowances were continued for each calf until it was slaughtered.

The blood plasma vitamin A level for each calf was allowed to decrease to 10γ% or less during the depletion period. At this time the depletion ration was supplemented with one of five levels of carotene from artificially-dehydrated alfalfa leaf meal or one of five levels of vitamin A from a dry carrier. These were fed at the rate of 12, 18, 24, 30, and 36γ of carotene or 2, 4, 6, 8, and 10γ of vitamin A per pound of body weight daily. The first 10 calves to arrive at the research barn were assigned to one of the 10 treatments according to a previously randomized allotment. This procedure was repeated until four groups of 10 calves each had been assigned to their respective treatments. The average age at the start of carotene or vitamin A supplement feeding, the comparison period, was 88.3 ± 8.5 days. The duration of the comparison period for each calf was 16 successive 7-day age intervals. At the completion of the comparison period, the calves were slaughtered.

The amount of artificially-dehydrated alfalfa leaf meal[4] for each calf was based on the carotene concentration of the leaf meal and the calf's anticipated average weight for the particular 7-day age interval calculated from the two previous 7-day increases in weight. It was weighed to the nearest 0.1 g. the afternoon prior to morning feeding period. To this amount of leaf meal was added sufficient depletion ration to equal 1.0 lb., and the ingredients were thoroughly mixed. The following morning prior to feeding, the remainder of the depletion ration allowance was added and the mixture was again thoroughly

[2] The aureomycin oblets were supplied by the Lederle Laboratories, Pearl River, N. Y., through the courtesy of Dr. Ralph Elliott.

[3] The fish liver oil contained 25% by weight of crude soybean lecithin and 25,000 U.S.P. units of vitamin A per gram. It was supplied by the Nopco Chemical Co., Harrison, N. J., through the courtesy of Dr. Melvin Hochberg.

[4] Artificially-dehydrated alfalfa leaf meal was obtained from the W. J. Small Co., Div. of Archer-Daniels-Midland Co., Kansas City, Mo.; it contained no additives, such as antioxidants.

dispersed. The dry carrier[5] containing the vitamin A was handled in a similar manner to the alfalfa leaf meal but was weighed to the nearest 0.01 g.

In order to minimize the possible effect of light exposure on the carotene requirement *(26)*, the calves were exposed only to artificial light from 6 A.M. to 6 P.M. daily. Average intensity of light at a height of 48 in. in the center of each calf stall was 4.8 foot-candles[6] with a standard deviation of 4.2. Temperature in the calf barn was maintained at a minimum of 46° F. Average daily minimum and maximum temperatures for the duration of all calves' comparison periods, January 28 through September 26, were 59 ± 8° F. and 69 ± 10° F., respectively.

Standard procedures followed at this station *(10)* were used in the treatment of diarrhea.

Observations and analyses. All feeds fed and refused were weighed to the nearest 0.1 lb. Body weights to the nearest pound were recorded on the 7th day of successive 7-day age intervals for each calf, as well as height at withers to the nearest ¼ in. on the 28th day of successive 28-day intervals during the comparison period. Venous blood samples for hemoglobin *(15)* and plasma carotenoid and vitamin A *(3, 23)* determinations were obtained by puncture of the jugular vein on the 4th and 7th days of each calf's 7-day age intervals during the depletion period and on the 7th day of successive 7-day intervals during the comparison period. Spinal fluid pressures *(27)* were observed during the last 7-day interval of the depletion period and during the last 7 days of successive 28-day intervals during the comparison period. Heart rates, determined by auscultation of the left chest wall, and rectal temperatures were obtained during two successive days each of the last three 7-day age intervals of the comparison period for each calf. At slaughter the liver was removed for carotenoid and vitamin A analyses *(8)*, and tissues were taken for histological examination *(20, 21)*.

Samples of the depletion ration were obtained at successive 4-week intervals during the course of the experiment. Samples of the artificially-dehydrated alfalfa leaf meal and of the dry carrier of vitamin A were taken at weekly intervals during all calves' comparison periods. These samples were analyzed for proximate constituents (Table 1) by A.O.A.C. procedures *(2)*, the alfalfa leaf meal for carotene by the official A.O.A.C. method *(2)* except extraction of the pigments employed the alternative extraction procedure of Method II, first action, in lieu of hot extraction, and the dry carrier for vitamin A by a modification *(1)* of the A.O.A.C. spectrophotometric method for fish liver oils *(2)*. The average carotene content of the depletion ration was 0.05 mg. per pound with a standard error of ± 0.03. The alfalfa leaf meal contained 0.24 ± 0.01 mg. per gram and the vitamin A content of the dry carrier expressed as vitamin A alcohol 3.37 ± 0.02 mg. per gram.

[5] Dry carrier of vitamin A was obtained from the Nopco Chemical Co., Harrison, N. J. It was NOPCAY "10" Type IV from Lot No. S228.

[6] Measured with a Weston foot-candle meter, Model 614 by H. C. Cornish of the Conn. Power and Light Co., Willimantic, Conn.

TABLE 1
Average chemical composition of feeds

	Per cent dry matter	Per cent of dry matter				
		Crude protein	Ether extract	Crude fiber	N.F.E.	Ash
Alfalfa hay, chopped	90.98 ±0.42	19.26 ±0.60	2.37 ±0.28	25.58 ±1.48	44.51 ±0.80	8.28 ±0.46
Calf starter	90.61 ±0.53	21.45 ±0.31	4.43 ±0.12	6.71 ±0.35	60.30 ±0.60	7.11 ±0.20
Vitamin A depletion mixture	89.15 ±0.57	15.53 ±0.49	2.63 ±0.18	14.35 ±0.21	61.19 ±0.37	6.30 ±0.13
Artificially-dehydrated alfalfa leaf meal	92.03 ±0.27	27.94 ±0.13	3.06 ±0.11	14.99 ±0.21	41.19 ±0.33	12.82 ±0.04
Dry stabilized vitamin A supplement	94.37 ±0.35	17.89 ±0.15	31.64 ±0.98	trace	48.21 ±1.01	2.26 ±0.05

In the statistical analysis *(6)*, variability due to the 10 treatments and four groups of calves was considered. When accounting for a significant reduction in variability ($P < 0.10$), observations taken during the comparison period were adjusted by regression methods *(6)* for observations taken the last 7-day interval of the depletion period to equalize insofar as possible individual calf differences. Functions of the various response criteria on the logarithm of carotene or vitamin A intake were derived by the method of least squares *(9)*. One calf, fed the 36-γ level of carotene, was removed from the experiment during its 10th comparison week because of an undetermined central nervous system infection. The missing values for the various criteria were estimated according to standard procedures *(6)*.

RESULTS AND DISCUSSION

Feed consumption and growth. Calves readily consumed their respective depletion ration allowances. Occasionally during the comparison period, refusals were observed which averaged per calf 1.4 days with a standard deviation of 1.4. Upon statistical analyses, these were found unrelated to level of either carotene or vitamin A fed.

At the beginning of the comparison period, the average calf weighed 223 ± 32 lb. and measured 35.6 ± 1.5 in. in height at withers. It increased during the comparison period 213 ± 22 lb. in body weight and 6.8 ± 0.6 in. in height at withers. Neither growth criterion was affected by treatment.

Blood and liver. Average hemoglobin at the start of the comparison period was 10.32 ± 0.88 g.%, and during the comparison period the average was 10.86 ± 0.56. The magnitude of the slight increases observed to occur during the comparison period was independent of the level of either carotene or vitamin A fed.

Plasma and liver carotenoids and vitamin A values are presented in Table 2, and regression of these criteria on the logarithms of the carotene or vitamin A intake are plotted in Figure 1. Concentration of carotenoids in the plasma and

TABLE 2

The effect of level of carotene or of vitamin A intake on plasma and liver carotenoid and vitamin A levels in the Holstein calf

	Plasma carotenoids		Plasma vitamin A		Liver[c]		
	Initial[a]	Comparison[b]	Initial[a]	Comparison[b]	Weight	Carotenoids	Vitamin A
	..... (γ%)				(g.)	..... (γ/g)	
Carotene intake *(γ/lb body weight/day)*							
12	7 ±1[d]	9 ±1	10.0 ±0.6	5.8 ±0.3	3522 ±160	0.33 ±0.04	0.08 ±0.01
18	13 ±4	13 ±1	8.8 ±0.5	7.1 ±0.3	3463 ±96	0.49 ±0.06	0.20 ±0.05
24	8 ±3	14 ±0	9.5 ±0.8	9.2 ±0.5	3445 ±237	0.44 ±0.05	0.30 ±0.09
30	12 ±3	20 ±2	8.0 ±0.5	9.4 ±1.1	3145 ±274	0.52 ±0.05	0.64 ±0.19
36	6 ±2	21 ±0	7.0 ±1.0	10.8 ±0.5	3162 ±203	0.59 ±0.00	0.78 ±0.09
Vitamin A intake *(γ/lb body weight/day)*							
2	5 ±2	2 ±1	7.6 ±0.3	6.2 ±0.6	3298 ±221	0.27 ±0.05	0.11 ±0.01
4	12 ±2	3 ±1	8.4 ±1.6	9.5 ±0.9	3079 ±140	0.29 ±0.05	0.69 ±0.24
6	8 ±2	2 ±1	8.4 ±0.6	13.1 ±1.4	3240 ±231	0.15 ±0.03	1.71 ±0.68
8	20 ±5	3 ±0	9.7 ±0.3	14.4 ±0.4	3154 ±50	0.18 ±0.06	4.12 ±0.59
10	8 ±3	3 ±0	7.6 ±0.8	19.2 ±1.0	3271 ±119	0.17 ±0.06	4.80 ±0.73

[a] Initial value equals that at start of comparison period.
[b] Comparison equals the arithmetic mean of the 16 blood plasma values obtained during the comparison period.
[c] Liver values represent those at the completion of the 16 week comparison period.
[d] Standard error of mean.

liver reflected carotene intake but, as would be expected, not that of vitamin A intake. In the latter case, no appreciable change was noted in plasma levels; however, some decrease in carotenoid concentration of the livers with an increase in vitamin A intake was observed, possibly due to the suppressing action of vitamin A on carotenoids *(30)*. Concentration of vitamin A in the plasma and liver was found to reflect carotene or vitamin A intake. Functions of the concentration of carotenoids and of vitamin A in the plasma or liver on the logarithm of either carotene or vitamin A intake were found to be linear. The rates of change with respect to vitamin A concentration were considerably greater for increases in vitamin A intake than for increases in carotene intake. With an increase in carotene intake, plasma carotenoid concentration increased at a more rapid rate than did plasma vitamin A concentration, whereas the opposite was found for the liver concentrations.

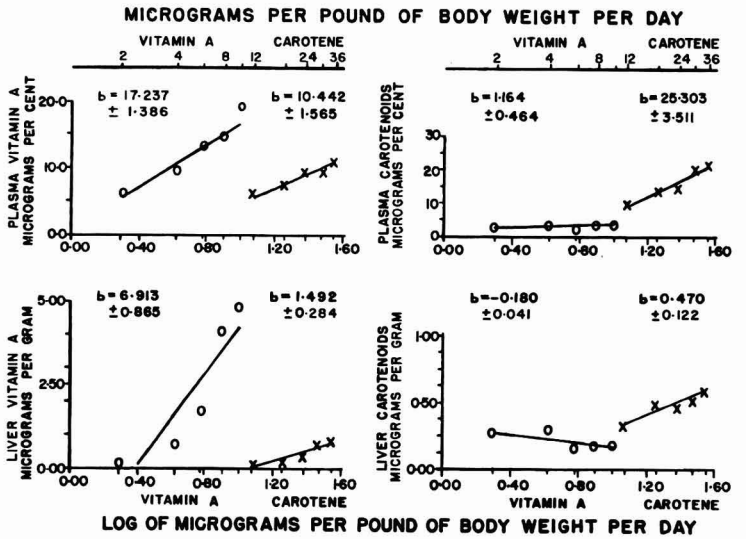

FIG. 1. Responses of plasma and liver concentrations of carotenoids and of vitamin A to levels of intake of carotene from alfalfa and of vitamin A from a dry carrier.

The data are in agreement with previous work as to trend *(5, 13, 14, 22, 24, 26, 27, 28, 29)*. As to the magnitude of the trends, some differences with previous reports *(13, 25, 27)* were apparent upon calculation of the data given in these. For example, the response of plasma carotenoids to the logarithm of the carotene intakes was approximately three times as great according to the data of Moore *et al. (25, 27)* as that reported in this study, whereas the response of plasma vitamin A was only slightly greater. The response of liver vitamin A concentrations on the logarithm of carotene intakes agreed well with Elliott's data *(13, 14)*. In plasma vitamin A response to the logarithm of vitamin A intakes, Lewis and Wilson *(24)* obtained results approximately one-half the magnitude reported herein, whereas the response of liver vitamin A concentrations was essentially the same. These differences may be due to dissimilarity in previous treatment of calves, sources of carotene and vitamin A, and chemical methods used.

Physiological and histological observations. Average heart rates and rectal temperatures observed two successive days of each of the last three 7-day age intervals of the comparison period for each calf were 102 ± 1 beats per minute and $101.8 \pm 0.1°$ F. Neither of these criteria was affected by carotene or vitamin A intake. Spinal fluid pressure expressed as logarithms (Table 3) decreased with an increase of carotene or of vitamin A intake. The regression of spinal fluid pressure expressed in logarithms of millimeters of H_2O on the logarithm of the carotene intake gave an average rate of decrease of 0.737 ± 0.142 and for the logarithm of vitamin A intake, 0.456 ± 0.060. Average days with diarrhea, 1.5 ± 3.3, and average number of treatments for scours, 0.4 ± 1.1, were

TABLE 3

The effect of level of carotene or of vitamin A intake on spinal fluid pressures and histological changes in the Holstein calf

	Spinal fluid pressure				Histological changes		
		Comparison[b]			Squamous metaplasia		
	Initial[a]	Unadjusted	Adjusted[c]	Geometric mean	Papillary edema	Stenson's duct	Parotid interlobular ducts
	(log mm. H_2O)			*(mm. H_2O)*	*(No. of calves)*		
Carotene intake *(γ/lb body weight/day)*							
12	2.031 ±0.052[d]	2.388 ±0.062	2.368	233	4	4	3
18	2.016 ±0.037	2.285 ±0.062	2.273	188	3	4	2
24	1.981 ±0.027	2.180 ±0.054	2.187	154	2	0	1
30	1.954 ±0.000	2.088 ±0.063	2.109	126	1	0	0
36	2.057 ±0.040	2.038 ±0.042	2.004	101	1[e]	0[e]	0[e]
Vitamin A intake *(γ/lb body weight/day)*							
2	2.019 ±0.022	2.352 ±0.060	2.338	218	4	4	2
4	1.978 ±0.024	2.079 ±0.030	2.088	122	2	1	0
6	1.994 ±0.040	2.159 ±0.040	2.159	144	0	0	0[e]
8	1.954 ±0.000	1.985 ±0.012	2.007	102	1[e]	0	0[e]
10	1.954 ±0.000	2.000 ±0.023	2.022	105	0	0	0

[a] Initial value equals that at start of comparison period.
[b] Comparison equals the arithmetic mean of the log of 4 observations obtained during the comparison period.
[c] Adjusted comparison equals the unadjusted value adjusted for initial by regression methods *(7)*.
[d] Standard error of the mean.
[e] Includes only 3 calves instead of 4.

unrelated to carotene or vitamin A intake. Muscular incoordination was observed in one calf fed the 12-γ level of carotene, in three calves fed the 2-γ level of vitamin A, and in one calf fed the 4-γ level of vitamin A. Convulsions occurred in two calves fed the 12-γ level of carotene.

Gross and histological changes observed upon completion of the comparison period related to either carotene or vitamin A intake were found to occur only in the eye and parotid gland (Table 3). The minimum daily microgram level necessary to prevent the occurrence of papillary edema of the eye was greater than 36 for carotene and greater than 8 for vitamin A. For the prevention of squamous metaplasia in the main duct (Stenson's duct) of the parotid gland, 24-γ level of carotene and 6.0-γ level of vitamin A were necessary. Similarly, for the interlobular ducts of the parotid gland 30-γ level of carotene and 4.0-γ level of vitamin A were required.

Of the physiological and histological criteria studied, it is apparent that spinal fluid pressure and papillary edema were the most sensitive to deficiences of carotene or vitamin A intake, with squamous metaplasia of Stenson's duct of the parotid gland and squamous metaplasia of the interlobular ducts of the parotid gland following in that order (see Table 3).

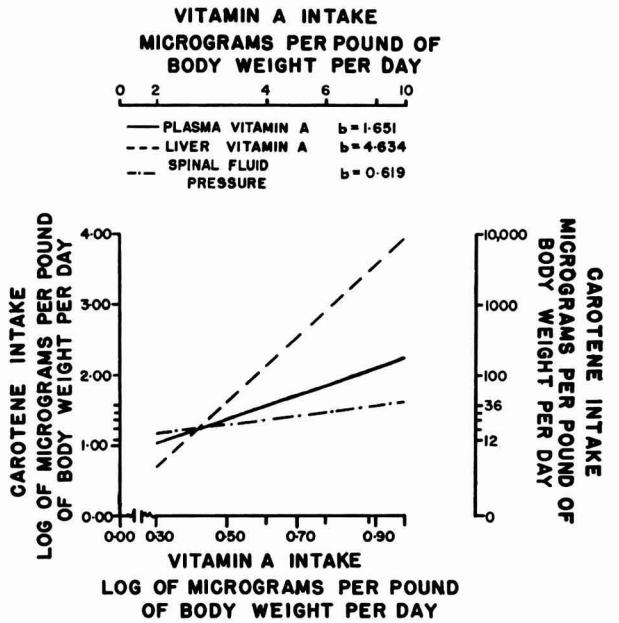

FIG. 2. Amounts of carotene from alfalfa necessary to obtain responses of plasma vitamin A, liver vitamin A and spinal fluid pressure equivalent to specified levels of vitamin A from a dry stabilized form.

Relative values of carotene and vitamin A. In Figure 2 are plotted response lines for plasma vitamin A, liver vitamin A, and logarithm of spinal fluid pressure against logarithm of carotene and vitamin A intakes. These represent amounts of carotene or of vitamin A necessary to result in similar response values for the above mentioned criteria.[7] It should be noted that the responses

[7] These response lines were derived from the logarithm of the carotene and vitamin A intake for which data are included in Tables 2 and 3. For example, plasma vitamin A on the logarithm of the carotene intake gave: $\hat{Y} = -5.6205 + 10.4421X_1$, where $\hat{Y}$ = plasma vitamin A and X_1 = the logarithm of the carotene intake; plasma vitamin A on the logarithm of the vitamin A intake gave: $\hat{Y} = 0.1245 + 17.2370X_2$, where X_2 = the logarithm of the vitamin A intake. To solve for the amount of carotene or vitamin A necessary to give a response in plasma vitamin A the following relationships were used *(9)*: $\hat{X}_1 = \frac{Y + 5.6205}{10.4421}$ and $\hat{X}_2 = \frac{Y - 0.1245}{17.2370}$

From these it was possible to solve for the amount of carotene, $\hat{X}_1$, and of vitamin A, $\hat{X}_2$, necessary to give a similar response in plasma vitamin A, Y.

to vitamin A intakes extended over a wider range than those to carotene intakes. Therefore, the relationships beyond the 36-γ level of carotene intake must be interpreted with caution since they extend beyond the limits of the experimental data. Within the limits of the data the relative values of carotene and vitamin A were as follows: To maintain a blood plasma vitamin A value of 10γ%, the 31-γ level of carotene and the 3.8-γ level of vitamin A were needed; to achieve a liver vitamin A concentration of 0.6 γ per gram, the 30-γ level of carotene and the 3.0-γ level of vitamin A were indicated, and to maintain a minimum spinal fluid pressure of 120 mm. of H_2O, the 33-γ level of carotene and 6.4-γ level of vitamin A were required. The above equivalencies of carotene and vitamin A agree with those in the literature *(5, 13, 14, 24, 27, 28)*. It has been pointed out by other investigators *(17)* that the equivalencies of carotene and vitamin A became wider as the intake of carotene was increased. This relationship was also apparent in the results of this study.

SUMMARY

Forty 63-day-old Holstein male calves previously raised on a limited whole milk–dry calf starter regime were fed an intake of a vitamin A depletion ration to give an anticipated 10-lb. increase in body weight per 7-day period until the blood plasma level of vitamin A for each calf decreased to 10.0γ% or less. At this time, when their average age was 88 ± 8 days and average weight was 223 ± 32 lb., each calf was fed in addition to the depletion ration one of five levels of carotene, 12, 18, 24, 30, and 36 γ from alfalfa or one of five levels of vitamin A, 2, 4, 6, 8, and 10 γ from a dry carrier per pound of body weight per day for 16 successive 7-day periods. Upon completion of this period, during which the average weight increase per calf was 213 ± 22 lb., the calves were slaughtered.

Regressions of plasma and liver concentrations of carotenoids and of vitamin A and of the logarithm of spinal fluid pressure on the logarithm of carotene and of vitamin A intakes were derived and found to be linear. From these relationships it was found that to maintain a plasma vitamin A value of 10γ%, a daily intake of 31γ of carotene or a daily intake of 3.8γ of vitamin A per pound of body weight was required. To achieve a liver concentration of 0.6γ per gram, a daily carotene intake of 30γ or a daily vitamin A intake of 3.0γ per pound of body weight was necessary, and to maintain a minimum spinal fluid pressure of 120 mm. of water a daily intake of 33γ of carotene or 6.4γ of vitamin A per pound of body weight was needed. In addition to these, carotene and vitamin A intakes in micrograms per pound of body weight per day required to prevent papillary edema were greater than 36 and 8, respectively; to prevent squamous metaplasia in the main duct of the parotid gland required 24 and 6.0, respectively; and to prevent similar lesions in the interlobular ducts of the same gland required 30 and 4.0, respectively.

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PEOPLE and EVENTS
in the Dairy Science World

Pioneers in the Dairy Industry

Forty-five years ago a young graduate from the University of Wisconsin dairy herdsmen's short course arrived at Montana State College to assume the duties of dairy and beef cattle herdsman. J. O. TRETSVEN, now better known to the dairy industry as Oscar Tretsven, immediately began to build up the dairy herd and improve the management practices. The beef herdsman responsibilities were soon transferred to another man, which allowed Oscar to devote more time to his "first love," the dairy herd. He proved to be more than a herdsman. He always has been, and still is, a keen observer and student of unsolved problems related to breeding, feeding, and management of dairy cows.

J. O. Tretsven

Because of his superior accomplishments, he was granted increases in salary to a level beyond which the State Board of Education would not pay a staff member without a degree. To offset this, the dean of agriculture permitted Oscar to take college work on a limited basis along with his full-time job — the slow, hard way to earn a degree. The B.S. degree was granted him in 1922. In 1933, after one year of leave for sabbatical study at the State College of Washington, he earned the M.S. degree. In 1922, immediately after graduation and at the unanimous request of the Montana Dairymen's Association, he became the first extension dairy specialist in Montana, the position he now holds.

While still a herdsman, Tretsven recognized that control of mastitis was primarily a problem of good herd management and milking practices. Subsequent studies and observations proved the truth of this theory, and later other investigators have come to the same conclusion. He was a pioneer in the introduction of sunflower and of grass silage. The latter has now been revived by many experiment stations as a means of increasing milk production and reducing feed costs. Open shed housing of the milking herd, the elevated milking stall to eliminate unnecessary "stoops and squats" of the milker, improved pasture management, and feeding calves by a metal nipple bucket are some of the many new innovations that Oscar devised while he had charge of the dairy herd.

As an extension specialist, Tretsven was one of the first to advocate open shed housing and parlor milking of cows. He organized extension feeding schools for dairymen, was very active in 4-H dairy work, and has made an outstanding contribution in improving the flavor of market milk through market milk schools.

Oscar also made real contributions in the teaching and research fields. Because of shrinking operating funds during the depression of the early thirties, he consented to become a triple-duty staff member by taking over the dairy production teaching and experiment station work in addition to his extension duties, and a very creditable job was done in each. His well rounded-out service and contribution to the development of the industry for 45 years have led his coworkers and dairymen in the state to refer to him as the "Father of Dairying in Montana."

With all the job responsibilities Professor Tretsven had in working his way through school and supporting a family of three children, he gained many honors. He was honored by being elected to Alpha Zeta, Phi Kappa Phi, and Phi Sigma. Later he became a member of Epsilon Sigma Phi, an honorary extension society, which recognized his accomplishments by honoring him at the last Montana State College extension workers' conference.

Oscar Tretsven is known in the dairy production field as a practical dairy scientist, one who has the ability to apply scientific findings in the field and get results. Montana has been greatly benefited by his earnest and diligent service to the dairy industry.

Eastwood Joins N.D.C.

RALPH EASTWOOD has accepted a position with the National Dairy Council as head of the Department of Affiliated Unit Services, a position formerly held by P. E. MAY, who has resigned. Eastwood, a graduate of the Univ. of Wyoming, took his advanced work at Cornell Univ. He served in the European theatre with the rank of Lt. Colonel in the Quartermaster Corps. Before joining N.D.C. he was on the Agricultural Economics staff at the Univ. of Illinois.

Nebraska Events

It is unusual when a plaque is dedicated in a dairy building in memory of a foreign statesman, yet that is what happened May 23, 1954, in Lincoln. On that date, a bronze plaque, in memory of KARLIS ULMANIS, a Latvian patriot, who served his country as Minister of Agriculture, then Premier, and finally as President, was unveiled in the dairy industry building by Lt. Governor Warner of Nebraska, on whose farm, Karl Ulman, as he was then known, worked for a period. A political refugee from the Czarist government, Ulmanis came to Nebraska, worked for Roberts Dairy in Lincoln and attended the Univ. of Nebraska, earning a B. Sci. in 1909 with a dairy major. He served for a time as a dairy instructor and then managed a creamery before a general amnesty allowed him to return to his native country. He fathered 4-H Club work in the Latvian Republic along with many other progressive agricultural policies. When the present Russian government overran Latvia, Ulmanis disappeared, undoubtedly a martyr.

L.S.U. Dairy Science Club Honors Malcolm Brian

MALCOLM BRIAN, Baton Rouge, La., has been selected as "Honorary Dairyman" of the year by the L.S.U. Dairy Science Club. Mr. Brian, president of the Santa Maria Dairy Products Co. in Baton Rouge, has been active as a leader in various dairy organizations throughout the state and is now president of the American Dairy Assoc. in Louisiana.

Student Annual Dairy Show at Virginia Polytechnic Institute

The annual VPI Dairy Show was one of the main features of the first Agricultural Exposition held May 15. E. A. DRINKWATER of Bayard won the grand champion award for dairy cattle fitting and showing with VPI Peerless Excelsior Mazie, a Guernsey cow. The Reserve Championship was won by C. N. LESTER of Glade Spring showing VPI Lilac Justice Buttergirl, a Jersey calf.

NORMAN TRAYLOR, a junior student of dairy manufacturing at VPI, won over 20 other contestants to take the annual dairy products judging contest. MASON HUTCHESON, a freshman in dairy production, won first place in a special contest for students who had not had a course in judging dairy products. The contest is an annual event sponsored by the Dairy Club and supervised by members of the judging team. The winners were presented trophies, donated by commercial firms, at the Agricultural Awards Banquet.

One of the highlights of the Agricultural Exposition Week were the displays sponsored by the different curricular clubs. The Dairy Club won the cup against nine other entries.

Parkhurst Named Head of A.F.M.A. Nutrition Council

R. T. PARKHURST, director of nutrition and research at Lindsey-Robinson & Co., Roanoke, Va., has been elected chairman of the Nutrition Council of the American Feed Manufacturers' Assoc., an 80-man committee composed of the industry's leading nutrition experts.

The Nutritional Council, under Dr. Parkhurst's direction, will encourage research into feeding requirements and proper nutrition of livestock and poultry, management practices, and ways to insure maximum profitable gains through the proper feed ingredients and quality. Membership on the Nutrition Council is limited to scientifically trained men of the feed industry who are responsible for feed formulation, nutrition research, and quality control of ingredients and manufactured feeds in their companies.

Completed Theses

M.S. Degree:

HOWARD L. FISHER — The grazing habits of lactating dairy cows under certain environmental conditions. Va. Polytech. Inst.

WIGGO F. AXELGARD — The use of corn syrup solids of different dextrose equivalents in the manufacture of frozen desserts. Utah Agricultural College.

J. P. COOPER — Consumer preferences for ice cream as affected by changes in composition or in chocolate flavor. The Ohio State Univ.

Ph.D. Degree:

DANIEL P. SCHWARTZ — Lipolytic enzymes in raw skimmilk. The Ohio State Univ.

Carl W. Larson Dies

CARL W. LARSON, 73, a life member of A.D.S.A., died June 13 from a heart attack suffered during services in Holy Trinity Lutheran Church, Buffalo, N. Y. Dr. Larson, a native of Iowa, obtained his B. S. degree from Iowa State in 1906, an M. S. from Pennsylvania State College in 1911, and a Ph. D. from Columbia in 1916. A former head of the department at Penn State (1913-15), he served with the Dairy Division, USDA, from 1917 to 1927 and became the first chief of the Bureau of Dairy Industry. He served on the Hoover Relief Commission in France and Belgium after World War I.

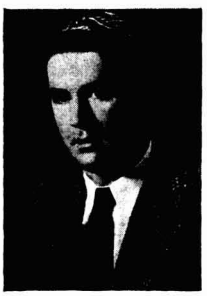
Dr. Larson was managing director of the General Ice Cream Corp. from 1930 to 1936. From 1936 to 1941 he was president of the Whiting Milk and Bashway-Whiting Ice Cream

Co., Boston. In 1942 he became president and general manager of the Bryant and Chapman Milk Co. and R. G. Miller and Sons in Hartford, Conn., and in 1944 he was made manager of the western division of the General Ice Cream Co., Buffalo. After his retirement in 1946, Dr. Larson organized the Dairy Products Improvement Institute, Inc. He retired from this group last year.

Dr. Larson was the author of a widely used textbook, *Dairy Cattle Feeding and Management*. He was active in scouting, a member of a number of honorary and scientific societies, a Rotarian, and a member of the Cosmos Club of Washington. He served as official delegate to the International Dairy Congress in London, in 1928. He was managing director of the National Dairy Council in 1928-29. Dr. Larson is survived by his wife, a daughter, Mrs. Charles A. Brody, and five grandchildren.

Ellsworth Joins Staff of M.I.F.

PERRY R. ELLSWORTH, a member of the extension staff of the Department of Dairy Technology at the Ohio State University and for several years secretary-treasurer of A.D.S.A., has become assistant to the executive director of the Milk Industry Foundation — R. J. WERNER. The position was recently created by the executive board. Mr. Ellsworth's duties will include editing *Milk Facts* and the Association *Newsletter*. He will represent the Association on the Plant Committee, which publishes the Association manual, and will serve on the Producer Relationship Committee, the Transportation Committee, the Statistical Studies Committee, and the Labor Relations Committee.



P. R. Ellsworth

Gleanings from the Annual Meeting

The 49th annual meeting was held at Pennsylvania State Univ. June 20-24. The total registration was 1,424, representing all states but three, Canada, and five foreign countries. Pennsylvania led in number of representatives, followed by New York, Ohio, and Illinois. The highlight of the meeting was the address by the Honorable Ezra T. Benson, Secretary of Agriculture, USDA, which is reproduced in the "Our Industry Today" section of this issue.

A. A. BORLAND, an honorary member of the Association and a former head of the department at Pennsylvania, attended several of the sessions. Other honorary members present were H. P. DAVIS of Nebraska, H. B. ELLENBERGER of Florida (formerly Vermont), J. H. FRANDSEN of Massachusetts, and E. S. GUTHRIE of Cornell. Professor Guthrie presented a paper before the Dairy Manufacturing section.

R. B. MAXCY has accepted a position with the Diversey Corp., Chicago, in the technical development service department.

At the business meeting the Association voted to raise the dues of members to $10 and that of subscribers to $15. The cost of reprints also was increased. Authors will now pay $5 per page for the first 100 reprints, $3 per page for the second 100, and $1 per page for all additional reprints. Thus, 300 reprints for an 8-page article would cost $72. These increases were necessitated by the increased cost of printing the Journal and the greater operating costs of the Association.

The 1955 meeting (the fiftieth anniversary of the founding of the Association) will be held at Michigan State College. In 1956 the meeting will be held at Connecticut, in 1957 at Oklahoma, in 1958 at North Carolina, in 1959 at Illinois (tentative), in 1960 at Utah, and in 1961 at Wisconsin.

The results of the questionnaire pertaining to the popular section of the Journal, which was sent to the members, were as follows:

1. Do you favor retaining the popular section:
   a. People and Events — yes, 91.0%; no, 4.8%
   b. Letters to the Editor — yes, 80.3%; no, 9.8%
   c. Our Industry Today — yes, 85.7%; no, 8.3%
2. Do you read the popular section?
   a. Regularly — 74.4%
   b. Occasionally — 21.0%
   c. Rarely — 2.8%
3. Which part of the popular section do you find most interesting?
   a. People and Events — 58.9%
   b. Letters to the Editor — 15.1%
   c. Our Industry Today — 45.3%
4. Which section would you like to see expanded?
   a. People and Events — 10.8%
   b. Letters to the Editor — 5.4%
   c. Our Industry Today — 25.8%
5. Do you read the abstracts of literature?
   a. Regularly — 71.8%
   b. Occasionally — 23.6%
   c. Rarely — 4.0%

The survey shows that among the 836 who replied, a large majority favor retaining the three new features, with "People and Events" being the most popular. Apparently, the amount of space devoted to each feature is about right for most members, with the possible exception of "Our Industry Today."

There was some indication that the members would like for the abstract section to be developed further and the abstracts to be made more complete.

The ballot tabulating committee announced the election of I. A. GOULD (Ohio) as vice-president and R. E. HODGSON (Washington, D. C.) and GEORGE HYATT, JR., (North Carolina) as directors.

PERRY R. ELLSWORTH resigned as secretary-treasurer of the Association. T. D. HARMON of Ohio State was appointed to serve as acting secretary-treasurer until arrangements are made for a permanent business management plan. A committee of the board is making a special study of the possibility of turning over the major duties of both the secretary-treasurer and the editor to a commercial organization specializing in this type of service.

Intelligent Disposal of Plant Wastes — A Challenge to the Industry

A Guest Editorial

Serious thinking on the subject of dairy plant wastes is the first step towards development of a satisfactory waste control and treatment program. When too much of the incoming milk leaves the plant through the sewers, the waste can represent an actual loss to the industry in terms of product as well as in terms of high cost for treatment facilities.

Control of losses within the plant should, therefore, be the first step in handling milk waste problems. Thorough study of the report of the Waste Disposal Task Committee of the Dairy Industry Committee, entitled "Waste Prevention in the Dairy Industry" is recommended to all dairy plant owners and operators. This bulletin, available from the Dairy Industry Committee, contains a wealth of material of value to the dairy industry. It should be reviewed in detail by everyone concerned with the processing of milk.

It is important also to understand that concentrated materials such as whey, skimmilk, and buttermilk cannot be treated in conventional waste treatment plants and should be utilized. Their discharge into streams often results in destruction of fish and other aquatic life with resultant payment of fines or damages by the industry. Most states prohibit discharge of these concentrated wastes into lakes or streams.

Several methods of treatment have been developed for process wastes which include can, floor, and equipment washings. These are mainly biological processes of treatment or soil absorption methods of disposal which official agencies after considerable research have recommended for installation at dairy plants.

Most of the research work has been done with the cooperation of individual plants which faced critical stream pollution problems that needed to be solved. The time has come, however, when the industry, as a group, needs to enter upon a full-scale detailed research and educational program involving the prevention of losses, development of more extensive uses for by-products such as whey, skimmilk, and buttermilk, and investigation of methods of waste treatment which can economically be applied to the treatment and disposal of milk plant wastes. An industry thoroughly acquainted with its processing problems is in a good position to develop solutions to its waste disposal problems. All that is needed is an organized approach, adequate financing and an intelligent staff of researchers.

T. F. Wisniewski

The industry can have all these if it will but realize that expenditures for research will represent only a fraction of the amounts saved in terms of waste loss prevention, improved markets for by-products, and lower cost treatment plants. It is estimated that thirty million dollars annually could be saved if milk losses were reduced to one-half of what they are today.

In its approach to the problem of disposal of wastes, the industry should look upon its expenditures in this field of activity as an investment that will bring about tremendous savings.

It is suggested that the industry start now to arrange through its industry-wide organizations for a voluntary assessment of a fraction of a cent per thousand pounds of milk intake so that ample funds will be available for research on its industrial wastes. The industry can then, at its own plants or laboratories or through the use of facilities of the university experiment stations, embark on an intelligent, well thought-out program of plant waste control and disposal and bring it to the attention of all dairy plant operators.

T. F. WISNIEWSKI, *Director*
Wisconsin Committee on
Water Pollution

LETTERS TO THE EDITOR

Observations on Organic Acids Formed During the Heat Sterilization of Milk[1]

One of the major chemical changes produced when milk is heated is an increase in titratable acidity *(1, 4)*. Both lactic and formic acids have been identified as contributing to this increase. Gould *(1)* found that approximately 5% of the developed acidity was lactic acid and about 60 to 80% of the volatile acids was formic acid *(2)*. The nature of other acids in milk which occur as the result of heat treatment has not been determined.

During a recent bacteriological investigation, chromatographic analyses of the sterilized milk controls revealed the presence of acids not reported previously to be present in heated milk. In this study, the milk was heated to 113° or 121° C. for 10 to 30 minutes. Then it was neutralized to pH 9.5 and concentrated to dryness by lyophilization. Analyses for pyruvic, acetic, propionic, and butyric acids were made on 5 g. of dry powder by a direct chromatographic procedure *(3)*. Careful acidification to pH 2.0 was necessary to free the acids from their salts.

TABLE 1

Effect of various sterilization procedures on the liberation of organic acids from skimmilk medium

Lot No.	Condition of heat treatment			pH of heated milk	Increase in total acid[a]	Percent of total acid produced by acids analyzed	Individual organic acids released in microequivalents per 100 ml. of milk			
	time	temp.	pressure				butyric	propionic	acetic	pyruvic
	(min.)	*(° C.)*	*(1 be.)*							
Control	—	—	—	6.6	—	—	6.0	—	—	—
1	10	121	15	6.5	166.5	60.2	15.5	28.5	6.5	50.40
2	20	121	15	6.25	333.0	33.3	15.5	29.0	12.5	63.50
3	30	113.3	10	6.5	no data	no data	7.0	101.5	0.70	37.5

[a] Increase expressed as microequivalents/100 ml. of milk.

The data in Table 1 show that all of the acids studied increased as a result of the heat treatment of the milk. Butyric acid, which was the only acid found in measurable quantities in the milk, showed the least increase in concentration. Pyruvic acid was produced in the greatest concentration with small amounts of propionic and acetic acids being formed. These acids contributed from 33 to 60% of the increase in titratable acidity. Although a greater total amount of these acids was formed at 20 minutes at 121° C. than at 10 minutes, it contributed less of the titratable acidity than when the shorter exposure was used. Increasing the heating time from 10 to 20 minutes directly affected the volumes of acetic and pyruvic acids (which were released). When the milk was heated for 30 minutes at 113° C., large quantities of propionic acid were formed with lesser amounts of acetic and pyruvic acids.

These observations reveal the presence in heated milk of at least four acids in addition to formic and lactic acids. The full significance of these findings cannot be realized until a more complete study is made of the acids formed during the heat treatment of milk to establish the relative concentration of each acid under standardized conditions of heating.

J. KERN, H. H. WEISER,
W. J. HARPER AND I. A. GOULD
The Ohio State University

[1] Supported in part by the Ohio Dairy Products Research Fund and by the Department of Bacteriology.

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OUR INDUSTRY TODAY

Brief Reviews of Current Topics

MAKING THE FARM FEED THE HERD FOR GREATER PRODUCTION AND HIGHER PROFIT

PAUL MONTAVON

Ex-English and chemistry teacher who operates 260-acre dairy and grain farm in De Kalb County, Ill., believes that it is possible not only to make the farm feed the herd but also to increase production and to gain an extra margin of profit in so doing. A 21-cow herd average of 525 lb. of fat without any protein supplement is good evidence that this 43 year old farmer, who learned scientific dairy farming by home study methods, is correct in his contention.

Since the cow was intended to consume roughage, and since good legume roughage is the cheapest source of protein, the key to making the farm feed the herd is a good roughage program — hay, pasture, and silage.

As the years have gone by and the feeding program at the Montavon farm has been improved, the amount of purchased protein supplement has been decreased until, in 1952, it was omitted from the ration altogether. Meanwhile the herd, through disease control and improved feeding and breeding, has continued to increase production, even in the past 2 years when no protein supplement was added to the winter ration. The peak was reached last year when the herd produced 525 lb. of butterfat per cow — all on home raised feed.

Table 1, compiled from Farm Bureau Farm Management records, shows the production and profit for the 2 years when no protein supplement was fed, compared with the preceding 5 years, when a rather small amount of soybean meal was added regularly to the grain mixture. The last column was added to show that the increased production was not due merely to better cows through improved breeding, but more probably was due to improved feeding, since these old cows, admittedly past their prime, also showed an increase. This column shows the production of four cows which were at least 3 years old in 1947, the first year considered in these figures, and are still in the herd, now ranging from 9 to nearly 12 years of age. In order to get this average, individual cows had to produce over 700 lb. of butterfat.

Although returns per $100 feed decreased in 1952 and 1953, the average returns of all farms in FBFM dropped considerably more, as shown by returns of the Montavon farm expressed as per cent of FBFM average. These data show the importance of a good roughage program to efficient operation. With the progress that has been made — and should continue to be made — in breeding and feeding, it is time for all dairymen to consider seriously the establishment of a 500-lb. herd.

A good dairy ration should be:

1. High in protein.
2. High in total digestible nutrients (TDN). In other words, it should contain enough carbohydrates to balance.
3. Low in fiber.
4. Ample in essential vitamins and minerals.
5. Highly palatable.

Obviously, the protein is needed for growth and milk production, the TDN to satisfy high energy requirements, the vitamins, especially A and C, and the minerals — calcium and phosphorus — for growth, milk production, and general health. Fiber content must be low if the animals are to be able to take in enough useful feed for high production, and palatability must be high to make them willing to do so.

Good, lush pasture most nearly meets all these requirements and is basic in a good feeding program. Good grass-legume silage should be rated next, and hay, which takes a lot of care if it is to be good, rounds out the roughage program. Grain is looked upon as a supplement and is needed to bring up the TDN to the proper level. High protein roughage is considered the basic part of the ration, with carbo-

TABLE 1

Production and profit of herd

Year	Protein fed in winter ration	Returns for $100 feed	Av. returns, all dairy farms in FBFM	Per cent farm returns were of FBFM average	Butterfat av. of herd	b.f. of 4 older cows
	(lb.)				*(lb.)*	*(lb.)*
1947-51	2,600	$253	$176	141	438	547
1952-3	none	$248	$165	153	500	579

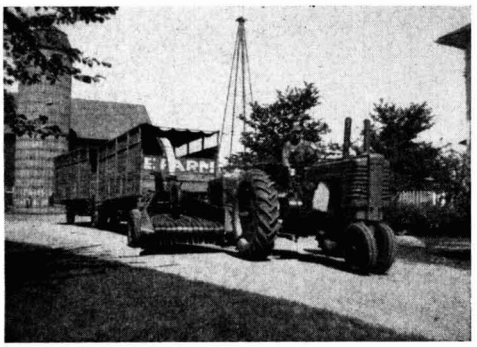


FIG. 1. On the way to the field to chop high protein hay. This same equipment is used to harvest hay, straw, and silage.

hydrates added as a supplement, thus reversing some traditional thinking.

Corn, because of its high yield and high available supply of TDN and the presence of certain vitamins, is probably the greatest all-round feed plant. But it is time to break away from tradition enough to recognize that feeding results, in the final analysis, come from total intake of TDN in a balanced ration, regardless of the source of those nutrients. In South America, for instance, a high quality of beef is finished on alfalfa and cereal grass pasture, not on corn.

Although pasture has been called the most nearly perfect feed for a dairy cow, most pastures are very imperfect. It was once the practice to turn the cows out on the old bluegrass creek pasture about May 10 and feel that the feeding worries were over until October. Of course, the cows nearly dried up in July, but the flies and the heat were blamed for that. Later, Sudan grass pasture was tried, and it was found that the cows would milk about as well in July and August as they did in the flush time of May and June if only the feed was as good.

Permanent pastures are still about the only way some land can be used, but the word "permanent" doesn't mean quite the same as it did once. Unless a permanent pasture is occasionally torn up, fertilized, and re-seeded with a legume mixture, its value is low. Nine acres of rotation pasture have furnished two or three times as much feed as 25 acres of creek bottom pasture, which is too rough and full of sloughs to renovate. The old creek pasture has one good use: it helps prevent bloat. Perhaps the fact that the cows have to walk through the creek pasture to get to the rotation pasture explains why there has been almost no trouble with bloat.

The hay and pasture seeding mixture is the same — alfalfa, brome grass, and ladino. The last boost in production, noted in Table 1, is attributed to the addition of ladino to the mixture. It definitely increases both the palatability and the protein content of the roughage. This grass-legume mixture is not necessarily the final word and may not be applicable to all cases. Ways for improving it are being looked for.

The Pasture Program on the Montavon Farm

The pasture program followed is briefly this: 10 acres of good grass-legume mixture, with the help of the creek pasture, will usually carry the herd from May 1 to some time in June. Successive 10-acre areas are added as needed, with each field being clipped when the cows are taken off it, and then reopened for grazing when the alfalfa begins to bloom. Silage and some hay are made from the first cutting. The bulk of the hay is made from the second cutting, and by the time the third cutting is ready most of it is usually needed for pasture. If the season is at all dry, it may take as much as 60 acres in September to do the work of 10 acres in June. The pasture is usually parceled out to the cows about 10 acres at a time, however. A little good hay is always offered the cows, together with a negligible amount of grain. This dry feed helps prevent bloat and is also a good indicator of the time to change pastures, for when the cows start cleaning up their dry feed, the pasture is probably too short or too tough.

Rye is occasionally used to stretch the pasture season a little at both ends, but sudan grass is not needed any more, for a conservation plan is being used on the farm that calls for ample grass. Sudan is too costly to produce unless it is really needed. All in all, pasture is the favorite feed, for not only is it the best, but it is the only way available for making the cows harvest the crop, feed themselves, and haul the manure.

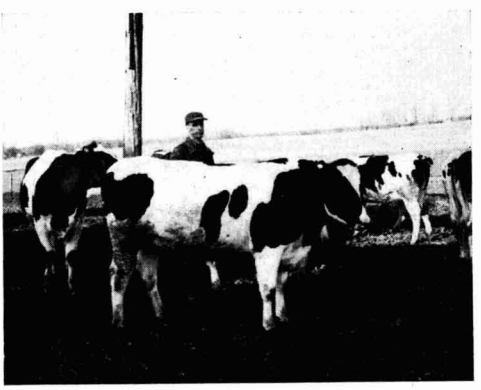


FIG. 2. The author is shown standing behind a heifer which will freshen this fall and which has attained a good size and condition on a ration of grass silage and hay throughout the winter.

Winter Feeding

Winter feeding is another matter. The basis of the winter feeding program is grass-legume silage, which has been used successfully for some 16 years. Grass silage is rated above hay because it is a succulent feed and therefore more palatable. It also contains more vitamins than hay, and it contains all the leaves and therefore more protein than hay, which normally loses perhaps 20% of the leaves through shattering. Furthermore, there is little weather risk in making grass silage. A comparison of grass silage with corn silage, which is a good feed, is shown in Table 2. These data explain why corn silage is no longer considered in this dairy program. Incidentally, the 5% protein shown for grass silage is not the ultimate figure.

TABLE 2

Comparative value of corn silage and grass-legume silage

	Dry matter	TDN	Protein	Calcium
	(%)	*(%)*	*(%)*	*(%)*
Corn silage	30	21	2.5	0.08
Grass-legume silage, corn pres.	30	20	5.0	0.48

One of the advantages of grass silage is that it can be made while the grass is still very high in protein, but it is also so high in water content that it would be difficult to cure as hay. A good alfalfa-brome-ladino mixture is cut for silage about June 1 or shortly after — before the alfalfa is in bloom. At this stage — especially if there is much ladino — it is advisable to mow five or more acres before beginning to chop. Providing the weather is favorable, the green material will be about half-dry as it goes into the silo. As this will be in the bottom of the silo, with this mixture there is no danger of excessive heating or any molding in the silo, for the weight of the silage above will press out the air. Ground ear corn is added at the rate of 150-200 lb. per ton of green material as a preservative and added source of TDN. If there is rainy weather, more preservative is used; if the silage is too dry, the amount of corn is reduced.

FIG. 3. Feeding the cows grain-corn and oats 50-50. Mr. Montavon Sr. has pioneered the feeding of grass silage in his section.

FIG. 4. A scene showing hay which has been cut through the middle. The little girl is pointing to the line between the hay which had air blown through it, and the hay above which did not. The hay below the line is actually much greener which accounts for the darker color. The box in the foreground is raised by the rope during the time the barn is being filled, providing a hole or flue through which the air is dispersed through the hay.

A green crop that would not make excellent hay or pasture cannot be expected to make excellent silage. A picked-over pasture, from which the cows have sorted out all the tender legumes, or a weedy or tough, overripe crop will make a silage much lower in protein and higher in fiber and must be handled accordingly, both in harvesting and in feeding. Ideal silage is probably just wet enough that the silo is on the point of leaking, but if it actually leaks the silage is too wet. Sometimes this too-wet silage cures admirably, and sometimes it cures cold, which means that it has a decayed odor. If the silo leaks, the green material was probably young enough to be high in protein.

The cows are fed all the silage and all the hay they will clean up twice a day, with grain at the rate of slightly less than 1 lb. to 4 lb. of milk daily. Many cows refuse to eat this much grain. All heifers over a year old and

TABLE 3

Protein content of rations containing grass-legume and corn silage

With grass-legume silage		With corn silage	
15 lb. good hay	× .16 = 2.4 (lb. prot.)	15 lb. good hay	× .16 = 2.4 (lb. prot.)
40 lb. silage	× .05 = 2.0 (lb. prot.)	40 lb. silage	× .025 = 1.0 (lb. prot.)
12 lb. grain	× .10 = 1.2 (lb. prot.)	12 lb. grain	× .18 = 2.2 (lb. prot.)
Total	5.6 lb. prot.		Total 5.6 lb. prot.

all dry cows are fed all the silage and all the hay they will clean up once a day. Calves are given a limited amount of milk for 60 days, and calf starter, hay, and silage from the time they will eat solid feed. At about 5 months the calf starter is replaced by grain, with 4 lb. per calf per day the maximum. Grain is discontinued at about a year. The grain mixture is corn and oats 50-50, with about 1.5% salt and a small amount of iodized lime added.

This feeding program has produced excellent results for every class of dairy cattle on the farm. The heifers are large and fat and ready to start milking at 26 months. Some heifers have had 500 lb. of fat to their credit before they passed their third birthday. The use of corn in the silage makes it unnecessary to feed grain to the dry stock. Cows have come off this dry ration and produced 100 lb. of butterfat a month, indicating that they freshened in good condition.

According to Morrison's *Feeds and Feeding*, a 1200-lb. cow giving 50 lb. of 3.5% milk a day should receive not less than 5 lb. total protein a day. Table 3 shows that the grass-legume silage ration provides that amount of protein. It also shows the grain mixture that would be required to furnish a like amount if corn silage were fed instead of grass silage.

It has been observed that when roughage is good the cows milk well, and when it is poor the cows do poorly. Poor roughage cannot be entirely compensated for by fortifying the grain ration.

The figures below show the difference in cost of feeding the two grain rations in Table 3, with soybean meal at $90 a ton:

With grass silage: 12 lb. grain @ 2.3¢ = 27.6¢
With corn silage: 12 lb. grain @ 2.8¢ = 33.6¢

The difference of 6¢ times 21 (No. of cows) times 180 (days winter feeding) is $225, amount saved on the grain alone. If it costs $225 extra to feed the 18% grain ration, remember that the cost of not feeding it would probably be

FIG. 5. Representative cows of the herd which averaged 525 lb. of fat without any protein supplement.

much greater. Of course, not all the cows in the herd get 12 lb. of grain a day, but the grain and supplement saved by feeding this high-value silage to the dry cows and heifers will more than make up for that. With grass silage figured $1.50 a ton cheaper than corn silage, the total saved by this ration is nearly $500, even assuming production to be the same in both cases.

How to Obtain Good Hay

The third part of the feeding program is good hay. On the average, even with favorable weather, 20% of the protein content of hay is lost in harvesting. This loss must be stopped if the farm is to feed the herd. On a dairy enterprise the size of the one under consideration, that would be the same as losing 5 tons of soybean meal a year. More and more farmers are turning to special methods of saving the protein-rich leaves so essential to good hay, and to ways of lessening the weather risk by shortening the field drying time. Some farmers use a stem crusher with good results. A blower can be used in the barn to finish curing so that the hay can be harvested while it is still tough enough for the leaves to hang on. The same chopper can be used for harvesting hay as for silage, with the cut set as long as possible for hay.

One reason for getting the first cutting hay off early is so that the second cutting can be made before oat harvest. By the time the oats are all combined, the hay is invariably too tough to be good. Even though it may be a beautiful pea green in color it can be so stiff and stemmy that the cows won't eat it, and most of the lower leaves will have fallen off, contributing greatly to the loss of protein. When the weather permits, it is possible occasionally to get some very good first cutting hay, particularly when the ladino is especially good, but the odds are against it.

Grass Essential for Sound Land Use Program

This whole idea of making the farm feed the herd is especially attractive, because with a sound land use program to stabilize soil fertility and increase production of all crops per acre, a generous acreage of grass is required. The job of the dairy herd is to convert that grass into cash. When the herd not only does this, but also shows an extra margin of profit over herds fed purchased concentrates, dairy farming is profitable.

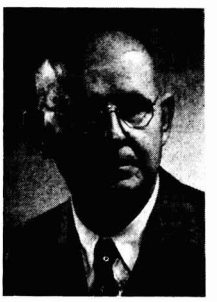
A neighbor who followed the same program as the one outlined above showed one of the best returns per $100 feed in the Farm Management service and had individual cows producing up to 600 lb. of butterfat. Let the cow be the judge — she probably knows best.

THE WISCONSIN CHEESE EXCHANGE

An institution where over 14,000,000 lb. of Cheddar cheese may be sold annually, as described by its president

R. W. LEFFLER
Plymouth, Wis.

Cheese has been produced in the state of Wisconsin for over 112 years, there being some record of an early cooperative venture in Jefferson County as early as 1841. Most of the early factories were operated on a cooperative basis, a cheesemaker being employed by a group of farmers who supplied a factory with milk. Early methods of distribution were inadequate from a standpoint of both producer and consumer, and the industry grew slowly.



R. W. Leffler

The early cheesemaker had no storage facilities at his factory. His cheese was perishable and he had to dispose of it promptly. He needed a buyer who would take his entire output from week to week, regardless of amount or grade. At the outset such buyers did not exist. The consumer bought from his grocer or possibly from a peddler, and the grocer, through whatever arrangements he could make, ordinarily dealt directly with the factory. Those arrangements were usually unsatisfactory. The factory might or might not be able to fill the grocer's order when received, and the cheese obtained might or might not be of the grade desired.

The Cheese Dealer Served an Important Function

Before long the cheese dealer entered the picture. His functions were those of a jobber or wholesaler. He maintained warehouse facilities which enabled him to buy cheese from the factory as it was made and hold it until a market could be found. He was able to grade, separate, and age the cheese purchased from factories with which he did business and sell to the grocer or other retail outlet the precise type of cheese for which that outlet had a demand.

The dealer solved some of the problems of the factory and many of the problems of the retailer. The cheesemaker, however, was still in a position where he had little bargaining power and little knowledge of general market conditions. Perhaps several dealers would periodically send buyers to his factory, but there was little the cheesemaker could do other than take the best price offered by them.

First "Call Board" Established in 1900

It was to improve the position of the factory operator that the so-called dairy boards were established, 1873 being the year in which they first made their appearance. A board was established in Plymouth, Wisconsin, on May 22, 1879, and Plymouth has continued from that date to occupy an important place in the marketing of cheese.

Essentially, a dairy board was no more than a market place where buyers and sellers of cheese might get together. The cheesemaker had an opportunity to come into contact there with more buyers than the limited number who might call at his factory. He had an opportunity to learn to some extent what other factories were getting for their cheese. Such boards were also time-savers for the buyer. He no longer had to travel from factory to factory in order to buy his cheese. For a while these boards attained considerable popularity. Over 50 were in existence at one time.

Originally, negotiations at a board meeting were conducted between an individual seller and an individual buyer. As time passed, however, the cheesemaker saw an opportunity to improve his position by disposing of his cheese through a form of auction. The cheesemaker or other factory representative would list his cheese upon a blackboard provided for that purpose, and then call for bids. Both the offers and bids were termed "calls" and the dairy boards soon began to be termed "call boards." The call board method of trading was adopted at Plymouth in 1900.

The call boards represented an improvement over earlier marketing practices but they still left much to be desired. A seller, for example, did not offer his cheese at a price which he named but simply offered cheese upon which buyers were asked to bid. Bids were firm only to a very limited extent and an offer could be withdrawn at any time. More importantly, the seller was not required to deliver cheese in strict accordance with his offer. State grades had not been developed at that time, and the seller on the early boards offered only to supply first class goods. If he failed to make such delivery, the buyer's only recourse was to reject such part of the cheese as was undergrade.

In order to insure themselves of cheese of the type and in the quantity desired, buyers began to establish direct connections with factories which were producing cheese of the particular type and quality for which the buyer had a market. A dealer would agree to take the entire output of a factory for a specified period of time, usually a year. The price the factory received would be based upon the prices paid from week to week on some specified call board in which both the dealer and the cheesemaker had some confidence. Such an arrangement saved the cheesemaker both time and trouble. He was able to spend all of his time at his factory instead of spending a part of it at the one or more boards which he formerly attended, and if he was guaranteed as much under a long-term contract as he would receive from a sale of his cheese on a call board he felt he had nothing to lose from such an arrangement. As this trend set in, the call boards declined rapidly in both importance and number.

Beginning of the Wisconsin Cheese Exchange

As buyers contracted for the output of more and more factories, new problems were created. Originally it was the cheesemaker who had to worry over the disposition of his cheese. When a dealer contracted for the entire output of a number of factories, however, he could no longer keep his receipts and sales in perfect balance. Particularly in periods of flush production he might receive more cheese than he could dispose of through his normal outlets or more than he could afford or had the facilities to store. Originally, the cheesemaker was the only one who had cheese available for sale on a call board. By 1918, the dealer frequently found himself in that same position.

On April 24, 1918, the Wisconsin Cheese Exchange was formed. It was an exchange in name only. In substance it remained a call board. Offers and bids were still made in accordance with customary call board procedure, and seller and buyer retained the right to withdraw offers and bids at any time. In one important respect, however, the Wisconsin Cheese Exchange marked a departure from its predecessor board, the Plymouth Central Call Board of Trade, and from other call boards. Dealers for the first time were accorded equal privileges with the cheesemakers. They were permitted to become members of the Exchange, to sell as well as to buy cheese upon it, and to have a voice in its management. The earlier call boards had been operated by and, it can no doubt be fairly said, solely for the benefit of the cheesemaker.

At the outset, substantial amounts of cheese were sold on the Exchange by factories and dealers. Cheesemakers, however, continued in ever increasing numbers to contract for the direct sale of their cheese, and factory offerings soon began to decrease in volume. By the end of 1921, factory offerings were virtually a thing of the past.

The disappearance of factory sales was a serious blow to the Exchange. Various new rules which were tried out and the adoption of official Wisconsin grades of cheese facilitating the settlement of disputes were all helpful, but the volume of trading continued on an unsatisfactory level for some 15 years.

A fairly comprehensive revision of the rules was made in 1936. The seller was then required for the first time to deliver according to specifications the full amount of the cheese offered by him. Bids were made firm, and the seller

was given only a limited right to withdraw his offer. The keeping of certain records was required, and provisions were added which enabled the Exchange to discipline members who did not observe its rules. The general tendency of this 1936 revision was to make the Exchange more attractive to the buyer. It did not solve all of the problems of the seller.

On August 12, 1938, the Wisconsin Cheese Exchange finally became an exchange in fact, as well as in name. After an exhaustive study of the operations of other commodity exchanges, and after consultation with various state and

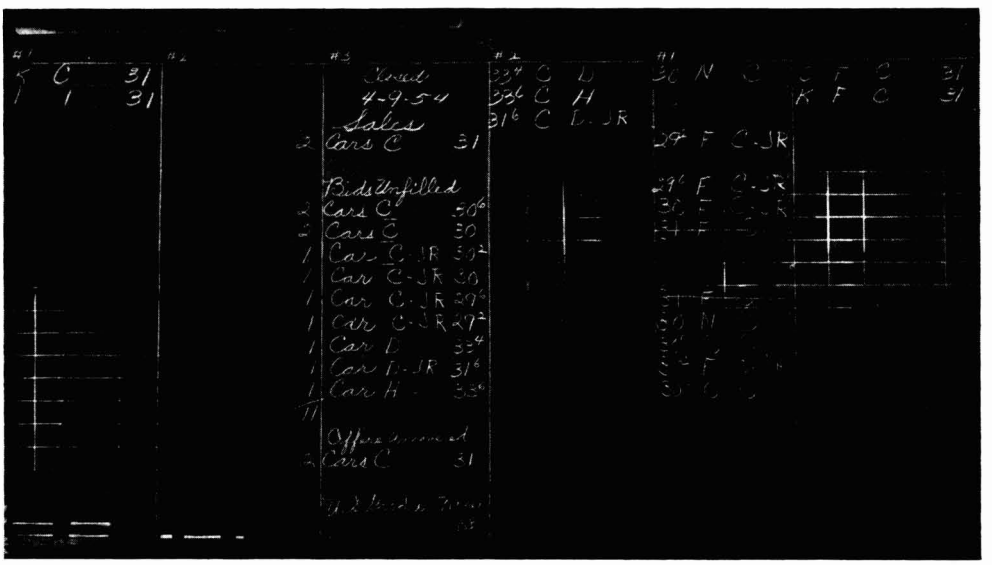


FIG. 1. The Exchange Board for Wisconsin-Grades at the close of trading. Completed sales are listed in the right hand column. Bids work from the sales column to the left and the offers are posted in the left column to the right. Bids and offers made during the session that were withdrawn are not shown. Where no grade is indicated, it is State Brand. Junior Grade is indicated by JR following the symbol for that style. Cheddars have the symbol C, Single Daisies D, and Longhorns H. A separate board is used for U.S. grades.

governmental agencies, an intelligible, workable set of rules was adopted. The assembly charge, a charge which enabled the seller to recover the costs incurred in assembling and grading factory cheese received by him, was increased to 5/8¢ per pound, which approximated the seller's actual costs and made it possible for him to sell as advantageously on the Exchange as through outside channels.

All offers and bids were required to be made at stated prices and registered in rotation. A bid was made against no particular lot of cheese. It was an offer to buy cheese of the type, in the quantity, and at the price specified in the bid, from the first person willing to sell on those terms. An offer was an offer to sell cheese on the terms specified therein to the first person agreeing to buy on that basis. No longer was it possible for the seller to choose the buyer to whom he wished to sell.

An entirely new rule had the general effect of broadening the trading base by application of the so-called freight differential charge. Since the general movement of cheese in Wisconsin was towards the south and east, the warehouses from which the freight rate to Chicago was the lowest occupied a favored position. All other factors being equal, a buyer would naturally purchase the cheese on which he had the lowest transportation charges to pay. By requiring the seller to absorb transportation costs to the extent that the rate from his warehouse to Chicago exceeded the rate from Plymouth to Chicago, this rule, in effect, enabled the buyer to purchase f.o.b. Plymouth. In the absence of such a rule, owners of warehouses which were unfavorably located from a freight rate standpoint found it difficult to sell their cheese on the Exchange.

All of these basic rules, with some slight modifications, remain in force today. The response to them was immediate and gratifying. Sales skyrocketed and trading has continued in large volume from the date they went into effect. Efforts to improve its practices and to make the Exchange even more attractive to buyers and sellers of cheese did not cease with the general revision of 1938. In that year a service charge was imposed upon both buyer and

seller, the charge being a fixed amount for each box of cheese sold. By the imposition of this charge, members were required to contribute to the support of the Exchange in proportion to their use of its facilities. Also in 1938, sellers were permitted to invoice the sales of certain styles of cheese on what was termed the "dry basis," which enabled the seller to obtain a premium for cheese having a low moisture content.

Special rules were adopted in order to enable the government to trade on the Exchange during the war, and as a result more than 134,000,000 lb. of cheese were purchased for Lend Lease and the Armed Services. In 1947, provision was made for the first time for the sale of cheese made in states other than Wisconsin. Although trading in out-of-state cheese remains relatively small, offerings have been made from Illinois, Indiana, Iowa, Kansas, Michigan, Minnesota, Missouri, and even Oregon. Also in 1947, the assembly charge was raised to 7/8¢ per pound in recognition of increased costs.

Membership in the Exchange

Membership on the Wisconsin Cheese Exchange is open to any person, partnership, association or corporation, including the operator of any licensed cheese factory for whom special rules have been established. Each member pays the same membership fee and the same annual dues. Each member has one vote and an equal voice in the management of the affairs of the Exchange. The interests represented by the members of the Exchange cover all phases of the cheese industry from the production of cheese to its ultimate distribution. Some of its most active members are cooperatives directly representing the interests of their producer members. Others own and operate factories themselves. Still others are producers who buy and convert large quantities of cheese into processed cheese or cheese foods. Large retail grocery chains also are represented. The members are located from the east coast to the west coast, throughout much of the mid-west and as far south as Tennessee. Members from distant points operate through trading members and maintain contact with them by means of telephones located on the floor of the Exchange.

The Exchange meets on Friday of each week. The trading session lasts for 30 minutes, with the presiding officer having the right to grant all necessary extensions of time — a right which is frequently exercised. No need has been found for holding more frequent meetings.

The question of futures trading has been considered on more than one occasion, but no interest in such trading has ever been shown by the Exchange members. Cheese is sold on the basis of age, grade, and type, with bids required to be made in multiples of 1/8¢. All transactions are on a cash basis.

The Exchange issues no official quotations or price interpretations. It is justifiably proud of the fact that Exchange transactions are generally regarded as an accurate barometer of the value of cheese and used on occasion as the basis of payment under long-term purchase contracts. The Exchange feels, however, that any question regarding the significance to be attached to these transactions is for determination by the parties interested therein.

As is true of all commodities which are produced on a year-round basis, the bulk of all cheese which is produced in Wisconsin and elsewhere throughout the United States is sold through direct trade connections which the producer establishes. The function of the Wisconsin Cheese Exchange, as is true of all commodity exchanges, is not to provide a means by which it is expected that all of a commodity will be marketed, but is rather to provide an alternate market where the producer, assembler, wholesaler, jobber, and distributor can buy, sell, offer, or bid for cheese if they choose to do so. It offers a safeguard to both buyer and seller, even though they may conduct most of their trade through other channels. As heretofore indicated, however, trading on the Exchange is far from being of an inconsequential character. It is in fact believed that a larger part of the cheese produced in the United States as a whole is sold on the Wisconsin Cheese Exchange than is the corresponding part of the total output of any other commodity which is sold on any other commodity exchange in the United States.

The Exchange is hopeful that its rules can be further improved and that even greater use will be made of the facilities which it offers. Suggestions to that end will always be welcome.

DAIRY RESEARCH TO THE RESCUE[a]

EZRA T. BENSON
Secretary of Agriculture
U. S. Dept. of Agriculture

It is a very real pleasure to be here for the annual meeting of the American Dairy Science Association because it gives me an opportunity to discuss the importance of research and education with people who believe just as fervently as I do in such programs.

Last Fall I asked the officers of this Association to request from the entire membership suggestions for strengthening American agriculture through a program of research and education. President W. V. Price recently presented me with a carefully prepared analysis of your suggestions. Approximately 300 members of the Association contributed to a list of 378 projects which are incorporated in this report.

It is most encouraging to me personally to

[a] Address by Secretary of Agriculture Ezra Taft Benson before American Dairy Science Association, State College, Pa., June 22, 1954.

know that this request brought such a wide response from you. I appreciate the thought and labor which has gone into this compilation of projects. The report is being made available to the Dairy Research Advisory Committee which counsels the Department of Agriculture on its research programs, as well as to the appropriate officials and agencies within the Department. I offer my sincere thanks to all of you who assisted in this undertaking.

The dairy industry needs the help of you men of science now as never before. It needs every push you can give it towards increased efficiency in production and marketing. It needs further outlets through new products. It needs critical re-examination of outmoded formulas which place too much emphasis on butterfat and not enough on nonfat milk solids.

The problem presents an enormous challenge to science. But the challenge to dairy farmers, to processors and distributors and to the government itself is fully as great.

Milk Should be Made More Available to Users

The industry has not done an adequate job of promoting and selling dairy products. Plain milk is the most nearly perfect food — and it tastes good. But do we tell that story? I'm afraid not. We don't even make milk readily available to many potential buyers.

Next time your travels take you to an airport or to a railroad station have a look at the banks of mechanical gadgets ringing the waiting room . . . soft drink, candy and cigarette dispensers . . . maybe even a few pinball machines. But up to now I have found just one airport in the United States where you can place a coin in the slot and obtain a drink of milk.

Generally you will not find milk dispensing machines in office buildings, factories, and public places where soft drinks are sold through mechanical devices. Until a few weeks ago, there were no milk dispensers in the Department of Agriculture buildings in Washington, which seems illogical enough in view of our current surplus. But even more fantastic is the situation in a midwestern farm machinery factory — deriving a substantial part of its business from sales to dairy farmers — where milk and all soft drinks but one are barred from dispensers by contract. I wonder how many hay-balers this favored bottler bought last year.

The various beverage makers spend millions of dollars each year plugging their products through every advertising device from sky-writing to match-covers. And after they have created a demand they make certain that the product is available where and when the public wants it.

Certainly no one would contend that milk dispensers alone will solve the dairy problem. They do, however, offer one means of stepping up consumption. They illustrate potential marketing possibilities. And in our present situation we must not overlook any chance to expand outlets for milk and other dairy products.

Milk Production Increasing

It is not exactly news to this audience that milk production hit an all-time high in 1953 and that production during the early months of 1954 was running ahead of last year. Despite the reduction in the level of government support, we are continuing to pile up butter, cheese and dried milk. While consumption has increased, most of this gain is being offset by higher production. It probably won't be until we have passed the flush spring production period that milk output will be reduced in response to the lower level of price support. We face the rather discouraging prospect of having to buy several hundred million dollars worth of dairy products during this marketing year.

Now if there were an export market for these items — even at a sharply reduced price — or if we could feed them back into domestic trade channels, even at some loss, without badly disrupting normal marketings, then our problem would be comparatively simple. But such disposal programs are extremely difficult. Moreover, subsidized disposal is at best a temporary solution of the problem which is popularly termed over-production but should rightfully be called under-consumption.

We know that if the American people were drinking as much milk per capita as they were only a few years ago, there would be no dairy surpluses. We also know that during those high consumption years in the 1940's, millions of our citizens, young and old, were not getting even the minimum amount of milk required for their own welfare.

Further Consumer Education Needed

While the job of promoting and selling is of course the responsibility of the dairy industry itself, research and education can play the major role in providing new and better products. During the war years, scientists made great progress in the utilization of nonfat milk solids. Further improvements have since come about. Yet we have somehow failed to stimulate the necessary consumption. We have not educated people sufficiently in the uses and advantages of these products, although high quality food proteins should find a ready market in a diet-conscious America which is steadily swinging away from such traditional foods as grain products and potatoes.

This change in the eating habits of our people is one of the more interesting phenomena of our times. It is forcing changes in our agricultural production patterns, even though our price support programs for the most part do not recognize its existence. Agriculture must inevitably adopt one of the first rules of mer-

chandising by giving the customer what he wants.

The customer today is eating about the same poundage of food as his grandfather did. But the dining table takes on a far different look at mealtime from that of a few decades ago.

The mountain of potatoes and the skyscraper stack of bread have been largely replaced by more beef, eggs, fish, certain dairy products, fruits and vegetables. Of course, these choice foods provide infinitely greater variety in our diets. They cost more per calorie, too. But the trend of consumer preference is unmistakable and the buyer is willing to pay the price.

Surpluses Continue to Grow

Yet we have gone merrily on our way with a farm program tied to another generation. Most of our current grain surpluses stem directly from the fact that government price supports encourage farmers to produce corn and many other grains for storage, rather than for livestock feed. We continue to pile up these surpluses in warehouses — far beyond our needs — through a pricing device which discourages their conversion into the type of food the American people need and will buy.

Our carryover of corn next October 1 is estimated at 950 million bushels — more than double what it was two years earlier. This increase, had it been consumed as feed, would have resulted in only 2 to 3% more meat and eggs.

From a nutritional point of view, the public could well have absorbed this without even letting out a notch in the national belt. In the form of corn, however, this same grain is giving both our markets and our storage facilities a bad case of indigestion.

With more flexibility in our support prices for corn, most of this current surplus would have disappeared. Increased marketing receipts from livestock would have largely offset the payments farmers now get by way of nonrecourse loans. Our agricultural economy would have been healthier. And the government currently would not have some $1.2 billion tied up in corn price supports.

In the case of wheat, we have stimulated even greater production for government storage. On July 1, as the new crop moves to market, we shall have on hand from previous years an estimated 875 million bushels of wheat — enough to supply our total domestic needs, plus our foreseeable exports, for the next 12 months.

The government now has nearly $2¼ billion committed to wheat price support operations in an effort to maintain the return to farmers at 90% of parity. Despite this huge outlay and our utmost efforts to make price supports effective, wheat is currently bringing only 80% of parity in the market.

Much of the wheat grown east of the Mississippi, which formerly was fed to livestock, now finds its way into government storage because we are outbidding the other users. As a result of these accumulations, wheat growers have voted to accept production controls this year. They face the necessity of further cut-backs in 1955. High price support, coupled with sharply-restricted output, can scarcely compensate the low-cost growers of the Great Plains states for the markets they are losing — markets which they are equipped to fill at competitive prices.

The Support Problem

There must be some significance in the fact that most of the farm commodities in serious difficulties today are the ones which we have attempted to support at 90% of parity. They include wheat, cotton, corn and dairy products. Of the approximately $6½ billion which Commodity Credit Corporation has committed to price support operations, more than $5 billion is tied up in these four items.

Producers of other crops, which enjoy no price supports at all or get help on a more limited basis, are beginning to express some concern over the effects of a farm program which places most of the emphasis upon a few selected commodities. They see particular danger that the acres diverted from such crops as wheat, corn and cotton may be planted to the non-supported commodities in which they have been specializing.

Despite the emphasis which our programs place upon the six basic commodities — and the present controversy centers mainly around the question of rigid versus flexible supports for these crops — they produce only 23% of farmers' cash marketing receipts. Of the Nation's farm income 56% is derived from commodities which have no direct price supports at all. The other 21% comes from products already supported on a flexible basis.

Farmers who buy price-supported feeds and sell hogs, cattle, poultry and eggs on a free market are often inclined to take a rather dim view of our current operations. For the most part, they don't want price support programs themselves and they feel that if other groups favor such aids there should at least be a realistic amount of flexibility in the operations.

The fact that there are obvious defects in the present programs does not prove by any means that the principle of price supports is either unsound or unworkable. I believe we need such programs. Soundly conceived and properly administered they can be of as much benefit to consumers as to farmers. They can help to assure an abundant, stable supply of food and fiber at reasonable prices. They can protect farmers against violent fluctuations in income. They can be of benefit to farmers in the orderly marketing of many commodities.

My own quarrel with high, rigid supports is not that they give the farmer too much but

that in actual practice they give him too little. In the long run they take from him more than he receives. They encourage him to deplete his soil. They saddle the markets with price-depressing surpluses which give him no opportunity to realize full parity for his production. They destroy the normal relationship of feed and livestock prices. They price him out of many natural markets both at home and abroad, at the same time encouraging the development of competitive synthetics and substitutes which further shrink agricultural outlets. They place him in such a tight production straitjacket that he loses most of his freedom to make management decisions.

When we pile up surpluses far in excess of normal reserve requirements, we are in effect borrowing from tomorrow's markets. Such practices can lead agriculture toward financial disaster just as certainly as borrowing against tomorrow's earnings can bankrupt an individual.

Today it is costing the federal government more than $30,000 an hour — over $700,000 per day — a quarter of a billion dollars a year — just to store the commodities owned by CCC. One of the safest predictions I can make here today is that the bill will be larger in the months ahead.

All of these things, I believe, are compelling reasons for the adoption of the President's farm program recommendations. The Administration's plan was not presented as a panacea — in fact there is no quick cure-all for agriculture's ills. In many respects the level of price support will not be greatly different from what we now have. But flexible supports will point our future farm programs in the direction we must take, toward better balanced production. Billy Sunday, the Evangelist, once said that he would rather be a foot from Hell, headed away from it, than 25 miles away and headed toward it.

In the case of dairy products, it would be especially unfortunate if we were to seek a solution through higher price supports, rather than through a program of vigorous sales promotion, research and education and sensible culling.

There are now recommendations that the support level for dairy products be pushed up to some higher percentage of parity. This would be after the farmers, the trade and the consumers have made the adjustment to a more realistic level. As a rule of thumb, every 5 point increase in the level of dairy price support would cost the government approximately $100 million and further postpone the time when the dairy industry could stand on its own feet.

If supports were raised to 80% of parity now, such action would have these additional probable results: Government acquisition of butter would increase to the extent of 100 to 150 million pounds. Consumption of butter would drop about 50 million pounds, with a corresponding increase in the use of substitutes. The incentive to maintain a high level of dairy production would be strengthened. Substantial windfall profits would accrue to the butter trade on inventories in stock.

We are painfully learning, through our experience with dairy products, some lessons that could have been more easily learned by a review of history. We are learning that if price supports are long maintained above their natural level, certain results follow as surely as the night follows the day. Production is stimulated. Consumption is retarded. Surpluses pile up. The problem of surplus disposal follows.

Even then we are not through. Surplus disposal is costly and offensive, so that we are driven to production control. In the first act of this melodrama, High Price Support is the hero who would thwart the villain. In Act Two, High Price Support must enlist the aid of that unpopular character, Surplus Disposal. In the final act, both of them must call for help from that obnoxious fellow, Production Control.

The audience should know all this before buying a ticket to the show. We should know that we cannot have high price supports, which are attractive, without having surplus disposal and production control, which are not.

The easy solutions to our dairy problems turn out to be illusions.

The Real Solution — Improved Marketing

I am convinced that the real solution to our problems — the hard solutions —lie in improved marketing. Here we have much to accomplish, especially for fluid milk. Although the present system of retail distribution of milk is the result of experience, it can and should be improved. Steps in this direction are important to consumers as well as producers.

Wages make up a high percentage of the costs of milk distribution. The problem of high labor costs in distribution lies not so much in high wages as such but rather in the failure to increase the output per man. Striking gains in labor efficiency have been made in producing, transporting, and processing milk, but, except for the change from every-day to every-other-day delivery, there has been little increase in output per man in delivery of milk to homes.

If milk is to meet successfully the growing competition with other foods, it will be necessary to make modifications in the marketing system. Consumers who wish to pay cash and do their own delivery should have the chance to do so at a price which represents the reduction in costs involved.

Fortunately, there are encouraging signs of united efforts by the entire dairy industry to stimulate consumption through improved marketing and merchandising programs. I am heartened at the magnificent response of so many food retail outlets throughout the Nation

in promoting sales of butter, cheese and milk during this June Dairy Month.

It is still too early, of course, to determine how much increased consumption of dairy products will be generated this year through the combination of aggressive selling and reduced prices. Our own field reports show that home sales of butter are running about 10% higher than they were in March, just prior to the adjustment in dairy price support levels. If we could obtain a 10% increase in consumption of all dairy products throughout this marketing year, the industry might look to the future with real confidence.

There is a Job to be Done

I believe this can be done through the combined efforts of all of us who have a vital stake in dairying. It is not a job which can be accomplished through one grand promotional flourish, however. It must be carried forward from one month to the next.

I know that the Department of Agriculture is not going to let down in this campaign. I am convinced that farmers and the industry generally will do their part. The contribution which dairy scientists can make here may be the most important of all and I look forward to results which will surpass even the accomplishments of the past.

Some day, in the years — possibly even in the months — ahead, science is going to perfect a frozen whole milk concentrate, or perhaps a whole milk powder. You know of the progress which has already been made in this field in our own laboratories and in those of industry. I am convinced, as I know you must be, that when this development comes it will bring with it a new era for the dairy industry.

We have seen what a similar process has done for citrus producers. It might do even more for dairymen.

Meanwhile, in an effort to meet some of our immediate problems, the Department of Agriculture in recent weeks has:

1. Launched a nationwide educational campaign to promote the culling of low-producing cows from our dairy herds.
2. Offered to sell for export at world prices government-owned stocks of butter, cheese and dried milk.
3. Developed a special program to facilitate the use of butter and nonfat dried milk solids for recombination and sale as fluid milk in friendly countries.
4. Sold more than 400 million pounds of dried milk at greatly reduced prices for use in mixed animal and poultry feeds.
5. Sought increased consumption of dairy products through school lunch programs, donations for domestic and foreign relief and other outlets.

The thorniest problem of all is posed by the more than 400 million pounds of butter which the government has in storage today. We have carefully examined and, in fact, are still studying several domestic disposal plans which would make this butter available to housewives at reduced prices. All of these plans would be costly — perhaps to the extent of half a billion dollars. None of them would be easy to administer.

Because any domestic disposal plan undertaken on the scale required here would disrupt normal markets, we would have to reconcile ourselves to a situation under which the government would be plunged deeply into the merchandising of dairy products.

In our concern over special problems such as the dairy situation, however, we must never lose sight of the greater and over-riding considerations which affect our very national existence. The matter of surpluses is insignificant along side the great question of our time: whether man shall at long last achieve peace among men.

Certainly I do not pretend to have the answer. But unless it is an affirmative one the consequences may well be the most serious since the dawn of history. The wrong answer might even slam shut the book of history.

The Threat of Communist Aggression

Our search for a solution must lead us toward a new and better understanding of the eternal moral and spiritual values which have been partially obscured by the smoke and fire of war and revolution throughout the world. Our leaders need Divine guidance as they strive to maintain peace.

There is no hope, of course, that the Communist masters will seek counsel from a higher source. But among the millions of human beings they have enslaved, many have not lost faith. Even in Russia, after nearly four decades of systematic attempts to wipe out all religion, there are still people who look to God, rather than the state, as the highest authority.

The threat of Communist aggression is ever-present. We may have to live with it for many more years. As a Nation, we must remain strong. And to remain strong we must remain productive.

No man is more deeply concerned than President Eisenhower with the dangers we face. Nor is any man more determined that we shall safeguard our own liberties while defending ourselves against the Communist threat from without and within.

We would be poor indeed if, in our zealousness to protect America's shores, we succumb to the hysterical preachings of those who would destroy our basic freedoms under the guise of anti-Communism. The trappings of the totalitarian state — neighbor spying against neigh-

bor, children informing against parents and government employees reporting undercover to self-appointed guardians of our security — such manifestations of moral decay must find no honored place in our way of life.

Our fundamental freedoms did not come easily. Eternal vigilance is still the price of liberty. We must guard, as we guard our skies and our shores, the guarantees set forth in the Bill of Rights. To me, freedom is a God-given, eternal principle, vouchsafed to us under the Constitution. It must be safe-guarded as something more precious than life itself.

The torch of freedom, burning bright and held high, must one day push back the shadows of ignorance and despair which now blanket the minds of Communism's slaves.

God grant it may be so.

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JOURNAL OF DAIRY SCIENCE

ABSTRACTS OF LITERATURE

W. O. Nelson, Abstract Editor

BOOK REVIEWS

665. Biochemistry and Physiology of Nutrition, vol. II. Edited by GEOFFREY H. BOURNE and GEORGE W. KIDDER. Academic Press Inc. 641 pp. $14.00. 1953.

The titles, authors and number of pages devoted to the various chapters follow: The Intracellular Localization by Histochemical Methods of Enzymes and Vitamins, by Geoffrey H. Bourne, (42); Structural Changes in Vitamin Deficiency, by Geoffrey H. Bourne, (85); Microbiology of Digestion, by D. P. Cuthbertson and A. T. Phillipson, (34); The Nutrition of Invertebrate Animals, by George W. Kidder, (35); Energetics and Metabolic Functions, by Eugene P. Kennedy, (35); Hydrolytic and Phosphorolytic Enzymes, by Warren H. Yudkin, (55); The Respiratory Enzymes, by B. L. Horecker and A. Kornberg, (41); Coenzymes, by G. David Novelli and Morris Soodak, (71); Iron Metabolism, by H. F. W. Kirkpatrick, (13); Calcium and Phosphorus Metabolism, by H. F. Kirkpatrick and J. Douglas Robertson, (16); Trace Elements, by E. J. Underwood, (79); Application to Human Nutrition, by Grace A. Goldsmith, (77); Author index, (32); Subject index, (26).

The objective of the editors was "to gather together into one work the most salient segments of the vast amount of research dealing with the field of nutrition." Like the preceding volume it is not to be regarded as a textbook in the usual sense of this term but presents excellent reviews of recent experimental work. In general the various chapters are well written although some readers would undoubtedly appreciate more extensive use of formulas, charts, and tables than is found in certain of the reviews.

Dr. Bourne is to be commended for a complete presentation of the information regarding Structural Changes in Vitamin Deficiency. This reviewer also found especially informative the novel chapter on Microbiology of Digestion, and the chapters on Trace Elements and Coenzymes.

The problems of galactose metabolism are of considerable interest to people concerned with the nutrition of milk. Some of the specific reactions of galactose are discussed under the uridine coenzymes. On page 389 a speculative reaction sequence for the interconversion of galactose-l-phosphate and glucose-l-phosphate is presented, and although it is of considerable interest, it is not supported with experimental evidence.

As with the preceding volume, the cost will unfortunately limit distribution of this fine work. R. G. Hansen

666. The Anatomy of the Bovine Foot. ROBERT F. WAY, V.M.D. University of Pennsylvania Press, Philadelphia. 58 pp. $4.00. 1954.

This book is a pictorial approach to the study of the bovine foot. The author has succeeded in vividly portraying the subject with 28 illustrations which are drawings very effectively indicating the third dimension. Accompanying each plate are pertinent observations directing the observer's attention to important structures and areas. The paper and printing are unusually effective in the presentation of the subject.

The book is intended for the clinician and practicing veterinarian, because the foot is a frequent seat of pathological conditions. Herdsmen and dairymen will also find it interesting and useful. E. E. Ormiston

BUTTER

667. Afproevning af nogle antioxidanter til smoer (Testing of some antioxidants for butter). K. P. ANDERSEN and A. P. FISKER, State Expt. Sta. Creamery, Hilleroed, Denmark. Bull. 4. 1953. (English summary).

The antioxidants metol, glycine, hydroquinone and ascorbic acid were added to butter in concentrations ranging from 0.025-0.04 p.p.m. The results proved none of these compounds satisfactory. Hydroquinone, ascorbic acid and metol showed an antioxidative effect but the first two compounds gave the butter an off-flavor and the last one caused a darkening of the butter. Glycine had very little antioxidative effect and when used in the higher concentrations gave the butter an off-flavor.

Eight commercial antioxidants also were tested. Ethyl gallate preparations were better than octyl and dodecyl gallates. Water-soluble preparations containing potassium or sodium iodide were superior to all others, giving better protection and less off-flavor.

T. Kristoffersen

668. Foersoeg og afproevninger i forbindelse med holdbarheden af detailpakket smoer (Experiments and tests in connection with keeping quality of printed butter). A. N. FISKER and T. THOMSEN, State Expt. Sta. Creamery, Hilleroed, Denmark. Bull. 83. 1953. (English summary).

Experiments were conducted to determine at which stage butter should be printed and the kind of wrapping material which should be used in order to obtain printed butter of the same high keeping quality as tub butter.

Even for home consumption, where the butter usually is eaten within 8-10 days after printing, it was found advantageous to print the butter directly from the churn rather than after it had been allowed to set. Free moisture was avoided by direct printing. For the European and overseas markets direct printing from the churn was necessary in order to maintain a satisfactory keeping quality.

Sweet cream butter with 2% salt kept better than the regular butter made from ripened cream and with a lower salt content.

Aluminum foil protected the butter better against light and air than parchment paper, and was considered a more satisfactory wrapping material although butter wrapped in aluminum foil showed a greater tendency to become moldy. Mold growth could be retarded by continuing working the butter in the churn for about 20 min. beyond the stage at which it usually was considered dry. This resulted in a considerable improvement in the size and distribution of the water droplets in the butter.

Butter, purposely inoculated with mold spores, worked in this manner showed no mold growth after incubation in darkness at 19° C. and 90-95% humidity for as long as 6 weeks. Keeping time for normally worked butter under the same conditions was about 4 weeks.

T. Kristoffersen

669. A buttermaker comments on starter butter. O. C. CAPPER, Clinton County Central Cry., DeWitt, Iowa. The Milk Prod. J., **45**, 6: 28. 1954.

A large percentage of butter manufactured 40 years ago was made from ripened cream. Starters often were used to achieve a better flavor and aroma.

This product has been replaced by sweet cream butter. The main reason for this transition was the better keeping quality of sweet cream butter. The author presents some arguments favoring the addition of starter to butter to improve its flavor and aroma and to help sales by making butter more appetizing.

J. J. Janzen

CHEESE

670. Meet the kaseferteger mechanical cheesemaker. Staff, Food Eng., **26**, 4: 89. 1954.

The German Steinecker machine is a cylindrical jacketed vat of 11,000 lb. capacity, elevated on a stand. It is equipped with a central shaft to which may be attached curd knives, agitators, or "swimming floats." It is designed so that vacuum can be applied to aid in separation of whey from curd. In emptying, curd flows from the vat to cheese hoops and whey runs to a holding tank. A half-ton of curd can be discharged in 11 min. The unit eliminates considerable manual labor and contributes to greater uniformity in moisture content of the cheese. Types of cheese made with the equipment include such as Port du Salut, blue, brick and baby Edam. T. J. Claydon

671. Keeping quality of cottage cheese. L. T. KESTER, Carnation Co., Van Nuys, Cal. Milk Plant Monthly, **43**, 4: 36. 1954.

The problem of cottage cheese spoilage amounts to a major financial loss to plant operators each year. Its shelf life may be improved by better sanitation of equipment, better treatment of water supplies, better chilling and refrigeration of curd, better education of consumers to the perishable nature of the product, better covers for cheese vats and better laboratory control machines. C. J. Babcock

CONDENSED AND DRIED MILKS; BY-PRODUCTS

672. Studies on improving the ease of reconstitution of skimmilk powder. D. L. GIBSON and J. W. RAITHBY, Univ. of Sask., Saskatoon. Can. J. Technol., **32**, 3: 60. 1954.

In an effort to improve the ease of reconstitution of skimmilk powder, methods of reconstitution and techniques of manufacture were studied. It was found that reconstitution at a temperature of approximately 50° C. gave the highest wettable value. Reconstituting above 60° C. markedly reduced reconstitutability. Powder particles with a diameter of less than 15 microns were more difficult to reconstitute than those having a diameter of 15 to 25 microns. The addition of up to 25% by weight of sugars or sugar derivatives produced no appreciable effect on wettability unless they were added after drying. Surface active agents of the Tween variety at concentrations as low as 0.1% by weight of the skimmilk powder improved wettability. O. R. Irvine

DAIRY BACTERIOLOGY

673. Substances in herd milks inhibiting acid production. C. K. JOHNS, Can. Dept. of Agr., Ottawa. Can. J. Agr. Sci., **33**, 6: 586. 1953.

Of 344 samples of herd milks collected in the spring, 7.3% showed inhibitory effects against lactic starter organisms, as against 5.4% of 298 summer samples. Those showing inhibitory action were then tested for the presence of antibiotics. Only 1.4% of the total showed zones of inhibition by the penicillin disk assay method. The much lower percentage of positive samples

by the disk assay method is attributed in part to the greater use in this area of antibiotics other than penicillin which are not detectable by the disk assay method at concentrations sharply inhibiting acid formation. Tests for the presence of residual quaternary ammonium compounds gave negative results, while bacteriophage action and naturally occurring heat-labile inhibitory substances were largely ruled out by preliminary heating. O. R. Irvine

674. Digestion by rumen microorganisms. Hydrolytic products of cellulose and the cellulolytic enzymes. W. D. KITTS and L. A. UNDERKOFLER, Chem. Dept., Iowa State Coll., Ames. J. Agr. Food Chem., **2**: 639. 1954.

The chemistry of cellulose digestion by rumen microorganisms was investigated with special reference to identification of the carbohydrates formed and the cellulolytic enzymes. In order to identify the carbohydrate intermediates, a number of organic and inorganic compounds were tested for their ability to arrest fermentation by rumen microorganisms at the reducing sugar stage. Among those found effective were toluene, thymol, and sodium fluoride. Inhibited cellulose-digesting cultures were prepared and the media analyzed at hourly intervals for the carbohydrate intermediates formed. Filter paper chromatography revealed the principal intermediate compound of cellulose degradation by rumen organisms to be glucose. The cellulolytic enzymes of rumen microorganisms are not present as such in rumen fluid, but are associated with the bacterial cells, as centrifuged and filtered rumen fluid failed to digest cellulose. Cell-free enzyme extracts of mixed rumen culture and of an isolated rumen organism were prepared which degraded cellulosic substrates. An optimum temperature of 40° C. and an optimum pH of 5.5 were found for the maximum activity of the cell-free preparations. The cell-free preparations are stable at low temperature, but are partially inactivated when allowed to stand at room temperature for 144 hours.

S. Patton

DAIRY CHEMISTRY

675. Nutritional assay. Relationship between in vitro enzymatic digestibility and in vivo protein evaluation of powdered whey. R. M. DEBAUN and W. M. CONNORS, Natl. Dairy Research Lab., Oakdale, N. Y. Agr. and Food Chemistry, 2, May 12, 1954.

The flavor, physical properties, and nutritive value of food products, such as dried milk, are often affected markedly by the so-called Maillard reaction between the carbonyl groups of sugars and the amino groups of protein and/or amino acids. Animal evaluation of the damage done to the nutritive value of the protein in such products due to the Maillard reaction is costly in both time and animals. Hence, it is difficult to assess the relative influence of processing variables and storage on such products. Measurement of the microbiologically available lysine liberated from resuspended whey powders by digestion with crystalline trypsin affords a satisfactory prediction of animal growth response on the powders. The method is also applicable to milk powders, milk concentrates, and protein fractions derived from whey powders. Digestion with a series of digestive enzymes (pepsin, trypsin, and chymotrypsin) does not give a good prediction. Processing variables — in particular drying methods and storage temperatures — affect the nutritive value of whey powders measured *in vivo* and by the enzymatic test. S. Patton

676. Alfalfa carotenoids. Xanthophylls in fresh and dehydrated alfalfa. E. M. BICKOFF, A. L. LIVINGSTON, G. F. BAILEY, and C. R. THOMPSON, Western Utilization Research Branch, Agr. Research Service, U.S.D.A., Albany 6, Calif. J. Agr. Food Chem., **2**: 563. 1954.

The carotenoids of fresh and dehydrated alfalfa were separated chromatographically. Five xanthophylls (lutein, violaxanthin, cryptoxanthin, zeaxanthin, and neoxanthin) comprised 99% of the xanthophylls in fresh material. In addition, seven minor bands were present. The same five pigments comprised 87% of the xanthophylls of a dehydrated alfalfa meal. A total of more than 40 xanthophyll bands were shown to be present in dehydrated meal. S. Patton

DAIRY PLANT MANAGEMENT AND ECONOMICS

677. Single product benefits — multi product plant. A. V. GEMMILL. Food Eng., **26**, 3: 52. 1954.

The article is a detailed description of plant layout, equipment, and processing at the new plant of Southern Dairies, Charlotte, N. C. Ice cream making and milk processing are carried out in the large central area. However, both operations are entirely separate with a minimum of interference to each other.

T. J. Claydon

678. How Kraft's package lab works. J. V. ZIEMBA and J. E. SLATER. Food Eng., **26**, 3: 82. 1954.

Recognizing the importance of packaging research, the Kraft Foods Co. provided a centralized laboratory at the research facilities at Glenview, Ill., and staffed it with experts on specific types of packaging. The laboratory staff develops, recommends, and specifies packaging materials for efficient and economical protection of the company products. Latest testing equipment is available for determining characteristics of packaging materials under various conditions. From the results specifications are established for particular materials and uses. Close contact is maintained with problems that

develop under field conditions. Among the many innovations developed by Kraft are portion-controlled packages for individual service in restaurants and institutions, and the method of packaging cheese slices in heat sealed, coated cellophane packs. T. J. Claydon

679. Tips for retailers. ANON. Ice Cream Rev., **37**, 9: 47. 1954.

Checking the cash register against the amount of custard mix used as well as checking the cone inventory has proved an effective method of controlling theft resulting from clerks failing to ring up all cash received on the cash register. One operator has found that a gallon of custard mix should yield a gross of $4.20. If the cash register fails to show this amount of cash based upon the mix used there is good evidence that all cash received is not being placed in the cash register. Experience has indicated that over a weekly period the cash receipts should check within 2% of the estimated returns.

In leasing buildings for new retail stores, an operator with a chain of approximately 20 stores follows the policy of leasing the building at a higher rate for the first year with an option to retain the lease in subsequent years at a lower rental.

If the location doesn't prove profitable, the store is out only the higher rental over a one year period. On the other hand, if the location is a profitable one the operator has the property tied up for a period of years at a reasonable rental. W. J. Caulfield

680. Putting the finger on branch accounting systems. D. W. GREEN, Biltmore Dairy Farms, Biltmore, N. C. Sou. Dairy Prod. J., **55**, 2: 34. 1954.

A system of branch accounting in use at Biltmore is described. All items are coded to indicate the branch, type of product, department within the branch and the nature of the expense. A Burroughs bookkeeping machine was used for grouping and regrouping items in small scale operations. Monthly expense analysis sheets are prepared. As volume of business increased the Burroughs machine was replaced by IBM. The expense of transportation to the branches is charged to the processing plant to equalize cost of the distributing plants. Administrative expenses are prorated.

F. W. Bennett

681. Sub-zero holding room on wheels. ANON. Food Eng., **26**, 4: 90. 1954.

Special ice cream transport trailers of Abbotts Dairies, Inc., Philadelphia, have unique features. The aluminum body construction gives light weight and permits maximum load. The self-contained refrigerator unit suspended below the body maintains a –20° F. temperature in the trailer, and the unit can serve as an ice cream holding room. It is loaded by conveyor, and a sectional arrangement in the interior prevents cargo shifting. The system has reduced labor and time involved in loading.

T. J. Claydon

682. Outer-market sales. ANON. Milk Plant Monthly, **43**, 4: 31. 1954.

Outer-market distribution of milk is the distribution outside the community or market in which the plant is located. It accounts for a very substantial portion of the total sales of milk plants in the North Central portion of the United States. The sparser the population of the surrounding area, the more important are such outer-market sales and the greater the distances the plant goes to distribute its milk.

Data obtained for May 1952 by experiment station representatives in Ill., Ind., Iowa, Kan., Ky., Mich., Minn., Mo., Neb., N.D., Ohio, S.D., and Wis., from 588 of the 608 milk plants in the region that were equipped with paper package machines showed that: (1) Outer-market shipments have become commonplace and separate markets for packaged milk have practically disappeared, (2) Though the period of most rapid expansion is probably past, outer-market distribution is 1 phase of trend toward fewer and larger plants, (3) Further study is needed of the effects of this expansion on local markets, volume of milk sold for fluid use, returns to various groups, and (4) All parties interested in dairying should know more about the effects of widening distribution on margins, cost, sales channels, and prices. C. J. Babcock

683. Expand your laboratory services. R. W. COLLINS, Weber Central Dairy Assoc., Inc., Ogden, Utah. Milk Plant Monthly, **43**, 4: 21. 1954.

The dairy products plant laboratory should guide all phases of the business by: (1) Keeping up with the literature and adopting new methods wherever possible, (2) Helping the purchasing department to evaluate new or competing processing supplies such as cleaning compounds, stabilizers, vitamin additives, anti-oxidants, etc., (3) Assisting in the advertising and selling by supplying accurate technical data for nutritional claims, etc., and (4) Conducting research. The research may deal with psychrophile problems, milkstone as a bacteria carrier, checking cartons for correct fill, etc. C. J. Babcock

684. Case-Handling the modern way. C. O. DAVIS, JR. Milk Plant Monthly, **43**, 4: 12. 1954.

The Slauson Ave. plant of Arden Farms Company in Los Angeles has six paper carton filling machines. It uses 3 "combiners," two automatic stackers, two elevators, and about 1,600 feet of conveyor. Two fillers are for pts. and ½ pts., two for qts., 1 for ½ gal. and 1 for standby purposes. The combiners assemble cases containing like packages into groups of 5 for automatic stacking. The method used in handling bottled milk is described and a diagram given

which shows the arrangement by which conveyors and automatic stackers route the flow of bottled milk from filler to truck.
C. J. Babcock

685. Vending milk builds "plus" sales for Michigan milk distributor through factory outlets. Anon. Milk Dealer, **43**, 7: 78. 1954.

Joppe's Dairy Co., Grand Rapids, Mich., has some 38 vending machines of the selective type installed in a variety of locations — mostly factories. Unit vended is a 10 oz. container for 10¢, and items carried include homogenized milk, chocolate milk, buttermilk, and orange or grape drink. Machines have a capacity of 105 units for direct vending and another 100 units in the storage compartment. Experience of the Joppe firm shows that a factory with 150 employees will average about 100 sales per day. In hot weather, fruit drinks such as orange and grape, or buttermilk are popular, but normally the sales run about 60% homogenized milk and 40% chocolate milk. Joppe chocolate sales run higher than in many markets because the firm puts out a particularly fine chocolate milk containing 3% butterfat. C. J. Babcock

686. Steps in plant design. G. R. Johnson, Hertel, Johnson, Eipper, Stopa & Culver, Chicago, Ill. Milk Dealer, **43**, 7: 42. 1954.

A case study of the basic steps in planning a new milk pasteurizing plant is presented. The plant consists of: (1) Dairy bar to accommodate 40 customers, (2) Bottle washing room for 10 wide washer, (3) Raw milk receiving by tank truck, with tank storage in processing room, (4) Processing room to accommodate 5,000 lb. per hr. HTST pasteurizer and auxilaries, two 300 gal. surge tanks, small paper bottle machine, a 10 valve glass filler, a hooder, and the necessary pumps, controls, and conveyor, (5) By-products room for cottage cheese, (6) Warehouse of 1,200 sq. ft., (7) Space for refrigeration equipment and boiler room, (8) Small laboratory, (9) Office facilities, toilets, and locker room, and (10) Garage of approximately 3,500 sq. ft. to accommodate two serving stalls, one trailer loading dock, one tank truck stall, and space for storage of six retail trucks. Diagrams showing the plant lay-out are given. C. J. Babcock

ICE CREAM

687. Flavor scoreboard. Ice Cream Trade J., **50**, 3: 36. 1954.

I.A.I.C.M.'s flavor analysis for 1952 production showed that the industry made 174 different flavors. 72% of the volume was vanilla (51.42%), chocolate (12.2%), and strawberry (8.66%). Var. chocolate, cherry vanilla, butter pecan, peach, maple nut, coffee and var. strawberry were next in order of popularity. A table is presented which shows the flavor preference by districts. W. H. Martin

688. Sherbet flavor ratings. Ice Cream Trade J., **50**, 3: 42. 1954.

The I.A.I.C.M.'s sherbet flavor rating study showed that 142 plants, representing 1,870,147 gal., made 32 sherbet flavors. Orange, pineapple, raspberry, lime and lemon were the most popular varieties. W. H. Martin

689. If profits are down, stop, look and think. H. Black, Howard Black Cherry Co., Traverse City, Mich. Ice Cream Rev., **37**, 9: 56. 1954.

The author suggests that the pricing of packaged ice cream sold through self service cabinets in food markets should be changed to reflect the actual cost of producing the different flavors of ice cream offered for sale. A high percentage of packaged ice cream sales in food markets is in special flavors. Since the use of fine flavors in adequate amounts costs more to produce, this added cost should be reflected in the retail price. If this practice were adopted, the author contends that the ice cream manufacturers would not have to skimp on the quantity or quality of flavor used and that the resulting ice cream would be improved to a point where people would go out of their way to purchase this ice cream of superior quality.

The practice of offering quantity rebates to ice cream retailers is outmoded and should be discontinued. The rebate system had its place when deliveries were made by horse and wagon and when ice and salt cabinets were used but today with mechanically refrigerated cabinets and trucks, the rebate system is not in keeping with the times. Under present conditions ice cream manufacturers should hold their dealers by means of a high quality product rather than with a rebate check. W. J. Caulfield

690. Some production problems. H. F. DePew, National Dairy Products Corp., New York, N. Y. Ice Cream Rev., **37**, 9: 50. 1954.

This is the first of series of two articles dealing with current production problems in the ice cream industry. The production problems of major importance as determined by contacting six production supervisors were personnel, excessive number of items produced, production planning, overrun variation, coliform organisms, ice cream turnover, and quality, sanitation and housekeeping.

Of the seven problems listed, the personnel problem was considered to be most important at the present time. In dealing with the personnel problem the author emphasizes the importance of: (1) Careful screening of applicants, (2) Adequate preparation for the job so that the employee will be familiar with company policies, the physical plant, his fellow employees and what will be expected of him in the work he is to perform, (3) Adequate on the job training so the employee understands the why and how of the job assigned to him,

(4) Development of promising young men to positions of greater responsibility, including graduates of dairy schools.

The production of an excessive number of items has been a factor in reducing plant efficiency. A wide variety of items produced in small volume makes for uneconomical production. Close cooperation and planning between the sales and production departments is essential in determining what items are to be produced. W. J. Caulfield

691. Some production problems. H. F. DePew, National Dairy Products Corp., New York, N. Y. Ice Cream Rev., **37**, 10: 69. 1954.

This is the concluding article by the author dealing with current production problems in ice cream plants. In the previous article the subjects of personnel and production of an excessive number of items were discussed. In the present article the subjects discussed include production planning, overrun variation, coliform bacteria, turnover of stock, and production losses. W. J. Caulfield

692. Efficient packaging cuts ice cream marketing costs. I. Hill, Ivan Hill, Inc., Chicago, Ill. Ice Cream Rev., **37**, 10: 104. 1954.

The linerless paraffined ice cream carton is considered to be the most desirable low cost package for ice cream. The cost of the linerless carton was estimated to be 5.4% of the retail selling price in the case of pts. and 5.6% in the case of the ½ gal. cartons.

Data are presented to show that in many other products the package and label cost may range from 2.7% of the retail price in the case of cigarettes to 23% in the case of baby foods. A carton cost representing only 5-6% of the retail price is well below the average for many packaged products. W. J. Caulfield

693. Dual operation of HTST pasteurization of milk and ice cream mix in the plant. D. A. Seiberling, M. J. Dotter, and L. C. Burkey. Ice Cream Trade J., **50**, 4: 36. 1954.

A dual operation of HTST pasteurization of milk and ice cream mix with a capacity of 300 gal. of mix or 600 gal. of milk per hr. has been developed for a small plant in Ohio. A schematic diagram of the system is presented.

W. H. Martin

694. Our experiences with HTST mix operation. F. Rasmussen, Jr., Rieck Ice Cream Co., Pittsburgh, Pa. Ice Cream Trade J., **50**, 3: 30. 1954.

Flexibility makes it possible to handle a wide variety of raw materials and a number of different types of mix in the new Rieck Ice Cream Co. plant in Pittsburgh, which has a capacity of 1,500 gal. of mix per hr. Controls have been installed to prevent ingredient losses and costly restandardization. These objectives have been reached without any sacrifice in quality. Short time high temperature pasteurization is used to save floor space, and less equipment and a more simplified process in terms of labor and control. Details of the processing are given. More stabilizer was found necessary when the short time high temperature pasteurization was used. W. H. Martin

695. New advances in sherbets. G. Illes, A. E. Illes Co., Dallas, Tex. Sou. Dairy Prod. J., **55**, 1: 56. 1954.

The two major advances in sherbets since the war have been increasing volume of sales and improved formulas. Sales of sherbet during the first 9 mo. of 1953 exceeded those for similar periods 1947-51 by 116%. Better formulas have provided for better stabilization, increases in sugar content from 20-22% to 28-34% including monosaccharides, and increases the total solids. Suggested formulas range from 22 to 23% sugar, 1 to 2% butterfat, 2 to 3% serum solids, 6 to 8% dextrose, 0.5% stabilizer, and 8 to 12 oz. of 50% citric acid per 10 gal. of mix. Sugar should be added just before freezing.

F. W. Bennett

696. Coliform in ice cream from the manufacturer to the consumer. J. M. Frayer, Vt. Agr. Expt. Sta., Burlington. Ice Cream Trade J., **50**, 3: 62. 1954.

In a study of coliform in ice cream, it was found that pasteurization presented no particular problem. Holding vats may be a source of increased counts if the mix is held for an extended time. Everything the mix touches should be thoroughly cleaned and sanitized. Products added at the freezer should be continually checked. Hand formed containers may cause trouble if operators are not properly instructed on the necessity of strict personal sanitation. Dipping operation was found to be the greatest source of coliforms in ice cream as served. Most moving water types of dipper wells seemed to be satisfactory while still water wells were uniformly bad. W. H. Martin

MILK AND CREAM

697. Effect of freezing rate on stability of frozen milk. D. Rose and H. Tessier, Nat. Research Lab., Ottawa, Can. Can. J. Technol. **32**, 3: 85-90. May, 1954.

Milk and concentrated milk stored at –12° C. (+10° F.) (a temperature selected as the upper limit of normal commercial storage and handling conditions) were most stable when frozen slowly over several hours than when frozen in less than one hour. The least stable milk was obtained by freezing at a very rapid rate in liquid nitrogen. Storage life at –12° C. was also increased by holding partially frozen samples at –3° C. for up to 48 hr. before storage. Storage life at –18° C., the lower limit of readily available commercial storage facilities, was not greatly affected by freezing rate.

It is suggested that slow freezing allows time for the establishment of new equilibria under the stress of temperature and concentration changes, thus increasing the stability of the colloidal suspension. O. R. Irvine

698. Estimation of protein denaturation in frozen milk. D. Rose, Nat. Research Lab., Ottawa, Can. Can. J. Technol., **32**, 3: 78. 1954.

The volume of milk solids settled by a standard centrifugation procedure gives a rapid and convenient measure of denaturation during frozen storage, but to obtain acceptable results, close control of temperatures and holding times during preparation of samples for centrifugation is necessary. Density of the precipitate, and therefore its volume, is also affected by the force and duration of centrifugation, pH, and other undetermined factors. For studies requiring more accurate data, a determination of total nitrogen in the supernatant milk is recommended. O. R. Irvine

699. The effect of K- and Λ-carrageenins on the viscosity of milk. D. B. Smith, Nat. Research Lab., Ottawa, Can. Can. J. Technol., **31**, 10: 209. 1953.

Commercial carrageenin was separated into two fractions, K-carrageenin being precipitated in KCl soln. This fraction resulted in greatly increasing the viscosity of pasteurized, homogenized milk, its action being dependent on K ion normally present in milk. O. R. Irvine

700. Some mechanical features of a plate-type HTST pasteurizer. S. A. Hansen, F. W. Wood, and H. R. Thornton, Univ. of Alberta, Edmonton, Can. Can J. Technol., **31**, 10: 231. 1953.

A 1000 lb./hr. plate pasteurization with a 15-sec. holding tube was subjected to study before and after rearrangement of the plates in the heating section. Time-temperature curves for the entire process are given. Air pockets in a downward-sloping holding tube decreased the holding time by as much as 50%. Holding time on diverted flow was 2 sec. less than on forward flow as a restrictor fitting was not installed. The pasteurizing effect of the heating up and cooling intervals is computed and the total pasteurizing effect is shown to be approx. double that of the holding section alone. Some of the public health aspects of HTST pasteurization are discussed. O. R. Irvine

701. Phosphatase inactivation in HTST pasteurization of milk. S. A. Hansen, F. W. Wood, and H. R. Thornton, Univ. of Alberta, Edmonton, Can. Can. J. Technol., **31**, 10: 240. 1953.

Phosphatase inactivation was studied using the pasteurizer described in the above abstract. With a 15 sec. holding tube phosphatase was inactivated to the 2 unit/0.5 ml milk end point when the control temp. was 157.7° F. The inactivation time (the total time the milk was not below the control temperature plus the heating up and cooling equivalents as computed by the Ball method) was 28.7 or 30 sec. depending on plate arrangement or approx. double the rated "holding time." When the control temperature was 160° F., the total inactivation time was 16.8 sec. and the "holding time" was 9.6 sec. The Ball method of determining the heating up and cooling equivalents was shown to be reasonably accurate. Inactivation was studied between 151.3° F. and 164.1° F. and the inactivation times when plotted semilogarithmically follow a straight line having a Z value of 9.7° F. O. R. Irvine

702. Creaming impairment in HTST pasteurization of milk. S. A. Hansen, F. W. Wood, and H. R. Thornton, Univ. of Alberta, Edmonton, Can. Can. J. Technol., **31**, 10: 250. 1953.

The impairment of creaming was studied under conditions similar to those for phosphatase. (See previous abstract.) The variability in the data is greater than in the case of phosphate. When the time-temperature requirements for impairment to a certain end point are plotted on semilogarithmic paper, the observed values follow a straight line having a Z value of 12.4° and intersecting the 160° F. abscissa at 15.02 sec. Detectable impairment occurred before phosphatase was inactivated, a cream vol. decrease of 1 ml. coinciding closely with phosphatase inactivation to the 2 unit end point. If the legal minimum is 161° F., most milk will be processed at approx. 162° F. and serious impairment of creaming is probable for milk held 15 sec. When cream vol. is of commercial importance, standards of 160° F. for 15 sec. should ensure pasteurization. O. R. Irvine

703. An analysis of flavor research on milk. D. R. Strobel and C. J. Babcock, Agr. Marketing Service, Dairy Div., U.S.D.A. Milk Dealer, **43**, 7: 80. 1954.

A review of literature indicates that no phase of the dairy industry has received more attention by research workers than the flavor of milk. Some necessary repetition is encountered in this research work. Additional work with feeds should be to improve the flavor and keeping quality of milk rather than to determine if the feed imparts abnormal flavor to milk. It has been definitely established that contamination with certain metals causes the development of an oxidized flavor. Many other causes reported have been contradicted or questioned by additional research. Future work on this flavor should, therefore, be to establish other definite causes for its development and methods for preventing or retarding it. More research is needed to determine the effect of bacterial action on the flavor and keeping quality of milk.

C. J. Babcock

SANITATION AND CLEANSING

704. Iodophors as sanitizing agents. C. K. JOHNS, Can. Dept. of Agr., Ottawa. Can. J. Technol., **32**, 3: 71. 1954.

Representative iodophors have been compared against chlorine and quaternary ammonium germicides. Test organisms included *M. pyogenes* v. *aureus*, *E. coli*, and *P. aeruginosa*. Solutions with and without 0.5% added skimmilk were tested by a modified Weber and Black method at 5°, 20°, and 45° C. The iodophors compared very favorably with a quick-acting hypochlorite, especially in the presence of skimmilk. Other products tested were appreciably slower. In the "capacity" test the iodophors showed up extremely well, frequently destroying many more "increments" of test organism than other germicides at eight times greater concentration. Exceptionally favorable results with spores of *B. subtilis* were subsequently found to be due to the bacteriostatic effect of certain nonionic constituents of the iodophors.

O. R. Irvine

705. Evaluation of sanitizing agents. P. R. ELLIKER, Ore. State Coll., Corvallis. Milk Plant Monthly, **43**, 4: 47. 1954.

Continuing trend toward more rigid bacterial standards plus merchandising practices which require longer keeping quality make problems of sanitation bacteriology of increasing importance. Improved procedures especially with chemical sanitizers have accomplished remarkable advances in flavor and keeping quality. The use of hypochlorites and related products, hypochlorite solutions containing wetting agents, quaternary ammonium compounds, and iodine compounds is described. C. J. Babcock

706. Dairy plant sanitation. C. W. ENGLAND, C. Y. STEPHENS Dairy Industries, Washington, D. C. The Milk Prod. J., **45**, 6: 31. 1954.

Good sanitation as applied to the dairy plant means the thorough cleaning after each use of all equipment, followed by a sanitizing treatment to render it practically free from microorganisms, without damage to the equipment.

Tremendous improvements in methods, materials and equipment for use in cleaning have been made in recent years. Consequently, the maintenance of good sanitation in a dairy plant is easier than it was a decade ago. Contributing factors include: (a) better materials for water softening, (b) better basic cleaning materials, synthetic detergents, surface active agents and better cleaning compound formulations, (c) better cleaning acids, (d) better sanitizing agents, (e) the work of the 3-A Sanitary Standards Committee, (f) the advent of in-place-cleaning with recent improvements in ferrule and gasket design and improved engineering, and (g) the O-R system of cleaning. These items and can washing and bacterial problems, i.e. coliforms, psychrophiles, thermophiles and thermodurics are briefly discussed in the article.

J. J. Janzen