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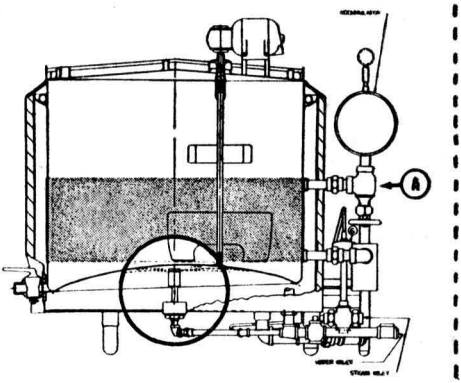

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BROWNING AND ASSOCIATED CHANGES IN MILK AND ITS PRODUCTS: A REVIEW[1]

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In many areas of the world today people are undernourished, malnourished, and even starved. At the same time in other parts of the world food is abundant to the point of substantial excess. Although many factors contribute to this imbalance, food spoilage is an important consideration. Some excess food can be processed and stored in a practical manner involving little or no deterioration, but some of it faces spoilage despite man's preventive efforts. One vexing problem of food spoilage is browning. Loss of palatability and nutritive value as well as undesirable changes in physical properties frequently attend the browning of a food.

On the other hand, browning is purposely sought in most baked and fried foods. Maple syrup owes its fine flavor and color to browning. The distinctive caramel and butterscotch flavors derived from dairy products result from the browning of milk or milk components. Thus, it is clear that browning has two aspects, both of which concern the acceptability of man's food. It is not surprising that browning is and has been an intensely active field of food research *(17, 43, 114)*.

There are two principal areas of interest with regard to browning and associated changes in milk. One is practical and concerns avoidance of the phenomena in certain dairy products and processed foods containing milk as an ingredient. The other is fundamental and concerns making the knowledge of milk and its behavior as complete as possible.

BROWNING OF MILK AND MILK PRODUCTS

Before considering the chemistry of browning in milk, it is of value to note the general nature of browning, the incidence of browning in milk products, and the magnitude of browning as a problem in the dairy industry. Authorities have classified browning into various types. The value of such a classification is

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limited, since mechanisms involved in these types show many similarities. Moreover, each food product offers its own unique browning system. For the purposes of this review, types of browning are classified as follows: *(a)* caramelization or non-amino browning of sugars, *(b)* amino-sugar or Maillard-type browning *(82)*, and *(c)* oxidative browning. A pure caramelization browning is rarely encountered in the food field. By definition, amino compounds would be absent and heat decomposition of sugars as a function of pH and buffers would be the essence of the reaction. Caramelization may occur at least to a limited extent in many foods undergoing browning, but by far the most important type of browning is that resulting from the interaction of sugars and amino compounds. As distinguished from caramelization, Maillard-type browning requires a relatively low order of energy for its initiation and exhibits autocatalytic qualities once it has started. Oxidative browning, or browning which requires oxygen at some critical step in its mechanism, is exemplified by the so-called enzymatic browning in fruits and vegetables. Browning of the cut or bruised surface of an apple is a case in point. Once the polyphenols (reductones) in the plant tissue have been converted enzymatically to quinones the essence of the browning mechanism is a Maillard-type *(43)*. Oxidative browning is not necessarily limited to enzyme-initiated systems. Autoxidation of reductones, such as ascorbic acid catalyzed by copper proteinate, also may lead to browning *(125)*.

Milk normally is a whitish opaque liquid showing no tendency to brown in the manner of an enzyme-catalyzed system. Although there are some indications that caramelization and oxidative browning may occur to a limited extent in milk and milk products, the primary mechanism in these media appears to be of the sugar-amino type. This point is discussed subsequently in detail (see section on mechanisms). When conditions are conducive, virtually all milk products will brown. However, processing, packaging, handling, and storage practices determine that browning is little or no problem in some products and a problem to varying degrees in others. Concentrated and dried products which are stored at room temperature for appreciable time are by far most susceptible. Products of this group in which browning has been investigated are evaporated milk *(5, 6, 62, 66, 90, 98, 115, 120, 121, 123, 131, 132)*, sweetened condensed milk *(52, 110)*, dried milks *(15, 19, 23, 42, 57, 58, 67, 84, 122)*, dried whey *(19)*, and dried ice cream mix *(68, 122)*. Sterilized, unconcentrated milks and creams and dried creams are also subject to browning, but the relatively low lactose and protein content of these products reduces both the incidence and degree of the defect. It seems that practical means are available to prevent browning in all milk products. Browning of a milk product, provided it is made from milk and ingredients of acceptable quality, can be traced in nearly all instances to unsatisfactory processing, packaging, or storage.

Concentrated fluid products. These products, particularly evaporated milk, have posed the most chronic problem with respect to browning. In 1951, evaporated milks from 11 manufacturers were evaluated for color *(98)*. Although considerable variation in color among the fresh products was noted, all exhibited some degree of browning. It has been suspected for many years and published

results *(5, 52, 115, 123)* have revealed that high-temperature, short-time (HTST) sterilization will produce evaporated milk essentially free of browning. In contrast to the conventional retort heat treatment of 117° C. for 15 minutes, Bell *et al. (5)* employed 135° C. for 30 seconds. The superior flavor and color of their product was offset by rapid thickening and cream separation during storage. Similar changes in HTST sterilized evaporated milk have been noted by others *(115)*. Tarassuk and Simonson *(123)* indicate that evaporated milks sterilized at 121° C. for 5 minutes or 129.5° C. for 2 minutes were far superior in color and flavor when compared with a conventionally processed (117.5° C. for 15 minutes) product. Recently it has been announced that an HTST sterilized evaporated milk has been placed on the market *(3)*. The claims concerning color and flavor of the product are impressive. Although it will require consumer experience with the product to bear out these claims, it seems that solution of the browning problem of evaporated milk may soon be realized with the aid of HTST sterilization procedures. It is important to note that application of ideal processing techniques may not necessarily prevent all deteriorative processes during storage.

Unquestionably, heat treatment during forewarming and sterilization is the most important factor relative to browning in evaporated milk *(5, 6, 66, 90, 115, 123, 131, 132)*. However, a number of other factors have been found to favor the defect. These are alkaline stabilizing salts *(66, 132)*, high solids content *(6, 62, 66)*, head space oxygen in the can *(120)*, unidentified characteristics of the raw milk *(6)*, high storage temperatures and long storage times *(98, 123, 131, 132)*. Temperatures of 0° to 5° C. will prevent further browning during the storage of evaporated milk, but refrigeration costs make this impractical. It seems to be common knowledge within the industry that traces of copper and iron salts promote, but tin salts inhibit, browning of evaporated milk. The findings of Webb support this point *(130)*.

Information on the browning of other concentrated fluid milk products is very meager. Corn sugar favors browning in sweetened condensed milk much more than does sucrose *(110)*. Dependence upon sucrose, a relatively inert sugar in the browning reaction, rather than sterilizing heat treatment for preservation helps to discourage discoloration in this product. The well-known practice of making caramel pudding by cooking a can of sweetened condensed milk in boiling water demonstrates that the product will brown under rigorous conditions. A closely comparable change in the product can be achieved by several years storage at room temperature. It seems probable that high acidity, by-products of bacterial metabolism, and handling and packaging conditions account for limited browning in some other fluid dairy products.

Dried products. Browning and related changes in dry milk systems have been reviewed by Coulter *et al. (15)*. According to them, browning is not a primary problem in dry milks. This point is emphasized by Henry *et al. (42)*, who found that dry milks with less than 5% moisture showed only very slight changes in color even after 2 years storage at 37° C. Unquestionably, moisture content (humidity) is the most important single factor in the browning of dry milk

products *(15, 19, 23, 42, 57, 67, 84, 122)*. The necessity of keeping moisture content of such products low can not be overemphasized. The forerunner of discoloration in high moisture dry milks is off-flavor, and even if browning is never reached, these products are usually unfit for human consumption. The maximum moisture content for good quality varies among the dry products, and probably there is an optimum for any given batch of product. Moisture levels of 4.0, 2.5, and 5.0% for nonfat dry milk, dry whole milk, and dried whey, respectively, appear to be reasonable upper limits. Ranking after moisture content, storage temperature is the next most important factor *(15, 19, 23, 42, 57, 67, 84, 122)*. In general, storage at room temperature is not conducive to browning in dry milks of reasonably low moisture levels. For practical purposes the lowest feasible storage temperature should be employed in order that flavor, as well as color changes, will be inhibited in so far as possible. To the extent that they influence moisture content, handling and packaging are factors in browning of dry milks. The hygroscopic nature of such products facilitates imbibing of water from humid atmospheres. Thus, quality of milk and care in its manufacture into a dried product can be completely offset by unsatisfactory packaging. In this connection an aggravated case of browning in dry milk stored in steel drums has been noted by Krienke and Tracy *(67)*. Some characteristic of the drums rather than of the milk was indicated as the cause. Oxygen level is not a significant factor in browning of dried milks *(15)*.

Dry ice cream mix appears to deteriorate and brown in much the same manner as do dry milks *(68, 122)*. However, dry whey darkens more rapidly and to a greater degree than do dry milks during storage *(19)*. As with dry milks, moisture, humidity, and storage temperature are critical factors in browning of dry whey. According to Doob *et al. (19)*, color development in dry whey is associated with high osmotically held moisture, particularly that not associated with lactose hydrate. The use of nonmoisture-proof containers for feed-grade dry whey is a common practice that aggravates browning. Such measures may be dictated by economy. However, some studies of the nutritional merits of the browned and uncolored product would seem worthwhile. Subsequent information (herein) confirms loss of essential amino acids and the possible formation of growth inhibitors under pronounced browning of sugar-protein systems. Perhaps packaging economies of dry whey for livestock and poultry feed are outweighed by loss of nutritive value during storage deterioration. In addition, investigation of sodium bisulfite or sulfur dioxide as browning inhibitors in feed-grade dry milks and dry wheys might prove fruitful.

COLOR MEASUREMENT

The study of browning, whether it be in milk and milk products or simplified systems thereof, requires a method of measuring color. Methods range from simple visual comparison of the color among samples to precision measurement with colorimeters and spectrophotometers. For determining whether a variable produces a difference in the color of a sample, it is hard to improve on side-by-side visual comparison of the treated sample with a control in a uniform environ-

ment. This procedure makes manifest very small differences and is the essence of simplicity. Significance data also can be derived through use of this method if sufficient numbers of observers are used. However, such a method is unsuitable when one wishes to make quantitative appraisal of color intensity. Quantitative evaluation by visual comparison necessitates the use of standards. Perhaps the ultimate in this type of method has been developed by Webb and Holm *(132;* see also *5, 6)*. Their method employs the Munsell color notation system, which takes into account chroma, hue, and lightness aspects of color. This method appears precise and capable of revealing differences in color characteristics that are not measured by conventional methods. Its main disadvantage appears to be a lack of simplicity. Since milk is essentially opaque, the most valid method of measuring its color is by reflectance. Reflectance methods have been applied to measurement of browning in milk with very satisfactory results *(9, 89, 123)*. However, such methods require specialized equipment and are limited as research tools since they cannot be applied to translucent or transparent systems. Doob *et al. (19)* have developed a procedure for extracting the brown pigment from dried milk or dried whey with a Na_3PO_4-NaCl solution. Optical density in the filtered extracts is determined in a photoelectric colorimeter. Although recovery of pigment by the method is not quantitative, color extraction appears to be uniformly proportionate to the amount of pigment originally present in browned samples. The method is not adapted to fluid milks. Color in the extracts is unstable and should be read within 30 minutes. These workers also offer an alternative aqueous extraction procedure, applicable only to dried whey, which yields stable color extracts.

A method that depends on tryptic digestion of the milk proteins to liberate brown pigment in dried milk products has been developed by Choi *et al. (11)*. This method has been adapted to evaporated milk, fluid milk, and simplified milk systems *(98)*. The versatility and comparative simplicity of the method are advantageous. Tryptic digestion of samples requires one hour, which would be considered a distinct limitation in routine analysis. However, the method shows a reasonable degree of precision and can utilize any conventional photoelectric colorimeter provided with a suitable filter. This method has shown good agreement with a reflectance procedure in evaluating the effect of pH on color development in heated milk *(9)*.

REACTANTS AND CHEMICAL MECHANISMS

A. *Reactants.* The literature indicates very clearly that the two principal reactants in the browning of milk and milk systems are lactose and casein. Neither casein nor lactose browns readily when heated alone but they do so when heated together *(38, 60, 84, 91, 98, 104, 110, 111, 118, 121, 137)*. It is known that casein is heterogeneous, but no evaluation of its components in the browning reaction has been made. Dephosphorylated casein browns as readily as intact casein *(60)*. Some question appears to exist as to the promotive effects of buffer phosphates. Heating lactose in phosphate buffers is accompanied by some browning *(130)*. However, Kass and Palmer *(60)* conclude that phosphate concentration in milk

is too low to induce any appreciable discoloration. In any case it is very difficult to draw defendable inferences regarding the effects on browning of the phosphates, or more generally the buffer system in milk, because it cannot be faithfully reproduced in a model system. The evidence that fluid rennet whey is very resistant to both heat- and storage-induced browning *(104, 110)* strongly suggests phosphates to be of secondary, if any, importance in the browning of milk products. A synergistic effect of phosphates and protein amino groups is possible, as shown in model systems by Pederson *et al. (106)*. In addition, β-lactoglobulin will brown with lactose under certain conditions *(25)*, and it has been suggested that urea is a probable participant in the heat-induced browning of milk *(1)*. These observations notwithstanding, lactose and casein are the reactants of first importance.

Implicit in the study of browning is measurement of the reactants after various times and conditions of reaction. This fact has hampered study of the problem as it applies to milk and has led to much confusion. Changes in both the protein amino-groups and the lactose content in milks undergoing browning have proven very difficult to follow. For lactose, adequately specific methods have not been available. Several groups of workers *(32, 38, 69, 108)* have made comparative studies of methods for measuring lactose and have concluded that application of such methods to highly heated milks yields conflicting results. With methods based on reducing power of lactose, the reducing substances generated in milk by heat undoubtedly contribute an error. Regarding the polarimetric method for lactose, it is a question whether the decomposition products formed in heated milk add to, subtract from, or have no effect on the optical activity of the lactose. The method of Fearon *(22)* for reducing disaccharides as adapted to blood by Horowitz *et al. (51)* has been suggested as an appropriate method, with suitable modifications, for lactose in heated milk *(100)*. The most specific procedure for lactose currently available is the paper chromatographic method of Honer and Tuckey *(50)*. Although inconvenient in a number of respects, their procedure gives the best assurance of measuring lactose specifically and has the advantage of permitting estimation of glucose and galactose as well. There is obvious need for further work in this area.

In the study of casein, the role of free amino groups in browning has been of first importance and much effort has been expended on showing destruction of these groups under various conditions. The formol titration has been used extensively for this purpose with questionable success. Under conditions of browning, results showing both increase *(30, 60)* and decrease *(32, 84)* in formol titration have been reported. The difficulty with this method seems revealed in part by the findings of Harland *et al. (38)*. In their study heated aqueous casein and lactose-casein systems were evaluated for amino nitrogen. Heating produced an increase in amino nitrogen of casein sols but no change in lactose-casein systems. It seems plausible that heating caused some protein hydrolysis of casein *(86)*, resulting in an increased value; however, this was about equally offset by destruction of amino groups in the lactose-casein system. Although somewhat more specific in measurement, the Van Slyke amino-nitrogen determination would

be subject to the same type of error. The value of the formol titration for measuring amino group change appears to be placed in further doubt by the statement of Hannan and Lea *(36)* that the amino group of casein is still basic after reaction with glucose in their "dry" state studies. They also found this to be true in the interaction of glucose with *a*-*N*-acetyl-L-lysine or poly-L-lysine. Many appear to think that basicity of the amino group is lost on reaction with reducing sugar. This point needs clarification, particularly with reference to aqueous systems.

Much of the difficulty and confusion resulting from studies of browning in heated milk can be traced to the dynamic state of the medium. It is apparent that in this medium the completion of one step is not essential to the initiation of the next. Many chemical reactions are proceeding at once. If one is looking for the disappearance of amino groups as a manifestation of the browning reaction in heated milk, one must also consider the possibility of their regeneration or action in the manner of a true catalyst. As is discussed subsequently, such regeneration seems more than a possibility and perhaps partially explains the inconsistent results obtained with the formol titration.

B. *Mechanisms.* The serious student of the chemistry of browning in milk is urged to consult the reviews of Hodge *(43)* and Danehy and Pigman *(17)*. The parallels and close analogies between browning of milk and of model systems are indeed striking. A principal difference is that much more information has been secured regarding the phenomena in model systems. Figure 1 characterizes

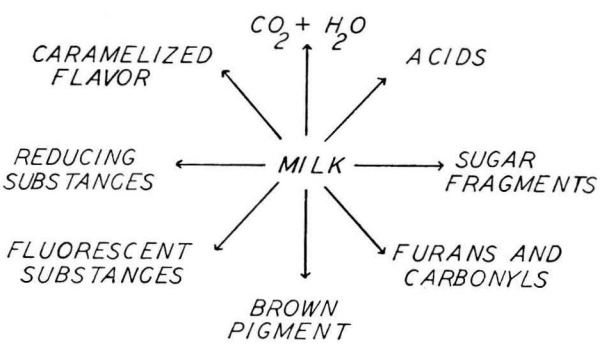


FIG. 1. A scheme of the more significant manifestations of browning in milk.

some notable manifestations of browning that have been observed in heated milk. These also are the concomitants of browning in model sugar-amino systems. There is considerable evidence supporting a Maillard (sugar-amino) type of browning in milk *(84, 100, 110, 118, 130)*. A number of studies have shown that browning in milk systems is independent of oxygen level *(42, 57, 58, 67, 68)*. The minor role which oxygen may play *(120)* seems best explained in terms of –SH inhibition of browning (see section on prevention). Thus, browning in milk does not appear to be of an oxidative type. A pure sugar caramelization browning is ruled out by the acknowledged importance of both lactose and casein in the discoloration. The classical work of Kass and Palmer *(60)* is cited frequently

as the principal support for a simple caramelization type of browning. Actually they concluded that browning ill heated milk results from "caramelization of lactose b." the casein and the adsorption of the lactocaramel by the colloidol caseinates." Thus, in this instance too the importance of casein to the pllenomena is admitted. Their objections appear to be leveled at a simple bifunctional reaction between lactose and casein as an explanation of browning in heated milk.

The question arises as to what chemical groups of the casein are primary reactants in the browning mechanism. Evidence from three distincth' different areas of study point to the <-amino groups of lysine (in caseiu) as key reactants. 1. Autoclaved systems containing casein and a reducing sugar consistently show the greatest amino acid losses in lysine (21, *92*, 93). Sigllificant losses of histidine. arginine. and tryptophane also usually accompany lysine destruction. 2. Lea and Hannan *(7f)* have demonstrated that in the "dry" state glucose reacts with the free amino groups of casein in a 1:1 ratio, that these amino groups are made np almost entirely of <-amino groups of lysine, and that the complex so formed browns readily under suitable conditions of temperature and humidity. Under these conditions in the presence of an excess of glucose other basic amino acids al'e involved secondarily in the reaction *(75)*. 3. On the basis of componnds isolated from heated milk, Patton (97, 98, *100)* has noted that the decomposition of lactose in this medium and in casein solutions appears to be base catalyzed and that free amino gl'onps of casein are the logical catalysts in this instance. It also is pertinent that browning in heated milk and related systems is a catalytic process (19,132) and that amino compounds catalyze sugar dehydration, fragmentation, and condensation (117, 135). Therefore, one might strongl.,· suspect that browning in heated milk involves catalytic decomposition of lactose b." the <-amino groups of lysine (in casein). It seems possible that under carefully controlled conditions a 1:1 reaction of casein amino groups ani! lactose could be demonstrated as the initial step in the browning of heated milk. Patton and Flipse *(100)* have isolated a colOl'less lactose-protein complex from heated milk which browned on reheating. The linkage between carbon 1 of the lactose and the protein was observed to be at least 67% disrupted by the reheating. These observations may help to explain the inconsistent relationship between lactose destruction and loss of amino groups that has been noted in lleated milk.

Many simple organic compounds appear to be dil'ecUy or indirectly involved in browning. Prominent among these are 5-hydroxymethylfurfural (7, 96, 116, 135,136), acetaldehyde (88, 118), and methyl glyoxal *(13, 20)*. Small additions of these compounds to milk did not significantly enhance the magnitude of heat-induced browning (98).

Conventional effects of pH on browning in sugar-amino systems have been shown to apply to heated milk (9, 98). H ions depress and OR ions enhance discoloration. It is of interest that the normal pH of milk (6.6 to 6.7) lies in a critical range with regard to color c1eYelopment (.98). Lowered pH and increased titratable acidity invariably accompany browning in milk (15, 18, *27*, *28*, *30*, 32,42,98, 134).

Beyond the involvement of lactose and amino groups there seems to be no

additional information regarding the mechanism of browning specifically in milk. In the domain of speculation, it is probable that the first formed lactose-casein condensate, among other things, undergoes the Amadori rearrangement, the product of which can lead to sugar fragmentation, formation of furfurals, reductone-like reducing substances, fluorescent substances, and melanoidins. Hodge *(43)* and Hodge and Rist *(44)* have shown and discussed at length the significance of this rearrangement in model systems. Lea and Hannan *(36, 75)* have indicated that such a rearrangement affords a logical explanation of some of their findings with casein-glucose systems.

The works of Lea *et al. (35, 36, 42, 70, 71, 72, 73, 74, 75, 76, 77, 78, 80)* constitute the outstanding contemporary contribution to the knowledge of browning in the area of milk systems. Although the investigations of this group have involved primarily work with casein and glucose in the "dry" state, their findings are correlated with earlier studies by them on dry milk. Coulter *et al. (15)* suggest that the basic mechanism of browning in dry milk does not differ from that in more dilute milk systems. This seems reasonable if one bears in mind preceding comments regarding the more dynamic activity of heated fluid systems. Whereas "dry" state studies by Lea *et al.* have shown evidence of stepwise progression toward browning, Kass and Palmer have aptly described the phenomenon in heated milk as a non-bifunctional, complex, indefinite, progressive formation of a melanoidin. In any event, confirmatory evidence is needed that the findings of Lea's group are extensively applicable to dry milk and that the basic mechanism of browning in dry milk systems does not differ materially from that in fluid milks.

Although model sugar-amino acid systems have been of indispensable value in the study of browning, there are some notable differences between such systems and milk which may be worth bearing in mind: *(a)* lactose and glucose do not react identically in browning systems *(80, 95, 97)*, *(b)* casein acts in the manner of a stronger base and a weaker buffer than a mixture of its amino acids *(98)*, and *(c)* the milk proteins introduce the concepts of surface catalysis and adsorption into the phenomenon of browning in milk.

C. *The brown pigment (melanoidin).* The most important manifestation of the complex and poorly understood chemistry of browning is the pigment. The brown substance (or substances) from milk and milk products has received very limited study. Binding of the pigment by milk proteins and its absence from solution in milk serum is characteristic *(60, 91, 98, 110, 137)*. However, if heat treatment of milk is sufficiently intense, discoloration of the serum also may be noted. Kass and Palmer *(60)* have viewed the association of the pigment with protein as the result of an adsorption process. They present extensive data on the positive capacity of sodium caseinate sols to adsorb lactocaramel, and similarities in behavior and properties of these complexes and the colored proteins of heated milk. Their conception of the mechanism is in agreement with that proposed earlier by Wright *(137)*. However, it is noteworthy that the pigment of heated milk can not be removed from the protein by any known methods without destroying the protein. This suggests that, at least in part, the binding is chemi-

cal rather than purely physical. Theoretically, one point of attack on this problem would be through nitrogen analysis of the brown material, the essential absence of nitrogen being indicative of a pure lactocaramel. This approach is fraught with difficulties. How could one be certain that the pigment had been recovered to the exclusion of protein fragments and other impurities? Moreover, at what level would nitrogen content take on significance? Reviews *(17, 43, 119)* suggest that catalytic decomposition of sugars by amino compounds leading to browning, as well as direct participation of amino groups in pigment synthesis, occurs in model systems. Since caramels and melanoidins are both brown and have many properties in common, one might view the presence of nitrogen in the melanoidin as somewhat incidental.

PHENOMENA ASSOCIATED WITH BROWNING

As shown in Figure 1, a number of chemical changes are closely associated with browning. These include the formation of various specific compounds, reducing substances, and fluorescent substances and the production of caramelized flavors. Flavor is treated separately in a following section.

A. *Compound formation.* One useful method of throwing light on the chemistry of browning and related flavor changes is through identification and study of the compounds formed. For the most part such compounds may be by-products and perhaps of secondary importance. However, they give definite indications concerning chemical mechanisms that may be operative. For example, the sugar fragmentation products that have been detected in heated milk have been demonstrated also in model systems, indicating the similarity of mechanisms involved.

Compounds formed in heated milk have been the subject of a review *(29)*. Those known to be formed in this medium are furfuryl alcohol *(103)*, 5-hydroxymethylfurfural *(96)*, maltol *(95)*, acetol *(61)*, methyl glyoxal *(13, 61)*; butyric *(64)*, propionic *(64)*, acetic *(61, 64)*, formic *(28)*, lactic *(27)*, and pyruvic acids *(64, 109)*; hydrogen sulfide *(126, 127, 128)* and carbon dioxide *(120, 121)*.[2] The formation of carbon dioxide and water has been noted in dry milks *(15)*. Butyric, propionic, and pyruvic acids and H_2S have not been established as lactose decomposition products. Presumably butyric acid results from fat hydrolysis and H_2S from heat denaturation of proteins. Pyruvic acid might be formed through either lactose decomposition or oxidative deamination of alanine. The balance of the above compounds is formed either substantially or completely from lactose. Information on the mechanism of their formation is limited. When lactose is destroyed in heated milk, galactose, but not glucose, accumulates *(49, 109)*. This, coupled with the fact that the glucose moiety of lactose contains the reactive hemiacetal structure, points to the glucose portion of the molecule as a primary origin of sugar fragments. Schemes accounting for the formation of furans *(97)* and some other compounds *(61)* in heated milk have been proposed. These are in need of confirmation, and the use of lactose labeled with C^{14} seems appropriate for the purpose. Both maltol and furfuryl alcohol appear to

[2] Some of these compounds have been detected only after application of heat treatments far in excess of those commercially employed in any milk processing.

require the intact lactose molecule as an origin *(95, 97, 98)*, whereas most of the other compounds are producible from glucose or lactose. Furfuryl alcohol results when lactose is degraded with $NaHCO_3$, but maltol does not *(97)*. Apparently amine catalysis is essential to maltol formation.

Unquestionably, an infinite number of decomposition products from heated milk and other browned milk systems are yet to be identified. The water soluble–ether insoluble sugar fragments and condensation products have received no attention to date. It has been reported also that heated milk contains a number of unidentified carbonyl compounds *(61)*.

B. *Reducing substances.* Heated and dried milks contain a complex reducing system involving –SH compounds, ascorbic acid, and substances associated with the browning reaction. A system in which a vitamin is involved is always of practical interest, and measurement of –SH and other reducing groups in milks is an aid in evaluating processing, quality, and antioxygenic activity. Methods for measuring these systems in milk independently of one another have been developed *(10, 16, 18, 37, 53)*. Of principal interest here are the browning-associated reducing substances. These have been detected or measured in a number of ways. Ramsey *et al. (110)* observed that casein recovered from a browned casein-glucose solution gives a strong reducing test with Benedict's solution. Kass and Palmer *(60)* have shown that browned systems of lactose and casein reduce methylene blue instantaneously. It has been demonstrated by Doan and Josephson *(18)* that 85% or more of the indophenol reducing substances in evaporated milk is associated with the proteins and that these must be removed for accurate determination of ascorbic acid. Acid ferricyanide has been used to quantitatively measure increases in the reducing substances of fluid and dry milk systems *(10, 14, 15, 16, 38, 57)*. Lea *(70)* and Crowe *et al. (16)* have established that such increases result from the interaction of lactose and casein and also from lactose decomposition catalyzed by buffer salts.

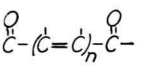
Reductone-like reducing substances consistently accompany browning in model sugar-amino systems. Although the structures of these materials are not known, the similarity in properties to the Amadori rearrangement products of *N*-substituted glycosylamines tabulated by Hodge *(43)* is of interest.

C. *Fluorescent substances.* Incident to browning in milk systems, fluorescent substances are produced *(15, 38, 56, 57, 58, 115, 123)*. Three types of fluorescent materials in browned milk systems have been recognized by Jenness and Coulter *(56)*. They are: 1) riboflavine and related substances, 2) lipid-associated substances, and 3) fluorescent materials resulting from lactose-protein interaction. They have devised a differential solubility procedure for separating these three groups. According to them, normal processing is without effect on the fluorescence of dry whole milk, but elevated moisture content and storage temperature substantially increase fluorescence of the groups 1 and 3. Tarassuk and Simonson *(115, 123)* have studied fluorescence in evaporated milk. Both sterilization and storage enhance fluorescence in this product, and practically all of the fluorescence increase is recovered with the proteins. These workers point out that although browning and fluorescence develop simultaneously and increase at a

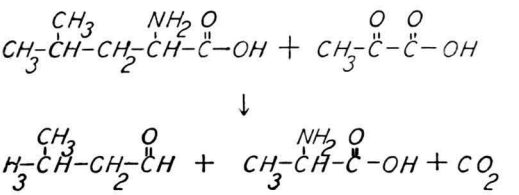
parallel rate, the materials involved in the two phenomena are not necessarily identical. They noted that all the fluorescing material could be released from the protein by much milder hydrolysis of the proteins than that required for liberation of the pigment. It would be of interest to know whether reducing activity is associated with both the fluorescent and brown materials. Hannan and Lea *(36)* have been successful in separating compounds with blue and purple fluorescence from a glucose-α-*N*-acetyl-L-lysine reaction mixture by paper chromatography.

THE STRECKER DEGRADATION

The Strecker degradation is an integral part of the various reactions involved in the browning of amino-reducing sugar systems *(43, 112)*. It occurs when an amino acid is heated with certain types of dicarbonyl compounds. The amino acid is degraded to an aldehyde of one less carbon. This reaction has been the subject of a review *(112)*, and Schönberg *et al. (113)* have established the requisite structure for the dicarbonyl compounds as:



where n is zero or an integer. The essence of the reaction with leucine and pyruvic acid to form 3-methylbutanal and alanine may be represented as follows:



Glucose and presumably lactose can serve in place of dicarbonyl compounds in this reaction *(4, 112)*. In all probability decomposition of the sugar to a suitable dicarbonyl precedes the reaction. It also has been shown that dehydro-(diketo) ascorbic acid can participate in this reaction *(107)*. The Strecker degradation is considered to be a principal route of CO_2 production during browning *(43, 79)*.

These observations appear important with regard to milk products. Milk contains the necessary reactants. Trace quantities of free amino acids are present in the protein-free serum of milk. Block *(8)* reports the presence of glutamic acid, glycine, alanine, valine, leucines, aspartic acid, and serine, with the first three predominating. Alanine, glutamic acid, glycine, leucines, and valine have been demonstrated by van der Zant and Nelson *(129)*. Unquestionably, these trace levels would be considerably enhanced by the hydrolytic effects of high temperature heat treatment on milk proteins. Dicarbonyls, such as methyl glyoxal and pyruvic acid, have been identified in heated milk, and precursors of such compounds, *i. e.*, lactose and ascorbic acid, occur in milk in the native state. Considering the variety of amino acids that are present in milk proteins, it seems

clear that great numbers of flavorful aldehydes could be produced by the Strecker degradation in milk.

From a practical standpoint it is of interest to know whether the Strecker degradation could occur in milk under practical processing conditions to an extent sufficient to cause off-flavors. Data by Schönberg *et al. (113)* show that substantial yields of aldehydes are obtained by heating dilute solutions of amino acids and dicarbonyls for 30 minutes at 100° C. The amount of heating necessary to produce quantities of aldehydes in excess of the flavor threshold would be considerably less. In this connection, Jackson and Morgan *(55)* have reported the aroma of 3-methylbutanal to be definitely noticeable in milk at 0.5 p.p.m. This aldehyde results from the Strecker degradation of leucine. Experience at this laboratory bears out the extreme potency of lower molecular weight aldehydes in imparting flavors to milk. A further practical point in this matter is the quality of milk for manufacturing purposes. Poor quality milk ordinarily makes poor flavored products. The potentialities of bacterial action in such milks are to produce both amino acids by proteolysis and dicarbonyls by sugar fermentation and various other mechanisms. Such action could be expected to make the milk a more suitable medium for the Strecker degradation. In the manufacture of evaporated milk, which involves very substantial heat treatments, these phenomena may be of considerable importance. Recent evidence indicates that light or certain varieties of bacteria, as well as heat, can bring about the essence of the Strecker degradation with certain amino acids *(55, 99)*.

The point of this discussion concerning the Strecker degradation is to emphasize the substantial indications of its importance to the flavor of dairy products and that it is a mechanism worthy of extensive study in connection with such products.

CARAMELIZED FLAVOR

A concomitant of the browning of milk is caramelized flavor. At times development of this distinctive flavor is promoted purposely; more often it is objectionable and coincident to faulty processing or product deterioration. At this point there is no clear or simple explanation of its nature and origin.

As milk is progressively heated toward 100° C. its flavor is altered. It is generally held by discriminating observers that pasteurization imparts a very slight change in flavor and that at or about 74° C., momentary heating, a distinct cooked flavor begins to develop. This flavor arises from sulfhydryl (–SH) compounds liberated by heat denaturation of β-lactoglobulin *(31, 53, 54, 59)*. Although the mechanism of cooked flavor formation is perhaps somewhat beside the point, it seems noteworthy that neither the so-called heat liberation of –SH groups in β-lactoglobulin nor the conversion of such groups to the flavorful volatile sulfides (primarily H_2S) has been adequately elucidated. However, an extensive study has been made of factors affecting the quantity of volatile sulfides produced in heated milk *(126, 127, 128)*. Intensity of cooked flavor increases with increasing heat treatment of the milk to a point determined in all probability by the number of –SH groups to be activated, the amount of oxidation of –SH groups by atmospheric oxygen, and the extent of irreversible reaction of

–SH groups with other components in the milk. It has been demonstrated that –SH groups slowly disappear when milk is held for prolonged periods at high temperatures and that cooked flavor in the milk seems to give way to caramelized flavor *(104, 126)*. Although –SH groups may serve as inhibitors of caramelized flavor (see section on prevention), there seems to be no good reason for presuming any cause-and-effect relationship between the two flavors at this time. At what point in the heat treatment of milk caramelized flavor becomes evident and whether browning is always co-present with the flavor is not clear from the literature. In general it is indicated that degree of caramelized flavor is correlated positively with degree of browning and related changes *(15, 104, 122, 126)*. The effect of varying casein content has not been studied in this connection; however, caramelized flavor (and browning as well) does not develop in heated fluid whey *(104, 110)*. It also has been observed that the addition of ascorbic acid to raw milk (1 g/l) will induce caramelized flavor at time-temperatures (90° C.–flash) lower than normally required *(101)*.

Although this limited information is hardly illuminating with reference to the mechanism of formation or the chemical identity of caramelized flavor, some evidence from microbiological studies is of more than passing interest. Hammer and Cordes *(34)* have observed that caramel off-flavors in milk and milk products are caused by *Streptococcus lactis* var. *maltigenes*. Similarly, Kelly *(63)* isolated caramel flavor-producing strains of bacteria from samples of milk rejected for having a cooked (caramel) flavor. These bacteria were classified as variants of *S. lactis*. Recently Jackson and Morgan *(55)* have shown that the malty flavor produced in milk by *S. lactis* var. *maltigenes* is due to 3-methylbutanal. The organisms in question were observed to synthesize the aldehyde from the amino acid leucine. In light of these findings, one is strongly tempted to speculate that the same caramel flavor compounds synthesized by these organisms may be generated in milk by intensive heat treatment and perhaps more specifically through Strecker degradation of amino acids. However, there is no substantiating evidence for such an assumption at this time. Moreover, this explanation probably greatly oversimplifies the nature of flavors in highly heated milk. The author proposes for what it may be worth that there are at least four flavor components in whole milk heated to the extent of noticeable browning: 1) caramel or malty, resulting from sugar decomposition and Strecker degradation of amino acids; 2) stewed meat, arising from methionine decomposition and the presence of H_2S; 3) hydrolytic rancidity, arising from fat hydrolysis; 4) coconut-like, resulting from the formation of lactones in the fat. The presence of these components is not without some support from the literature *(44, 47, 64, 105, 126)*.

PREVENTION OF BROWNING

There appears to be a natural inhibiting mechanism to heat-induced browning in milk. Data have indicated that lactose-casein systems brown more readily than does milk under comparable conditions *(98, 100)*. Nelson *(90)* has observed a lag in the discoloration of evaporated milk during processing. Townley and Gould *(126)* have noted that substantial browning occurred at the time of marked

decrease in labile sulfide liberation. According to Patton and Josephson *(104)*, the onset of browning in heated milk coincides with disappearance of sulfhydryl (–SH) groups as measured by the nitroprusside test. In their study heated whey exhibited a positive nitroprusside test and no browning even after 11 months storage. Tarassuk *(120)* has shown the beneficial effect on color in evaporated milk of removing head-space oxygen from the can prior to sterilization. This procedure is known to have a stabilizing effect on –SH groups in heated milk. Guss *(33)* has reported the inhibiting effects of –SH compounds on browning in model systems, and the prevention of browning by reduced sulfur compounds is known *(66, 118, 130)*. In essence it appears that –SH groups inhibit heat-induced browning in milk, and measures which preserve such groups should help prevent discoloration. The mode of action of –SH compounds in this connection is not known. An interesting speculation concerns the addition of –SH compounds at the double bond of the Amadori rearrangement product (enol form). Hodge *(43)* has suggested that mechanisms of this type might be effective in blocking the browning reaction in certain systems.

Several other agents are known to inhibit browning in milk systems. These include formaldehyde *(60, 110, 118, 130)*, sodium bisulfite *(66, 118, 130)*, sulfur dioxide *(66, 118)*, and hydrogen peroxide *(66, 118)*. Brominating, nitrating, or iodinating casein, as well as treating it with formaldehyde, was observed to prevent browning on subsequent heating with lactose *(118)*. None of these materials or procedures has received consideration for practical purposes in dairy products. The most important preventive for browning in milk and milk products is to keep moisture, heat treatment, and storage times and temperatures at acceptable minima.

NUTRITIONAL IMPLICATIONS OF BROWNING

One important reason for the interest of the food industry in the phenomenon of browning is its relation to nutrition. There is sufficient evidence in the literature to suggest three separate considerations in this regard: 1) lowered consumption of browned foods because of poor palatability, appearance, and physical properties; 2) loss of nutritional value due to vitamin and essential amino acid destruction and loss of biological value and digestibility of protein; 3) production of toxic substances or metabolic inhibitors. These considerations extend beyond the scope of this paper. The interested reader should consult an exhaustive review of the nutritive value of milk and milk products by Kon and Henry *(65)*. Nevertheless it seems desirable to show some of the possible relationships between browning and the field of nutrition.

At the outset one should bear in mind that research on browning frequently has involved reaction conditions that seem improbable even under the worst conditions of food processing and storage. Moreover, actual food products have been used in very few instances. Much of the work on amino acid destruction and loss of nutritive value during browning has utilized mixtures of casein and glucose *(26, 41, 71, 73, 74, 75, 76, 81, 92, 93)*. Although casein and glucose conceivably may occur together in some processed foods in appreciable quantities,

they, as such, form no known food product. In addition, lactose is a much more logical attendant of casein. Lactose appears to brown less readily than glucose in the presence of casein *(71, 80)* and presumably causes less destruction of amino acids than glucose under conducive conditions. In order to amplify and thus facilitate characterization of chemical changes during browning, the use of aggravated conditions is desirable. However, findings under such conditions are not necessarily of immediate practical importance. Further, one is inclined to consider with some reservation the applicability to man of findings on test animals and from in vitro protein digestibility studies.

Destruction of essential amino acids, particularly lysine, has been demonstrated in both heated aqueous and stored dry casein-glucose systems. It is notable that small losses of lysine and histidine during sterilization of evaporated milk have been shown *(48)*. In a study by Henry *et al. (42)* much of the reduction in biological value of stored nonfat dry milk of high moisture content was traced to lysine becoming biologically unavailable through combination with lactose. It is significant here that essential amino acids are destroyed in milk systems which have undergone browning.

Hodson *(45, 46)* discusses the conflicting results that have been obtained on the nutritive value of evaporated milk. It appears that processing and storage for reasonable periods at room temperature may have very minor effects on the nutritive value of the product for the rat and the dog *(2, 40, 47, 133)*. A recent paper by Hodson *(47)* indicates that the amino acid deficiency of evaporated milk proteins for rat growth involves mainly the sulfur-containing amino acids (cystine, cysteine, and methionine). The formation of volatile sulfides in heated milk also suggests destruction of these amino acids *(126, 127, 128)*.

In addition to the findings on amino acid destruction, it is reported that 5-hydroxymethyl-furfural arrests the growth of rats *(39)*. This compound has been isolated from milk which has undergone drastic heat-induced browning *(96)*. With reference to foods which are toasted brown, the cariogenic effect of heated nonfat dry milk noted by McClure and Folk *(83)* is of interest. Dental caries closely resembling those found in humans were produced in rats fed heated dry milks as part of the ration. The detrimental qualities of heated (115° C. for more than 20 minutes) milks as a culture medium for lactic acid bacteria have been noted *(24)*. Milks which have received too rigorous heat treatment do not give optimum results when used as diluters for bovine semen *(12, 124)*. Losses in motility of sperm and lower conception rates commonly result under such conditions.

This brief summary makes clear that lowered nutritional and biological value is a distinct possibility in food products which have undergone a noticeable degree of browning. Toxic or growth-inhibiting effects of browned foods seem worthy of much more extensive investigation than they have received to date. Dried wheys the color of coffee are not uncommon ingredients of livestock and poultry feeds. In view of the available knowledge and equipment, browning is by no means inevitable in milk and milk products, but prevention of the defect will involve application of suitable processing, packing, and storage conditions.

In summing up the problem of browning in dairy products it is evident that with the notable exception of evaporated milk, measures for controlling the defect have been known and used for many years. Occurrence of browning in dairy products has resulted from accidental or purposeful omission of adequate control measures. Even in evaporated milk the problem appears to be yielding to processing innovations. Thus, on a practical level, the problem of discoloration is more one of control and development than research. However, when browning becomes evident in a dairy product the point of good palatability is long since past. It is the early (colorless) stages of browning which are in need of much additional research, not only to unravel the chemistry of pigment formation but also to elucidate the mechanisms and means of prevention of various prebrowning off-flavors.

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MILK LIPASE.
I. THE LIPOLYTIC ACTIVITY OF SEPARATOR SLIME[1,2]

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Many different approaches have been made to describe the phenomenon of lipolysis in milk and other dairy products, and numerous methods have been used to measure lipase activity.

The data accumulated in the literature as the result of these varied methods of experimentation appear to be somewhat confusing and in some disagreement. A partial explanation for the diversity in results lies in the variety of methods used, as well as inadequate control of experimental conditions in some instances. Of perhaps greatest importance in regard to the latter point is the poor control of enzyme source and concentration. Raw milk has most often been used as the source of lipase, and it is natural to assume that the enzymes in milk, like other constituents, are subject to wide fluctuations in amount from one sample to another. Therefore, it seemed necessary, particularly for a study of enzyme kinetics, to develop a potent and stable lipase preparation to permit comparison of experiments under a variety of conditions over relatively long periods of time.

The work of Pfeffer *et al. (3)* indicated that separator slime was an excellent source of lipase and usually showed about three times as much lipolytic activity as normal milk. Accordingly, this work was undertaken to procure from separator slime highly concentrated lipase preparations in a physical state that would provide ease of handling and a source of enzyme that would remain constant in concentration and other properties.

EXPERIMENTAL METHODS

Source and preparation of milk products. All milk products used in this study were obtained from milk produced by the University of Minnesota dairy herd. The cream, skimmilk, and separator slime were prepared by warming approximately 30 gal. of raw milk to a temperature of 32° to 35° C. and passing it through a warm milk separator. The cream and skimmilk were passed over a surface cooler and cooled to 4.5° C., and all three materials were stored at 0° C. until tested. The separator slime was collected immediately after completion of separation and held at 0° C. until utilized in experiments.

The method employed to test lipase activity was very similar to that used by Peterson *et al. (2)*. One change was the use of 5.8 ml. rather than 2.0 ml. of 0.6 *M* sodium barbital in order to increase the buffering capacity of the substrate suspension. The titration was done with aqueous 0.1 *N* sodium hydroxide.

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[2] The data in this paper are taken from a thesis presented by the senior author in partial fulfillment of the degree of Doctor of Philosophy, University of Minnesota.

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The substrate preparation consisted of 0.3 ml. of tributyrin, 5.8 ml. of the barbital buffer, and 4.9 ml. of pasteurized skimmilk. These three ingredients were mixed together and passed through a hand homogenizer four times to bring about a uniform suspension.

After the substrate preparation had been measured into test tubes, resuspended separator slime, cream, or skimmilk was added. The amount of inoculum varied, depending on the source of lipase. The sample was then shaken vigorously 20 times, and a pH determination was made before the sample was incubated at 37.7° C. for 1 hour.

The titration procedure consisted of mixing 5.0 ml. of sample to be tested with 5.0 ml. of 95% ethyl alcohol containing 0.4 g. thymolphthalein per 1,000 ml. of alcohol. Two ml. of ethyl ether was next added, and the mixture was titrated with 0.1 *N* NaOH to a permanent dark blue end point. This procedure was repeated on another 5-ml. portion after the 1-hour incubation period. The difference between the initial titration and that obtained after the incubation period was considered to be a measure of the amount of hydrolysis of the substrate by the lipase present. Controls were run to check for bacterial activity by substituting pasteurized skimmilk for the tributyrin.

The lipolytic properties of blood and various blood constituents were tested in a manner similar to the method used for skimmilk and separator slime. Blood was drawn from lactating cows and defibrinated by shaking in a flask with glass beads. Samples of the defibrinated blood and blood constituents were mixed with the tributyrin substrate, and titrations were made as described previously.

RESULTS

In Table 1 are shown the results of preliminary work in which the lipase activities of fresh separator slime, raw skimmilk, and cream were measured. The data indicate that separator slime was considerably higher in lipolytic activity than was either of the two other products tested.

The fact that the cream showed considerably less lipolytic activity than did either separator slime or skimmilk would seem to indicate that the lipolytic activity of milk is associated with the milk plasma to a greater extent than with the butterfat. That the milk separation process tends to concentrate the lipase

TABLE 1

A comparison of the lipase activity of raw skimmilk, cream, and separator slime as measured by the hydrolysis of tributyrin at pH 8.5

Enzyme source	Amount	0.1 *N* NaOH		
		Initial titration	Final titration	Increase
	(ml.)	*(ml.)*	*(ml.)*	*(ml.)*
Raw skimmilk	1.0	2.30	2.46	0.16
Raw skimmilk	5.0	2.50	2.88	0.38
Cream, 20%	1.0	2.45	2.50	0.05
Cream, 20%	5.0	2.30	2.37	0.07
Separator slime[a]	1.0	2.64	3.16	0.52

[a] Fresh separator slime reconstituted with water in a ratio of 1:1.

in the separator slime, and to a lesser extent in the skimmilk, suggests further that the lipases are associated with some of the heavier milk constituents. These heavier constituents, affected by the centrifugal force of the separation process, are concentrated in the separator slime and skimmilk, leaving only a slight amount of lipolytic activity in the lighter cream.

Additional comparisons were made between the activity of the enzymes of separator slime and raw skimmilk, both obtained from the same milk, by testing their abilities to hydrolyze tributyrin. The results are recorded in Table 2. There

TABLE 2

A comparison of the lipase activity of raw skimmilk and separator slime produced from the same whole milk as measured by the hydrolysis of tributyrin

Trial No.	Source of enzyme	Amount	Initial pH	0.1 *N* NaOH		
				Initial titration	Final titration	Increase
		(ml.)		*(ml.)*	*(ml.)*	*(ml.)*
1	Raw skimmilk	10.0	8.50	2.64	3.14	0.50
	Separator slime[b]	1.0	8.50	3.80	4.02	0.22
2	Raw skimmilk	10.0	8.43	2.36	2.62	0.26
	Separator slime[b]	1.0	8.50	3.34	3.56	0.22
3	Raw skimmilk	10.0	8.56	2.74	3.50	0.76
	Separator slime[b]	1.0	8.50	3.87	4.22	0.35
4	Raw skimmilk	10.0	8.53	2.08	2.54	0.46
	Separator slime[b]	1.0	8.58	2.69	2.76	0.07
5 (Control)	Raw skimmilk[a]	10.0	8.50	2.00	2.00	0.00
	Separator slime[b]	1.0	8.50	2.15	2.15	0.00

[a] Pasteurized skimmilk used as substrate.
[b] Fresh separator slime reconstituted with water in the ratio of 1:1.

was a wide variation in the lipolytic activity of different samples of skimmilk and among the different lots of separator slime. Furthermore, the lipase activity of the separator slime did not increase or decrease in direct proportion to the amount of lipase activity in the skimmilk from which the separator slime was obtained.

In one of these trials pasteurized skimmilk was used in place of tributyrin. When this change was made, there was no increase in titratable acidity after an incubation period of 1 hour. This observation was considered as evidence that the increase in titratable acidity was due to hydrolysis of substrate and not to bacterial fermentation.

After fresh separator slime was shown to possess relatively strong tributyrin hydrolyzing activity, tests were made to determine variation in enzyme activity in individual lots of slime. Wide variations in the amounts of tributyrin hydrolyzing enzymes in individual lots of separator slime were observed and are recorded in Table 3. For example, the separator slime used in Trial 2 produced more than three times the lipolytic activity produced by the slime used in Trial 6.

The effect of Waring blendor and (or) homogenization treatments on separator slime. The rubbery, sticky texture of the separator slime made weighing

TABLE 3

The variation in lipase activity of six lots of separator slime[a] as measured by the hydrolysis of tributyrin

Lot No.	Amount	Initial pH	0.1 *N* NaOH		
			Initial titration	Final titration	Increase
	(ml.)		*(ml.)*	*(ml.)*	*(ml.)*
1	1.0	8.50	2.00	2.40	0.40
2	1.0	8.45	2.46	3.28	0.66
3	1.0	8.50	2.64	3.16	0.52
4	1.0	8.54	2.70	3.20	0.50
5	1.0	8.50	2.20	2.50	0.30
6	1.0	8.50	2.15	2.35	0.20
7 Control[b]	1.0	8.50	2.10	2.10	0.00

[a] Fresh separator slime reconstituted with water in ratio 1:1.
[b] Pasteurized skimmilk used as substrate.

and uniform dispersion difficult. Efforts to extract or redisperse the lipases in the separator slime in an aqueous suspension were made by homogenizing the separator slime after mixing it with water or by mixing separator slime and water with a Waring blendor. The resulting heavy suspension was then centrifuged.

The vigorous mixing action of the Waring blendor transferred only a small percentage of the total lipase activity of the separator slime to the aqueous supernatant. But when the water and slime were homogenized together the supernatant liquid always exhibited greater lipase activity than did the residue resulting after centrifugation.

Repeated treatments with the homogenizer or the Waring blendor or both did not increase the lipolytic activity in the aqueous phase beyond that resulting after a single treatment.

The effect of concentrating enzyme preparations under vacuum. When the separator slime was mixed with water and homogenized, the aqueous supernatant preparations resulting from centrifugation were always more dilute so far as the lipase activity was concerned than were the original separator slime samples. However, the removal of a large portion of the water by condensation under a high vacuum reduced the volume of the aqueous suspensions without destroying the lipase activity. By this means (as is shown in Table 4) it was possible to obtain a high concentration of lipase dispersed in an aqueous solution. Although it was possible by means of condensing the lipase solutions under vacuum to produce a highly concentrated lipase solution, the yield was too small to justify the use of this method.

Lyophilizing separator slime. The separator slime was spread on parchment paper and frozen in a blast of air at –28° C. The frozen slime was placed in a lyophilizing apparatus which was equipped with a heating element, a condenser, and a vacuum pump. A high vacuum was drawn on the lyophilizing apparatus and sufficient heat was applied to dry the slime in 12 hours. At the end of the drying period the slime had reached a temperature of approximately 30° C.

TABLE 4

The effect of vacuum concentration on the lipase activity[a] of the supernatant obtained after the centrifugation of homogenized separator slime

Trial No.	Enzyme source	Amount	Ratio of concentration	0.1 *N* NaOH		
				Initial titration	Final titration	Increase
		(ml.)		*(ml.)*	*(ml.)*	*(ml.)*
1	Separator slime	1.0	—	1.80	2.16	0.36
	Supernatant	5.0	—	1.80	1.92	0.12
	Concentrated supernatant	5.0	7-1	1.80	2.56	0.76
2	Separator slime	1.0	—	1.82	2.88	1.06
	Supernatant	5.0	—	1.80	2.30	0.50
	Concentrated supernatant	5.0	7-1	1.86	5.70	3.90

[a] Determined by hydrolysis of tributyrin at pH 8.5.

Before being tested, the dried separator slime was soaked in distilled water for ½ hour and then homogenized three times.

The lipase activity of separator slime dried by lyophilization was compared with that of the undried slime. The wet slime was tested while fresh and also after 24 hours of storage of 0° C., a period equivalent to that necessary to dry the slime. The dried slime was prepared and tested 1 day after it had been produced. The results recorded in Table 5 reveal that the powder resulting from the drying of the slime was rich in lipase activity. The freeze-drying operation produced a light powder that was easily dispersed in distilled water and that made a fine, uniform suspension resembling reconstituted milk. The fact that the dried separator slime formed a uniform suspension may account for the fact that the dried separator slime samples showed more hydrolysis of the substrate than did the fresh separator slime when compared on a total solids basis. The storage of wet slime seemed to be detrimental to the lipases. A decrease in lipase activity of approximately 15%, as measured by change in titratable acidity, was noted after the wet slime had been stored in a refrigerator for 24 hours.

TABLE 5

The lipase activity of fresh, lyophilized and day-old separator slime from the same lot as measured by hydrolysis of tributyrin at pH 8.5

Enzyme source	Amount	Total solids	Total solids of inoculum	0.1 *N* NaOH			
				Initial titration	Final titration	Increase	Increase per gram solids
	(ml.)	*(%)*	*(g.)*	*(ml.)*	*(ml.)*	*(ml.)*	*(ml.)*
Separator slime	10.0	0.058	0.58	3.04	3.63	0.59	1.01
Lyophilized slime	10.0	0.083	0.83	3.57	4.71	1.14	1.37
Day-old separator slime	10.0	0.058	0.58	3.10	3.60	0.50	0.86

Studies were made of the storage life of dried separator slime. The results indicated that enzymes in dried separator slime stored for 3 days at 0° C. caused about 3% less increase in titratable acidity in the tributyrin emulsion than did the enzymes in the freshly dried slime.

Additional comparisons were made to determine the storage effect on the lipases in separator slime stored wet and separator slime that had been dried by lyophilization. The enzymes of the undried slime caused considerably less hydrolysis after 4 days storage at 0° C. than they had prior to storage. The storage seemed to have little or no effect, however, on the lipase in the separator slime that had been dried and then stored for 4 days at 0° C.

Blood components as a source of lipase. Efforts were made to determine the possible reasons for the relatively high lipolytic activity of separator slime. Various body cells and cell debris are present in separator slime in higher concentrations than in skimmilk. Accordingly, bovine blood constituents, both serum and various types of cells, were tested for lipolytic activity.

The results of these studies indicated that no hydrolysis of tributyrin was produced by whole blood or several of its components, including serum, a leucocyte preparation containing predominantly monocytes, and one containing predominantly polymorphonuclear cells. From these results it appears that under the condition of these experiments neither blood serum nor leucocytes are a source of the lipolytic activity in separator slime. It should be emphasized that the cellular debris of udder origin has not been ruled out as a possible factor.

DISCUSSION

In agreement with Pfeffer *et al. (3)*, the results obtained in this study indicated that separator slime was by far the richest source (on a volumetric or weight basis) of milk lipase when compared with skimmilk and with 20% cream. The reason for the high concentration of lipase in separator slime was not established, but it might be postulated that the enzymes apparently are closely associated with some milk constituents, such as one of the milk proteins or the cellular material that is concentrated in the separator slime.

All lipases in separator slime must come from the milk, provided, of course, that the numbers of bacteria are not excessive. Therefore, it would seem that milk containing high concentrations of lipase would produce either separator slime, skimmilk, or both that would exhibit considerable lipase activity. The data obtained from the above experiments indicate that the separator slime and skimmilk from the same sources exhibit considerable difference in lipase activity.

The fact that the lipase concentration of separator slime does not vary in direct proportion to the lipase content of skimmilk produced in the same operation might be attributed to several factors. The type of feed, stage of lactation, and season of the year presumably influence the lipolytic activity of milk. A variation in the lipase concentration in milk to be separated would probably influence the amount of lipase accumulating in the separator slime. However, the separation process itself seems to play some role in determining the amount of lipolytic activity present in the separator slime. Any factor influencing the

age and total solids content of the slime may exert some influence: speed of separator, duration of separation process, and method of collecting and handling the slime.

Furthermore, it is becoming apparent that there is more than one lipase in milk *(4)* and in separator slime *(1)*. Therefore, during the separation process one lipase may be preferentially removed from the milk, and any treatment affecting the physical state of the lipase(s) in milk would in all probability affect the efficiency of their removal. Accordingly, separator slime, under certain conditions, may exhibit high lipolytic activity, and skimmilk may vary, depending on relative concentrations of the several components of the milk lipase system.

The higher lipolytic activity of the aqueous supernatant resulting after homogenization and centrifugation either would indicate a finer dispersion of slime by homogenization and better extraction of lipase or would indicate that the very vigorous action of the Waring blendor resulted in considerable denaturation of lipase. The rather large differences in activity between the two types of preparations would be difficult to explain solely on the basis of differences in extraction.

Vacuum concentration of an aqueous extract of slime resulted in increased activity almost in direct proportion to the decrease in volume. Such a method provides a lipase preparation of high potency but yields are small, and so much time was expended in processing the preparation that it was not considered to be practical for routine experimentation. However, these results indicate the ability of the lipase(s) of separator slime to withstand temperatures up to 57° C. (maximum temperature of concentration) for considerable periods of time without significant loss of activity.

Freeze-drying separator slime resulted in a product which was very easy to handle, the powder being nonhygroscopic and readily dispersible in water. The process was very simple and apparently not severe enough to cause any decrease in lipolytic activity—in fact, there appeared to be some activation, since the powder showed proportionally more activity in some cases than the increase in solids would indicate. Furthermore, the stability of this enzyme preparation was excellent and storage life appeared adequate. This process[4] has proved suitable in providing enzyme source material for several lipase studies and has served as the basis for a series of experiments describing in some detail the pH optima and substrate specificity characteristics of separator slime *(1)*.

SUMMARY AND CONCLUSIONS

Separator slime is a source of much more concentrated lipase than is either skimmilk or cream. The process of separation seems to play some role in determining the amount of lipase in separator slime and skimmilk since there is considerable variation in the amount of lipase activity found in separator slime and skimmilk. The causes of these variations were not determined.

[4] Schwartz *et al. (4)* have used lyophilized raw skimmilk as an enzyme source to demonstrate evidence of several lipases based on pH optima, substrate concentration, and formalin inhibition.

At least some of the lipase of separator slime can be transferred to an aqueous solution by means of homogenization and centrifugation. Condensation under high vacuum will concentrate lipase activity in direct proportion to the reduction in volume of the aqueous solution.

By means of lyophilization it was possible to obtain the separator slime in a dry powder form. This powder was soluble in water, possessed high lipase activity, and retained its lipolytic properties over a considerable length of time when stored at 0° C.

Blood serum and leucocytes do not seem to play a part in contributing to the high lipase activity of separator slime.

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A STUDY OF THE VOLATILE FRACTION ISOLATED FROM OXIDIZED MILK FAT. II. FURTHER CHARACTERIZATION OF COMPOUNDS RESPONSIBLE FOR THE OXIDIZED FLAVOR[1]

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In a previous paper *(11)* a method was presented for the chromatographic separation of the volatile fraction from oxidized milk fat, and preliminary characterization was made of the nature of the compounds responsible for oxidized flavor.

This study, dealing with further identification of the compounds that contribute to oxidized flavor, is based on hydrogenation of the volatile compounds and their reaction with semi-carbazide. As in the previous paper, determination of the absorption characteristics in the ultraviolet region of the spectrum is the main diagnostic tool. Saturated carbonyl compounds exhibit characteristic maxima *(1, 2, 8, 9)*, and the molar extinctions are ca. 0.001 in magnitude of that of carbonyl compounds with conjugated double bonds. Semi-carbazones exhibit maxima with high molar extinctions *(3, 10)*.

MATERIALS AND METHODS

Skellysolve solutions of carbonyl compounds from the volatile fraction were obtained from milk fat oxidized at 100° C., as described in the preceding paper *(11)*. Methods for ultraviolet absorption, chromatographic separation, and organoleptic evaluation were those used previously.

Hydrogenation. The catalyst was prepared by the method of Thomas *(12)*, modified as follows: A solution of 0.5 g. of platinum in 100 ml. of aqua regia was mixed homogeneously with 20 g. of Celite. The aqua regia was evaporated and 150 g. of sodium nitrate dissolved in 100 ml. of hot water was added and mixed thoroughly. After evaporation of the water the mixture was fused in ½ hour under stirring in a Vicor beaker on a Meeker burner. The mixture was held in the liquid state for ½ hour and then poured into 300 ml. of water. The salt solution was decanted and the solid material was washed with hot water and dried in a filter crucible, first by suction and finally in a 100° C. oven. The catalyst was then ground in a mortar and passed through a 150-mesh platinum screen. The Kaufmann-Baltes *(5)* apparatus was employed for the hydrogenation procedure. Flasks were modified to contain about 160 ml., and a separatory funnel was set in the upper side, through which solvent and sample could be introduced. Skellysolve and catalyst were shaken for 10 minutes for equilibration. Then Skellysolve solution of carbonyl compounds was introduced, and the separatory

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funnel was rinsed by introducing more Skellysolve into the flask. The apparatus was shaken until it was again equilibrated. The reaction was carried out at a constant temperature of 25° ± 0.1° C. Blanks were run. Volumes, temperatures, and pressures were read at definite intervals during the hydrogenation. Optimum conditions for hydrogenation were established from preliminary data obtained by hydrogenating mixtures of saturated octaldehyde and $\alpha\beta$-unsaturated dimer octaldehyde in such a way that unsaturation could be hydrogenated without significantly attacking the carbonyl group. The procedure adopted was as follows: Equilibration of 50-200 mg. of catalyst with 5 ml. of solvent and introduction of 25 ml. Skellysolve solution of carbonyl compounds and 5 ml. of solvent for rinsing. Shaking time was about 20 hours. The starting pressure without solvent was ca. 50 cm. of mercury; with the solvent it was higher than atmospheric pressure. The last part of the liquid was forced into the flask by nitrogen pressure. The final pressure was about atmospheric. In this way the pressure during the later, long hydrogenation period was as close as possible to atmospheric pressure, reducing chances of leakage.

Reaction with semi-carbazide. Semi-carbazide HCl and sodium acetate in weight ratio of 1:1.5 were dissolved in water. Skellysolve solution of carbonyl compounds was added, and the mixture was shaken in a glass-stoppered bottle filled with nitrogen for 2-5 hours on a Fisher-Kahn fast shaker at room temperature. The following Skellysolve solutions of the reaction mixture were then examined for ultraviolet absorption:

1. The original Skellysolve layer of the reaction mixture.
2. The Skellysolve extract of the aqueous layer of the reaction mixture.
3. The water layer in the above, extracted with chloroform, and the chloroform evaporated to dryness in vacuum under nitrogen flow and the residue redissolved in Skellysolve and (when necessary) mixed with some alcohol to increase the solubility.

Residue weights of the three types of Skellysolve solutions were determined by evaporating the solvent under nitrogen flow to constant weight.

It was established from exploratory work that the reaction with semi-carbazide leads to an equilibrium: A large excess of semi-carbazide forms more semi-carbazone; removal of semi-carbazide with HCl directs the reaction in the opposite direction. Semi-carbazones could be hydrolyzed by shaking a Skellysolve or Skellysolve + alcohol solution with ca. 7.5% HCl in water.

RESULTS

Hydrogenation changed the oxidized flavor into a rather pleasant, aromatic flavor. This would indicate that the compounds responsible for oxidized flavor are unsaturated. Upon hydrogenation, the ultraviolet absorption decreased to approximately 1-2% of the original value. The maxima at 265 and 215 mμ disappeared, hydrogenated solutions exhibiting low maxima at 270-280 mμ. The

curves showed still lower maxima, or even discontinuity, under more vigorous hydrogenation conditions or when chromatographic fractions were hydrogenated. The maxima of the hydrogenated solutions decreased during storage at 4° C. This was particularly true for maxima at 270 mμ. Partial hydrogenation caused an increase in the ratio of optical density at 215 mμ to optical density at 265 mμ, indicating a faster rate of hydrogenation of the diene fraction relative to the monene fraction.

Table 1 presents the typical absorption characteristics of the hydrogenated volatile fraction and hydrogenated chromatographic fractions obtained from the volatile fraction, as described previously *(11)*. These indicate that the carbonyl compounds present were very likely not aldehydes but ketonic in nature, because of the single maximum exhibited at 280 mμ after hydrogenation. The shifting to 270 mμ of the maximum for aged solutions may be explained by formation of ketoacid *(1, 7)*. The diene chromatographic fraction 5 contributed most to the ketone maximum of the hydrogenated mixture because the monene fraction 1 exhibited a low maximum and the other fractions even showed a discontinuity in the curve. From the hydrogen uptakes and optical density values of chromatographic fractions 1 and 5 (the purest monene and diene compounds available), "molar extinction values" for conjugated monene compounds and conjugated diene compounds were calculated to be 10,900 and 10,400, respectively. In these calculations uptakes of 1 mole of H_2 per mole of monene compound and 2 moles of H_2 per mole of diene compound were assumed because the hydrogenation procedure did not significantly attack the carbonyl group (*Methods* section). A "molar extinction value" was then calculated as the optical density (extinction) per liter solution at which the quantity of H_2 required for 1 mole of the particular compound would be taken up, therefore representing 1 mole of that (average) compound. Correction was made for the diene absorption present in the monene fraction, and vice versa, by treating the calculation as two equations with two unknowns. The molar extinction values seem to be on the low side compared with similar values in the literature *(3, 10)*, indicating too high a hydrogen uptake per optical density unit and the presence of unsaturation not shown in the ultraviolet absorption. From the previous calculations, based on the data of fractions 1 and 5, it followed that a 1-liter solution of optical density 10,900 at 215 mμ would take up 1 mole of H_2 and a 1-liter solution of optical density 10,400 at 265 mμ, 2 moles of H_2. Subsequently, hydrogen uptakes were calculated per unit of optical density per 25 ml. volume for both maxima, and with these values the hydrogen uptakes of the solutions in Table 1 were calculated from the optical densities. The hydrogen uptakes actually determined were expressed as percentages of the calculated values in Table 1, last column. Hydrogen uptakes determined for mixtures were generally lower than those calculated from fractions 1 and 5. Although this was not true for fractions 2 and 4, they were close to 1 and 5 and contained mainly one class of unsaturated compounds. The high values calculated for mixtures may be the result of mutual influence, with consequent increase of both maxima.

TABLE 1

Absorption characteristics and ratio of hydrogen uptake of carbonyl compounds upon hydrogenation

Skellysolve solutions of volatile fraction	Nonhydrogenated solutions			Hydrogenated solutions		Hydrogen uptake detnd[c] × 100
	O.D.[a] 265 mμ	O.D. 215 mμ	O.D. 215 : 265 mμ	Max. (mμ)	O.D. max.[b]	Hydrogen uptake calc[d]
1 : 4 dilution, aged	25.3	279.5	11.00	270	4.01	79
1 : 4 dilution, fresh	86.0	237.3	2.80	280	2.18	68
Chromat. fraction 1	3.0	276.8	92.30	280	0.69	100
Chromat. fraction 2	10.0	154.6	15.50	no max.	—	120
Chromat. fraction 3	57.5	222.0	3.90	no max.	—	74
Chromat. fraction 4	34.5	17.8	0.51	no max.	—	128
Chromat. fraction 5	88.1	12.0	0.14	280	0.88	100

[a] Optical density.
[b] Recalculated to the original volume 25 ml.
[c] Determined.
[d] Calculated from chromatographic fractions 1 and 5.

Semi-carbazones. Reaction with semi-carbazide was studied on four types of Skellysolve solutions of carbonyl compounds:

1. Volatile fraction unhydrogenated.
2. Chromatographic fractions unhydrogenated (obtained from unhydrogenated volatile fraction).
3. Volatile fraction hydrogenated.
4. Chromatographic fractions (obtained from unhydrogenated volatile fraction) hydrogenated.

Oxidized flavor, if present, disappeared by reaction with semi-carbazide and was produced again upon hydrolysis of the semi-carbazones. This would indicate that carbonyl compounds are responsible for oxidized flavor, confirming the observations of Keeney and Doan *(6)*.

1. *Semi-carbazones of the volatile fraction unhydrogenated.* The volatile fraction from 3.65 kg. of oxidized milk fat in 70 ml. of Skellysolve (optical densities for 10 ml. volume at 215 and 265 mμ, 6,412 and 2,156, respectively) was reacted with 300 ml. of 1.7% aqueous solution of semi-carbazide. The semi-carbazone fractions prepared are listed in Figure 1. Upon completion of the reaction the Skellysolve layer was separated. After aging the Skellysolve layer over night at 4° C., crystalline material separated from the layer was washed with a small quantity of cold Skellysolve, redissolved, and examined for ultra-

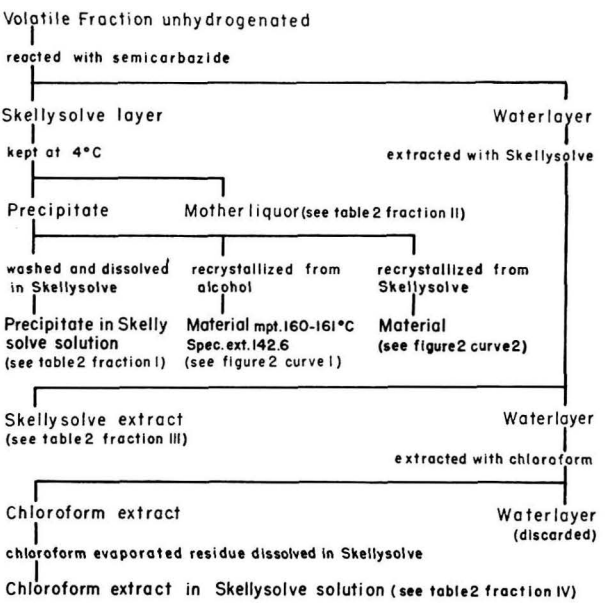

FIG. 1. Semi-carbazone fractions obtained from the volatile fraction unhydrogenated.

violet absorption. Further Skellysolve solutions were prepared as described in the *Methods* section.

Data on various semi-carbazone fractions are presented in Table 2. No ultraviolet absorption maximum was left at 215 mμ. Reaction products exhibited maxima at 265 and 230 mμ, indicating semi-carbazones of conjugated monene carbonyl compounds and of saturated carbonyl compounds, respectively *(3, 10)*. Absorption curves showed discontinuity at ca. 290-300 mμ, indicating semi-carba-

TABLE 2

Optical densities and weights of semi-carbazones formed from the volatile fraction

Skellysolve solutions of semi-carbazones	O.D.[a] 265 mμ		O.D. 230 mμ		Residue weight	
	per 10 ml. volume	% of total	per 10 ml. volume	% of total	*(mg.)*	*(% of total)*
Precipitate (I)	4,848	71	no max.	0	457	25
Mother liquor (II)	1,435	21	2,955	63	976	55
Skellysolve extract (III)	160	2	333	7	93	5
Chloroform extract (IV)	431	6	1,390	30	261	15
Total	6,874	100	4,678	100	1,787	100

[a] Optical density.

zones of conjugated diene carbonyl compounds. These compounds, present in much smaller quantities, probably react more slowly with semi-carbazide, as will be shown later with hydrogenated compounds. The total weight of the reaction products was ca. 0.05% of the fat. Carbonyl compounds represented a large part of the volatile fraction. (Fatty acids may also form a considerable part of the volatile fraction. Acidity was always present: The number of acid groups varied from ca. 0.5 to 2.0 times the number of carbonyl groups. The possibility of keto acids was not further investigated.)

Attempts were not successful to separate and purify saturated semi-carbazones from the reaction mixture by precipitation from various solvents, by distillation, or by chromatography.

Monene semi-carbazone was recrystallized from alcohol to a specific extinction of 142.6, melting point 160-161° C. It was moderately soluble in alcohol and very slightly in Skellysolve. Absorption curves for monene semi-carbazone, recrystallized from alcohol and from Skellysolve, are presented in Figure 2. Later results with chromatographic fractions will show that the curve from material recrystallized from alcohol indicates the presence of some diene semi-carbazone. Recrystallization from Skellysolve is better in this respect but shows a higher absorption below 250 mμ, probably the result of contamination with saturated semi-carbazone.

Hydrolysis of recrystallized monene semi-carbazone did not produce oxidized flavor, which indicates that conjugated monene carbonyl compounds are not important in this respect.

2. *Semi-carbazones of chromatographic fractions unhydrogenated.* The volatile fraction representing 65 g. of milk fat was chromatographed and the eluate was recovered in 25-ml. fractions numbered 1 to 21. The fractions were reacted

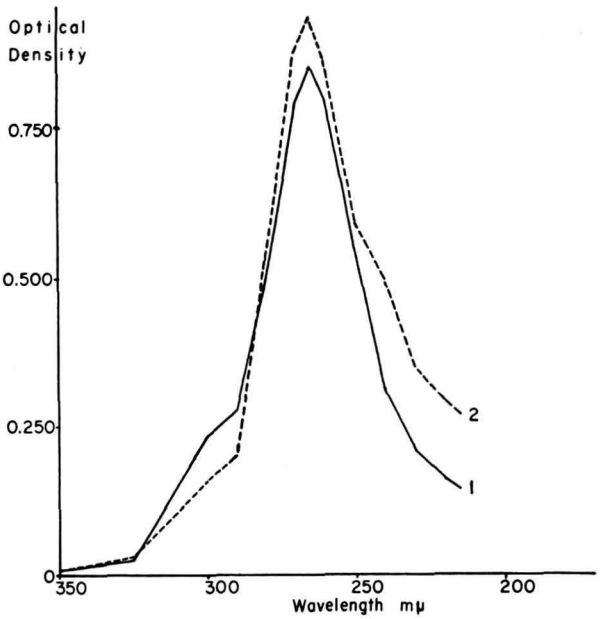

FIG. 2. Ultraviolet absorption of semi-carbazone of conjugated monene carbonyl compounds in Skellysolve solution. 1 = recrystallized from alcohol, 2 = recrystallized from Skellysolve.

with 15.6% aqueous solutions of semi-carbazide (8 ml. per fraction). Upon completion of the reaction, the Skellysolve layers were separated and examined for ultraviolet absorption. The water layers were discarded without extraction. Optical densities in the ultraviolet absorption maxima of the various fractions before and after reaction with semi-carbazide are presented in Figure 3. Ultraviolet absorption curves of three fractions representing saturated, monene, and diene carbonyl compounds are presented in Figure 4. Data show that semicarbazones of the three classes of carbonyl compounds were obtained. Saturated carbonyl compounds, when eluted, came out before and with the monene compounds. They did not show up in the ultraviolet absorption; fraction 6 undiluted had no maxima in the region 270-300 mμ; after reaction with semi-carbazide there was a maximum at 230 mμ. Oxidized flavor was found in fractions 6, 7, and 8, which indicates that saturated carbonyl compounds are the important constituents involved in oxidized flavor. "Saturated" here includes the carbonyl compounds that are unconjugated unsaturated, which do not show in the ultraviolet absorption. Crystalline semi-carbazone residues were obtained from all fractions, but quantities were too small for recrystallization. Diene fractions after storage for 2 weeks at 4° C. showed slight absorption decrease at 265 mμ and slight absorption increase at 215 mμ. However, after reaction with semicarbazide these aged fractions, compared with the fresh fractions, showed a large decrease in absorption at 290 mμ and large increases with maxima at 230 and 270

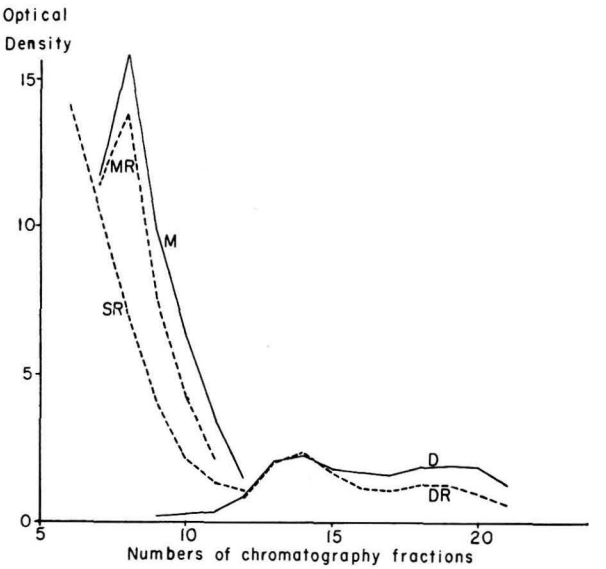

FIG. 3. Optical densities in maxima of chromatography fractions, before and after reaction with semi-carbazide. M = max. at 215 mμ, before reaction (monene carbonyl), D = max. at 265 mμ, before reaction (diene carbonyl), MR = max. at 265 mμ, after reaction (monene carbonyl semi-carbazone), DR = max. at 290 mμ, after reaction (diene carbonyl semi-carbazone), SR = max. at 230 mμ, after reaction (saturated carbonyl semi-carbazone).

mμ. This indicates that saturated and monene carbonyl compounds can be formed from the diene compounds.

3. *Semi-carbazones of the volatile fraction, hydrogenated.* The hydrogenated volatile fraction from 180 g. of milk fat in 10 ml. of Skellysolve was reacted with 25 ml. of 0.2% aqueous solution of semi-carbazide. Skellysolve solutions of the reaction mixture were prepared as described in the *Methods* section. The ultraviolet absorption of the Skellysolve layer, before and after reaction (Figure 5), indicates the formation of semi-carbazones of saturated carbonyl compounds. Attempts failed to separate and crystallize saturated semi-carbazones from the Skellysolve layer by precipitation from various solvents or by distillation. Skellysolve and chloroform extracts showed the same type of absorption curves as did the Skellysolve layer. Residues from these extracts solidified. From Skellysolve the material had a moist appearance, melting point 83° C. (unsharp) with specific extinction ca. 43 at 230 mμ. From chloroform the crystals looked dry, melting point 85.5-86.5° C., with specific extinction at 230 mμ, 67.7-75.5. Quantities were too small for recrystallization to constant values. The material probably was semi-carbazone of hydrogenated monene carbonyl compounds because this semi-carbazone, when not hydrogenated, was only slightly soluble and present in large quantities.

4. *Semi-carbazones of chromatographic fractions, hydrogenated.* Two chromatographic fractions—with only one maximum, obtained from 365 g. of milk

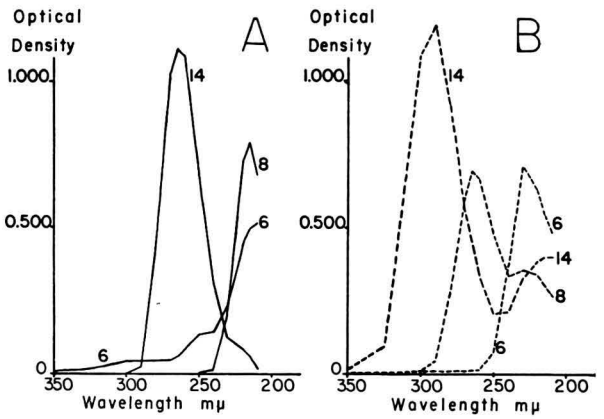

FIG. 4. Ultraviolet absorption of chromatography fractions, before and after reaction with semi-carbazide. A = before reaction: 6 = fraction 6, undiluted, 8 = fraction 8, diluted 1:20, 14 = fraction 14, diluted 1:2; B = after reaction: 6 = fraction 6, diluted 1:20, 8 = fraction 8, diluted 1:20, 14 = fraction 14, diluted 1:2.

fat—the "monene fraction" (maximum at 215 mμ) with saturated carbonyl present and the diene fraction (maximum at 265 mμ) were hydrogenated and then reacted (in 35 ml. of Skellysolve) with 175 ml. of 0.2% aqueous solutions of semi-carbazide. Skellysolve solutions of the reaction mixtures were prepared as described in the *Methods* section. The ultraviolet absorption of the Skellysolve layers, before and after reaction, is shown in Figure 5. Semi-carbazones formed easily from the hydrogenated monene fraction, and with difficulty from the hydrogenated diene fraction, even though the latter showed a much better "ketone" maximum before reaction. This indicates that the diene fraction is less

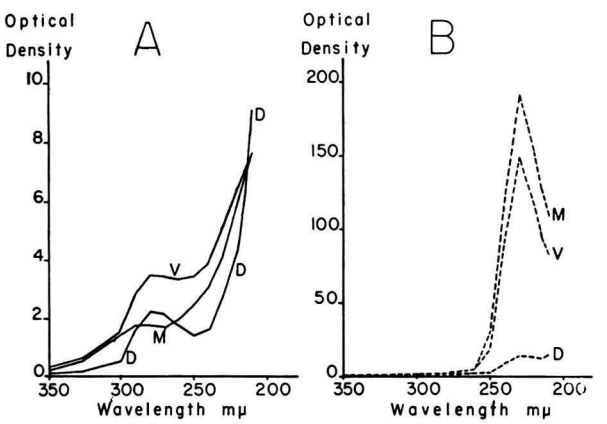

FIG. 5. Ultraviolet absorption of hydrogenated carbonyl compounds, before and after reaction with semi-carbazide. A = before reaction, B = after reaction, V = volatile fraction, hydrogenated, M = monene fraction, hydrogenated, D = diene fraction, hydrogenated.

reactive, probably because of longer chain length and less solubility in water. Skellysolve and chloroform extracts showed the same type of absorption curves as did the Skellysolve layers. Residues of these extracts solidified in the "monene fraction." Residues of the Skellysolve and chloroform extracts had specific extinctions at 230 mμ of 55 and 63, respectively. Crystalline material precipitated from the Skellysolve layer of the "monene fraction," when held at 4° C., had a specific extinction at 230 mμ of 64. This material was probably semi-carbazone of hydrogenated monene carbonyl compounds. Quantities were too small for further purification. No solid semi-carbazone could be obtained from the diene fraction.

DISCUSSION

Hydrogenation studies indicated that compounds responsible for oxidized flavor were unsaturated. Studies based on ultraviolet absorption of the semi-carbazones showed that these compounds were carbonyl compounds located in the saturated class. This indicates that oxidized flavor is caused by unsaturated unconjugated carbonyl compounds, which is supported by the fact that the conjugated monene carbonyl fraction mixed with the fraction responsible for oxidized flavor used more hydrogen for hydrogenation than would be expected from the ultraviolet absorption at 215 mμ.

Carbonyl compounds from the volatile fraction were probably ketonic in nature, because after hydrogenation the mixture of compounds showed maximum absorption in the ketone region. The conjugated diene fraction in particular had a good ketone maximum after hydrogenation. This maximum was less pronounced for the conjugated monene fraction, and no maximum was found for the unconjugated fraction. Semi-carbazone yield was very good for the monene fraction (hydrogenated and not hydrogenated) and for the unconjugated fraction, although these two fractions seemed to contain little carbonyl compound as far as the maximum after hydrogenation is concerned. This suggests tautomerism, in which the carbonyl maximum does not have to show because the compound may be present largely in a tautomeric form without carbonyl group (enol?). Upon reaction with semi-carbazide the tautomeric form will react via the real carbonyl compound. The possibility of tautomerism was further supported by the fact that the ketone maximum of the hydrogenated mixture (particularly the aged mixture with little "diene fraction" present) decreased in storage.

Results of this study show the direct contribution of unconjugated unsaturated carbonyl compounds. Van der Waarden *(13)* postulated these as unsaturated C_6 up to C_8 aldehydes, whereas Keeney and Doan *(6)* located an unsaturated C_7 ketone of this type. Results support Keeney and Doan as to the ketonic character of the volatile mixture of carbonyl compounds.

Chromatography on Celite, with semi-carbazone formation in the eluate, appears promising as a technique for isolation and purification of the three classes of carbonyl compounds because both separation and crystalline residues resulted. Unconjugated and diene compounds were obtained free from contamination with

the other classes. Monene compounds were contaminated with unconjugated compounds, but the latter can be removed easily because semi-carbazones are more soluble in alcohol. This procedure should be repeated in large-scale operation, and pure carbonyl compounds of the three classes should be analyzed and studied: the unconjugated for oxidixed flavor properties, by mixing in fat and milk; the conjugated for their possible role as precursors in oxidized flavor formation. It must be understood that the term "oxidized flavor" is used in this paper for the flavor developed in milk fat after exposure to air at 100° C. This flavor was organoleptically similar to the oxidized flavor that develops in whole milk and milk products. This does not necessarily mean that the mechanism and compounds formed are the same in both cases.

Swift *et al. (10)* demonstrated the presence of conjugated diene, conjugated monene, and saturated aldehydes, with 10, 8, and 6 C-atoms, respectively, in oxidized cottonseed oil. They isolated semi-carbazones with melting point of 156°-160° C. and specific extinction of 147.0 for the conjugated monene fraction, and semi-carbazones with melting point of 85°-100° C. and specific extinction of 71.0 for the saturated fraction. In the present study the semi-carbazones of the conjugated monene fraction had a melting point of 160°-161° C. and specific extinction of 142.6, the semi-carbazones of the hydrogenated conjugated monene fraction had a melting point of 85.5-86.5° C. and specific extinction of 67.7-75.5. The fact that the semi-carbazone constants of the compounds are in the same range may indicate a similarity between the mechanisms of oxidation of cottonseed oil and of milk fat.

SUMMARY AND CONCLUSIONS

Three classes of carbonyl compounds were found in the volatile Skellysolve soluble fraction from milk fat oxidized at 100° C. They are: *(a)* unconjugated unsaturated, *(b)* conjugated monene, and *(c)* conjugated diene carbonyl compounds. The first two classes constituted the major portion of the volatile fraction; the third was present only in minor quantities. The first class of carbonyl compounds appears to be responsible for oxidized flavor; carbonyl group and unsaturation both were essential in this respect.

Carbonyl compounds, particularly of Class *c*, were ketonic in nature; Classes *a* and *b* may exhibit tautomerism. Compounds of Class *c* were very unstable and could form conjugated monene and unconjugated or saturated carbonyl compounds.

The weight of the total semi-carbazones was ca. 0.05% of the fat. Reaction with semi-carbazide was most difficult with Class *c*. From Class *b* a semi-carbazone was isolated of specific extinction 142.6 and melting point of 160°-161° C. This carbonyl compound did not contribute to the oxidized flavor.

A procedure was developed that seems promising for separation and purification of the three classes of carbonyl compounds. It involves three steps:

1. Chromatography of volatile fraction on Celite.
2. Reaction of the eluate fractions with semi-carbazide.
3. Recrystallization of the semi-carbazones.

ACKNOWLEDGMENT

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THE STANDARD PLATE COUNT OF MILK AS AFFECTED BY THE TEMPERATURE OF INCUBATION

Report of the Committee of the Manufacturing Section of A.D.S.A. (1953)

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G. H. WATROUS,[5] AND M. L. SPECK,[6] *Chairman*

The purpose of the study was to ascertain the reliability of the present temperatures (32° or 35° C. for 48 ± 3 hours) of incubation in the Standard Plate Count of Milk. The study was prompted by observations that other temperatures (usually lower than 32° or 35° C. for longer incubation times) occasionally detect bacteria which are unable to grow at the standard temperatures.

In approaching the problem, the following were considered to be primary questions on which information was needed:

1) The reliability of the present temperatures and time of incubation.

2) The temperature and time, if other than the standard, which gave the highest plate count of milk.

In designing the experimental procedure for suitable statistical analysis, temperatures were selected over a range wherein the extremes would normally be expected to give minimum counts and the intermediate temperatures would provide data whereby the optimum temperature for obtaining maximum counts could be determined. At each temperature four periods of time of incubation were selected which, it was hoped, would provide information as to the time when the maximum count at each temperature would be obtained. In order to obtain data on these factors the following protocol for times of incubation at the different temperatures was selected:

Temperature of incubation (° C.)	Time of incubation (days)			
10	4	6	8	10
20	2	4	6	8
26	1	2	3	5
32	1	2	3	5
35	1	2	3	4
37	1	1.5	2	3
45	1	1.5	2	3

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Each committee member selected locally one raw and one pasteurized milk sample once a month (two laboratories examined two of each per month) and made the Plate Count on each sample according to the technique outlined in *Standard Methods for the Examination of Dairy Products*, 9th edition. A separate set of plates was prepared for each time of incubation at each temperature. Naturally, an exception to the standard technique was the inclusion of the additional temperatures and times of incubation. Another exception was the inclusion of plates showing less than 30 colonies, which was necessary, primarily in the shorter incubation periods at the lower temperatures, for the purposes of this study.

The ages of the raw milk samples varied from 3 to 29 hours after milking, and those of the pasteurized milk varied from 0 to 24 hours (one was 48 hours) from the time of pasteurization.

The study was made from November, 1951, through August, 1952. Seventy-eight samples equally divided between raw and pasteurized milk were examined.

The individual counts for each time at each temperature from the individual members were converted to logarithms. The mean values were obtained by averaging the logs of the counts at a given temperature and time for all months and stations; these are presented in Tables 1 and 2. The values for each incubation temperature at the different incubation times are also presented graphically in Figures 1 and 2.

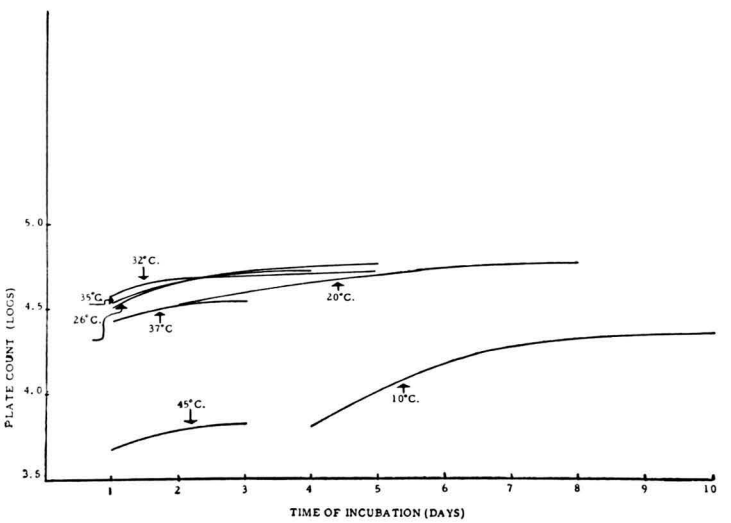

FIG. 1. The plate count of raw milk obtained by incubation of plates at different temperatures for varying times.

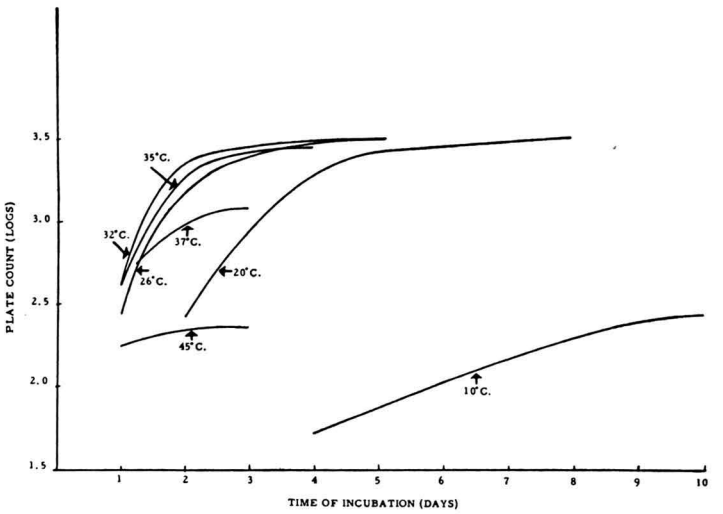

FIG. 2. The plate count of pasteurized milk obtained by incubation of plates at different temperatures for varying times.

RESULTS

Some of the pertinent observations that can be made from the analysis of the data are listed below:

Incubation of plates for raw milk samples (Table 1 and Figure 1):

1) 20° C. for 8 days gave the highest count, although this was only slightly higher than 26° C. for 5 days.
2) The mean count at 32° C. was generally higher than that at 35° C.
3) Mean counts at 32° C. were higher than at 26° C. after 1 day of incubation and were essentially the same after 2 days, but after longer incubation plates at 26° C. gave the higher counts.

Incubation of plates for pasteurized milk samples (Table 2 and Figure 2):

1) 32° C. for 5 days gave the highest count, although only slightly higher than 20° C. for 8 days.

TABLE 1

The mean plate counts (expressed as logarithms) of raw milk obtained by incubation of plates at various temperatures for varying times

Temp. of incubation	Time of incubation *(days)*								
	1	1½	2	3	4	5	6	8	10
(° C.)									
10					3.808		4.176	4.301	4.350
20			4.528		4.656		4.718	4.754	
26	4.516		4.675	4.732		4.747			
32	4.584		4.689	4.688		4.715			
35	4.523		4.664	4.653	4.732				
37	4.424	4.502	4.531	4.522					
45	3.680	3.734	3.801	3.822					

TABLE 2

The mean plate counts (expressed as logarithms) of pasteurized milk obtained by incubation of plates at various temperatures for varying times

Temp. of incubation	Time of incubation *(days)*								
	1	1½	2	3	4	5	6	8	10
(° C.)									
10					1.724		2.025	2.268	2.419
20			2.406		3.268		3.432	3.463	
26	2.421		3.070	3.377		3.430			
32	2.586		3.318	3.436		3.485			
35	2.594		3.265	3.351	3.458				
37	2.627	2.855	2.974	3.064					
45	2.241	2.284	2.326	2.348					

2) Although the counts at 32° and 35° C. increased at about the same rates after the various incubation times, the counts at 32° C. were higher.

3) The development of colonies from pasteurized milk samples was slower than from raw milk. (Compare slopes of curves between 1 and 2 days in Figures 1 and 2.) It is generally agreed that the bacterial flora of pasteurized milk is slower growing than the flora of raw milk, and this is confirmed by these data.

In considering the counts obtained at the different temperatures after a 2-day incubation period (Figure 3), certain observations should be made, viz.:

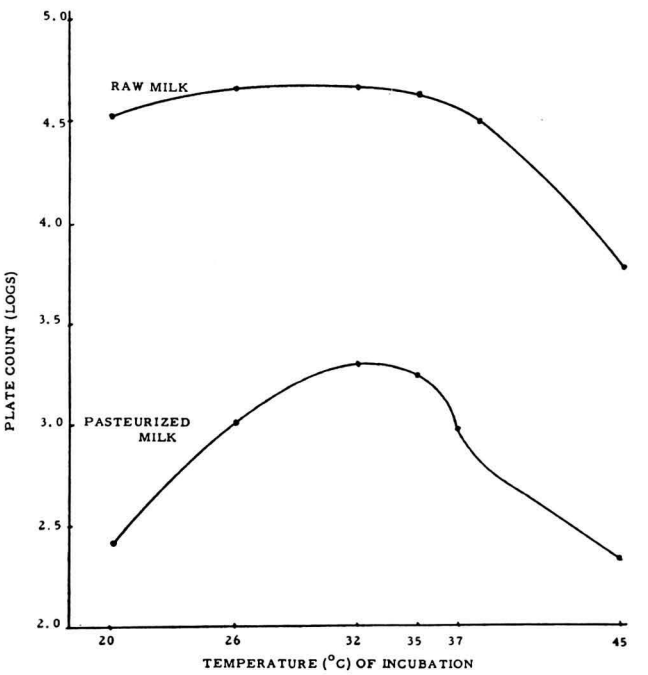

FIG. 3. The plate count of milk obtained by incubation of plates for two days at different temperatures.

EFFECT OF OROTIC ACID AND METHIONINE SUPPLEMENTATION ON FEED CONSUMPTION AND GROWTH OF YOUNG DAIRY CALVES[1]

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Early life usually is regarded as the most critical period in development of the dairy calf. Thus, accelerated growth of the young calf is especially desirable. The presence in whey of an unidentified growth factor for chicks is recognized *(2, 4)*. The response of dairy calves to reconstituted milks containing dried whey product is variable, however, and often results in severe scours, especially if whey solids constitute over 5% by weight of the milks *(8)*. It has been suggested that the whey factor may be a combination of orotic acid and methionine *(1)*. Orotic acid is essential to growth of certain microorganisms *(3, 6)*. Thus, the benefits from whey feeding may occur indirectly through gastric and/or intestinal bacteria. Orotic acid is used directly by rats, however, in the synthesis of nucleic acids *(5, 7)*. The effect may result from direct utilization, or it may be of a dual nature. In either event it seems possible for the whey factor to be utilized without the deleterious effects of whey feeding. The purpose of the present work was to investigate the effect of method and level of administration of various combinations of nucleic acid, methionine, and orotic acid on young dairy calves.

EXPERIMENTAL PROCEDURE

Pilot work to aid in design of the experimental ration was accomplished with 40 new-born Jersey calves. From 110 to 880 mg. per 100 lb. of body weight daily of methionine, nucleic acid, and orotic acid, alone and in combination, were dispersed in the milk, which was fed between the ages of 4 and 60 days. Observations included body weight, height at withers, feed consumption, and general thriftiness.

Calves which were fed a supplement containing equal amounts by weight of methionine and orotic acid at a level of 440 mg. per 100 lb. of body weight daily gained an average of 37 lb. in body weight and 8.2 cm. in height at withers. This compares with 29 lb. and 5.5 cm. for controls. This is the only pilot supplement which warranted further investigation. Methionine, orotic acid, or nucleic acid alone or in other combinations appeared ineffective.

Since bacterial action may be involved, it was decided that administration should be directly into the rumeno-reticular cavity, rather than by adding the supplement to milk. Three calves were fed gelatin capsules containing bromcresol green and were immediately sacrificed. In each calf the rumen contents were stained, showing that the capsule contents entered the rumen. In the second phase, therefore, supplementation was by capsule.

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[1] Florida Agricultural Experiment Station, Journal Series No. 287.

Twelve new-born Jersey calves (six of each sex) were assigned to two groups which were comparable in body weight, height at withers, and sex. All the calves were removed from their dams immediately after birth and were confined in individual pens. They were fed colostrum for 4 days, whole milk through 21 days, and reconstituted skimmilk until 60 days old. All milks were fed at the rate of 9 lb. per 100 lb. of body weight daily. Chopped alfalfa hay and a mixture of 50 parts oats, 60 parts corn, 40 parts wheat bran, 20 parts nonfat dry milk solids, 26 parts peanut meal, 2 parts salt, and 2 parts bone meal were offered free choice. Fifteen thousand I. U. of vitamin A per 100 lb. of body weight daily were administered by gelatin capsule to each calf. The six calves in the experimental group also received supplementary orotic acid and methionine. Body weight and height at withers were determined weekly, and feed consumption was recorded daily.

RESULTS AND DISCUSSION

Incidence of scours was negligible (one calf in each group scoured for 2 days) and all subjects exhibited excellent health. The calves which received orotic acid and methionine gained an average of 51 lb. in body weight (Figure 1). This compares with an average gain of 35 lb. made by the control group. These differences in body weights are statistically significant at $P = 0.01$.

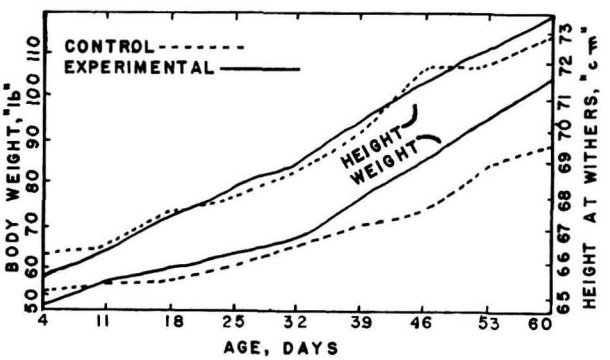

FIG. 1. Effect of orotic acid and methionine on body weight and height at witchers.

Supplemented calves increased in average height at withers by 7.9 cm., as compared to an average gain of 6.7 cm. for the control group. Because of individual variations, however, the difference in height is not statistically significant.

Neither group was significantly superior in tendency to consume feed (Table 1). The difference in growth, therefore, apparently was not caused by simple stimulation of appetite by supplementation. A comparison of feed intake per pound of increase in body weight (Table 2), however, reveals a 41% superiority in efficiency of feed utilization for the supplemented group. This difference is statistically significant at $P = 0.01$.

TABLE 1

Total consumption of dry feeds weekly by groups of calves, in addition to whole or skimmilk

Group	Type of dry feed	Weeks on experiment 1	2	3	4	5	6	7	8
					(lb.)				
Control (6 calves)	Hay	0.2	0.9	2.8	5.9	10.7	13.4	22.2	45.6
	Concentrate	0.0	1.1	3.5	5.8	8.4	11.4	21.3	28.5
Experimental (6 calves)[a]	Hay	0.0	1.4	3.8	4.8	12.6	16.8	25.8	51.7
	Concentrate	0.0	1.4	3.7	5.9	8.9	10.1	16.5	20.1

[a] Equal amounts of methionine and orotic acid were fed daily by capsule in proportion to body weights of calves.

TABLE 2

Relation of methionine and orotic acid supplementation to efficiency of feed utilization[a]

Group	Feed intake per pound gain in body weight: Hay	Concentrate	Whole milk	Skimmilk	Calculated TDN[b]
			(lb.)		
Control (6 calves)	0.39	0.42	2.7	6.8	1.58
Experimental (6 calves)	0.22	0.32	1.8	4.8	1.12

[a] Although maintenance is concerned in feed use, these computations are charged solely against gains in weight.

[b] MORRISON, F. B. *Feeds and Feeding.* 21st ed. 1948.

Better efficiency of feed utilization and greater stimulation of growth apparently result from supplementing calf rations with orotic acid and methionine. The scope of the present work, however, does not justify conclusions relative to the specificity of these supplements in calf nutrition. Further work should include observations on blood constituents and rumen organisms.

Perhaps attention should be given also to the orotic acid content of dried whey. It seems possible that with methionine supplementation, growth stimulation could result from levels of whey which can be tolerated by the dairy calf.

SUMMARY

Young Jersey calves whose rations were supplemented with orotic acid and methionine gained 46% more in body weight than did comparable controls. A 41% superiority in efficiency of feed utilization also was observed in the treated group. Both observations are statistically significant at $P = 0.01$. No significant difference was found in increased height at withers.

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THE EFFECTS OF VARIOUS LEVELS OF SODIUM CITRATE, GLYCEROL, AND EQUILIBRATION TIME ON SURVIVAL OF BOVINE SPERMATOZOA AFTER STORAGE AT -79° C.[1]

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Much work has been undertaken to determine optimum conditions for processing bovine semen for frozen storage to retain maximum survival *(4, 5, 6, 7, 8, 9, 10, 12,* and *13)*. It is generally conceded that further refinement is needed before the technique can be adapted to wide field usage. In particular, research appears to be needed on per cent sodium citrate and glycerol per cent (by volume) in the diluent and on the length of the equilibration period.

Smith and Polge *(12)* used a diluent containing a buffer of 3.92% sodium citrate adjusted to a pH of 6.7. Stewart *(13)* indicated the use of a 3.6% sodium citrate solution. Apparently the optimum level has not been established for a given set of conditions.

At the time these experiments were undertaken there were no reports of critical experiments having been conducted to determine the optimum level of glycerol to use in the freezing of bull spermatozoa. Smith and Polge reported the use of 10 to 15% glycerol as giving best results. Emmens and Blackshaw *(5)* successfully used 7.5 to 10% glycerol with 1.25% of a pentose. In the experiments of Polge and Rowson *(8)* 10% glycerol (by weight) was used. Miller and VanDemark *(6)* used levels of glycerol of 4, 6, 8, and 10% (by volume) and stated that approximately 7% (6-8) glycerol resulted in optimum spermatozoan survival after freezing and storing at -79° C.

Polge and Rowson showed that the number of spermatozoa which survived could be substantially increased if semen were equilibrated for several hours with the glycerol diluent before freezing. They reported an equilibration time of 15 to 20 hours as being optimum, whereas Miller and VanDemark reported that 6 hours of equilibration with glycerol was satisfactory; however, in this study only 2, 6, and 18 hours equilibration periods were used, and it was found that 6 hours was better than either 2 or 18 hours.

Since optimum levels of sodium citrate, glycerol, and equilibration time were not clearly established, this investigation was undertaken to determine the optima of these factors when bull semen was frozen in a sodium citrate–egg yolk diluent.

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EXPERIMENTAL PROCEDURE

Semen. The semen used in this study was obtained from Holstein bulls which were 2 to 4 years of age. Five series of experiments were conducted in this study. In Series 1, 2, and 3 one ejaculate per bull was used, and in Series 4 and 5 two ejaculates per bull, collected 2 weeks apart, were used. Thirty-two ejaculates with a mean spermatozoa count of 1.244 billion per milliliter and a mean estimated motility of 64% were studied. The final dilution rate in Series 1, 2, and 3 was one part semen to 20 parts diluent. In Series 4 the final dilution rate was 30 million spermatozoa per milliliter of diluted semen and in Series 5, 20 million spermatozoa per milliliter.

Methods. Immediately after the semen was collected, the sample was evaluated microscopically for progressive motility and rate of motility. A haemacytometer count was made to determine the number of spermatozoa per milliliter. Semen was used if the volume of the ejaculate was sufficient, if estimated progressive motility was equal to or greater than 60%, and if the rate of motility was the maximum rating of 4+ (where 0 is equal to no motility). The undiluted semen was pipetted into diluents containing egg yolk and sodium citrate of various concentrations, the ratio of yolk to citrate being held at 1:1. Dihydrostreptomycin was added at the rate of 500 γ per milliliter of diluted semen in Series 5 only. The diluted semen was cooled to 5° C. over a period of 4 hours. At this time an equal volume of glycerol-sodium citrate solution at 5° C. was added in two equal portions ½ hour apart. The final diluent contained approximately 24% yolk. One-ml. samples of the final dilution were frozen in 2-ml. screw-capped glass sample vials at the end of each of the various equilibration periods at the rate of 3° C. per minute from 5° C. to –20° C. The rate of temperature decrease was uncontrolled from –20° to –50° C., at which time the samples were transferred to a Dewar flask containing a 95% alcohol–dry ice bath at approximately –77° C. The Dewar flask and contents were then placed in a chest containing dry ice for a 5-day storage period. Samples were then thawed in a 35° C. water bath and examined microscopically, and the per cent motility and rate of motility were recorded.

Per cent survival. This was calculated as follows:

$$\% \text{ survival of motile spermatozoa} = \frac{\% \text{ motile after 5 days at } -79^\circ \text{ C.}}{\% \text{ motile at time of collection}} \times 100$$

Design. A three-dimensional central composite design was used to assign treatments in these experiments *(2, 3)*. For example, using the coded values for treatment combinations (Table 2), the treatments may be graphically represented (Figure 1).

It should be noted that Treatments 1 through 8 form a 2^3 factorial experiment, and the additional treatments, 9 through 15, form a fractional 3^3 factorial experiment. The 2^3 factorial experiment allows good estimates to be made of the main effects and the two-factor interactions. The fractional 3^3 factorial experiment, as used in the three-factor composite design, allows a good estimate to

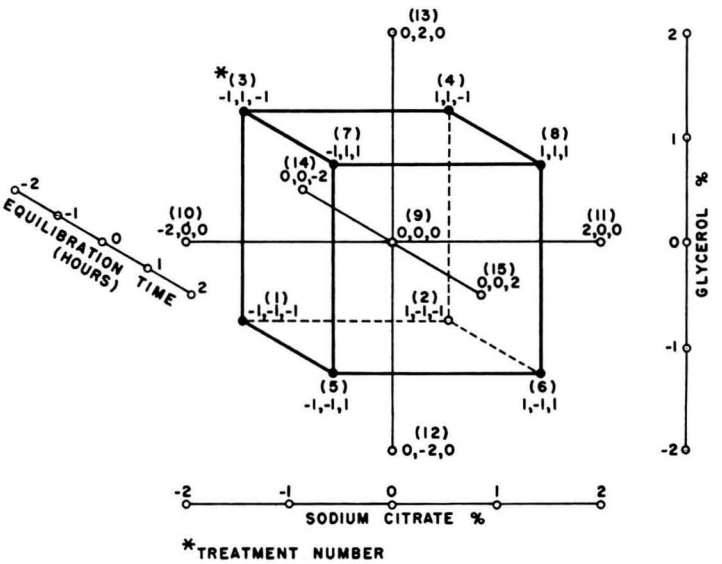

FIG. 1. The three-dimensional central composite design.

be made of the quadratic effects. It should be noted that in the complete 3^3 factorial experiment, estimates can be made for four groups of two-factor interaction effects. They are linear × linear, linear × quadratic, quadratic × linear, and quadratic × quadratic. In the composite type design the assumption is made that the quadratic × quadratic (fourth degree) effects are negligible, as also may be the linear × quadratic and quadratic × linear (third degree) effects *(1)*. In this experiment a multiple regression model was set up to estimate only the linear, quadratic, and the linear × linear interaction effects.

$$Y = B_0 + B_1X_1 + B_2X_2 + B_3X_3 + B_{11}X_1^2 + B_{22}X_2^2 + B_{33}X_3^2 + B_{12}X_1X_2 + B_{13}X_1X_3 + B_{23}X_2X_3,$$

where Y is the estimated response and the B's are the partial regression coefficients. The composite design is adapted for estimating a point of maximum survival of spermatozoa in terms of the levels of sodium citrate, glycerol, and equilibration time which correspond to that point. In these experiments, given levels each of per cent sodium citrate, glycerol per cent (by volume), and equilibration hours were combined to form the 15 treatment combinations for each ejaculate studied.

Procedure. Semen from four bulls was used in each of the five series. A sequential procedure was employed to find the optimum levels of sodium citrate, glycerol, and equilibration time *(2, 3)*. For example, after each of the first four series of experiments, levels of the factors being studied were changed for the next series. This was done to attain the point of maximum survival of spermato-

zoa well within the range of each factor. Therefore, since only the last series of experiments met this requirement, data from this series were analyzed to determine the levels of the factors studied which resulted in maximum survival of spermatozoa. To obtain the estimates of treatment effects in this last series, three vials of diluted semen were frozen from each sample. At the time these samples were thawed, two observers recorded motility estimates for each of the three vials for any given sample. These six observations were averaged to give the estimated response for each treatment for each ejaculate from each of the four bulls; hence, a total of 120 average responses were obtained.

In order to simplify the presentation and calculation, a system of coding was used for values of sodium citrate, glycerol, and equilibration time *(2, 3)*. The levels of the factors used in the first and fifth series only are given with their coded values in Table 1.

TABLE 1

Levels of factors and coded values used in Series 1 and 5

Coded value	−2	−1	0	1	2
			Series 1		
Sodium citrate *(%)*	2.9	3.3	3.7	4.1	4.5
Glycerol *(%)*	5.0	9.0	13.0	17.0	21.0
Equilibration time *(hr.)*	4.0	6.5	9.0	11.5	14.0
			Series 5		
Sodium citrate *(%)*	1.6	2.3	3.0	3.6	4.4
Glycerol *(%)*	2.0	5.0	8.0	11.0	14.0
Equilibration time *(hr.)*	4.0	10.0	16.0	22.0	28.0

TABLE 2

Treatment combinations (with code numbers indicated) and the resulting per cent survival for Series 5 (Average of 2 ejaculates from each of 4 bulls)

Treatment No.	Sodium citrate	Glycerol	Equilibration time	Survival[b]
	(%)	*(%)*	*(hr.)*	*(%)*
1	2.3 (−1)[a]	5.0 (−1)	10 (−1)	57
2	3.7 (1)	5.0 (−1)	10 (−1)	40
3	2.3 (−1)	11.0 (1)	10 (−1)	19
4	3.7 (1)	11.0 (1)	10 (−1)	40
5	2.3 (−1)	5.0 (−1)	22 (1)	54
6	3.7 (1)	5.0 (−1)	22 (1)	41
7	2.3 (−1)	11.0 (1)	22 (1)	21
8	3.7 (1)	11.0 (1)	22 (1)	43
9	3.0 (0)	8.0 (0)	16 (0)	63
10	4.4 (−2)	8.0 (0)	16 (0)	28
11	1.6 (2)	8.0 (0)	16 (0)	11
12	3.0 (0)	14.0 (−2)	16 (0)	2
13	3.0 (0)	2.0 (2)	16 (0)	18
14	3.0 (0)	8.0 (0)	28 (−2)	56
15	3.0 (0)	8.0 (0)	4 (2)	46

[a] Coded values presented in parentheses.

[b] Per cent survival based on the average of 8 responses (2 ejaculates on each of 4 bulls).

RESULTS AND DISCUSSION

The treatments and results of Series 5 are given in Table 2.

A response equation was derived from the data collected in this series. This equation was found to be:

$$Y = 66.3889 - 1.4400X_1 - 2.2812X_2 - 1.0950X_3 - 11.3561X_1^2 - 13.6798X_2^2 - 3.4972X_3^2 + 9.1000X_1X_2 + 0.6075X_1X_3 + 0.8125X_2X_3$$

where Y is the estimated per cent survival and X_1, X_2, and X_3 are the coded values for sodium citrate, glycerol, and equilibration time, respectively. The original calculations were carried to six decimal places but are rounded to four in this paper.

The standard error for this experiment, based on the variation between Y from one ejaculate of semen to another from the same bull for the same treatment, was 16.1862 (60 degrees of freedom). Here the coefficient of variation was 45%.

If we let b_i be the estimate of B_i in the response equation, the standard errors of the b_i were as follows:

Constants	*Standard error*
Linear (b_1, b_2, b_3)	1.4307
Quadratic (b_{11}, b_{22}, b_{33})	1.7195
Interaction (b_{12}, b_{13}, b_{23})	2.0233
Intercept (b_0)	5.0469

The point of maximum per cent motility is calculated by taking the derivatives of the equation of the response surface with respect to X_1, X_2, and X_3 and equating them to zero. Then the equations with X_1, X_2, and X_3 are solved simultaneously to produce the estimated co-ordinates of the point of maximum per cent motility. The equations are:

$$\frac{\partial Y}{\partial X_1} = -1.4400 - 22.7122X_1 + 9.100X_2 + 0.6075X_3 = 0$$

$$\frac{\partial Y}{\partial X_2} = -2.2812 - 27.3597X_2 + 9.100X_1 + 0.8125X_3 = 0$$

$$\frac{\partial Y}{\partial X_3} = -1.0950 - 6.9945X_3 + 0.6075X_1 + 0.8125X_2 = 0$$

The solutions of these equations are:

$$X_1^0 = -0.1198; \quad X_2^0 = -0.1286; \quad X_3^0 = -0.1819$$

These are the coded values for maximum per cent motility. Converting these optima to actual values, sodium citrate = 2.9%, glycerol = 7.6%, and equilibration time = 14.9 hours. Although 14.9 hours was estimated as the optimum time to give maximum survival of motile spermatozoa with a survival of 67%, it was estimated by inserting the proper coded values into the response equation that 10 and 4 hours equilibration time gave estimated survivals of 64% and 55%, respectively.

Although it was found that the constants in the response equation differed from bull to bull ($P = 0.001$), the relative magnitudes were very similar. In other words, if the average survival of spermatozoa from one bull was higher than that of another bull, it was generally higher over all treatments. As a result, the optima were fairly constant from bull to bull. A significant interaction between sodium citrate and glycerol ($P = 0.001$) was found. As per cent sodium citrate and per cent glycerol are increased simultaneously beyond the point of maximum survival of spermatozoa, survival falls off less rapidly than if only one of these variables is increased. The same relationship appears to exist with a simultaneous decrease of these two variables. The interactions between sodium citrate and equilibration time and between glycerol and equilibration time were not significant.

An eosin-aniline blue stain for live-dead determinations (Shaffer and Almquist, *11)* was used on the samples of diluted semen in Series 5 shortly after they were thawed. The results obtained were not consistent with averages for visual observations and were therefore discarded.

SUMMARY

Experiments were designed to determine the optimal combination for per cent sodium citrate, per cent glycerol (by volume), and equilibration hours which would allow maximum survival of motile spermatozoa after being frozen for 5 days by use of dry ice. A sodium citrate–yolk glycerol diluent was used which contained approximately 24% yolk in the final dilution. A dilution rate of 20 million spermatozoa per milliliter of diluted semen was used. A three-dimensional central composite experimental design was used, which is adapted to estimating the optimal level of the factors studied. The estimated optima were: sodium citrate, 2.9%; glycerol, 7.6%; equilibration time, 14.9 hours.

As equilibration time was decreased, per cent survival decreased only slowly. An interaction between sodium citrate and glycerol was noted. Although semen from bulls was different, the pattern of response to treatments was the same with only absolute magnitude of response varying. An eosin-aniline blue live-dead stain was not satisfactory for estimating live spermatozoa when used on freshly thawed semen samples.

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USE OF CHROMIC OXIDE AS AN INDICATOR OF FECAL OUTPUT FOR THE PURPOSE OF DETERMINING THE INTAKE OF PASTURE HERBAGE BY GRAZING COWS[1]

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Considerable attention has been given recently to the estimation of the herbage intake of grazing animals by the combined use of two indicators. In this kind of procedure, the amount in the feces of an indicator occurring naturally in plants serves as an index of indigestibility and that of an indicator administered to grazing animals in known and constant amounts per unit of time provides a measure of the total output of feces. The amount of dry matter in the herbage consumed per unit of time then may be readily computed from a knowledge of the indigestibility of the herbage and the amount of feces voided during the same unit of time. Accurate estimates of intake have been derived from the use of Cr_2O_3 as an indicator of fecal output and plant chromogen *(3, 4)* or nitrogen *(2)* as an indicator of indigestibility.

The results of recent studies *(4, 7)* in which clipped pasture herbages were fed in conventional digestion trials support the validity of the fecal-chromogen procedure *(10)* for the measurement of indigestibility. Forty forages ranging in digestibility of dry matter from 55 to 81% were examined by Raymond *et al. (7)* with sheep. The mean difference between the measured and estimated digestibility was 3.2%. However, Raymond *et al.* assumed that digestibility is a linear function of the fecal chromogen content, whereas their data suggest curvilinearity. A study of the data of Raymond *et al.* revealed that the average difference between the measured and estimated digestibility is reduced to 2.9% when the following equation is employed: $Y = 38.75 \log X - 0.0046X - 32.12$; where Y = per cent digestibility of dry matter and X = chromogen concentration of feces (units per gram of dry matter). The extent to which the use of this equation reduces the error incurred by the use of the straight-line equation proposed by Raymond *et al.* depends upon the level of digestibility of the forage being studied. Use of the linear regression equation introduces considerable error into the prediction of the digestibility of forages of low or high digestibility. Both equations give about the same value for forages of intermediate digestibilities. In view of the fact that Raymond *et al.* found differences as great as 3.1 percentage units between the average digestibility of a forage and the lowest or highest digestibility for the same forage when measured by the conventional digestion trial technique, it would appear that the fecal chromogen procedure for estimating the digestibility of herbage grazed by sheep is very accurate.

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[1] A portion of the data reported here was taken from the thesis presented by A. M. Smith to the Graduate School, Cornell University, in partial fulfillment of the requirements for the Master of Science degree, 1954.

The regression of digestibility on the chromogen content of the feces in the study of Raymond *et al.* is strikingly similar to that reported by Reid *et al. (10)*. The constants in the equations are different because the units of chromogen used in the two laboratories have a different potency. Therefore, the equation computed by Raymond *et al.* gives estimates of digestibility which are approximately four percentage units higher than those derived from the equation reported by Reid *et al.* Since these equations are empirical in nature, as the value of the constants depends upon the potency of a unit of chromogen, this apparent discrepancy does not affect the accuracy of the method. Raymond *et al. (6, 7)* have suggested that when the spectrophotometer employed in their studies *(7)* is adjusted for the transmission of light of a wave length of 406 mμ it may, in reality, be transmitting light of a wave length of 403 mμ. This explanation would account for the difference, as the spectrophotometer employed by Reid *et al.* has been carefully checked and the wave length adjustment has been found to coincide precisely with the wave length of light actually transmitted in this range. In addition, the difference between the two equations might have arisen from the use of sodium chromate as a standardizing agent as this compound has been found to give different results, depending upon the degree of hydration *(9)*. These observations show that the equation proposed by Reid *et al.* may be used only when the unit of chromogen employed has the same value as that used in the Cornell laboratory. This was emphasized in an earlier report *(10)*, but unfortunately chromate has since been found to be a hazardous standardization agent *(9)*.

Chromic oxide has been used successfully as an indicator of fecal output by grazing steers *(1, 3)* and grazing cows *(2, 4, 5)*. A considerable intraday variation in the concentration of Cr_2O_3 voided in the feces of grazing cattle makes it imperative that feces be sampled at specified times of the day *(3)*. As a result of experiments conducted with grazing steers, Hardison and Reid *(3)* proposed that the bulking of equal weights of feces sampled at 6 A.M. and 4 P.M. during periods of seven or more days would provide samples of which the Cr_2O_3 concentration would allow accurate estimates of the total fecal output. The results of subsequent experiments conducted by Brannon *et al. (1)* with steers supported those *(3)* reported earlier. Lancaster *et al. (5)* and Kane *et al. (4)* secured accurate estimates of the fecal output of grazing cows by employing essentially the same sampling procedure *(3)* found to be satisfactory for use with steers. These findings support those reported earlier by Coup *(2)*, in whose experiments Cr_2O_3 was determined in feces taken from cows in the morning and afternoon (hours of the day were not reported).

Although the data obtained to date indicate that Cr_2O_3 is a useful agent for the estimation of the output of feces by grazing cows, the great intraday variation in the excretion of Cr_2O_3 by steers *(3)* and the number of factors which might contribute to the magnitude of this variation *(3, 8)* require that much further attention be given to the application of the method to experiments conducted with grazing cows. The objectives of the experiments summarized in this report were to study: *(a)* the Cr_2O_3-excretion patterns of grazing dairy

cows, *(b)* the accuracy with which the amount of feces voided can be determined using Cr_2O_3 as an indicator, and *(c)* the effects of number of dosages per day and the time and mode of administration of Cr_2O_3 upon the accuracy of measuring the output of feces.

EXPERIMENTAL PROCEDURE

During three grazing seasons a series of five trials was conducted in which dairy cows were permitted to graze continuously throughout the period of experimentation except for brief periods when they were confined to stalls specially constructed for the sampling of feces and the administration of Cr_2O_3. The same pasture, consisting largely of alfalfa, was used in all trials. The total amount of feces voided by the individual cows during each trial was collected in fecal bags. In certain trials, samples of feces were taken rectally at specified times. Chromic oxide was administered one or two times per day either in capsule form or as a portion of a concentrate feed, but the amount administered per day during a given trial was practically constant for each cow. A summary of the times and modes of administering Cr_2O_3 and the amounts of Cr_2O_3 administered during all trials is shown in Table 1.

Three lactating Holstein cows were employed in Trial 1, conducted during an 18-day period of the 1952 grazing season. Feces were totally collected during the last 7 days of the period. Ten grams of Cr_2O_3 was administered in a capsule at 7 A.M. on each day of the grazing period. No feeds other than the pasture herbage were given to these cows. On the second and sixth day of the fecal-collection period, feces samples were taken rectally at 2-hour intervals. On each of the five other days, samples of feces were taken in the same manner at approximately 6-hour intervals. These samples were employed in a study of the Cr_2O_3-excretion pattern of grazing cows.

In 1953, Trials 2, 3, and 4 were conducted primarily to study the effect of number of dosages and mode of administration of Cr_2O_3 upon the accuracy with which the total fecal output can be estimated from the Cr_2O_3 content of feces taken at convenient sampling times (6 A.M. and 4 P.M.). The four treatments imposed consisted of administering Cr_2O_3 in: *(a)* gelatin capsules in one dosage per day (6 A.M.), *(b)* gelatin capsules in two dosages per day (6 A.M. and 4 P.M.), *(c)* a concentrate feed in one meal per day (6 A.M.), and *(d)* a concentrate feed in two meals per day (6 A.M. and 4 P.M.). Approximately the same total amount (17.5 to 20 g.) of Cr_2O_3 was administered per day to each cow regardless of treatment. The amount administered to a given cow was constant throughout a given trial except in a few cases in which a small amount of the Cr_2O_3-containing concentrate was refused. The amount of concentrates fed on both a per-meal and per-day basis was the same for all treatments. The cows allotted to the capsule-treatment groups received the same amount of the same concentrate feed, though devoid of Cr_2O_3, as the cows composing the two other groups. Ten cows were employed in the conduct of Trials 2, 3, and 4. The two cows (No. 1 and 2) administered Cr_2O_3 in the concentrate feed in Trial 2 were the same animals (No. 3 and 4, respectively) given Cr_2O_3 in capsules in Trial 4. Each of Trials 2,

TABLE 1

Time, rate, and mode of administration of Cr_2O_3 and amounts of concentrates offered to cows

Cow No.	Time[a]	Concentrates (dry basis) offered containing		Cr_2O_3 in capsules	Total Cr_2O_3 consumed
		No Cr_2O_3	Cr_2O_3		
		(g.)	*(g.)*	*(g.)*	*(g/day)*
		Trial 1			
1	7 A.M.	0	0	10	10
2	7 A.M.	0	0	10	10
3	7 A.M.	0	0	10	10
		Trial 2			
1	6 A.M.	329.9	264.7	0	18.4
	4 P.M.	329.9	264.7	0	
2	6 A.M.	67.4	525.9	0	18.3
	4 P.M.	595.9	0	0	
3	6 A.M.	595.9	0	10	20.0
	4 P.M.	595.9	0	10	
4	6 A.M.	595.9	0	20	20.0
	4 P.M.	595.9	0	0	
		Trial 3			
1	6 A.M.	329.9	264.7	0	19.2
	4 P.M.	329.9	264.7	0	
2	6 A.M.	67.4	525.9	0	19.0
	4 P.M.	595.9	0	0	
3	6 A.M.	595.9	0	10	20.0
	4 P.M.	595.9	0	10	
4	6 A.M.	595.9	0	20	20.0
	4 P.M.	595.9	0	0	
		Trial 4			
1	6 A.M.	329.9	264.7	0	17.6
	4 P.M.	329.9	264.7	0	
2	6 A.M.	67.4	525.9	0	17.5
	4 P.M.	595.9	0	0	
3	6 A.M.	595.9	0	10	20.0
	4 P.M.	595.9	0	10	
4	6 A.M.	595.9	0	20	20.0
	4 P.M.	595.9	0	0	
		Trial 5			
1	6 A.M.	0	0	0	25.4
	4 P.M.	0	795.3	0	
2	6 A.M.	0	397.6	0	25.4
	4 P.M.	0	397.6	0	
3	6 A.M.	0	0	0	25.9
	4 P.M.	0	795.3	0	
4	6 A.M.	0	397.6	0	23.9
	4 P.M.	0	397.6	0	

[a] Times when concentrates were fed and/or Cr_2O_3 was administered.

3, and 4 was conducted with four cows and consisted of a 21-day grazing period. The total outgo of feces was measured during the last 7 days of each period. In addition, samples of feces were taken rectally at 6 A.M. and 4 P.M. on each day of the fecal collection period.

Trial 5 was conducted with four nonlactating cows during the 1954 season. This trial consisted of a 22-day grazing period in which feces were collected during the last 7 days. On each day, small samples of feces were taken rectally

at 6 A.M. and 4 P.M. and samples representing the total feces voided during a 24-hour period were procured. Two pounds of concentrates containing 26.2 g. of Cr_2O_3 were offered daily to each cow throughout the grazing period. The concentrate feed offered was not always completely consumed. The orts were weighed, analyzed, and taken into account in the computation of fecal outputs. This experiment was conducted to examine further the effects of the time of administration, and the number of administrations, of Cr_2O_3 upon the accuracy with which the fecal output may be estimated. Each of two cows received 2 lb. of the concentrate feed at 4 P.M. and each of the other two cows was fed 1 lb. of the feed at 6 A.M. and 1 lb. at 4 P.M.

RESULTS AND DISCUSSION

Intra-day variation in excretion of Cr_2O_3. The average Cr_2O_3 excretion-time pattern observed in Trial 1 is shown in Figure 1. Expression of the concentration of Cr_2O_3 in the "grab" samples of feces as a percentage of the concentration of Cr_2O_3 in feces compounded from total collection during the entire collection

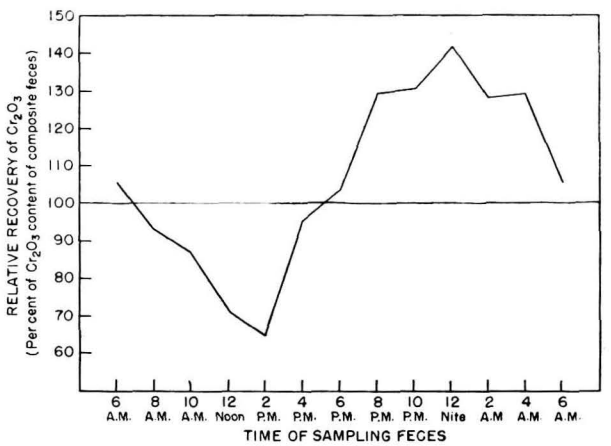

FIG. 1. Chromic oxide excretion-time pattern for grazing cows administered Cr_2O_3 in capsules at 7 A.M.

period allows the rates of excretion of Cr_2O_3 by animals consuming different quantities of herbage and Cr_2O_3 to be compared. The excretion patterns for the three cows were of the same general form and resembled those reported earlier for steers *(3)*. However, the lowest and highest rates of recovery of Cr_2O_3 occurred later in the day (2 P.M. and 12 midnight, respectively) for cows than for steers (12 noon and 7 P.M., respectively). The range in the rate of recovery of Cr_2O_3 from the feces of cows (65 to 141%) was not as great as that from the feces of steers (52 to 183%). This suggests that the intake of herbage by cows is more evenly distributed through the day than that by steers. Nevertheless, the variation in the excretion of Cr_2O_3 by cows is sufficiently great to preclude the indiscriminate sampling of feces without consideration for time of day.

Use of convenient fecal sampling times. Since accurate estimates of fecal output by steers *(1, 3)* and cows *(5)* have been derived from the Cr_2O_3 content of feces samples taken at 6 A.M. and 4 P.M. and since these sampling times are convenient from the standpoint of animal management, the application of this sampling scheme was studied further with cows in the present experiments. A comparison of the amount of feces voided daily as estimated from the Cr_2O_3 content of bulked samples of feces taken at 6 A.M. and 4 P.M. of seven consecutive days with that measured during the same period of time by bag collection is shown in Table 2. The coefficient of correlation between the measured and

TABLE 2

Comparison of measured and estimated outputs of feces by grazing cows

Cow No.	Fecal output (Lb. D.M./day)	
	Total[a]	Estimated[b]
	Trial 1	
1	7.96	7.68
2	10.48	10.26
3	10.10	10.76
	Trial 2	
1	9.84	9.53
2	10.76	10.64
3	5.23	5.22
4	4.95	3.51
	Trial 3	
1	8.71	8.67
2	8.95	8.80
3	9.43	9.25
4	12.82	13.87
	Trial 4	
1	9.72	9.54
2	9.56	10.19
3	9.92	9.80
4	12.74	12.53
	Trial 5	
1	10.26	9.82
2	5.83	6.00
3	6.17	5.86
4	8.03	8.19

[a] Collected in bag.

[b] Based upon Cr_2O_3 concentration of combined feces samples taken rectally at 6 A.M. and 4 P.M.

estimated outputs of dry matter was 0.983, and the relationship between the output values determined by the two procedures is expressed by the equation: $Y = 1.10\ X - 0.98$; where Y = estimated output (lb. D.M./day) and X = measured output (lb. D.M./day). The standard error of estimate of the daily output of dry matter determined from the use of Cr_2O_3 was 0.47 lb. in these trials in which the mean measured output was 9.02 lb. per day. These data indicate that satisfactory estimates of fecal output by cows may be effected from the Cr_2O_3 concentration of feces taken at 6 A.M. and 4 P.M. of seven consecutive days.

In general, the concentration of Cr_2O_3 in the feces voided at 6 A.M. was greater than that in the feces voided at 4 P.M. [These observations are in accord with those reported by Lancaster *et al. (5)*, who made an extensive study of the fecal output by three cows during eight consecutive 5-day periods.] It appeared that a compensatory relationship existed between the concentration of Cr_2O_3 in the feces sampled at 6 A.M. and that of feces taken at 4 P.M. which resulted in a recovery of approximately 100% of the ingested Cr_2O_3. This was examined further in Trial 5, in which the Cr_2O_3 concentration of feces taken at 6 A.M. and 4 P.M. was studied in relation to that of feces representing individual 24-hour periods. As a percentage of the Cr_2O_3 concentration of feces representing the total amount voided during the 7-day period, the range (lowest and highest values) in Cr_2O_3 concentration of the feces taken at 6 A.M. and 4 P.M., respectively, from the four cows was: Cow 1, 112 to 142 and 78 to 95; Cow 2, 86 to 120 and 84 to 99; Cow 3, 99 to 128 and 83 to 117; and Cow 4, 89 to 117 and 76 to 104. These data reflect a considerable interday variation in herbage consumption.

Despite the marked variation in the Cr_2O_3 concentration of individual samples taken at 6 A.M. and 4 P.M., the Cr_2O_3 concentrations of feces compounded from collections at these two times were very similar to those of the total feces collected during corresponding 24-hour periods. These two variables were highly correlated (0.97), and the regression of the Cr_2O_3 concentration (Y) of feces taken at 6 A.M. and 4 P.M. of a given day upon that (X) of feces representing the corresponding 24-hour period resulted in the equation, $Y = 1.13X - 0.64$. The standard error of estimate and the mean concentration of Cr_2O_3 in the feces were 0.45 mg. and 7.71 mg., respectively, per gram of dry matter.

In this experiment (Trial 5), the mean recovery of Cr_2O_3 from the totally collected feces and that from the 6 A.M. and 4 P.M. composite samples were 97.5 $\pm$ 1.73 and 101.2 $\pm$ 2.15%, respectively. The average recovery rate (93.6%) from the totally collected feces of Cow 2 was somewhat lower than that (97.6%) from the 6 A.M. and 4 P.M. composites. This disparity was to a considerable extent responsible for the regression coefficient (1.13) being greater than 1.0. Nevertheless, the ratio between the Cr_2O_3 concentration of the 24-hour composite samples and that of the 6 A.M. and 4 P.M. composite samples was sufficiently close to 1.0 to suggest that an influence exerted upon the concentration of Cr_2O_3 in the feces voided at the one sampling time effects a change of similar magnitude, though of opposite direction, in the concentration of Cr_2O_3 in the feces voided at the other sampling time. This phenomenon seems to assure the success of intake-measurement studies in which feces are sampled at 6 A.M. and 4 P.M.

Although 136% of the ingested Cr_2O_3 was recovered from the feces of Cow 4 in Trial 2, the dry matter intake of this cow was so low that a difference of only 1.44 lb. of dry matter existed between the measured and estimated fecal outputs. When the datum for this cow is excluded from the total data, a mean recovery of 100.58 $\pm$ 0.87%[2] of the ingested Cr_2O_3 from combined samples of feces taken

[2] Standard error of mean.

at 6 A.M. and 4 P.M. for seven consecutive days from the remaining animals was effected. As a consequence, the average estimated output of feces was within 0.58 ± 0.87% of the measured output. This means that the estimated intake of herbage would be within 0.58 ± 0.87% of the true intake provided that the indigestibility of the consumed herbage is determined free from error.

Effect of time and mode of administration and number of dosages per day. Fecal output was estimated with about the same degree of accuracy regardless of whether Cr_2O_3 was administered in gelatin capsules or in a concentrate feed and whether it was administered once daily (6 A.M.) or twice daily (6 A.M. and 4 P.M.) by either means (Trials 2, 3, and 4 in Table 2). In another experiment (Trial 5), the effectiveness of administering Cr_2O_3 in a concentrate feed at 4 P.M. was compared with that of feeding Cr_2O_3 in the same concentrate feed at both 6 A.M. and 4 P.M. Satisfactory estimates of fecal output resulted from both of these treatments. The data obtained in Trials 2, 3, 4, and 5 show that the time of day (either morning or afternoon or both) when Cr_2O_3 is administered does not influence the accuracy with which the total outgo of feces may be derived from the Cr_2O_3 content of feces sampled at 6 A.M. and 4 P.M.

Application of procedure to measuring intake of pasture herbage. In applying the Cr_2O_3-indicator method to the measurement of pasture intake, the administration of Cr_2O_3 in a concentrate feed requires less time and has more mechanical convenience than that of Cr_2O_3 in capsules. However, if the nature of an experiment requires that animals receive no feed other than pasture herbage, gelatin capsules offer a satisfactory means of administration, particularly when care is taken to assure positively that the Cr_2O_3 is ingested and not regurgitated and lost. A problem of similar consequences is sometimes encountered when Cr_2O_3 is administred in a concentrate feed. Occasionally animals may not consume all of the Cr_2O_3-containing feed allotted to them, or the Cr_2O_3 may not be evenly distributed in the feed. Obviously, these situations could result in irregular intakes of Cr_2O_3 and could contribute to an erroneous estimate of fecal output and, therefore, of herbage intake.

In the conduct of pasture-intake trials, it would appear that the daily administration of Cr_2O_3 (in concentrates or capsules) should be commenced a week or more prior to the first sampling of feces, though good results were obtained by Lancaster *et al. (5)* when a period of only 5 days was allowed to elapse. Although it has not been determined whether Cr_2O_3 may be administered less frequently than once per 24-hour period, one dosage per day appears to be adequate when the feces are sampled daily at approximately 6 A.M. and 4 P.M. Sampling of feces at these times effects a recovery of approximately 100% of the ingested Cr_2O_3 and eliminates the need for fecal-collection apparatus. As shown previously *(3)*, the accuracy of the intake estimate for a given period increases at a decreasing rate as the number of days during which the feces are sampled is increased. Since the interday variation in the intake of herbage may be considerable, the mean daily intake for a period of several days generally is more meaningful than the intake for single day. However, the nature and objectives of a particular experiment may decide the length of the fecal-sampling

period employed. The results of the present experiments demonstrate that reliable estimates of fecal output may be derived from the sampling of feces during seven consecutive days and that a relatively accurate estimate may be made of the output for even a single day.

The amount of Cr_2O_3 to administer daily is arbitrary, but the analytical operations in measuring Cr_2O_3 may be made more conveniently if the dosage is determined on the basis of an expected range in herbage intake and of a desirable concentration of Cr_2O_3 in a sample of feces of an analytically convenient size. Dosages ranging from approximately 10 to 30 g. per day have been employed successfully.

When concentrate feeds are employed as a carrier for Cr_2O_3 and the forage intake, rather than total feed intake, is desired, the total amount of feces voided has to be corrected for the amount of dry matter contributed by the concentrate. The amount of dry matter contributed to the total feces by the concentrate feed may be computed from data on the amount of concentrates consumed and the indigestibility of the dry matter in the concentrates. Generally, the digestibility values for concentrate feeds recorded in tables of feed composition are sufficiently accurate to be used for this purpose. If greater accuracy is desired, the indigestibility of the concentrate feed in question may be determined in a conventional digestion trial. The concentration in the feces of the indicator (chromogen or nitrogen) employed to measure the indigestibility of the herbage consumed also must be corrected for the amount of indicator contributed by the concentrate feed.

The mechanics of deriving the dry matter intake of grazing cattle may then be reduced to the following equations:

$$\text{(a)}\quad \text{Fecal output (g. D.M./day)} = \frac{Cr_2O_3 \text{ consumed (g/day)}}{Cr_2O_3 \text{ concentration of feces (g/g D.M.)}}$$

$$\text{(b)}\quad \text{Herbage intake (g. D.M./day)} = \frac{\text{Fecal output (g. D.M./day)}}{\text{Indigestibility of D.M. (\%)}}$$

The data obtained to date demonstrate that this method provides an objective approach to the evaluation of pastures in which intake and digestibility may be derived simultaneously.

SUMMARY

A series of five trials was conducted to determine the adequacy of Cr_2O_3 as an indicator of fecal output by grazing cows. Seventeen individual cows were employed in these studies.

The Cr_2O_3 concentration of feces taken rectally at 6 A.M. and 4 P.M. on seven consecutive days and bulked on an equal weight basis provided accurate estimates of the total fecal output. The mean rate of recovery of the ingested Cr_2O_3 from the combined feces sampled at 6 A.M. and 4 P.M. was 100.58 ± 0.87%. A study of the Cr_2O_3 concentration-time excretion patterns of grazing cows revealed that from 65 to 141% as much Cr_2O_3 is voided in the feces at various hours of the day

as is found in well-mixed feces representing the total amount of feces voided during a 7-day period. This precludes the indiscriminate adoption of times for the sampling of feces for the purpose of measuring the total output of feces by grazing cows.

No difference in the accuracy of the estimated output of feces was found between the administration of Cr_2O_3 in capsules and that in concentrate feeds. Also, the effectiveness of the procedure for measuring fecal output was the same regardless of whether Cr_2O_3 was administered at 6 A.M. or 4 P.M. or at 6 A.M. and 4 P.M. It is suggested that the time and mode of administration of Cr_2O_3 employed in the conduct of grazing studies should be determined by the nature of the experiment and the convenience of the operation.

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SOME EFFECTS OF INBREEDING AND EVIDENCE OF HETEROSIS THROUGH OUTCROSSING IN A HOLSTEIN-FRIESIAN HERD

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Inbreeding, although one of the most useful systems of mating available to the animal breeder, is not generally recommended for average herds. Calculated risk is always taken in mating closely related animals even of high quality; average stock almost always shows signs of deterioration. Inbreeding results only in an increased number of homozygous loci. Unfavorable results, then, must be due to the uncovering of undesirable recessives and to a reduction in the number of heterozygous loci. The exposure of deleterious recessives probably accounts for a greater part of the deterioration, since in some lines decline can be prevented by selection.

Inbreeding experiments with dairy cattle have been carried out at the New Jersey *(2, 3)*, California *(1, 18, 19)*, and Beltsville *(22, 24)* Experiment Stations. Other inbreeding analyses have made use of data collected outside formal experiments. These include the studies of Tyler *et al. (23)*, Nelson and Lush *(17)*, and Laben and Herman *(15)*.

The development of inbred lines of farm animals and the results of crossing inbred lines have been investigated mostly with swine *(6)* and poultry *(12)*. Significant heterosis in productive characteristics of swine has been well established. The amount of heterosis, however, is variable; some crosses may show little or none and others may show pronounced effects. Two recent studies are those of Sierk and Winters *(21)* and Chambers and Whatley *(5)*. It is evident that some of the important factors involved in the performance of line crosses are the merit of the lines, genetic diversity, and degree of inbreeding. Good producing lines of diverse origin appear more likely to exhibit a useful level of heterosis.

The terms heterosis, nicking, and hybrid vigor are sometimes used synonymously. All describe essentially the same genetic phenomena of a higher level of performance than would be predicted in a strictly additive scheme of inheritance. Nicking is a term used in animal breeding to describe the resulting heterosis through the mating of animals within a breed.

Dairy cattle have been studied to determine if heterosis occurs and whether it is frequent enough to bias sire provings as usually conducted. Heizer *et al. (11)* studied production records of groups of daughters of seven sires in three herds. In four instances daughters of a given sire out of dams by one sire showed distinctly superior production to his daughters out of dams by another sire. The differences between group means in the case of one of the sires were found to be highly significant and were attributed to nicking. Johnson *et al. (14)* reported evidence of nicking in the matings of one Jersey bull. They concluded, "while

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nicking may exist, it seems quite logical that it has been used far too much as a convenient term to explain differences in production of the daughters of a bull that may be due to other causes.'' Woodward and Graves *(24)* concluded there was no evidence of heterosis in their data. Seath and Lush *(20)* studied the Dairy Herd Improvement Association records of daughters of 13 sires. Differences in production between groups segregated according to maternal grandsires ''gave no indication that 'nicking' is generally important enough to need much attention when proving sires.'' No evidence of heterosis was found in an analysis of the Missouri Station Holstein herd *(15)*.

MATERIAL AND METHODS

The data presented here are from the purebred Holstein herd of the University of California Dairy Cattle Breeding Experiment. The herd was entered on the inbreeding project in 1928, the foundation animals being mildly line-bred to Sir Aaggie De Kol Acme 185724. Six of his sons were used and all sired one or more daughters. Another sire, Sir Colantha Juliana 67203, had influence on the herd, particularly through a son and grandson. The average relationship between the foundation cows and Acme was estimated to be 0.19, and for Juliana it was 0.17. Single daughters of five other essentially unrelated sires were also among the foundation cows. Intense inbreeding appears to have been purposely avoided. The first sire used in the breeding experiment was 100A, Sir Aaggie De Kol Mead 7th 367061, a grandson of Acme. He was selected on the basis of a pedigree indicating high production. The average relationship between 100A and the foundation cows was 0.14. He was used from April, 1922, to September, 1933. Only a limited amount of inbreeding was accomplished with him since the formal experiment was not initiated until 1928.

Sire 100B, Bear Valley Ormsby Esther 518683, was selected to follow 100A on the basis of a pedigree indicating inheritance for high butterfat test and production. As 100B was essentially unrelated to 100A or to the few foundation cows that were left, his introduction represented an outcross. The line was closed and sire-daughter matings followed. He remained in active service from April, 1928, until March, 1941.

The third sire was 100D, Los Robles Ormsby Lyons Gerben 830220. He was selected to outcross on the inbred 100B daughters. He had an inbreeding coefficient of 0.25 and was essentially unrelated to the University herd. He was used from February, 1942, until July, 1951. The University herd consisted of 21 inbred daughters of 100B at the time 100D was introduced as a young, unproved sire. To obtain some estimate of the performance of his daughters out of non-inbred, unrelated cows, he was loaned to a local artificial insemination cooperative. Sixty daughters of 100D by artificial service were located, but only nine of these and their dams were found to have records.

The present study deals with the production of 164 cows. The records were obtained from daily milk weights and monthly butterfat tests conducted under A. R. supervision. Only a single lactation record under reasonably standard

conditions is available on each cow. This is usually the first record; subsequent lactations involved other types of research unlikely to interfere with reproduction. In an effort to compensate for the lack of additional records, an attempt has been made to maintain the environment for the "test" record constant throughout the experiment. Animals have been raised and fed to time of freshening on a high plane of nutrition. For the most part they have been dry-lot managed from birth until first freshening to control feeding conditions more closely and to avoid fluctuations due to good and poor pasture seasons. During their first lactation, pasturing was limited to 2 hours per day on irrigated alfalfa. Composition of the concentrate ration and level of feeding has been kept constant. A few of the early records were made on four times a day milking. Most of the records prior to 1942 were on three times a day milking, and since 1942 all records have been made on twice a day milking. This has been the only management change. The herd has been under care of the same herdsman throughout the period of the study. All of the records used have been corrected to a 305-day, junior 2-year-old, twice daily milking basis. The use of this age standard required a minimum of record conversion. Milk production was expressed in actual pounds and on the constant energy basis of fat-corrected milk (FCM) *(8)*.

Persistency of production was measured by the slope of a straight line fitted to the mean daily production of FCM per month from the third through the eighth month of lactation. In testing these points for linearity, it was found that a straight line described this segment of the curve very well. This period was selected because most lactations reach their peak before the third month and the effect of gestation is not important until after the eighth. The linear regression of production on a definite time period is suggested as a measure of persistency by Johansson and Hansson *(13)*. The regression coefficient was estimated by a method described by Bartlett *(4)*. This estimate may be expressed as:

$$\text{Persistency index} = \frac{(\text{3rd mo. av.} + \text{4th mo. av.}) - (\text{7th mo. av.} + \text{8th mo. av.})}{8}$$

In a test using 131 lactation records the correlation between this estimate and the least squares estimate was +0.99. Ludwin *(16)* used a similar type of regression estimate as a measure of persistency. In the present study only one record of the 164 had an ascending lactation curve. Ludwin *(16)* and Gaines *(7)* found 2.9% and 5.4% of the records in their respective studies had ascending curves.

Since the records studied were compiled from daily milk weights, it was possible to study two other characteristics of the lactation curve. These were the height of the maximum daily milk production and the length of time in days from the start of the record (3 days postpartum) to the maximum.

RESULTS AND DISCUSSION

The intrasire regression coefficients for the various measures of productivity on degree of inbreeding *(25)* were calculated as estimates of the effect of inbreeding. The 164 cows available for this study were the progeny of 22 sires. The

TABLE 1

Average measures of productivity and their intrasire regression coefficients on inbreeding

Characteristic of production	Mean	Standard deviation	Intrasire regression on inbreeding
Pounds of milk	8,784	3,131	−209.8[a] ± 9.95
Percentage of fat	3.73	0.37	+0.008[a] ± 0.0023
Pounds of fat	321	99	−4.88[a] ± 0.49
Pounds of FCM	8,338	2,663	−131[a] ± 13.23
Persistency *(lb. FCM)*	−2.21	1.30	−0.0054 ± 0.0084
Maximum prod. *(lb. FCM)*	46.0	11.3	−0.47[a] ± 0.06
Days to maximum prod.	39.2	17.3	+0.02 ± 0.11

[a] $P < 0.01$

average coefficient of inbreeding ranged from 0 to 0.44, with a mean of just under 0.13. The results of this study are presented in Table 1.

The size of the standard deviations indicates rather wide variation in production. The analysis of variance of these data revealed nonsignificant differences between sires for milk production when uncorrected for fat content. Differences between sires were significant at the 1% level for FCM, butterfat test, pounds of butterfat, maximum daily FCM production, and time period from start of the record to the maximum daily yield. Differences between sires were significant at the 5% level for the slope of the lactation curve in its declining phase.

The regression of pounds of milk on inbreeding was −209.8. The greatest inbreeding effect on productivity is the depression of total milk secretion. When the rise in fat percentage with declining production is accounted for by expressing production of all cows on an equal energy basis, the depression with inbreed-

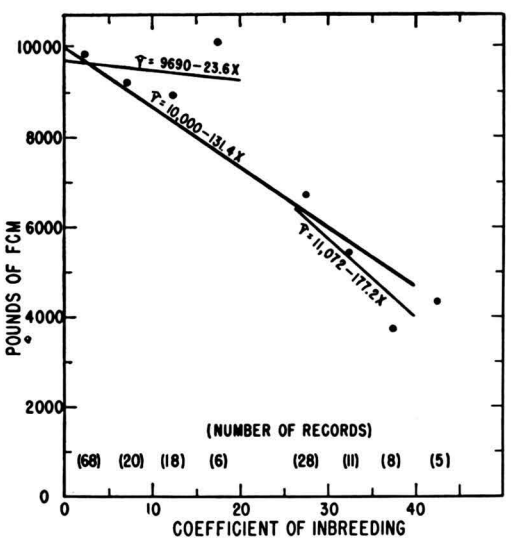

FIG. 1. Mean yield of FCM at 5% class intervals of inbreeding and the intrasire regressions of FCM on inbreeding.

ing is –131.0 lb. of FCM. These effects are greater than those reported in other studies. Tyler *et al. (23)* reported a regression of –4.5 lb. of butterfat. This would be equivalent to about –121 lb. of 3.7% milk. Laben and Herman *(15)* reported a figure of –66 lb. of milk. These studies were all in Holstein herds.

Butterfat test, when studied as a separate characteristic of production, shows a significant increase of +0.008% per degree increase in inbreeding. This is the first study to show a definite effect of inbreeding on test. Whether the rise in test with increased inbreeding results from true negative relationships between milk production and test, random sampling errors, or a concentration of genes for higher test has not been determined. Estimates of a negative genetic correlation between milk yield and fat per cent of –0.20 to –0.50 have been reported *(15)*.

Figure 1 gives the mean FCM yields at 5% class intervals of inbreeding. The intrasire linear regression equations are indicated and plotted for the entire group and for the low and high inbreeding groups. The six records in the 0.15 to 0.19 class averaged 10,067 lb. of FCM, a figure above the mean production of the non-inbred group. Three of these records were made by daughters of 100A out of granddaughters of 100A sired by 100B. The others were by daughters of three different sires. The dams of the six cows were not high producers; they averaged 8,852 lb. FCM, with a range of 6,100 to 10,230 lb. There is evidence that inbreeding may have had a lesser effect on production within the range of 0 to 0.15 or 0.20 than it had in the range above a coefficient of 0.25. The number of records is small, however, and except for the above-mentioned group of six, a single straight line would describe the entire range very well. The true relationship may be a curvilinear one. Bartlett and Margolin *(2)* observed no serious decline in production with inbreeding up to 20%.

TABLE 2

Regression coefficients of yield on inbreeding within the three major sire groups

	Sire 100A		Sire 100B		Sire 100D	
	20 dtrs. av. Fx. 0.10		61 dtrs. av. Fx. 0.19		40 dtrs. av. Fx. 0.10	
	Mean	Coef. of reg.	Mean	Coef. of reg.	Mean	Coef. of reg.
FCM *(lb.)*	9,330	+14.0 ± 40.7	6,751	−129.7[b] ± 16.5	9,188	−166.5[b] ± 26.5
Test *(%)*	3.77	−0.008 ± 0.008	3.91	+0.009[a] ± 0.004	3.73	+0.008 ± 0.004
Persistency	−1.40	−0.041 ± 0.026	−2.31	−0.015 ± 0.012	−2.32	+0.014 ± 0.013

[a] $P < 0.05$
[b] $P < 0.01$

The regression coefficients for FCM, test, and persistency on inbreeding for the daughters of the three major sires are given in Table 2. No inbreeding depression was apparent in 100A daughters up to 0.20. The inbred progeny of 100B, particularly those resulting from first and second generation sire-daughter matings, showed a significant decline in production. These inbred daughters had also shown a definite decline in size and growth rate *(1)* and an increase in calf mortality *(18)*; in addition, there was evidence that they carried genes for sterility *(9)*. It should be noted that since most of the inbreeding was brought about by the mating of 100B to his daughters, relationship to him was built up

TABLE 3

Comparative FCM yields of outcross 100D daughters with their inbred 100B dams and other groups of cows

Description of group	No. of cows	Mean FCM	Group I minus Group II	
			Difference	Std. error
		(lb.)	*(lb.)*	*(lb.)*
Group I				
Outcross 100D dtrs.	26	10,910	—	—
Group II				
Dams of the outcross dtrs.	26	5,366	+5,544[a] ±	667
100A and 100B dtrs., Fx < 0.05	40	8,891	+2,019[a] ±	364
All cows prior to 100D	123	8,024	+2,886[a] ±	566
All prior to 100D less inbred 100B	88	9,107	+1,803[a] ±	459
"Control" 100D dtrs.	9	8,776	+2,134[a] ±	777

[a] $P < 0.01$

along with the estimated increased inbreeding. If the breeding value of 100B were lower than that of his unrelated mates, the depression noted in his sire-daughter matings could be due to increased relationship to 100B as well as to inbreeding or to the joint effect of the two. The daughter-dam comparisons presented in Table 4 indicate that 15 outbred 100B daughters averaged some 365 lb. of FCM below their dams. Although these daughter-dam differences have very large standard errors, it is still possible that his actual transmitting ability was lower than that of his unrelated mates.

Sire 100D had only outbred daughters and daughters resulting from sire-daughter matings. Thus, the same situation as above, involving a correlation between inbreeding and relationship to the sire, was involved. The estimate of

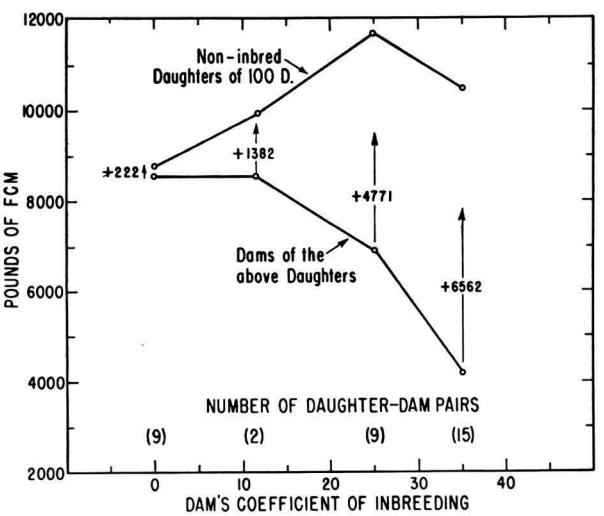

FIG. 2. Non-inbred daughters of 100D compared with their dams. Comparisons are plotted by degree of dam's inbreeding.

the breeding value of 100D obtained from the nine daughter-dam comparisons obtained outside the University herd (Table 3, Figure 2) does not suggest a value much different from that of his first mates in the University herd. Although separation of inbreeding and relationship effects cannot be conclusively made, the major part of the decline in production of his inbred progeny is interpreted as the effect of inbreeding. Decreases in yield of FCM were highly significant for 100B and 100D daughters. Butterfat test varied inversely with production, but only in the 100B group did the change in test reach significance. The responses of daughters of 100A to inbreeding appear to be distinctly different from those of 100B or 100D daughters. Important differences between sires in the responses of their daughters to inbreeding have been noted by Bartlett *et al. (3)* and Swett *et al. (22)*.

Persistency, measured by the slope of the lactation curve as previously described, was found to be highly variable. The coefficient of variation of the persistency index was 59%. This index of persistency is not influenced by level of production as are indices based on the ratios of the amount of milk produced in different periods of lactation. The sign of the regression coefficient as shown in Table 1 indicates there may be a slight decline in persistency as inbreeding increases; however, it did not approach significance. Maximum daily yield declined significantly with increased inbreeding (Table 1). The decline in total yield with increased inbreeding was due more to a reduction in the height of maximum yield than to any important change in the slope of the lactation curve in its declining phase.

It is not surprising that the effect of inbreeding on persistency is nonsignificant. Extremely low-producing cows can be milked over a long period with no marked change in production. The slope of such lactations then tends to be very flat. High persistency is of little real value unless a reasonably high maximum is reached. It must be realized, however, that a linear regression over only a part of the lactation curve does not reflect the entire picture.

The one other characteristic of the lactation curve studied, time interval from start of record to maximum production, did not change significantly with inbreeding. The average length of the period was 39.2 days, and the standard deviation, 17.3 days. This particular characteristic is probably not normally distributed. The frequency distribution of the records in this study was skewed toward the shorter time periods with a long tail running out to periods of longer than 90 days. The ascending lactation curve and six other periods over 90 days long were dropped from these calculations. This study seems to indicate that although production declines with inbreeding, the shape of the lactation curve is probably not greatly influenced by inbreeding.

Sire 100D was selected as an inbred, unrelated bull to mate to the inbred daughters of 100B. The results of this outcross are presented in Figure 2. All of the records except the nine pairs at 0.0 inbreeding coefficient of dam were made in the University herd. These nine pairs were collected from two DHIA herds in an adjoining county.

The 222-lb. increase in yield of FCM of the 100D daughters out of the unrelated, non-inbred dams has a standard error of ±620 lb. It was hoped that a much larger number of records would be available for proving this bull outside the University herd. No indication could be found that other 100D daughters were much better or worse than the ones for which records were available. In some cases, daughters had left the herd and records had been lost or destroyed. The owner's memory or opinion could be of no value except to gain a general impression that the daughters were not outstanding but few had been extremely poor.

The inbred dams to which 100D was mated were all daughters of 100B. The increase of 1,382 lb. of FCM in Figure 2 at the 0.125 inbreeding level of dams had a standard error of ±2,213 lb. The other increases of 4,771 lb. and 6,562 lb., with respective standard errors of ±924 and ±806, represent highly significant differences between dams' and their daughters' production. It is unfortunate that a group of non-inbred 100B daughters was not available for mating to 100D. Such matings would have helped to determine whether the large and important increase in the outcross 100D daughters was the result of exceptionally high general breeding value of 100D or of specific combining ability of 100D with inbred daughters of 100B. His record in the two outside herds does not indicate exceptional breeding value. An analysis of variance of the individual daughter-dam differences comprising the mean differences shown in Figure 2 (+1,382 lb., +4,771 lb., and +6,562 lb.) indicates that only the first and third groups differ from each other, and this at the 5% level of probability. This gives some evidence that the pattern of the production spread, widening steadily with higher inbreeding of the dams, was not due to chance alone. Figure 2 shows the downward trend in yield of the daughters of the most highly inbred dams. This mean decline from the peak of 11,675 lb. of FCM amounts to 1,094 ± 877 lb. If this were a significant decline, it would be interesting to speculate why the maximum response of the daughters was shown at the 0.25 inbreeding level of dams.

Evidence of heterosis in the yield of non-inbred 100D daughters out of inbred 100B dams is based on comparisons tabulated in Table 3. The 26 non-inbred daughters of 100D out of inbred 100B dams averaged 10,910 lb. of FCM. They were large, vigorous animals of very acceptable dairy type. Their mean production was over twice the mean production of their dams, as is indicated in the first comparison of Table 3. The average yield in FCM of the 26 outcross daughters is compared with the mean yield of various groups of cows that should represent the average productive capacity of the population from which they came. The mean inbreeding coefficient of the 40 100A and 100B daughters in the second comparison in Table 3 was under 0.05. This average of 8,891 lb. of FCM should furnish the best estimate of the productive level of the herd before the inbred 100B daughters lowered the herd average drastically. The third comparison of 123 cows averaging 8,024 lb. includes all the cows that made records in the herd prior to the outcross daughters. When these data are corrected for the effect of inbreeding, the mean approaches 9,000 lb., or, by omitting the inbred 100B daughters, the fourth comparison of 88 cows averaging 9,107 lb. is obtained. In

all cases the "outcross" 100D daughters show highly significant increases over their dams or other groups representing the productive level of the herd.

These increases could be due to Sire 100D's carrying exceptionally good inheritance for production or to some specific genetic interaction between 100D and inbred 100B daughters. The outside proving of 100D, as mentioned earlier, is the best estimate of his productive capacity in matings with non-inbred dams. The nine "control" daughters are used in the fifth comparison of Table 3. This comparison contains uncorrected herd differences, as the control daughters made their records in two outside herds. The management practices and other environmental factors in the outside herds were observed and seemed not to differ greatly from those of the University herd. However, since there were only nine control daughters, this estimate of 100D's inherent productivity is not as reliable as would be desired. Chance variation in sampling the inheritance of 100D cannot be ruled out with this small sample. No significant difference could be demonstrated between the average of the control cows and the average of the non-inbred cows in the University herd. It is proposed that the superiority of the outcross University daughters is due at least in part to an expression of heterosis.

When the high-producing outcross daughters of 100D were bred back to him, the production of the resulting offspring dropped precipitously. Thirteen 100D daughters from such matings averaged 6,146 lb. of FCM, a decrease of 4,636 lb. below their dams (Table 4). This behavior might be interpreted as further evidence that 100D's superior performance in the outcross was not due entirely to the additive effects of genes for unusually high productivity carried by him but that heterosis or specific combining ability was an important factor in the

TABLE 4
FCM yields of the major sire groups

	Daughters' records				Dams' records					
					Dam's					
Sire	No.	Av. Fx.	Av. FCM	Coef. of var.	Sire	Gr. sire	Av. FCM	Coef. of var.	Difference & std. errors	
			(lb.)	*(%)*			*(lb.)*	*(%)*	*(lb.)*	*(lb.)*
100A	11	.06	9,048	17	Foundation		9,262	14	− 214	± 599
"	3	.06	9,220	30	100B	—	9,784	28	− 564	± 2274
"	3	.16	11,060	7	100B	100A	9,925	9	+1135	± 684
"	3	.26	8,759	20	100A	—	10,273	14	−1514	± 1299
100B	7	.0	9,069	31	Foundation		10,176	13	−1107	± 1173
"	8	.0	9,476	11	100A	—	9,192	17	+ 284	± 659
"	3	.02	8,527	13	100A	100A	9,209	22	− 682	± 1304
"	7	.12	7,283	23	100A	100B	10,369	12	−3086[a]	± 808
"	35	.31	5,301	43	100B	—	8,148	29	−2847[a]	± 557
100D	2	.0	9,931	30	100B	100A	8,548	11	+1383	± 2213
"	24	.0	10,991	19	100B	100B	5,101	49	+5890[a]	± 666
"	13	.29	6,146	53	100D	—	10,782	21	−4636[a]	± 1098
Total 100A	20	.10	9,332	16	—	—	9,592	15	− 260	± 468
Total 100B	60	.20	6,690	40	—	—	8,836	25	−2146[a]	± 450
Total 100D	39	.10	9,322	36	—	—	7,171	50	+2151[a]	± 787

[a] $P < 0.01$

outcross. Yearly differences remain in these comparisons as outcross and inbred daughters made their records in different years. There is no reason to believe that year-to-year environmental change was very great.

The production records of daughters of the three sires in the University herd are grouped in Table 4 by maternal grandsires. The groups of 100A daughters are all small. It is interesting to note the average production of the three 100A daughters out of his own granddaughters; these were the highest producers listed. The 100B daughters show no evidence of unusual interaction. The very drastic effects of inbreeding are quite evident in the matings of 100B to his own granddaughters and especially to his daughters. Some of the latter group represent two generations of sire-daughter matings. The two fair-sized 100D groups show the large increase in yield of his daughters out of inbred 100B dams and the decided decline in yield when he was mated back to his own daughters.

The Holstein herd at present consists largely of the low-producing inbred daughters of 100D. These are being outcrossed to selected unrelated sires.

SUMMARY AND CONCLUSIONS

The analysis of the effect of inbreeding on yield in the University of California Holstein-Friesian herd is based on the records of 164 cows. Daughters of 22 sires are represented; however, the majority are the progeny of three sires (100A, 100B, and 100D). The pooled intrasire regression coefficients of production on inbreeding indicate significant declines of –209.8 lb. of milk, –4.9 lb. of butterfat, and –131.0 lb. of FCM. A significant increase in butterfat test of +0.008% per degree increase in inbreeding was found to accompany the decline in milk yield.

A significant reduction in height of maximum daily yield occurred as inbreeding increased. No significant inbreeding effect was demonstrated on the time required to reach maximum production or upon the slope of the lactation curve. It would thus appear that no important change in the shape of the lactation curve accompanied the depression of yield with inbreeding. There is some evidence that inbreeding may have a somewhat lesser effect on production in ranges up to 0.20 than in ranges above 0.25. Significant differences were found between sires in the response of their daughters to inbreeding.

One of the sires (100B) remained in active service for 13 years. His 43 inbred daughters showed a marked decline in production. Twenty-one of them, averaging 5,366 lb. of FCM, were outcrossed to a third unrelated, inbred sire (100D). The 26 resulting daughters from this outcross averaged 10,910 lb. of FCM. This yield was significantly greater than that of the inbred dams and of any group or estimate representing the productive capacity of the herd prior to the outcross. The increase noted in the outcross daughters is interpreted to be due, at least in part, to heterosis.

ACKNOWLEDGMENTS

The Holstein-Friesian sire 100B was donated by Col. Langdon of Olema, California. The assistance of Mrs. Dorothy H. Brown in the analysis of the records is gratefully acknowledged.

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MANGANESE IN THE NUTRITION OF YOUNG DAIRY CATTLE FED DIFFERENT LEVELS OF CALCIUM AND PHOSPHORUS[1,2]

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In several species of animals differences in the manganese contents of diets have been related to various responses, including gains of body weight, concentrations of manganese in blood and blood serum, activity of phosphatase of blood serum and of bone, development of bone, composition of soft tissues, and metabolism of calcium and phosphorus. Excessively high intakes of these two minerals by swine *(19)* and by poultry *(28)* have accentuated the dietary needs for manganese, thus indicating metabolic interrelationships.

The expanding usage of mineral mixtures of increasing complexity in cattle feeding, the growing interest in the role of supplemental manganese in ruminant nutrition, and the increasing recognition of metabolic disturbances from mineral imbalances incited initiation of a study to ascertain the effects of supplemental dietary manganese on young dairy cattle consuming different levels of calcium and phosphorus.

GENERAL PROCEDURE

Experimental design. The design was based on the assumption that the needs of growing cattle for dietary manganese are increased, as for poultry and swine, by feeding excessive amounts of calcium and phosphorus. Hence, supplements *(a)* of calcium and phosphorus and *(b)* of manganese were the two ingredient variables of the diet. Each of these supplements was fed at two different levels, thus forming a factorial arrangement of four experimental dietary regimes. A fifth regimen, a conventional herd type, was added for comparative purposes. The experimental subjects, 15 healthy male dairy calves, represented three different breeds—Guernsey, Jersey, and Holstein. Five calves from each of these breeds constituted a replication in a randomized block design. Thus, three calves, one from each breed, were subjected to each dietary treatment. As calves

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became available, they were assigned, at random, to diets within replications. The duration of the study was determined by the survival period of the calves subjected to the experimental regimes.

Test diets. The dietary sequence was: colostrum from birth to 3 days of age, whole milk only during the subsequent 10-day period, and the experimental and the control diets (Tables 1 and 2) thereafter. The basal experimental diets used

TABLE 1

Constituents of the basal diets (B) fed to calves at various ages

Age of calf	Whole milk[a b] fed	Concentrates[b] fed		
		Nonfat dry milk solids	Hominy grits[c]	Lard
(days)	*(lb/cwt of calf)*	*(%)*	*(%)*	*(%)*
14 to 56	10	0	0	0
57 to 95	10	100	0	0
96 to 219 ± 25	10[d]	50	50	0
219 ± 25 to death	d	50	40	10

[a] Daily supplements of 10,000 I. U. of vitamin A and 2,667 I. U. of vitamin D/cwt of calf were mixed with the milk.

[b] Basal minerals were added to provide in p.p.m., on dry matter basis, of diet: Mg as $MgSO_4$, 600; Fe (carbonyl iron powder), 100; Cu as $CuNO_3$, 10; and Co as $CoCl_2$, 0.1.

[c] Table quality; low in Mn (av. 1.35 p.p.m.); purchased from the Quaker Oats Company.

[d] Amount of whole milk fed daily to each calf was limited to maximum levels of 16 lb. for Jerseys and Guernseys and 20 lb. for Holsteins.

TABLE 2

Test diets fed to calves in the experimental and the control groups

Group classification	Diet designation	Components of test diets
Experimental	*B*[a]	Basals (see Table 1)
	*B*Mn	Basals + 50 p.p.m. of Mn (manganous sulfate)
	*B*CaP[b]	Basals + 5% monocalcium phosphate[c]
	*B*MnCaP	Basals + 50 p.p.m. of Mn + 5% monocalcium phosphate[c]
Control (normal)	Herd[d]	Milk (fortified with vitamins A and D) + commercial calf pellets and alfalfa hay

[a] Maximum concentration of manganese, on a dry-matter basis, was less than 1 p.p.m.

[b] Manganese content of diet did not exceed 1.17 p.p.m.

[c] Fed at 3% level of dry matter of diet from 14 to 56 days of age; thereafter at 5% level. From 96 days of age to end of experiment monocalcium phosphate replaced an equal amount of hominy grits in diet.

[d] Milk feeding and vitamins A and D supplementation were discontinued at 135 days of age. Mn content of calf pellets was 66 p.p.m. and of hay was 39 p.p.m.

in different stages of the investigation are presented in Table 1. Whole milk was the principal component of the basal diets throughout the study, but when the calves were 8 weeks of age, concentrates were introduced for the purpose of increasing energy intake. Basal minerals and vitamins were incorporated in sufficient quantities to provide at least the minimum amounts of these nutrients recommended *(17, 20)*. Experimental diets (Table 2), other than the basals, contained either monocalcium phosphate or manganous sulfate, or both.

The sequential changes in the herd diets (Table 2), fed to the control group, were similar to those commonly used in conventional calf husbandry.

Feeding procedures. All calves receiving experimental diets were fed twice daily. Milk was consumed from nipple pails, and concentrate mixtures from glazed earthenware crocks. Intakes of the concentrates at each feeding were limited to the amounts consumed in 30 to 60 minutes, during which time the calves were stanchioned in such a manner that extraneous matter could not be consumed. Distilled water in lieu of tap water was offered daily. Except during the periods of either feed or water consumption, the calves were muzzled to prevent ingestion of wood shavings used for bedding.

Calves assigned to the herd diet were subjected to the same general feeding schedules as commonly recommended in modern dairy husbandry but otherwise managed the same as the experimental groups.

RESULTS

Criteria of responses to various dietary treatments included: 1, growth; 2, changes in several constituents of the blood; 3, clinical symptomology at different periods, and, 4, lesions upon autopsy.

1. *Growth.* Calves in all groups grew, as indicated by changes in average body weight (Figure 1), at approximately the same rate during the first month.

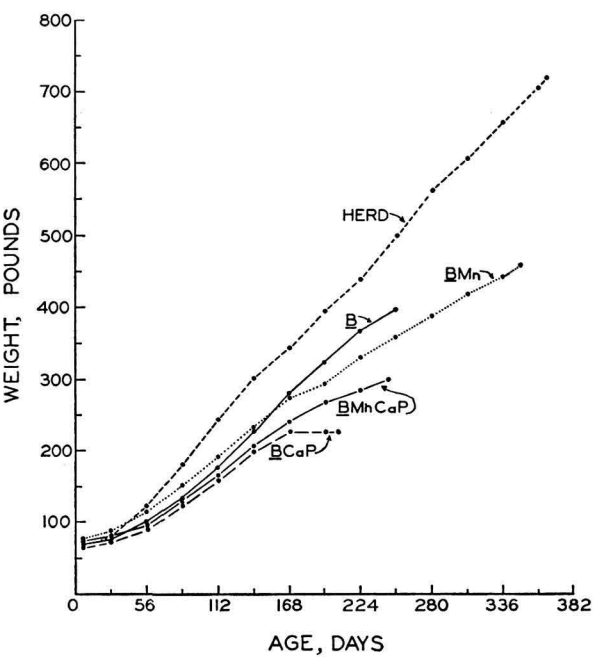

FIG. 1. Average periodic weights of calves in control group, receiving a conventional herd-type diet, and of calves in various experimental groups, receiving diets differing in supplements of manganese and of calcium and phosphorus.

Thereafter, the animals subjected to the herd regimen grew more rapidly than those receiving the various experimental diets. All experimental groups sustained a similar rate until they were about 140 days of age, when the ceiling on whole milk allocation was reached. Subsequently, the average gains for the various experimental groups also tended to diverge, as a consequence of the retarded growth in the calves receiving the experimental mineral supplements. The only group, however, in which weight gains became nil was the *B*CaP.

The weight increases were correlated positively with TDN intakes. The feed efficiency in terms of gains per unit of TDN was similar for all dietary groups except for *B*CaP, in which the efficiency was significantly lower than in the others.

Thus, observations indicated that prolonged feeding of excessive amounts of calcium and phosphorus adversely affected feed intake, which was reflected in a reduction in the rate and the efficiency of gains. Supplemental manganese, *B*MnCaP, tended to counteract the deleterious effects of the high dietary levels of calcium and phosphorus, *B*CaP. Though the responses of the *B*Mn group were complicated by respiratory infections, the data do not indicate that a manganese deficiency per se was involved in the weight gains of the basal group *(B)*.

2. *Constituents of the blood.* Within 1 hour either before or after the morning feeding, venous blood was collected periodically for determinations of *(a)* manganese in whole blood and *(b)* calcium, *(c)* phosphorus, *(d)* alkaline phosphatase, and *(e)* magnesium in serum. Samples for manganese determinations were collected at approximately 50-day intervals, whereas samples for other analyses were collected biweekly.

(a) Manganese. Concentrations of this element in whole-blood samples were determined by a spectrographic procedure developed by one of the authors.[4] Trends of the mean values for the respective groups are presented in Figure 2. Comparisons of *B*Mn with *B* and of *B*MnCaP with *B*CaP indicate that supplemental dietary manganese increased the concentrations of this element in the blood; the maximum levels attained, however, did not persist. Further comparisons (*B*CaP vs. *B*, and *B*MnCaP vs. *B*Mn) revealed a tendency of high intakes of calcium and phosphorus to suppress the manganese of the blood.

(b) Calcium. Blood serum was analyzed for calcium by the method of Weybrew *et al. (27)*. All values were between 9.2 mg. and 14.9 mg. per 100 ml., which range is normal for young cattle. Although there were no definite trends in the concentrations of this mineral, the highest levels occurred most frequently in the initial periods of the experimental treatments and the lowest in the terminal. At no stage, however, were differences in the diets reflected in the concentrations of serum calcium.

(c) Inorganic phosphorus. This was determined in the serum samples by using the molybdivanadate method of Simonsen *et al. (24)*. General trends and concentrations of phosphorus in blood serum of calves of all groups were similar during the first 6 months of the experiment. The levels increased during the first 3 months from an initial mean of 8.2 mg. per 100 ml. (range, 6.2-9.1) to 10.1

[4] W. L. Lott.

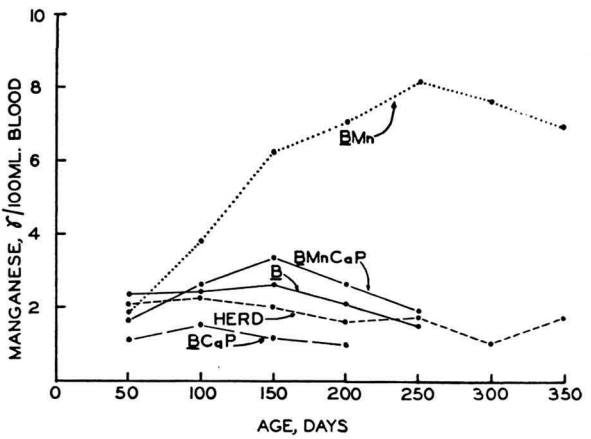

FIG. 2. Average concentration of manganese in whole blood from calves in control group, receiving a conventional herd-type diet, and from calves in various experimental groups, receiving diets differing in supplements of manganese and of calcium and phosphorus.

mg. per 100 ml. (range, 7.8-12.8). From 3 months to 5 months the concentrations tended to plateau, averaging 10.5 mg. per 100 ml. and ranging from 8.3 to 13.9 mg. per 100 ml. During the ensuing month, the values declined slightly to a mean of 9.0 mg. per 100 ml. (range, 6.5-10.8). Although this decrease followed immediately after the ceiling on the milk ration was reached, the relationship of this drop in serum phosphorus to the milk intake is obscure.

From 6 months to the conclusion of the experiment, the concentrations of inorganic phosphorus in the blood serum of all groups, except those receiving supplemental calcium and phosphorus, fluctuated only slightly. The values for Group *B*CaP during the terminal stages of life were extremely variable, ranging from 6.5 to 21.6 mg. per 100 ml., but were significantly higher than observed in the serum from calves in any other group at the corresponding pre-death stages. The highest concentrations were detected in serum of blood samples collected from calves shortly after convulsive seizures and during the rapidly degenerating stages immediately preceding death. The levels of inorganic phosphorus in the serum from Group *B*MnCaP were low and variable during the terminal period but less extreme, 5.7 to 10.6 mg. per 100 ml., than for Group *B*CaP.

(d) Alkaline phosphatase. Activity of this enzyme in the serum was measured by the Bodansky procedure *(8)*, modified to utilize the molybdivanadate color reaction. The general trends of the mean values for the respective groups showed an increase in activity from a low (between 5.2 and 7.9 units) after the first 2 weeks to a high (between 8.1 and 13.3 units) at 4 to 10 weeks. The highest mean occurred in the *B*MnCaP group and the lowest in the *B*CaP. During the subsequent 16 to 22 weeks, the trends in all groups were downward to mean values between 2 and 5 units. In this low plateau stage of the trends, the activity in serum from Groups *B* and *B*Mn was similar to that in the herd group but

greater than in Groups *B*MnCaP and *B*CaP. The supplemental calcium and phosphorus thus appeared to suppress the alkaline phosphatase activity, particularly during the terminal months in the lives of the calves, and manganese supplementation tended to enhance activity in the calcium and phosphorus supplemented groups but not in the others. The differences, however, were not significant statistically, probably due to the high degree of variability within groups.

(e) Magnesium. Concentrations of this element in the blood serum were measured by the method of Simonsen *et al. (25)*. Inasmuch as magnesium determinations were not initiated until convulsive seizures indicated the possibility of hypomagnesemia *(10)*, the ages of the calves on test ranged from 118 to 186 days at the time of the initial analyses. Since data are available on all the calves only during the final stages of the experiment, the common reference point on the time scale of Figure 3 is the terminal period rather than the initial.

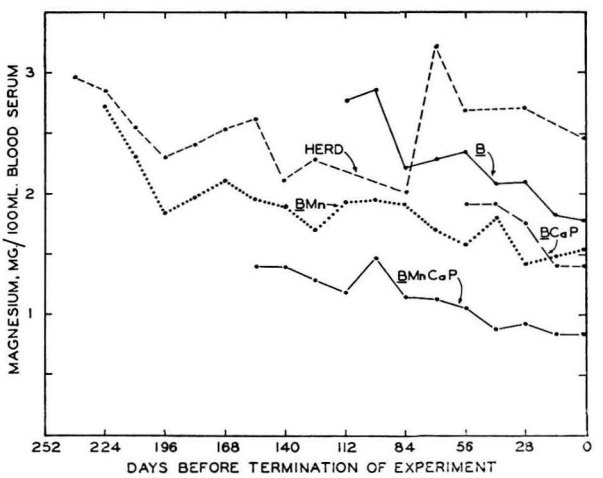

FIG. 3. Average concentration of magnesium in blood serum from calves in control group, receiving a conventional herd-type diet, and from calves in various experimental groups, receiving diets differing in supplements of calcium and phosphorus.

The concentrations of magnesium in the blood serum were highest in the herd group and lowest in the *B*MnCaP group. The trends indicated that the addition of either monocalcium phosphate or manganous sulfate to the basal diets, *B*, tended to decrease the levels of magnesium in the serum. This suppression was accentuated significantly when a combination of the two supplements was fed, diet *B*MnCaP.

3. *Clinical observations.* All calves receiving the herd diet survived in a healthy, vigorous condition throughout the study, but all calves fed the experimental diets died after manifesting a variety of nonspecific symptoms characteristic of general malnutrition. Clinical symptoms common to all the experimental

groups were diarrhea, excessive salivation and lacrimation, nasal discharges, arching of back, enlarging and buckling of knees, weakening of pasterns, and spreading of phalanges. These abnormalities were manifested more prominently during the terminal 2 to 6 weeks in the life of the calf than at any other stage.

Hyperirritability in varying degrees was detected in one or more calves of the respective dietary groups among the experimental animals. Convulsions of the tonic-clonic type, characteristic of those observed in calves restricted to a milk diet *(10)*, also were common. The youngest age at which these seizures were first detected in the various dietary groups were: *B*CaP, 170 days; *B*, 219 days; *B*MnCaP, 244 days; and *B*Mn, 373 days. Thus, it appears that the calcium and phosphorus supplements accelerated and the manganese delayed the onset of the seizures. The incidence and the severity of the convulsions also were greater in the first three groups than in the last.

All calves receiving the supplemental calcium and phosphorus manifested, during the terminal weeks of life, abnormal locomotion characterized by stiffness of the legs and a stilted movement in Group *B*CaP and a spastic gait in Group *B*MnCaP. Calves in this latter group also exhibited opisthotonus—muzzle extended forward and upward and twisted to the left followed occasionally by the head's drawing against the right side.

All the calves in the basal group, *B*, were vigorous until the occurrence of convulsions, terminating in death. Vitality of calves in other groups declined gradually over several weeks. During the terminal days, many of them became prostrate.

Comparisons of the average periods of survival of calves in the various dietary groups, *B* with *B*Mn and *B*CaP with *B*MnCaP (Table 3), indicated that the manganese supplementation increased the life span, whereas corresponding comparisons involving the calcium-phosphorus supplementation suggested that these

TABLE 3

Effect of supplements of manganese and of calcium and phosphorus on the average period of survival of calves

Supplemental calcium and phosphorus	Supplemental manganese		Mean
	Absent	Present	
	(days)		
Absent	276 (*B*)	382 (*B*Mn)	329
Present	211 (*B*CaP)	269 (*B*MnCaP)	240
Mean	244	327	[a]

[a] L.S.D., P = 0.01 is 59 for both treatments, i.e., Mn and Ca + P.

elements reduced longevity. When both of the foregoing supplements were added to the same diet (*B*MnCaP), the counteracting effects were such that the mean survival period of the calves was about the same as for those fed the basal diets *(B)*. When the last calf receiving an experimental diet died, 421 days of age (*B*Mn diet), the control group receiving the herd diet was discontinued.

4. *Lesions.* The only consistent abnormalities observed in post-mortem exam-

inations of calves receiving the basal diets, *B*, were petechiations of the epicardium, hemorrhages of muscles and lungs, abomastitis and enteritis. Calcified lesions were found on the endocardium, the diaphragm, the lungs, and the spleen of calves in the other experimental dietary groups. Although there were variations in the degrees of calcification of the endocardium, the general condition was as shown in Figure 4. Surface plaques were grayish-white in color and granular in texture. Most endocardial lesions were scar-like, whereas others were sub-intimal and rod-like. Myocardial depositions were extra-cellular, white, and granular. Osseous-like deposits typical of those found in the lungs of calves fed the mineral-supplemented diets are shown in Figure 5. A sample of lung deposits contained 24.0% calcium and 13.9% phosphorus. Spectrographic analysis of the material revealed high concentrations of magnesium and of nickel.

Spleens of calves fed the *B* diets appeared to be normal, but those of calves fed other experimental diets were enlarged and/or wrinkled and permeated with hard fibers. There was a close positive correlation between the extensiveness of calcification observed in other organs and the degree of wrinkling of the spleen.

DISCUSSION

The basal diets used in this investigation presumably supplied all nutrients reported to be needed by calves *(17)*, but the responses indicated a nutritional inadequacy.

Manganese supplementation enhanced weight gains only in calves receiving excessively high dietary levels of calcium and phosphorus. The failure of the addition of manganese to the basal diets to stimulate growth is in accord with

FIG. 4. Part of heart showing endocardium having typical calcified lesions (white plaques on surface) observed in calves fed large amounts of supplemental minerals.

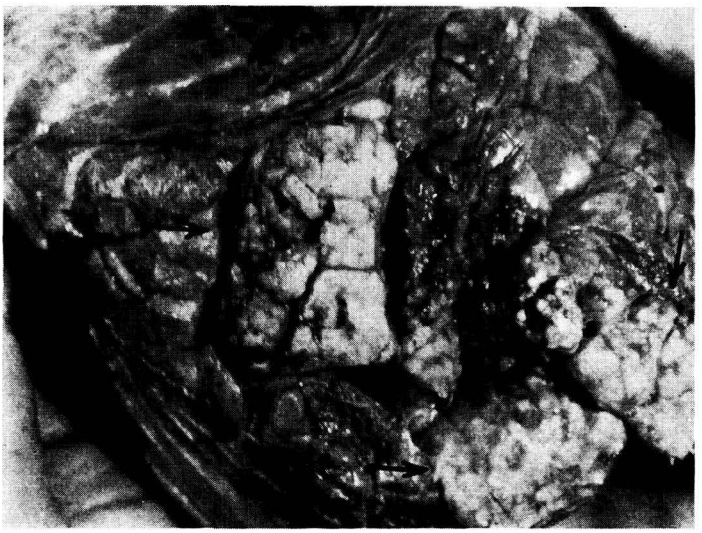

FIG. 5. Area of lung showing a calcified deposit observed in the calves receiving diets containing large amounts of supplemental minerals.

other observations on cattle *(4)* but contrary to responses noted in rabbits *(11)*, chicks *(14)*, and mice and rats *(23)*. The question of whether or not there are basic differences among species in requirements of manganese for growth cannot be answered without further definitive experiments. The results from this study might have been due either to the adequacy of manganese in the basal diets of the calves or to a masking of their maximum growth potential by some deficiency other than manganese.

The significant increase of this element in whole blood following increased manganese intake is in harmony with observations by Blakemore *et al. (5)* but at variance with reports by Bentley and Phillips *(4)*. This discordance probably is due primarily to differences in analytical methods.

Factors other than the quantity of manganese in the diet also affect the levels of this element in the blood of calves. The tendency of high intakes of calcium and phosphorus to suppress manganese of the blood is in accord with the premise that the metabolic interrelationships of these minerals in the calf are similar to those reported for other species *(19, 22, 23, 28)*. Evidence of the involvement of additional factors is found in the observation that concentrations of manganese in diets *B*Mn and herd (control) were similar, but blood manganese values were significantly higher in calves of the former group than in the latter. Furthermore, manganese in the herd diet was higher than in the basal *(B)*; yet the blood manganese in the two groups of calves was about the same. The extent to which these blood values reflected manganese retention was not ascertained.

Of the constituents studied in blood serum, only magnesium seemed to be related significantly to differences in diets. Although Blaxter *et al. (6)* stated that

blood serum magnesium concentrations in calves are determined to a large extent by dietary intake, the amounts of other minerals in the diets apparently are determinants also. The results reported herein indicate that supplementation of the diets with manganese and with calcium and phosphorus reduced the magnesium in the blood serum. Blakemore *et al. (5)* noted similar responses in cows grazing pastures high in manganese. Fain *et al. (12)* also detected decreases in serum magnesium of cattle when the manganese concentration was 100 p.p.m. but no change at either lower levels, 75 p.p.m., or higher, 150 p.p.m. and 200 p.p.m. The manner in which these metabolic alterations are effected merits investigation.

Since hypomagnesemia and short life are salient characteristics noted in calves fed magnesium-deficient diets *(6, 26)*, the suppression of blood serum magnesium in and increased longevity of calves fed supplemental manganese appear to be discordant. Conceivably, however, a compensatory role may be played by manganese by partial substitution for magnesium in certain enzymatic reactions *(16, 18)*. This functional replacement, however, would not explain the longer life span of the calves receiving the supplemental manganese. The specific role of this mineral in extending survival under the conditions of this study remains to be established.

The cardiovascular and pulmonary lesions detected in calves receiving either the manganese or the calcium-phosphorus supplemented diets in this study were similar to the gross pathology observed in calves fed either whole milk diets *(21)* or semi-synthetic diets having a mineral composition similar to that of milk *(15)*. Magnesium insufficiency has been implicated in the pathological calcification of the vascular system *(21)*, but Blaxter *et al. (6)* did not detect such lesions in calves dying of uncomplicated magnesium deficiency at 50 to 120 days of age. Whether or not calcified lesions might have developed in calves having a deficiency over a longer period or in older cattle remains to be resolved. In addition to magnesium deficiency, other dietary conditions that have been related to the incidence of calcified-like lesions in tissues are improper ratios of calcium to phosphorus *(13)*, excesses of calcium *(1, 2)* and of vitamin D *(3)*, and inadequate vitamin E *(7, 9)*. The variety of dietary regimes under which metastatic calcification has been observed suggests that the pathology is the result of nonspecific imbalances.

The experimental diets fed in this study probably supplied inadequate amounts of vitamin E and perhaps of other nutrients that generally are not considered to be essential for calves. The inadequacy of available information on the nutritive requirements of calves emphasizes the need for comprehensive investigations of metabolic problems relating to excesses and to nutrient ratios in diets of calves as well as to insufficiencies.

SUMMARY

1. In a study of manganese in the nutrition of dairy calves five test diets, four experimental and one conventional type (control), were fed to a total of 15 calves randomly assigned within breeds to dietary groups. The experimental

basal diets *(B)* contained less than 1 p.p.m. of manganese. Three other experimental diets were formulated by adding, respectively, to the basal diets manganous sulfate supplying 50 p.p.m. of manganese (*B*Mn), 3 to 5% monocalcium phosphate (*B*CaP) and a combination of the preceding supplements (*B*MnCaP). A conventional type calf diet was fed for comparative purposes.

2. Growth rate and serum magnesium levels of the herd-diet group were significantly greater than for calves fed experimental diets.

3. Supplemental manganese did not affect significantly the levels of calcium, inorganic phosphorus, or the activity of alkaline phosphatase in blood serum.

4. Supplemental manganese apparently effected the following physiologic responses of the calves:

a. Partially counteracted the deleterious effects of monocalcium phosphate on weight gains during the latter stages of the feeding periods.
b. Depressed serum magnesium levels, particularly when the manganese was fed in conjunction with monocalcium phosphate.
c. Increased blood levels of manganese, but increases were not statistically significant when the diet contained supplemental monocalcium phosphate.
d. Increased life span while monocalcium phosphate decreased it, but neither affected mortality, which was 100% for calves fed experimental diets.

5. Calcified deposits were found on post-mortem examinations in the cardiovascular or pulmonary system of calves in all experimental groups except *B*.

6. The results of this study suggest that the need for dietary manganese by calves is extremely low and that increased amounts of calcium and phosphorus intensify the needs for dietary supplements of manganese.

ACKNOWLEDGMENTS

The authors wish to record their appreciation to H. L. Lucas, Department of Experimental Statistics, for advice in designing the experiment and in analyzing the data; to J. C. Osborne, Animal Pathologist, for post-mortem examinations of calves, and to the American Dry Milk Institute, Chicago, for providing nonfat dry milk solids used in the diets.

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TECHNICAL NOTES

THIAMINE AND RIBOFLAVIN IN VARIOUS FRACTIONS OF RUMEN CONTENTS OF YOUNG CALVES[1]

Recently, Usuelli and Piana *(4)* have reported that thiamine is synthesized in the sheep rumen by bacteria but not by protozoa. Observations made in this laboratory on the B-complex vitamin content of various fractions of rumen contents indicate that this is also true in young calves fed high roughage rations.

Rumen samples, which were obtained from rumen-inoculated, high-roughage-fed calves *(2)* ranging in age from 4 to 6 months, were fractionated by straining through two layers of cheesecloth, which yielded a residue of partially digested feedstuff and rumen juice. A portion of the strained rumen juice was then centrifuged at 1,000 r.p.m. for 5 minutes, which layered the protozoa in the bottom of the centrifuge tubes. The supernatant liquid was decanted and saved. The protozoa were washed in physiological saline and allowed to stand 4 to 6 hours. During this procedure they settled as a white mass at the bottom of the containers *(3)*. The supernatant liquid remaining after removal of protozoa was then centrifuged at 3,000 r.p.m., which removed a large proportion of the remaining bacteria. The bacterial fraction was obtained after decanting the supernatant liquid. However, this decanted supernatant liquid still contained some bacterial cells; therefore, it was passed through a Seitz bacterial filter to obtain the bacteria-free filtrate fraction. Results of thiamine and riboflavin assays *(1)* of these various fractions of rumen contents are presented in Table 1 with similar assays of the feed given the calves at the time of sampling.

These data show that rumen bacteria provide a relatively rich source of thiamine and riboflavin and that these vitamins are principally bound in the bacteria cells. Although rumen protozoa provided a relatively rich source of riboflavin, their thiamine content was not much higher than that found in the feed. These results suggest that thiamine is synthesized by bacteria and not by protozoa in the rumen. This is in agreement with the results reported by Usuelli and Piana *(4)*.

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[1] Approved by the director of the Ohio Agricultural Experiment Station as Journal Article No. 53-54.

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TABLE 1

The content of thiamine and riboflavin in various fractions of rumen contents

	Thiamine	Riboflavin
No. of samples	6	6
Rumen contents *(γ/g of D.M.)*	2.61	22.0
Residue after straining *(γ/g of D.M.)*	1.77	10.1
Strained rumen juice *(γ/100 ml.)*	8.28	112.8
Centrifuged rumen juice *(γ/100 ml.)*	4.77	54.9
Bacteria-free filtrate *(γ/100 ml.)*	2.87	21.1
Rumen bacteria *(γ/g of D.M.)*	9.71	39.2
Rumen protozoa *(γ/g of D.M.)*	3.34	59.0
Feed *(γ/g of D.M.)*	2.53	6.9

THE FERTILITY OF INSEMINATIONS MADE IN COWS SHOWING POSTESTROUS HEMORRHAGE

Weber *et al. (4)* observed 68 complete estrous cycles of 22 normal virgin heifers and noted that 81% of these showed visible bleeding at the vulva. Bleeding was seen on the average between 50 to 60 hours after onset of estrus.

The 60-90 day nonreturn data indicate that the fertility of first service inseminations made when cows are showing a bloody discharge is 26%. This may be compared to the University bull stud average for the particular period

TABLE 1

Fertility of inseminations made in cows showing postestrous hemorrhage

Method of estimating fertility	1st services		2nd services		3rd services		4th-8th services		All services	
	No. of insemi-nations	Per cent fertile	No. of insemi-nations	Per cent fertile	No. of insemi-nations	Per cent fertile	No. of insemi-nations	Per cent fertile	No. of insemi-nations	Per cent fertile
60-90 day nonreturns	99	26.3	56	37.5	26	15.4	30	30.0	211	28.4
Pregnancy examination	49	22.4	30	23.3	14	0.0	23	26.1	116	20.7

Trimberger *(3)* observed 400 cows and noted that 303 showed a bloody discharge. His study also established that cows can show a bloody discharge and still be pregnant.

Cows reported for artificial insemination sometimes show a bloody discharge when inseminated. Dairymen and persons working in artificial breeding often have expressed the opinion that such inseminations are made too late for the cow to conceive. Autrup and Rasbech *(1)* reported that of 293 inseminations made while the cows were showing a bloody discharge, 29.7% were fertile. The following study was carried out to obtain further information regarding this problem.

The data were collected during the period from Nov. 1, 1948, through Oct. 31, 1949. The inseminators of the University of Wisconsin bull stud, who were working in Dane County, marked the breeding receipts for cows inseminated while showing a bloody discharge. All services, repeats as well as first services, were considered.

Two hundred eleven inseminations were reported as made when the cows showed bloody discharge. Of these, 154 were inseminated with semen from Holstein bulls, 49 with semen from Guernsey bulls, and 8 with semen from bulls of other breeds. The inseminations represented 0.9% of the breedings made by the bull stud during the period and in the area of the study. Sixty-ninety day nonreturn information was available on all the inseminations. Pregnancy examination *(5)* information was available on 116 of the 211. The results are shown in the accompanying table.

and area of 65.4%. The pregnancy examination estimates showed a lower percentage of fertility than did the nonreturns. However, the difference in the results is comparable to the difference reported by Barrett *et al. (2)* between 60-90 day nonreturn and pregnancy examination data. The fertility estimated for all services was 28% and 21% for nonreturns and pregnancy examinations, respectively.

The results of this study are similar to those of Autrup and Rasbech *(1)*. One may conclude that inseminators could breed cows showing a bloody discharge with approximately one chance in four of saving another trip to the farm.

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PROGRAM

FIFTIETH ANNUAL MEETING

of the

AMERICAN DAIRY SCIENCE ASSOCIATION

MICHIGAN STATE COLLEGE

EAST LANSING, MICHIGAN

JUNE 20-23, 1955

Program Committee

R. E. ERB, Washington
G. H. HARTMAN, Wisconsin
C. F. HUFFMAN, Michigan
E. T. ITSCHNER, Missouri
W. V. PRICE, Wisconsin, *Chairman*

GENERAL PROGRAM

Sunday, June 19

9:00 A.M. **Meeting of the Executive Board,** *Room 106, Kellogg Center*

Monday, June 20

9:00 A.M. **Meeting of the Executive Board,** *Room 106, Kellogg Center*

10:00 A.M. **Registration,** *Lower Lounge, Brody Hall*

1:00–5:00 P.M. **Campus Tours**—Buses leave all afternoon from *Kellogg Center*

7:30 P.M. **Informal Get-Together,** *Brody Hall Area*

8:00 P.M. EXTENSION SECTION SESSION (open to all), *Recreation Room, Brody Hall*

Progress Report and Discussion: Handling D.H.I.A. Records by Modern Machines

J. F. KENDRICK, *Dairy Herd Improvement Section, USDA*

Tuesday, June 21

8:00 A.M. **Registration,** *Brody Hall*

9:45 A.M. OPENING SESSION, *Lower Lounge, Brody Hall*
DR. EARL WEAVER, *Head, Dairy Department, Michigan State College, presiding.*

Prelude
WENDELL WESTCOTT, *Music Department, Michigan State College.*

National Anthem
Leader—DR. N. P. RALSTON, *Dairy Department, Michigan State College.*

Invocation
DR. N. A. MCCUNE, *Minister Emeritus, The Peoples Church, East Lansing.*

Welcome
DR. THOMAS COWDEN, *Dean, School of Agriculture, Michigan State College.*

Presidential Address
DR. LANE A. MOORE, *Dairy Husbandry Research Branch, U. S. Department of Agriculture.*

Introductory Remarks
DR. JOHN A. HANNAH, *President, Michigan State College.*

Address
DR. DURWARD B. VARNER, *Vice-President, Michigan State College.*

Postlude
WENDELL WESTCOTT

SECTION MEETINGS

1:30–4:30 P.M. MANUFACTURING SECTION A
Lactic Acid Cultures—Papers and Symposium
North Lower Lounge, Brody Hall

1:30–4:30 P.M. MANUFACTURING SECTION B
Concentrated and Dry Milk—Papers and Symposium
South Lower Lounge, Brody Hall

1:15–5:00 P.M. PRODUCTION SECTION A
General Physiology and Reproduction
Auditorium, Kellogg Center

1:15–5:00 P.M. PRODUCTION SECTION B
Rumen Physiology
Ballroom, Kellogg Center

1:30–4:30 P.M. EXTENSION SECTION
Opening Business Session, Teaching Methods and Exhibits
Recreation Room, Brody Hall

4:30–6:30 P.M. STUDENT AFFILIATE AND FACULTY ADVISER MEETING
Discussion of Problems and Plans for a More Comprehensive Student Affiliate Program
Room 103-4, Kellogg Center

7:30 P.M. **Reception** (Informal)
Union Building

Wednesday, June 22

SECTION MEETINGS

8:30–10:45 A.M. MANUFACTURING SECTION A
Cheese
North Lower Lounge, Brody Hall

8:30–10:45 A.M. MANUFACTURING SECTION B
Market Milk and Ice Cream
South Lower Lounge, Brody Hall

11:00 A.M.–NOON MANUFACTURING SECTION BUSINESS MEETING
North Lower Lounge, Brody Hall

8:00–11:00 A.M. PRODUCTION SECTION A
Endocrine Physiology
Auditorium, Kellogg Center

8:00–11:00 A.M. PRODUCTION SECTION B
Management and Forage Utilization
Ballroom, Kellogg Center

11:00 A.M.–NOON PRODUCTION SECTION BUSINESS MEETING
Auditorium, Kellogg Center

8:00–9:00 A.M. EXTENSION SECTION AND PRODUCTION SECTION B
Management and Forage Utilization
Ballroom, Kellogg Center

9:00–11:00 A.M. EXTENSION SECTION
Dairy Records
Recreation Room, Brody Hall

11:00 A.M.–NOON EXTENSION SECTION BUSINESS MEETING
Committee Reports
Recreation Room, Brody Hall

1:30–4:00 P.M. MANUFACTURING SECTION A
Dairy Chemistry
North Lower Lounge, Brody Hall

1:30–4:00 P.M. MANUFACTURING SECTION B
Market Milk
South Lower Lounge, Brody Hall

2:30–4:00 P.M. DAIRY PRODUCTS JUDGING CONFERENCE
Room 103-4, Kellogg Center

4:00 P.M. MANUFACTURING SECTION BUSINESS MEETING
North Lower Lounge, Brody Hall

1:15–3:20 P.M. PRODUCTION SECTION A
Genetics
Auditorium, Kellogg Center

1:15–3:20 P.M. PRODUCTION SECTION B AND EXTENSION SECTION
Symposium: Ratios of Forages and Grain in the Milking Ration
Ballroom, Kellogg Center

3:30–4:30 P.M. PRODUCTION AND EXTENSION SECTIONS
Joint Committee Reports
Auditorium, Kellogg Center

5:30 P.M. **Barbecue**
Across Red Cedar River from Kellogg Center

8:00 P.M. **Recognition Program**
Presentation of Honor Awards and Installation of Officers
Lower Lounge, Brody Hall

Thursday, June 23

SECTION MEETINGS

8:30–10:00 A.M. MANUFACTURING SECTION A
Microbiology
North Lower Lounge, Brody Hall

8:30–10:00 A.M. MANUFACTURING SECTION B
Milk Fats
South Lower Lounge, Brody Hall

10:00 A.M.–NOON GENERAL ASSOCIATION BUSINESS MEETING
Lower Lounge, Brody Hall

8:00–10:00 A.M. PRODUCTION SECTION A
Milk Secretion
Auditorium, Kellogg Center

8:00–10:00 A.M. PRODUCTION SECTION B
Calf Nutrition
Ballroom, Kellogg Center

8:00–10:00 A.M. EXTENSION SECTION
Dairy Cattle Health Problems
4-H Club Work
Recreation Room, Brody Hall

1:15–4:45 P.M. MANUFACTURING, PRODUCTION, AND EXTENSION SECTIONS
Symposium: Dairy Products in Human Nutrition
Lower Lounge, Brody Hall

1:15–2:45 P.M. PRODUCTION SECTION A AND EXTENSION SECTION
Report of Progress—Regional Dairy Cattle Breeding Projects
Auditorium, Kellogg Center

2:50–4:30 P.M. PRODUCTION SECTION A AND EXTENSION SECTION
Frozen Semen
Auditorium, Kellogg Center

1:15–4:30 P.M. PRODUCTION SECTION B
Nutrition
Ballroom, Kellogg Center

ENTERTAINMENT

GENERAL PROGRAM

Monday, June 20 — 7:30 P.M.—**Informal Get-Together**
Brody Hall Area

Tuesday, June 21 — 7:30 P.M.—**Reception** (Informal)
Union Building

Wednesday, June 22 — 5:30 P.M.—**Barbecue,** Butter Charcoal-Broiled Chicken
Across Red Cedar River from Kellogg Center

8:00 P.M.—**Recognition Program and Entertainment**
Lower Lounge, Brody Hall

WOMEN'S PROGRAM

Tuesday, June 21

8:00 A.M. and 12:30 P.M.
—**Oldsmobile Tours**
Kellogg Center, Parking Lot

9:00 A.M. and 1:15 P.M.
—**Campus Tours**
Kellogg Center, Parking Lot

9:30 A.M.—**Talk,** "Fair, Fat, and Forty"—DR. MARGARET OLSON, *Michigan State College*
Music Auditorium

12:15 P.M.—**Luncheon**
Ballroom, Union Building
Floral Table Arrangements—MRS. FRANCES K. PATCH, *Kesel's Flower Shop, East Lansing*

Thursday, June 23

9:30 A.M.—**Talk,** New Fabrics and Their Care—MRS. LEONA MACLEOD, *Michigan State College*
Music Auditorium

2:00 P.M.—**Bridge, Canasta, and Chatting**
Parlors A, B, and C, Union Building

CHILDREN'S PROGRAM

Tuesday, June 21

9:00 A.M.–NOON
—**Nursery**
Quonset Village, Harrison Road

9:30–11:30 A.M.
—**Campus Tour** (6-12 years)
Assemble in North Lobby, Brody Hall

9:30 A.M.—**Hike to Pinetum** (12 years and older)
Assemble in North Lobby, Brody Hall

1:00 P.M.—**Oldsmobile Tour** (12 years and older)
Assemble in North Lobby, Brody Hall

2:00–4:00 P.M.
—**Swimming** (6-12 years, bring suit and towel)
Women's Gymnasium

7:15 P.M.—**Movies** (6 years and older)
Union Building

7:15 P.M.—**Games and Dancing** (12 years and older)
Union Building

Wednesday, June 22

9:00 A.M.–NOON
—**Nursery**
Quonset Village, Harrison Road

9:30–11:00 A.M.
—**Recreation** (6-12 years)
Assemble in North Lobby, Brody Hall

11:30 A.M.—**Picnic at Potter Park**
Assemble in North Lobby, Brody Hall

2:00–4:00 P.M.
—**Swimming** (12 years and older, bring suit and towel)
Women's Gymnasium

7:30 P.M.—**Movies**
Recreation Room, Brody Hall

8:30 P.M.—**Dancing** (12 years and older)
Forestry Cabin

Thursday, June 23

9:00 A.M.–NOON
—**Nursery**
Quonset Village, Harrison Road

9:30–11:30 A.M.
—**Recreation or Handicraft** (6-10 years)
Assemble in North Lobby, Brody Hall

2:00–4:00 P.M.
—**Recreation** (6-12 years)
Assemble in North Lobby, Brody Hall

Special Recreation: Golf courses and tennis courts are available. Bring your own clubs or rackets.

MANUFACTURING SECTION

Tuesday, June 21

1:30–4:30 P.M.

SECTION A. **Lactic Acid Cultures**
W. M. ROBERTS, *presiding*
North Lower Lounge, Brody Hall
Contributed Papers:

M1 The activity of lactic acid starters in reconstituted nonfat dry milk solids and fluid milks. V. W. GREENE and J. J. JEZESKI, *University of Minnesota.*

M2 Preparation of starter cultures by combining separate strains of a lactic Streptococcus and an associate at the final propagation. D. D. DEANE and F. E. NELSON, *Iowa State College.*

Symposium:

P. R. ELLIKER, *Chairman, Oregon State College.*
North Lower Lounge, Brody Hall

Species involved. J. M. SHERMAN, *Cornell University.*

Nutrition of the cultures. M. L. SPECK, *North Carolina State College.*

Growth and action of cultures in milk. F. E. NELSON, *Iowa State College.*
Bacteriophage, antibiotics, and other inhibitory substances. F. J. BABEL, *Purdue University.*

Problems in the manufacture and storage of cultured products. M. E. POWELL, *Knudsen Creamery Co., Los Angeles, Calif.*

1:30–4:30 P.M.

SECTION B. **Concentrated and Dry Milk**
H. L. TEMPLETON, *presiding*
South Lower Lounge, Brody Hall

Contributed Papers:

M3 Viscosity of reconstituted nonfat dried milks. W. D. RUTZ and C. H. WHITNAH, *Kansas State College.*

M4 Studies on sterile milk. E. O. HERREID and LALITHA KADABA, *University of Illinois.*

M5 The effect of HTST heat treatment of separated milk on the quality of spray-dried nonfat dry milk solids for manufacture of cottage cheese. W. K. STONE, E. J. FINNEGAN, and G. C. GRAF, *Virginia Agricultural Experiment Station.*

Panel:

GEORGE HOLM, *Moderator, USDA, Washington, D. C.*

Factors that affect the stability of frozen concentrated milk. R. W. BELL, *Eastern Utilization Research Branch, USDA, Washington, D. C.*
What's new in evaporated milk? E. H. PARFITT, *Evaporated Milk Association, Chicago.*

Destabilization of the caseinate in relation to the utility of concentrated and dry milk products. R. JENNESS, *University of Minnesota.*

Theory and practice of spray drier operation. S. T. COULTER, *University of Minnesota.*

Evaluation of nonfat dry milk solids for the final use of the product. A. M. SWANSON, *University of Wisconsin.*

Wednesday, June 22

8:30–10:45 A.M.

SECTION A. **Cheese**

H. L. TEMPLETON, *presiding*
North Lower Lounge, Brody Hall

M6 The manufacture of Ricotta cheese from whole milk. H. M. WINDLAN and F. V. KOSIKOWSKI, *Cornell University.*

M7 A method for determining time for cutting curd for cottage cheese. D. B. EMMONS, W. V. PRICE, and A. M. SWANSON, *University of Wisconsin.*

M8 Cheddar cheese made from pasteurized milk homogenized at various pressures. I. I. PETERS, *Texas Agricultural Experiment Station.*

M9 Studies in the ripening of surface-ripened cheese. S. L. TUCKEY and R. M. SAHASRABUDHE, *University of Illinois.*

M10 Effect of fatty acids in pasteurized Liederkranz cheese on the growth of *Clostridium botulinum* added to the product. N. GRECZ, R. O. WAGENAAR, and G. M. DACK, *Food Research Institute, University of Chicago.*

M11 Preliminary studies of the Australian short-time method of making Cheddar cheese. P. A. DOWNS, *University of Nebraska.*

M12 Determination of free amino acids in Cheddar cheese by one-dimensional paper chromatography. D. H. BULLOCK, O. R. IRVINE, and W. H. SPROULE, *Ontario Agricultural College.*

M13 Formulation and stability of three new flavor varieties of Cheddar cheese spread for use by the Armed Forces. R. I. MEYER and J. M. MCINTIRE, *Quartermaster Food and Container Institute for the Armed Forces, Chicago.*

M14 Free fatty acids produced during Blue cheese ripening. H. A. MORRIS, J. J. JEZESKI, W. B. COMBS, and S. KURAMOTO, *University of Minnesota.*

8:30–10:45 A.M.

SECTION B. **Market Milk and Ice Cream**

W. M. ROBERTS, *presiding*
South Lower Lounge, Brody Hall

M15 High-temperature short-time pasteurization of ice cream mixes. J. TOBIAS, O. W. KAUFMANN, and P. H. TRACY, *University of Illinois.*

M16 A critical study of 2,6-dichloroquinonechloroimine as an indicator in the phosphatase test. R. W. HENNINGSON and F. V. KOSIKOWSKI, *Cornell University.*

M17 Observations on raw milk quality before and after conversion to bulk tank pick-up at the farm. H. V. ATHERTON, *University of Vermont.*

M18 Butterfat tests of milk from farm bulk tanks. ALEC BRADFIELD and P. E. GOTTHELF, *University of Vermont.*

M19 Study of several methods for the determination of total solids in fluid milk. W. R. DAVEY and STUART PATTON, *The Pennsylvania State University.*

M20 Effects of age upon the viscosity of whole milk. C. H. WHITNAH, W. D. RUTZ, and H. C. FRYER, *Kansas State College.*

M21 Some properties of "subnormal milk." W. A. KRIENKE, L. E. MULL, and E. L. FOUTS, *Florida Agricultural Experiment Station.*

M22 An evaluation of a centrifugal method for determining the efficiency of homogenization. D. A. SEIBERLING, *The Ohio State University.*

11:00 A.M.–NOON

SECTION BUSINESS MEETING
North Lower Lounge, Brody Hall

1:30–4:00 P.M.

SECTION A. **Dairy Chemistry**
H. L. TEMPLETON, *presiding*
North Lower Lounge, Brody Hall

M23 Analysis of milk for free volatile fatty acids. F. V. KOSIKOWSKI, *Cornell University.*

M24 Isolation and identification of δ-decalactone as the compound responsible for the coconut-like flavor of butter oil. P. G. KEENEY and STUART PATTON, *The Pennsylvania State University.*

M25 Prevention of sunlight flavor in milk by removal of riboflavin. H. J. VELANDER and STUART PATTON, *The Pennsylvania State University.*

M26 The isolation and identification of the carbonyl compounds resulting from the oxidation of butter oil. E. G. HAMMOND and E. W. BIRD, *Iowa State College.*

M27 Breed and individual variations in the specific protein constituents of milk. G. D. ROLLERI, B. L. LARSON, and R. W. TOUCHBERRY, *University of Illinois.*

M28 Water-insoluble acid content of farm-separated cream produced in Kentucky. T. R. FREEMAN, L. A. RICHARDSON, and J. O. BARKMAN, *Kentucky Agricultural Experiment Station.*

M29 The separation of the free fatty acids of mold hydrolyzed butterfat by partition and displacement chromatography. S. KURAMOTO, J. J. JEZESKI, and H. A. MORRIS, *University of Minnesota.*

M30 The effect of several organic and inorganic acids on the heat precipitation of calcium hardness from water. D. A. EVANS and G. H. WATROUS, JR., *The Pennsylvania State University.*

M31 Direct chromatographic determination of lactic, pyruvic, and C_1 to C_6 fatty acids in biological materials. M. KEENEY, *University of Maryland.*

M32 Acids formed by the high-heat treatment of milk. C. V. MORR, I. A. GOULD, and W. J. HARPER, *The Ohio State University.*

1:30–4:00 P.M.

SECTION B. **Market Milk**

W. M. ROBERTS, *presiding*
South Lower Lounge, Brody Hall

M33 Synthetic vitamin K and oxidized flavors in milk. M. RECHCIGL, JR., V. N. KRUKOVSKY, and L. H. SCHULTZ, *Cornell University.*

M34 Organoleptic study of oxygenated and copper-treated milk prior to pasteurization. V. N. KRUKOVSKY, *Cornell University.*

M35 The occurrence of rancidity in milk from pipeline milkers. G. W. GANDER, *Cornell University.*

M36 Development of oxidized flavors in unhomogenized milk and homogenized milk. E. S. GUTHRIE, *Cornell University.*

M37 The effect of mineral fortification on lipase activity in pasteurized milk. R. A. REISFELD, W. J. HARPER, and I. A. GOULD, *The Ohio State University.*

M38 Observations on the extent of lipolysis in raw milk supplies as related to various milk handling procedures. E. L. THOMAS, A. J. NIELSEN, and J. C. OLSON, JR., *University of Minnesota.*

M39 The relationship of processing variables to the feathering of coffee cream. O. B. CURLEY, I. A. GOULD, and D. A. SEIBERLING, *The Ohio State University.*

M40 The stability and role of aureomycin in milk. K. M. SHAHANI, I. A. GOULD, H. H. WEISER, and W. L. SLATTER, *The Ohio State University.*

M41 A test for keeping quality of pasteurized milk. E. A. DAY and F. J. DOAN, *The Pennsylvania State University.*

2:30–4:00 P.M.

DAIRY PRODUCTS JUDGING CONFERENCE
G. M. TROUT, *presiding*
Room 103-A, Kellogg Center

4:00–5:00 P.M.

SECTION BUSINESS MEETING
North Lower Lounge, Brody Hall

Thursday, June 23

8:30–10:00 A.M.

SECTION A. **Microbiology**
W. M. ROBERTS, *presiding*
North Lower Lounge, Brody Hall

M42 Some characteristics of the natural antibacterial properties of milk. A. G. WOLIN and F. V. KOSIKOWSKI, *Cornell University.*

M43 Observations on bacterial growth stimulants present in pancreas tissue. W. E. SANDINE, L. HANKIN, M. L. SPECK, and L. W. AURAND, *North Carolina State College.*

M44 The application of the 2,3,5-triphenyltetrazolium chloride test for inhibitory substances in milk. C. E. NEAL and H. E. CALBERT, *University of Wisconsin.*

M45 Microbiological fermentation of dairy wastes. J. E. EDMONDSON and A. R. BRAZIS, *University of Missouri.*

M46 Effects of ultrasonic waves on the bacterial flora of milk. J. A. ELLIOTT and W. C. WINDER, *University of Wisconsin.*

M47 HTST pasteurization for the control of psychrophilic organisms in plant water supplies. D. A. SEIBERLING and W. J. HARPER, *The Ohio State University.*

M48 Interactive phenomena among several bacteria of dairy origin. W. C. VAN DER ZANT, *Texas Agricultural Experiment Station.*

8:30-10:00 A.M.

SECTION B. **Milk Fats**
G. H. HARTMAN, *presiding*
South Lower Lounge, Brody Hall

M49 Effect of phospholipids and unsaponifiable matter on the flavor stability of milk fat. E. N. FRANKEL, L. M. SMITH, and E. L. JACK, *University of California.*

M50 Characteristics of pure fats compared to the same fats as extracted from frozen desserts. C. L. BURTON, M. LOEWENSTEIN, and J. B. MICKLE, *Oklahoma A. & M. College.*

M51 Study of the effect of extraction methods on composition of fat taken from frozen desserts. J. B. MICKLE and M. LOEWENSTEIN, *Oklahoma A. & M. College.*

M52 Improved fractionation and fat number determination for detecting butterfat adulteration. W. A. KRIENKE, *Florida Agricultural Experiment Station.*

M53 A modified hydroxamate method for determining the short-chain fatty acids in fats. W. F. SHIPE, *Cornell University.*

M54 Studies on nonwashing of butter. A. H. WHITE and R. R. RIEL, *Department of Agriculture, Ottawa, Canada.*

M55 Tocopherol content of the fat of dairy products as an index of adulteration. J. H. MAHON, C. ANGLIN, and R. A. CHAPMAN, *Food and Drug Laboratories, Ottawa, Canada.*

M56 Tortelli-Jaffe reaction for detecting marine fats in butterfat. J. H. MAHON and R. A. CHAPMAN, *Food and Drug Laboratories, Ottawa, Canada.*

M57 Acetic acid turbidity temperature as an index of butterfat adulteration. J. H. MAHON and R. A. CHAPMAN, *Food and Drug Laboratories, Ottawa, Canada.*

10:00 A.M.–NOON

GENERAL ASSOCIATION BUSINESS MEETING
Lower Lounge, Brody Hall

1:15–4:45 P.M.

Joint Session—
MANUFACTURING, PRODUCTION, AND EXTENSION SECTIONS

Symposium: Dairy Products in Human Nutrition

G. H. HARTMAN, *presiding*
D. H. JACOBSEN, *Chairman, American Dairy Association, Chicago*
Lower Lounge, Brody Hall

Dairy products in our food economy. H. DEGRAFF, *Cornell University.*
Milk in adult diets. MARGARET OHLSON, *Michigan State College.*
Milk in infant diets. D. B. COURSIN, *St. Joseph's Hospital, Lancaster, Pa.*
Milk proteins in human nutrition. C. A. ELVEHJEM, *University of Wisconsin.*
Milk fat in human nutrition. F. A. KUMMEROW, *University of Illinois.*
Nutrition in relation to dairy science. ZOE ANDERSON, *National Dairy Council, Chicago.*

PRODUCTION SECTION

Tuesday, June 21

1:15–5:00 P.M.

SECTION A. **General Physiology and Reproduction**
R. E. ERB, *Chairman*
Auditorium, Kellogg Center

P1 Further studies on the influence of dietary calcium and phosphorus on the incidence of milk fever. J. M. BODA, *University of California.*

P2 Relative reactions of cattle and man to higher temperatures. SAMUEL BRODY, *University of Missouri.*

P3 Growth comparisons for Holstein, Ayrshire, Guernsey, and Jersey males for the first six months of life. H. P. DAVIS, *University of Nebraska.*

P4 The relationship of nutrition to reproductive performance of dairy cattle—a field study. W. A. HARDISON, S. L. KALISON, N. O. PRICE, R. W. ENGEL, and W. B. BELL, *Virginia Agricultural Experiment Station.*

P5 Factors affecting pregnancy interruption in artificial insemination of dairy cattle. T. Y. TANABE, C. E. HEIST, and J. O. ALMQUIST, *The Pennsylvania State University.*

P6 Diverticula in the bovine oviducts. H. J. WEETH and P. R. TERNEN, *University of Nevada.*

P7 How do cow families develop? MOGENS PLUM and M. G. A. RUMERY, *University of Nebraska.*

P8 The occurrence of estrus during pregnancy in several Holstein herds. H. R. DONOHO and H. E. RICKARD, *The Ohio State University.*

P9 Thyroidal influence on semen production and the behavior of bulls used in artificial breeding. W. S. GRIFFITH, CECIL BRANTON, H. C. KELLGREN, and G. F. D'ARENSBOURG, *Louisiana Agricultural Experiment Station.*

P10 Seasonal trends in plasma protein-bound iodine levels, semen production and fertility of bulls. CECIL BRANTON, W. S. GRIFFITH, T. E. PATRICK, J. E. JOHNSTON, and G. F. D'ARENSBOURG, *Louisiana Agricultural Experiment Station.*

P11 Semen production by a bull ejaculated three times per week for three consecutive years. N. L. VANDEMARK, L. J. BOYD, and F. N. BAKER, *University of Illinois.*

P12 The effect of supplementary adenosine mono- and tri-phosphate on the metabolism of bovine spermatozoa. F. J. GRUNFELD and C. P. MERILAN, *University of Missouri.*

P13 The effect of phosphate-containing and saline diluents on the aerobic metabolism of bull semen. G. W. SALISBURY and N. T. NAKABAYASHI, *University of Illinois.*

P14 Influence of dilution rate on metabolism of semen in phosphate buffer and in milk diluter. M. H. EHLERS and R. E. ERB, *State College of Washington.*

P15 Study of possible factors related to freezability of spermatozoa. J. I. OHMS and E. L. WILLETT, *American Foundation for the Study of Genetics, Madison, Wis.*

P16 The use of mechanical refrigeration for storage of frozen semen. W. M. ETGEN and T. M. LUDWICK, *The Ohio State University.*

P17 Electroejaculation in the bull. H. J. HILL, F. S. SCOTT, NORMAN HOMAN, and F. X. GASSNER, *Colorado A & M College.*

1:15–5:00 P.M.

SECTION B. **Rumen Physiology**
N. P. RALSTON, *Chairman*
Ballroom, Kellogg Center

P18 Effect of concentrate level on in vitro incorporation of S-35-labeled sulfate by rumen microbiota. R. S. EMERY, C. K. SMITH, R. L. SALSBURY, and C. F. HUFFMAN, *Michigan State College.*

P19 Studies on the dissimilation of purines and pyrimidines by bovine rumen bacteria. P. JURTSHUK, JR., and F. G. HUETER, *University of Maryland.*

P20 Further studies on the influence of diet on the development of the ruminant stomach. R. G. WARNER, C. H. GRIPPIN, W. P. FLATT, and J. K. LOOSLI, *Cornell University.*

P21 Lactic acid production in the rumen. D. R. WALDO and L. H. SCHULTZ, *Cornell University.*

P22 Relation between age of calf, blood glucose, blood and rumen levels of volatile fatty acids, and in vitro cellulose digestion. R. D. MCCARTHY and E. M. KESLER, *The Pennsylvania Agricultural Experiment Station.*

P23 The effect of protein level on rumen volatile fatty acids. N. S. WOODHOUSE, R. F. DAVIS, and G. H. BECK, *University of Maryland.*

P24 Studies on the bloat syndrome. J. T. BLAKE, N. L. JACOBSON, and R. S. ALLEN, *Iowa State College.*

P25 The effect of alfalfa saponin on rumen activity in sheep. H. W. COLVIN, JR., P. T. CUPPS, and C. R. THOMPSON, *University of California.*

P26 Ingesta volume increase in rumen contents of cattle on normal and bloat-producing rations. DON JACOBSON, *University of Maryland* and IVAN LINDAHL, *Agricultural Research Center, USDA.*

P27 Volatile fatty acids and pH in rumen contents of cud inoculated and uninoculated calves fed high roughage pellets. H. R. CONRAD, J. W. HIBBS, J. H. VANDERSALL, and W. D. POUNDEN, *Ohio Agricultural Experiment Station.*

P28 Methods of feeding and rumen inoculation as they affect the growth and development of young dairy calves. R. A. ACKERMAN and J. E. FIKE, *University of West Virginia.*

P29 Further studies on an iodine staining substance produced by bovine rumen bacteria. R. J. GIBBONS and R. N. DOETSCH, *University of Maryland.*

P30 The development of the flora and fauna in the rumen of growing calves. M. P. BRYANT, NOLA SMALL, and L. A. BURKEY, *Dairy Husbandry Research Branch, USDA.*

P31 Observations on the use of mixed suspensions of bovine rumen bacteria as a technique of the rumen microbiologist. R. N. DOETSCH, J. C. SHAW, J. J. MCNEILL, and P. JURTSHUK, JR., *University of Maryland.*

P32 Studies on the methane and hydrogen metabolism of bovine rumen bacteria. J. J. MCNEILL and D. R. JACOBSON, *University of Maryland.*

P33 The rates of cellulose digestion in vitro using different sources of cellulose. R. L. SALSBURY, C. K. SMITH, and C. F. HUFFMAN, *Michigan State College.*

P34 Cellulolytic activity of bovine rumen liquid upon a soluble cellulose derivative. J. L. CASON and W. E. THOMAS, *North Carolina State College.*

Wednesday, June 22

8:00–11:00 A.M.

SECTION A. **Endocrine Physiology**
R. E. ERB, *Chairman*
Auditorium, Kellogg Center

P35 Experimental udder growth and lactation in infertile dairy heifers. H. YAMAMOTO and C. W. TURNER, *University of Missouri.*

P36 Mammary gland development in heifer calves. R. P. REECE, *New Jersey Agricultural Experiment Station.*

P37 The effects of the hormones progesterone and estrogen in initiating lactation in dairy cows. RALPH WILLIAMS, O. A. CHILDS, and DAN SMITH, *Southern State College,* and C. W. TURNER, *University of Missouri.*

P38 Milk production of first-calf heifers following prepartal administration of growth hormone. A. C. CHUNG, *University of Maryland.*

P39 The effect of various hormones upon udder development and milk secretion in dairy cattle. H. L. DALTON, *University of Georgia.*

P40 The association of lactogenic hormone labeled with radioactive iodine with the cytoplasmic nucleoprotein of the mammary gland cell. W. F. WILLIAMS and C. W. TURNER, *University of Missouri.*

P41 Effects of cortisone, hydrocortisone, and ACTH on mammary growth and pituitary prolactin content of rats. R. M. JOHNSON and JOSEPH MEITES, *Michigan State College.*

P42 Estimation of the L-thyroxine secretion rate of dairy animals. G. W. PIPES and H. RUPPERT, *University of Missouri.*

P43 Thyroxine and iodine determination in the bovine using a paper chromatography-photoelectric colorimetry technique. J. F. LONG, J. W. HIBBS, and L. O. GILMORE, *Ohio Agricultural Experiment Station, Wooster.*

P44 Eosinophil count and glucose level in blood of dairy cattle during the first pregnancy. A. B. SCHULTZE, *University of Nebraska.*

P45 Interrelationships among plasma 17-hydroxycorticosteroid levels, plasma protein-bound iodine levels, and ketosis in dairy cattle. W. G. ROBERTSON, H. D. LENNON, JR., and J. P. MIXNER, *New Jersey Agricultural Experiment Station, Sussex.*

P46 Additional studies on the etiology and treatment of bovine ketosis, including an evaluation of metacortisone and fluorocortisone. J. C. SHAW, A. C. CHUNG, and R. A. GESSERT, *University of Maryland.*

P47 The effect of synthalin A on blood glucose levels of dairy calves. C. R. RICHARDS and H. G. WEAVER, *University of Delaware.*

P48 The effect of cortisone on the semen of a normal bull. P. T. CUPPS, R. C. LABEN, and S. W. MEAD, *University of California.*

8:00–11:00 A.M.

SECTION B. **Management and Forage Utilization**
N. P. RALSTON, *Chairman*
Ballroom, Kellogg Center

P49 Response of herd infections of mastitis to various incident factors and therapeutic treatments. L. A. BURKEY and CECELIA R. BOUMA, *Dairy Husbandry Research Branch, USDA.*

P50 How hay feeding to cows on pasture affected their milk production, dry matter intake, and body weight. D. M. SEATH, C. A. LASSITER, CARL DAVIS, J. W. RUST, and MAURICE COLE, *Kentucky Agricultural Experiment Station.*

P51 The utilization of ammonium nitrate by bromegrass pastures. I. L. HATHAWAY, *University of Nebraska.*

P52 Performance of dairy cows on immature oat forage. G. E. HAWKINS, JR., and K. M. AUTREY, *Alabama Polytechnic Institute.*

P53 Influence of grazing on persistency of ladino clover. J. W. COBBLE, I. H. STUCKEY, and B. W. HENDERSON, JR., *Rhode Island Agricultural Experiment Station.*

P54 Comparison of alfalfa hay and wilted alfalfa silage as a roughage for growing dairy heifers. J. F. SYKES, L. A. MOORE, and H. T. CONVERSE, *Dairy Husbandry Research Branch, USDA.*

P55 A comparison of results obtained by different measures used in studying temporary summer pastures. L. M. UNDERWOOD, W. J. MILLER, and T. H. ROGERS, *University of Georgia.*

P56 Effects of various hay: concentrate ratios on production responses and nutrient utilization of dairy cows. S. BLOOM, N. L. JACOBSON, L. D. MCGILLIARD, E. O. HEADY, and P. G. HOMEYER, *Iowa State College.*

P57 Comparative grazing performance of purebred Brown Swiss, Brown Swiss × Red Sindhi crossbreds and Jersey cows under Louisiana conditions. E. J. STONE and D. M. JOHNS, *Louisiana Agricultural Experiment Station.*

P58 A comparison of an immature corn silage with alfalfa hay as the forage for dairy cows. E. A. KEYES and E. P. SMITH, *Montana Agricultural Experiment Station.*

P59 Self-feeding of Bermuda grass hay-grain mixtures to lactating cows. F. N. BAKER, *North Louisiana Hill Farm Experiment Station.*

P60 The effect of nursing calves on milk production of identical twin heifers. E. W. SWANSON, *University of Tennessee.*

P61 A sanitary study of detergents and detergent-sanitizers used in the circulation cleaning-in-place of a pipeline milker installation in a stanchion dairy barn. J. B. LINDAMOOD, E. J. FINNEGAN, and G. C. GRAF, *Virginia Polytechnic Institute.*

P62 Normal variations in rate of milking. W. E. STEWART and L. H. SCHULTZ, *Cornell University.*

11:00 A.M.–NOON

PRODUCTION SECTION BUSINESS MEETING
R. E. ERB, *Chairman*
Auditorium, Kellogg Center

1:15–3:20 P.M.

SECTION A. **Genetics**

N. P. RALSTON, *Chairman*

Auditorium, Kellogg Center

P63 Preliminary report comparing cellular antigens with type defects in dairy cattle. P. G. NAIR, T. M. LUDWICK, E. J. LAZEAR, and L. C. FERGUSON, *The Ohio State University.*

P64 Estimated changes in the environment and average real producing ability in a Holstein herd from 1901 through 1954. W. M. DILLON, JR., W. W. YAPP, and R. W. TOUCHBERRY, *University of Illinois.*

P65 The relationships between heritability and twin efficiency values calculated from twin uniformity trials. R. LAIRD and L. O. GILMORE, *Ohio Agricultural Experiment Station, Wooster.*

P66 Physical changes in young dairy heifers as indicated by type evaluation studies. C. M. CLIFTON and F. ELY, *The Ohio State University.*

P67 A type and production study of Holstein-Friesian cattle in Canada. J. B. STONE, J. C. RENNIE, and G. E. RAITHBY, *Ontario Agricultural College.*

P68 An analysis of the components of type of Holstein-Friesian cows in Canada. J. C. RENNIE and G. E. RAITHBY, *Ontario Agricultural College.*

P69 An evaluation of the American Jersey Cattle Club star bull program. W. J. BRAKEL, *The Ohio State University.*

P70 Udder palpation in heifers as a basis for estimating their milk yield as cows. W. W. YAPP, W. M. DILLON, JR., and W. R. SMITH, *University of Illinois.*

P71 Phenotypic relationships among climatic conditions and performance of lactating cows. J. E. JOHNSTON, G. D. MILLER, J. B. FRYE, JR., and J. J. VIZINAT, *Louisiana Agricultural Experiment Station.*

1:15–3:20 P.M.

SECTION B. JOINT SESSION WITH EXTENSION SECTION

Symposium: Ratios of Forages and Grain in the Milking Ration

R. E. ERB and E. T. ITSCHNER, *Co-Chairmen*

Ballroom, Kellogg Center

3:30–4:30 P.M.

JOINT SESSION WITH EXTENSION SECTION

Committee Reports

Auditorium, Kellogg Center

Dairy Cattle Health. G. H. WISE, *North Carolina State College*

Dairy Cattle Breeding. R. A. CORBETT, *University of Maine*

Dairy Cattle Type. HILTON BOYNTON, *University of New Hampshire*

Breeds Relations. G. H. BECK, *University of Maryland*

Thursday, June 23

8:00–10:00 A.M.

SECTION A. **Milk Secretion**
R. E. ERB, *Chairman*
Auditorium, Kellogg Center

P72 Effect of heavy concentrate feeding prior to calving upon lactation and upon mammary edema. K. E. GARDNER and J. F. D. GREENHALGH, *University of Illinois.*

P73 The resistance of milk samples from cows on silage to the action of *S. agalactiae.* W. D. POUNDEN, NORMA A. FRANK, R. W. BROWN, and R. K. SCHERER, *Ohio Agricultural Experiment Station.*

P74 A comparison of milk yields and estimated secretion rates when between-milking intervals were varied. J. D. DONKER and H. L. DALTON, *University of Georgia.*

P75 "Subnormal milk"—its production and correction. R. B. BECKER, P. T. DIX ARNOLD, J. M. WING, JACK MCCALL, and G. K. DAVIS, *Florida Agricultural Experiment Station.*

P76 Bromide content of milk when cows are fed forage grown on ethylene dibromide-treated soil. R. W. YOUNG, L. I. MILLER, W. A. HARDISON, and R. W. ENGEL, *Virginia Agricultural Experiment Station.*

P77 The secretion of I^{131} in milk. F. W. LENGEMANN, R. A. MONROE, and E. W. SWANSON, *UT-AEC Agricultural Research Program.*

P78 The acid-soluble nucleotide content of mammary gland and of tissues from animals fed galactose. R. G. HANSEN and R. A. FREEDLAND, *University of Illinois.*

P79 Milk protein production in the bovine. B. L. LARSON, G. D. ROLLERI, and K. A. KENDALL, *University of Illinois.*

P80 The fat and solids-not-fat content of milk of individual cows. T. N. COMBS, P. M. REAVES, and G. C. GRAF, *Virginia Polytechnic Institute.*

8:00–10:00 A.M.

SECTION B. **Calf Nutrition**
N. P. RALSTON, *Chairman*
Ballroom, Kellogg Center

P81 A preliminary report on the optimum protein level of calf starters. L. D. BROWN, C. A. LASSITER, and J. W. RUST, *University of Kentucky.*

P82 Interrelationships between carotene from artificially dehydrated alfalfa and vitamin A from a dry carrier when fed simultaneously to Holstein calves. K. L. DOLGE, J. E. ROUSSEAU, JR., R. TEICHMAN, H. D. EATON, and G. BEALL, *Connecticut Agricultural Experiment Station.*

P83 Plasma tocopherol levels of dairy animals receivign different diets. J. W. THOMAS and M. OKAMOTO, *Dairy Husbandry Research Branch, USDA.*

P84 Study of pregastric esterase observed in the alimentary tract of the calf. H. A. RAMSEY, G. H. WISE, and S. B. TOVE, *North Carolina State College.*

P85 The use of antipyrine in aureomycin-fed dairy calves. D. L. MACFADDEN, and C. R. RICHARDS, *University of Delaware.*

P86 Effect of chlortetracycline (aureomycin) on young dairy calves in a new environment. F. T. LANDAGORA, L. L. RUSOFF, and R. M. CROWN, *Louisiana State University.*

P87 Interaction of para-amino salycilic acid and aureomycin in the feed of young calves. J. M. WING, *Florida Agricultural Experiment Station.*

P88 Effect of feeding high levels of chlortetracycline (aureomycin) and tetracycline (achromycin) to newborn calves. L. L. RUSOFF, E. J. STONE, A. H. CUMMINGS, and J. B. FRYE, JR., *Louisiana State University.*

P89 A comparison of feeding methods as they affect veal production and carcass quality. R. P. NIEDERMEIER, N. N. ALLEN, and R. W. BRAY, *University of Wisconsin.*

10:00 A.M.–NOON

GENERAL ASSOCIATION BUSINESS MEETING
L. A. MOORE, *Chairman*
Lower Lounge, Brody Hall

1:45–4:45 P.M.

JOINT SESSION—
MANUFACTURING, PRODUCTION, AND EXTENSION SECTIONS

Symposium: Dairy Products in Human Nutrition
G. H. HARTMAN, *presiding*
D. H. JACOBSEN, *Chairman, American Dairy Association, Chicago*
Lower Lounge, Brody Hall

Dairy products in our food economy. H. F. DEGRAFF, *Cornell University*

Milk in adult diets. MARGARET OHLSON, *Michigan State College*

Milk in infant diets. D. B. COURSIN, *St. Joseph's Hospital, Lancaster, Pa.*

Milk proteins in human nutrition. C. A. ELVEHJEM, *University of Wisconsin*

Milk fat in human nutrition. F. A. KUMMEROW, *University of Illinois*

Nutrition in relation to dairy science. ZOE ANDERSON, *National Dairy Council, Chicago*

1:15–2:45 P.M.

SECTION A. JOINT SESSION WITH EXTENSION SECTION

E. T. ITSCHNER, *Chairman*
Auditorium, Kellogg Center

Reports of Progress—Regional Dairy Cattle Breeding Projects:

Southern—J. E. LEGATES, *North Carolina State College.*
Northeastern—J. O. ALMQUIST, *Pennsylvania State University.*
North Central—W. J. TYLER, *University of West Virginia.*
Western—R. E. ERB, *State College of Washington.*

2:50–4:30 P.M.

SECTION A. JOINT SESSION WITH EXTENSION SECTION

Frozen Semen

N. P. RALSTON, *Chairman*
Auditorium, Kellogg Center

P90 Results obtained through the use of frozen bovine semen in field trials. J. W. SNYDER, W. D. RUTZ, and G. B. MARION, *Kansas State College.*

P91 Equipment for technicians' storage and field handling of frozen semen. H. H. BRUGMAN and M. E. POORE, *University of Maine.*

P92 Variation in survival of bovine spermatozoa when stored at subzero temperatures as affected by differences in breeds, bulls, and ejaculates. M. E. POORE and H. H. BRUGMAN, *University of Maine.*

P93 The effect of glycerol equilibration time on the freezing of bovine spermatozoa in egg yolk–citrate and skimmilk semen diluters. G. D. O'DELL and VICTOR HURST, *Clemson Agricultural College.*

P94 The influence of added lipoprotein on the freezing of bovine spermatozoa. G. BIALY and E. A. HESS, *The Ohio State University.*
Discussion and Summary on Field Use of Frozen Semen. N. L. VANDEMARK, *University of Illinois.*

1:15–4:30 P.M.

SECTION B. **Nutrition**

G. W. TRIMBERGER, *Chairman*
Ballroom, Kellogg Center

P95 The relation of carotene intake to the carotene and vitamin A values of the plasma, liver, and milk fat of dairy cattle. I. R. JONES, P. H. WESWIG, J. F. BONE, and B. F. MAGILL, *Oregon State College.*

P96 Effect of previous ration on lactation response to substitution of corn for alfalfa according to net energy and total digestible nutrients systems of estimation. R. TEICHMAN, H. D. EATON, G. BEALL, and R. E. JOHNSON, *Connecticut Agricultural Experiment Station;* H. L. LUCAS, JR., *North Carolina State College;* and L. A. MOORE, *Dairy Husbandry Research Branch, USDA.*

P97 Urea versus cottonseed meal as the chief source of protein for young dairy steers, with a study of methods used to determine tthe efficiency of urea utilization. I. LEVY and J. D. DONKER, *University of Georgia.*

P98 Comparison of cottonseed meal, molasses containing urea, and ammoniated molasses as protein supplement for dairy heifers. W. A. KING, G. D. O'DELL, J. P. LAMASTER, and D. B. RODERICK, *Clemson Agricultural College.*

P99 Variability in the quality of untreated high moisture silages. C. H. GORDON, C. G. MELIN, H. M. IRVIN, and H. G. WISEMAN, *Dairy Husbandry Research Branch,* and L. E. CAMPBELL, *Agricultural Engineering Research Branch, USDA.*

P100 Effects of sodium metabisulfite and degree of compaction on composition of legume-grass silage in miniature silos. R. S. ALLEN and RUTH M. WARD, *Iowa State College.*

P101 Effect of herbicides on in vitro cellulose digestion by rumen microorganisms. R. G. JENSEN and C. P. MERILAN, *University of Missouri.*

P102 The influence of the lignin content of long hay on the passage of nutrients through the rumen. O. T. STALLCUP, J. L. CASON, and B. J. WALKER, *Arkansas Agricultural Experiment Station.*

P103 The effect of light on optical density of extracts of fecal pigments in digestibility studies. E. A. KANE and W. C. JACOBSON, *Dairy Husbandry Research Branch, USDA.*

P104 Digestibility of certain carbohydrate fractions of forages by ruminants. R. E. ELY and L. A. MOORE, *Dairy Husbandry Research Branch, USDA.*

P105 Value of identical twin cattle in digestibility studies. CLARENCE CHESNUTT, JR., and I. R. JONES, *Oregon State College.*

P106 Effect of feeding various levels of fluorine, calcium, phosphorus, and grain to dairy heifers from four months to thirty-two months of age. G. E. STODDARD, J. L. SHUPE, M. L. MINER, L. E. HARRIS, D. A. GREENWOOD, W. BINNS, G. Q. BATEMAN, H. NIELSON, and D. STRONG, *Utah State Agricultural College.*

P107 The effect of added calcium and trace elements on mineral balance in dairy cows in early lactation. R. E. MATHER, *New Jersey Agricultural Experiment Station, Sussex.*

P108 Trace mineral deficiencies in cattle resulting from heavy fertilization of the soil. H. A. KEENER, F. E. ALLEN, H. A. DAVIS, *New Hampshire Agricultural Experiment Station*, K. C. BEESON and E. J. THACKER, *U. S. Plant, Soil and Nutrition Laboratory, Ithaca, N. Y.*

P109 The effect of pulverized limestone and dicalcium phosphate on the nutritive value of dairy cattle feed. N. F. COLOVOS, H. A. KEENER, and H. A. DAVIS, *New Hampshire Agricultural Experiment Station.*

EXTENSION SECTION

Monday, June 20

8:00 P.M.

Progress Report and Discussion: Handling D.H.I.A. Records by Modern Machines. J. F. KENDRICK, *Dairy Herd Improvement Section, USDA.*

Recreation Room, Brody Hall

Tuesday, June 21

1:30–4:30 P.M.

Opening Business Session, Teaching Methods, and Exhibits
E. T. ITSCHNER, *Chairman*
Recreation Room, Brody Hall

E1 Effective techniques in extension teaching. K. F. WARNER, *Personnel Training Branch, USDA.*

E2 Television for dairy extension work. G. H. AXINN, *Michigan State College.*

E3 Role of dairy extension activities in the farm and home development program. J. E. CROSBY, JR., *Division of Agricultural Programs, USDA.*

Presentation and Discussion of Exhibits
C. W. REAVES, *Chairman-in-charge*

Wednesday, June 22

8:00–9:00 A.M.

Grassland Farming
Ballroom, Kellogg Center
See Production Section B, page 566, for papers.

9:00–11:00 A.M.

Dairy Records
GEORGE WERNER, *presiding*
Recreation Room, Brody Hall

E4 Using D.H.I.A. records as an effective teaching tool.
Moderator, J. D. BURKE, *Cornell University.*
Panel members:
H. G. GILMORE, *The Pennsylvania State University*
C. H. PARSONS, *University of Massachusetts*
D. E. VOELKER, *Iowa State College*

E5 Standardized procedures for the Babcock test for milk. E. O. HERREID, *University of Illinois.*

E6 Detergent mixtures for testing fresh milk samples. E. O. HERREID, *University of Illinois.*

E7 Comparison of the B.D.I. detergent test with the Babcock test under herd conditions. FRED GRANT, *Dairy Husbandry Research Branch, USDA.*
Discussion

11:00 A.M.–NOON

EXTENSION SECTION BUSINESS MEETING
Committee Reports
E. T. ITSCHNER, *Chairman*
Recreation Room, Brody Hall

1:15–3:20 P.M.

JOINT SESSION WITH PRODUCTION SECTION B
Symposium: Ratios of Forages and Grain in the Milking Ration
R. E. ERB and E. T. ITSCHNER, *Co-Chairmen*
Ballroom, Kellogg Center

Grain feeding and roughage consumption in relation to input-output considerations. J. T. REID, *Cornell University.*
Forage production and utilization in feeding dairy cattle. R. E. HODGSON, *Dairy Research Branch, USDA.*
Economics in producing and feeding roughage and grain to dairy cattle. C. R. HOGLUND, *Michigan State College and Production Economics Research Branch, USDA.*

3:30–4:30 P.M.

JOINT SESSION WITH PRODUCTION SECTION
Joint Committee Reports
Auditorium, Kellogg Center

Dairy Cattle Health. G. H. WISE, *North Carolina State College*
Dairy Cattle Breeding. R. A. CORBETT, *University of Maine*
Dairy Cattle Type. HILTON BOYNTON, *University of New Hampshire*
Breeds Relations. G. H. BECK, *University of Maryland*

Thursday, June 23

8:00–10:00 A.M.

Dairy Cattle Health Problems
4-H Club Work
GEORGE WERNER, *presiding*
Recreation Room, Brody Hall

E8 Dairy cattle health problems. B. T. SIMMS, *Animal Disease and Parasite Research Branch, USDA.*

E9 Enlisting, developing, and using 4-H Club leaders. P. A. MILLER, *Director of Extension, Michigan State College.*

Panel Discussion: Enlisting, developing and using 4-H Club Leaders.
Moderator: GEORGE HYATT, *North Carolina State College*
Panel members:

IRVING WYETH, *County Agricultural Agent, St. Clair County, Michigan.*
A Michigan local leader.
R. D. STEWART, *American Guernsey Cattle Club.*
NEVELS PEARSON, *Assistant State 4-H Leader, Michigan State College.*
J. D. GEORGE, *North Carolina State College.*
E. R. BONEWITZ, *Kansas State College.*

10:00 A.M.–NOON

GENERAL ASSOCIATION BUSINESS MEETING
Lower Lounge, Brody Hall

1:15–4:45 P.M.

JOINT SESSION—MANUFACTURING, PRODUCTION, AND EXTENSION SECTIONS

Symposium: Dairy Products in Human Nutrition

G. H. HARTMAN, *presiding*
D. H. JACOBSEN, *Chairman, American Dairy Association, Chicago*
Lower Lounge, Brody Hall

Dairy products in our food economy. H. F. DEGRAFF, *Cornell University*

Milk in adult diets. MARGARET OHLSON, *Michigan State College*

Milk in infant diets. D. B. COURSIN, *St. Joseph's Hospital, Lancaster, Pa.*

Milk proteins in human nutrition. C. A. ELVEHJEM, *University of Wisconsin*

Milk fat in human nutrition. F. A. KUMMEROW, *University of Illinois*

Nutrition in relation to dairy science. ZOE ANDERSON, *National Dairy Council, Chicago*

1:15–2:45 P.M.

JOINT SESSION WITH PRODUCTION SECTION A

E. T. ITSCHNER, *Chairman*
Auditorium, Kellogg Center

Reports of Progress—Regional Dairy Cattle Breeding Projects:
Southern—J. E. LEGATES, *North Carolina State College*
Northeastern—J. O. ALMQUIST, *The Pennsylvania State University*
North Central—W. J. TYLER, *University of Wisconsin*
Western—R. E. ERB, *State College of Washington*

2:50–4:30 P.M.

JOINT SESSION WITH PRODUCTION SECTION A.

Frozen Semen

N. P. RALSTON, *Chairman*
Auditorium, Kellogg Center

PEOPLE and EVENTS
in the Dairy Science World

Pioneers in the Dairy Industry

High among the top-ranking pioneers in the agriculture of the world is HENRY HERBERT KILDEE, Dean Emeritus of Agriculture at Iowa State College. He was born on a farm in Iowa, March 4, 1884, and received his early education in a country school, later attending Cedar Valley Seminary and Iowa State College. He was a member of the Iowa State College livestock judging team that participated in the American Royal Livestock Show and Intern. Livestock Exposition judging contests in 1908. At the latter he was high man. As a senior student he was selected to serve as laboratory instructor in animal husbandry and soils. The B.S.A. degree was granted him by Iowa State College in 1908 and the M.S. degree in 1917. In 1940 the Doctor of Agriculture degree was awarded him by North Dakota State College.

H. H. Kildee

Immediately after receiving his first degree from Iowa State, Kildee joined the staff of the College and for 2 years worked in animal husbandry. In 1910 he was appointed professor of dairy husbandry and superintendent of the College dairy farm. During this period he became one of the foremost United States authorities on type in dairy breeds. He left Iowa State in 1916 to assume a professorship and to head the Division of Dairy Husbandry at the Univ. of Minnesota. He returned to Iowa 2 years later to head the work in animal, dairy, and poultry husbandry and has been on the staff ever since.

Professor Kildee was appointed vice-dean of agriculture in 1923 and dean in 1933, after the retirement of C. F. CURTISS. He became director of the extension service in agriculture and home economics in 1946 as the first step in the integration of activities in research, teaching, and extension. The consolidation was completed in 1948, when he became director of the Experiment Station. He retired in 1949.

Dean Kildee has judged all breeds of dairy cattle, beef cattle, hogs, and draft horses at leading shows throughout the country. He has done a great deal in formulating the conception of dairy type that is distinctly correlated with production. He aided in developing the Unified Dairy Cattle Score Card adopted by the Purebred Dairy Cattle Assoc. and the American Dairy Science Assoc.

In 1938 his portrait was hung in the gallery of leading livestock men in the famed Saddle and Sirloin Club at the Union Stockyards in Chicago. His portrait was the first to be placed in the newly organized Dairy Shrine Club at Waterloo, Iowa, in 1949.

Dean Kildee is a member of A.A.A.S., A.D.S.A., American Society of Animal Production (of which he is a past president), Sigma Xi, Alpha Zeta, Phi Kappa Phi, Gamma Sigma Delta (of which he is past national president) and the Saddle and Sirloin Club. In 1950 he was awarded a gold medal and citation for distinguished service to organized agriculture by the American Farm Bureau Federation; in 1952 he received the national Distinguished Service Award from Gamma Sigma Delta, a citation and plaque from the Iowa State Soil Conservation Committee for "outstanding leadership and service to soil conservation," and a Certificate of Honor citation from the American Guernsey Cattle Club.

Throughout his career Dean Kildee has maintained a great interest in the conservation of natural resources, along with his work in dairy and animal husbandry. In the latter field he has conducted research in breeding, feeding, and management of dairy cattle, swine, and beef cattle. He also has studied livestock production, especially of swine, and marketing in Continental Europe and Great Britain. He is the author of several bulletins on feeding, breeding, and management of livestock, as well as on land use and planning. He has served as a member of the Board of Trustees and of the Board of Deacons of the Congregational Church of Ames and has been a member of the Lay Committee of the National Council of Churches of Christ since 1950. He is a former member of the board of the Iowa Christian Rural Fellowship. He served as honorary chairman of the Iowa College Alumni Achievement Fund from 1951 to 1953.

The father of two married daughters, Dean Kildee with his wife lives on the Iowa State College campus, where he has the reputation of being a most likable person and one who makes friends wherever he goes. He has been an inspiration to thousands of students, friends, and acquaintances. He is truly a pioneer of the dairy industry and of all agriculture.

A. H. PORTER

Reproduction and Infertility Symposium to be Held

A symposium on reproduction and infertility, sponsored jointly by the Michigan State College School of Veterinary Medicine and the Michigan Agricultural Experiment Station, will be held at the Kellogg Center for Continuing Education, East Lansing, June 27-29. Major objectives will be the review and evaluation of recent progress in the field of reproduction and discussion of problems requiring further study. National authorities in their respective areas will present the formal papers on the program.

The session on June 27 will be devoted to problems of diagnosis of reproductive diseases and a survey of progress in the specific disease areas of vibriosis, leptospirosis, trichomoniasis, and brucellosis. On the second day five lectures will be presented on various aspects of the physiology and anatomy of male and female reproductive processes. In the evening a panel discussion will be held on ova transplantation. The final session will be devoted to papers on the endocrine control of reproduction in the male. The program has been scheduled to allow ample time for questions and discussion after each formal paper.

Arrangements have been made for lodging and meals for out-of-town guests at the Kellogg Center. Printed programs can be obtained by writing to E. P. REINEKE, symposium chairman, Dept. of Physiology and Pharmacology, Michigan State College.

Student Affiliate Chapters Scheduled for June Meeting

A Student Affiliate and Faculty Adviser meeting is scheduled for Wednesday, June 22, 1:15 p.m. in Room 103, Kellogg Center, Michigan State College, during the annual A.D.S.A. meeting. Student problems, objectives of student activities, and a program for the coming year will be among the topics discussed. All interested students and faculty members are invited to attend.

Special Breakfast Meetings to be Held

Three special breakfasts are scheduled for the annual meeting of A.D.S.A. in June as follows:

Eckles Club
Wed., June 22, 7:00 A.M.
Red Cedar Room, Kellogg Center
Reservations:
E. L. ANTHONY, School of Agriculture, Michigan State College, East Lansing

Michigan State College Breakfast
Thurs., June 23, 7:00 A.M.
Brody Hall Cafeteria
Reservations:
J. A. MEISER, Dairy Dept., Michigan State College, East Lansing

Breakfast honoring DR. and MRS. W. E. PETERSEN
Thurs., June 23, 7:00 A.M.
Red Cedar Room, Kellogg Center
Reservations:
J. D. DONKER, Dairy Dept., Univ. of Georgia, Athens

Ohio Legislative Committee Considers Important Milk Control Bill

A Milk Control Bill with possible far reaching effects is now being considered by a committee in the Ohio Legislature. The bill proposes that a three-member commission appointed by the governor be given the power to supervise, investigate, and regulate the production, transportation, disposal, manufacture, processing, storage, distribution, delivery, handling, bailment, brokerage, consignment, purchase, and sale of milk and milk products in Ohio. This includes establishment of reasonable trade practices, systems of production control, and marketing areas.

The commission and its employees would have the right to enter plants, inspect facilities, and audit all books and documents. The bill would require that all milk dealers be licensed to the commission, the fees to provide money for operating expenses. The commission could control the marketing area of any individual dealer. Manufacturers are not exempt from licensing requirements but are exempt from minimum producer price control.

The state would be divided into marketing areas, and the price of milk would be set in each area. Such prices would guarantee a profit to producers and dealers. Bottle deposits would be mandatory. Paper-glass differentials may be established. Costs would be determined by the use of a cross section representative of average producers and dealers. At its discretion, the commission would also set prices for skimmed milk, condensed or concentrated whole or skimmed milk, bulk cream, and ice cream mix. Outlawed would be discounts, premiums, rebates, free service, trading stamps, advertising allowance, extension of credit, or combination of milk and other items priced at less than normal. Penalties for violation would be provided.

Proponents of the bill have had a hearing before the committee considering the new legislation. A steady stream of proponents stressed the economic plight of Ohio's dairy farmers. A hearing for opponents of the bill was held April 19.

1955 International Dairy Products Judging Tourney Scheduled for St. Louis

The 34th Collegiate Students' Intern. Contest in Judging Dairy Products will be held in October in St. Louis, according to an announcement from American Dairy Science Assoc. and Dairy Industries Supply Assoc., cosponsors of the contest. Winning college teams and individuals will be announced at a gala awards presentation. Top prizes include graduate fellowships, worth $1,380, $1,280, and $1,180, awarded by DISA; and silver cups, and gold, silver, and bronze medals awarded by American Butter Inst., Intern. Assoc. of Ice Cream Mnfrs., Milk Industry Fdn., National Cheese Inst., and DISA.

Superintendent of the contest is C. J. BABCOCK, a USDA official. D. R. STROBEL, also of the USDA, serves as assistant superintendent. Product judges are H. L. WILSON, cheese; J. HOFFMAN ERB, ice cream; N. E. FABRICIUS, butter; and D. A. PETTEE, milk.

Citation Awarded Herzer by Southern Division of A.D.S.A.

FREDERICK HERMAN HERZER was born May 6, 1893, in Marion, Ohio, where he received his early education. He graduated from Ohio State Univ. with a B.S. degree in dairy technology in 1914 and received an M.S. degree from Iowa State College in 1935.

From his graduation through most of 1919 he was employed for short course instruction at Ohio State Univ., was instructor in dairying at the Univ. of Arkansas, enlisted for naval aviation training, and was employed by Nestles Food Company. In the fall of 1919 Herzer became associate professor in the Dairy Department of Mississippi State College and organized the dairy manufacturing curriculum. Since his appointment as head of the department in 1949 he has guided the reorganization of the production division, the expenditure of $350,000 for new buildings, and an expanded program in teaching and research.

F. H. Herzer

In the field of research his attention has been centered on such subjects as the effect of feeds on the fat constants; flavor and texture of cheese and butter; the vitamin A potency of Mississippi butter; weed flavors in dairy products; the effect of straining on the quality of manufactured milk products; and the chemical composition of milk.

Professor Herzer has established an outstanding record with the college dairy products judging team, which he coached for 17 national contests. In tabulating the placings of the various teams, Mississippi has the highest over-all record for a 21-year period, winning sweepstakes four times, and placing second four times. Twelve scholarships were won, another top record for the contest.

In 1938 he was elected vice-chairman and in 1939 became chairman of the Manufacturing Section of A.D.S.A. He has been a member of the Dairy Products Judging Committee of the Association since 1940. He served as secretary, 1942-46, and was chairman, 1948, of the Southern Section of A.D.S.A. He served 3 years on the National Feed Survey Committee of the American Feed Mnfrs. Assoc. He was instrumental in establishing Mississippi A.D.A. in 1946 and continues to serve as a director. Since 1944 Herzer has been secretary of the Mississippi Dairy Products Assoc. and was presented a Buick car in 1951 by this organization in appreciation of his service to the industry. He was appointed dairy products grader by the Agricultural Marketing Service in 1942 and through this medium has helped to improve the quality of Mississippi butter and cheese.

Professor Herzer is an Episcopalian, Mason, and Alpha Zeta and is a member of the Assoc. of Southern Agricultural Workers and the Mississippi Farm Bureau.

News from North Carolina State College

M. W. CARTER, graduate student in the Nutrition Section of the Animal Industry Dept., has been awarded a predoctoral fellowship by the National Science Foundation. Mr. Carter, who completed work for his M.S. degree in June, 1954, will continue work on estrogenic substances extracted from soybean meal and their effect upon growth and reproduction in animals.

DOAK FINCH, owner of Wheatmore Dairy Farms, Thomasville, has created a $2,000 "Talent for Service" scholarship to be awarded to a 4-H Club or F.F.A. boy who has exhibited outstanding leadership, scholarship, and character and has had at least 2 years of creditable work with a purebred Holstein heifer or cow as a 4-H Club or F.F.A. project. This scholarship will be awarded on the basis of $500 per year for a 4-year period.

Among the "Talent for Service" scholarships is one in honor of A. C. KIMREY, retired extension dairy specialist, established by his sons. The scholarship award will be given annually to an outstanding 4-H Club boy who must enroll in agriculture at N. C. State College. The first recipient is WILLIAM J. LINDLEY, JR., Snow Camp, N. C. He was a member of the state 4-H Dairy Cattle Judging Team in 1954 and had an outstanding record in 4-H activities.

Louisiana Milk Plant Dedicated

The dedication of a $500,000 milk processing plant at Franklinton, La., was held April 6. A large crowd was present, and after a free barbecue at noon conducted tours were made through the plant. Speakers on the program included the Mississippi secretary of state, the mayor of New Orleans, and the Louisiana commissioner of agriculture.

The plant, which is now handling about 260,000 lb. daily, should be able to provide a market for the surplus fluid milk in the New Orleans area. This is the first approved project built under the Louisiana Industry Inducement Act.

Bulk Milk Handling Conference Held at Illinois

A total of 104 representatives of industry attended the 3-day conference on bulk milk handling held April 12-14 at the Univ. of Illinois. B. L. HERRINGTON of Cornell Univ. was the speaker at the banquet. He discussed the relation of pipe line milking and bulk handling of milk to the development of rancidity. University staff members and technical experts from commercial companies and health regulatory groups served as discussion leaders for the four panel sessions, which were operated simultaneously.

Dairy Technology Activities at Ohio State

To support the efforts of the Dept. of Dairy Technology to increase enrollment and to serve otherwise in an advisory capacity to the Department, the Ohio Dairy Products Assoc. has appointed an Educational Committee with members from various sections of the state and under the chairmanship of CARL BROUGHTON, president, Broughtons Farm Dairy, Marietta. A similar committee has been organized by the Ohio Dairy Boosters Assoc. under the chairmanship of LESTER DRUSENDAHL, Drusendahl Sales Co., Cleveland. This association has made available funds for supporting the high school recruitment program. A joint meeting of the two committees was held in Columbus recently to lay plans for a state-wide program aimed at attracting larger numbers of superior high school graduates into the field of dairy technology.

A series of dinner meetings has been held in the state at which high school principals and counselors were guests of the local dairy industry and at which staff members from the Dept. of Dairy Technology described the opportunities in this field of work.

I. A. GOULD has been asked to serve as a member of a newly established committee on Milk, Food, and Nutrition of the National Research Council and attended the first meeting in Chicago on March 31.

Pennsalt Appoints Representatives

R. E. WILSON was recently appointed resident sales and service representative for the Pennsylvania Salt Mfg. Co. He will have charge of the company's Florida territory with headquarters in Orlando. Mr. Wilson attended the Univ. of Florida. He served in the navy during World War II and prior to joining Pennsalt was manager of Miller Machinery & Supply Company's Tallahassee branch.

R. J. EGGERT has been named regional sales supervisor for the company's south-central territory. Memphis will be his headquarters. Mr. Eggert, a native of Ohio, joined the Pennsalt Co. in 1953. He attended Fenn College in Cleveland and served as an infantry officer during the war.

Minnesota News

W. E. PETERSEN left March 30 for a 2½ month trip to New Zealand and Australia. He will confer with research workers in those countries and will lecture to farmer and scientific groups.

A. L. BRUNDAGE, after completing the requirements for the Ph.D. degree, has returned to the Alaska Agricultural Experiment Station, Palmer, where he is dairy husbandman.

C. B. A. Bryant Retires

After 25 years of service to the dairy industry C. B. A. (BILL) BRYANT, field service director of Dairy Filters Dept., Johnson and Johnson, has retired. In announcing Mr. Bryant's retirement Feb. 15, G. W. WILLITS, general manager of Filter Products Division, recalls a few of the many highlights of Bill's illustrious career: his "Never-Be-Broke" Club; his numerous writings on dairy subjects; his amateur color movie travelogues; his 25th anniversary party, at which time he received a gold watch.

Bill has staged a wonderful come-back after a recent illness and will be available, after his retirement, as a consultant on matters pertaining to quality milk production at the farm level and for a limited number of speaking engagements.

Brown Swiss Cattle Project in Iran

Brown Swiss cattle from more than 40 American dairy herds are helping Iran in one of the most rapid dairy improvement programs ever attempted by an entire nation. The cattle, many raised by 4-H boys and girls, were furnished to Iran by Heifer Project, Inc., an association of

American church groups interested in foreign assistance, and the program was developed by the U. S. Foreign Operations Mission to Iran in cooperation with Iran's Ministry of Agriculture.

W. E. WARNE, director of the mission to Iran, says that whereas Iranian native cattle produce only about 1,400 lb. of milk a year, the offspring from a cross with Brown Swiss are averaging more than 5,000 lb. Mr. Warne states that this is only a part of the results that may come from American aid to Iranian farmers. He says: "On top of this is the great contribution to a friendly nation that will come from greater health, vigor, and vitality of an Iranian people when they are able to have the dairy products necessary for good health."

Live-stock stations with small foundation herds have been established in Iran's various provinces. Working out of these stations are trained inseminators who used bicycles and motorcycles to answer the calls from dairy cattle owners. There are now more than 80 purebred Brown Swiss bulls and bull calves in Iran and more than 50 purebred females in the foundation herds. The natural increase from these animals will be put into the program as they mature each year, and with the growth of the artificial insemination program the Iran Livestock Bureau expects that in the next 5 years one-fourth of the dairy cattle will be at least half Brown Swiss and producing an average of four times as much milk per cow as the animals they have replaced.

1956 Short Course Changed at MSC

The winter dairy manufacturers short course at Michigan State College will consist of four 2-week training sessions beginning in 1956. The schedule will be as follows: Jan. 9-20, market milk; Jan. 23-Feb. 3, laboratory methods; Feb. 6-17, ice cream; Feb. 20-Mar. 3, cottage cheese, cultures, and fermented milks. Enrollment may be for any one or more of the 2-week courses.

Wilster to Spend Year in Denmark

G. H. WILSTER, professor of dairying, has been awarded an educational exchange grant under the Fulbright act to be lecturer in dairy science in Denmark for the coming academic year. He will be affiliated with the Royal Agricultural and Veterinary College at Copenhagen during his stay in that country. Dr. and Mrs. Wilster plan to leave Oregon July 1, 1955, and embark for Denmark August 1. They will return to Oregon June 30, 1956.

The Royal Agriculture College and the Danish Dairy Technical Society asked Dr. Wilster to apply for the award and were instrumental in his receiving the grant.

A sales promotional campaign is being planned for Oregon to reacquaint the public with the economical food value of dairy products. Spearheading the promotion program will be the Oregon Dairy Products Commission, Oregon Dairy Council, and the Oregon State College Extension Marketing Specialists. The campaign is being initiated because of a 16% milk price drop from October to February, combined with a 2% increase in cost of production.

BEN SIMONSON, manager of the Oregon Dairy Breeders Assoc. for 6½ years, has recently resigned. He will become sire analyst and office manager for Roger Jessup Farms Artificial Breeding Service in California. FRED ROBERTS, former farm and laboratory supervisor, has taken over as acting manager for the Oregon group.

Washington State Meeting Attracts 200

Approximately 200 representatives of the dairy industry from the Pacific Coast, Canada, Alaska, and the Midwest completed a session of lectures, demonstrations, panel discussions, product judging, conducted tours, and entertainment features at the 24th annual Washington State College Institute of Dairying, March 7-11.

The program included discussion of the operation of federal milk-marketing orders; advancements in detergents and sanitizers; work simplification; cause and prevention of milk flavor defects, especially in connection with modern bulk handling methods; sanitary standards; waste disposal; and the importance of psychrophils.

Completed Theses

M.S. Degree:

H. E. STRUSS—A survey of the utilization of uniform labeled C^{14} glucose in the production of lactose by the perfused bovine mammary gland. Univ. of Minnesota.

ROBERT LAIRD—A study of the influence of inheritance and environment on the growth of young heifers. The Ohio State Univ.

Ph.D. Degree:

E. F. GRAHAM—The effect of different planes of nutrition and frequencies of collection on bovine semen production. Univ. of Minnesota.

S. H. LOMBARD—Investigations of the stale flavor in sterile and dried whole milk. Univ. of Illinois.

Do Dairy Students Understand Dairy Arithmetic?

A Guest Editorial

When commercial dairy manufacturing groups are asked to suggest changes which, in their opinion, would improve the dairy manufacturing curriculum of a college, somewhat standard replies are received. One suggestion is that business administration be added along with the technical courses, and, in particular, that specialized bookkeeping procedures be given, so that profit and loss statements on many highly individualized items may be secured. As an illustration, the management of an ice cream plant making specialties could well use information on the cost, profits, and losses experienced in the making of tarts or popsicles or fancy molds; a milk plant needs information concerning profits and losses on chocolate milk and by-products. Most types of bookkeeping show the profits or losses on an over-all operation.

P. S. Lucas

A second criticism is one that concerns the lack of engineering training. This lack has been remedied to a great extent in several of the larger universities. In the past, dairy mechanics or engineering has been concerned chiefly with the study of ice machines, but it could just as well be concerned with the entire dairy engineering field.

A third criticism is scarcely, if ever, mentioned and yet it is painfully evident to any teacher of dairy plant management or to any employer who questions his recently hired college graduate. This is concerned with the new employee's ability to apply the principles of dairy arithmetic. A recent problem, which was very simple in itself, but which, when given to a class of seniors, few were able to solve, simply asked the pounds of milk that a plant would require in a day to put out a certain number of quarts of milk, chocolate milk, whipping and coffee cream, condensed milk, and ice cream. The same confusion is experienced when a student is attempting to apply the principles of proportion, which, supposedly, he learned in the fourth or fifth grades of common school. The average student finds it difficult to use his imagination in reasoning out the intricacies of standardizing an ice cream mix. The very basis of good management lies in the adept use of the tip of a pencil. The manager may be throwing away thousands of dollars each year by not figuring sharply. The day has passed when carelessly compiled cost figures are acceptable.

The manager of the moderate sized creamery may feel that he can hire a trained man to do the necessary figuring which absolves him of the need of understanding all the technicalities involved. Therein lies an advantage for the college graduate: If he understands the mathematics involved in the dairy manufacturing industry he has one more arrow in his quiver.

Where and how this information should be given to the student is a matter that each department must decide. The problems of standardizing milk for fat and solids so as to meet the standards set for condensed and sweetened condensed milk can be taught in a course on condensed milk. Probably ice cream standardization should be taught in the ice cream course. Seldom does one find a student starting a cheese course who knows how to reduce milk with skimmilk in such manner that it will meet the fat as well as the fat-free solids standards required for legal cheese. Surely, if a student aspires to a managerial job he ought to understand also how to calculate the value of overrun in either butter or ice cream.

Although students can be counted on to perpetrate many surprises for their instructors, it is a safe prediction that many a conscientious instructor will be surprised, if he will take the time, to learn how confused a student may be in trying to solve many of the simple mathematical problems that commonly occur in the operation of the dairy plant. Dairy arithmetic merits more attention than it has been getting.

P. S. Lucas
Dairy Department
Michigan State College

OUR INDUSTRY TODAY

Brief Reviews of Current Topics

Ingredients for Successful Dairy Farming[1]

How Henry Beland, A Young Michigan Farmer, Achieved Success

C. R. HOGLUND

Production Economics Research Branch, Agricultural Research Service, USDA, and Michigan State College

Modern dairy farming is becoming a highly commercialized, skilled business which requires a large capital investment. Returns are seldom phenomenal and they are often discouraging. Many farmers have found it a highly satisfying, profitable venture; others have barely made a go of it. The more successful dairymen earn as much as $1.50 to $2.00 per hour for their labor and management. Others receive as little as $0.30 per hour.

Dairymen Must Consider Many Factors

What are the essential ingredients for success in dairy farming? How should these ingredients (success factors) be combined to yield the greatest success? These are two important questions faced by prospective dairy farmers. At least five ingredients or factors are basic to success in dairy farming. The dairy farmer, like the good cook, must combine these ingredients in the right proportion. The more important success factors for dairy farming are as follows:

1. Adequate volume of business
2. Efficient cropping program
3. High producing livestock
4. Labor saving methods
5. Meaningful farm records

HENRY BELAND, JR., a 36-year-old Ionia County, Michigan dairyman, has combined these success ingredients in about the right proportion. An average of more than 360,000 lb. of milk annually from 30 cows is his record for the last 2 years. Milk production per worker is better than 200,000 lb. Beland had one of the lowest feed costs in producing 100 lb. of milk in a recent dairy cost study conducted by Michigan State College.

Beland Not Always Interested in Farming

Henry grew up as a farm boy but decided to leave the farm for city work when he was a high-school student. He attended business college for 1 year to prepare himself for what he thought was his life's work. It was near the end of his first year at college that an event occurred which permanently changed his viewpoint about farming. Because of poor health, his father asked him to help operate the dairy farm. Henry welcomed the opportunity to return to the farm as the glamour of city life was largely gone and real wages there were lower than he had expected.

Henry Beland entered into a partnership with his father in 1937 after completing the year of business training. Practically all of his earnings in the first 2 years went to pay for a share of his father's cattle and machinery. Later, he and his father went into debt to buy additional machinery.

In 1945 Henry bought the farm he is now operating. The same year he bought out his father's share in the livestock and machinery. The buildings and cropland on the 100 acres were badly run down. Eighty acres of land have been rented since 1946, and in 1952 he bought 17 more acres.

Beland has gone a long way in developing a successful dairy unit. He recalls that the gross income from an entire year's production when he and his father started out with 10 or 12 cows was very little higher than the value of a recent 2 weeks' milk production. He owned 16 cows when he moved on his present farm in 1945. Milk production since that date has more than doubled.

Large Milk Output Is Important for Efficient Production

An adequate volume of business is one of the chief factors that contribute to successful dairying. Only the dairy farmer with a large output can take full advantage of mechanization and other improved techniques that reduce costs. An annual production of at least 200,000 lb. of milk (3.5 test basis) appears to be a desirable goal.

How do typical dairymen score on volume of output? A detailed study was made in 1954 of the production practices used and the ad-

[1] The second of a series of success stories by the author to show that scientific dairy farming makes for better living.

justments made in the farm operations of 40 young dairy farmers in southern Michigan. Their milk production ranged from a low of 110,000 to a high of 374,000 lb. annually. About a third of these dairymen produced less than 200,000 lb. of milk annually. These were full-time dairy farmers who had operated their farms for about 10 years. They averaged 35 years in age.

A major problem of most beginning farmers is to acquire enough land to support a minimum-sized dairy herd. To produce 200,000 lb. of milk will require about 20 good cows. A dairyman who produces 3 tons of hay per acre will need about 60 acres of meadow to supply the hay and pasture for a 20-cow herd. If hay yields are only 2 tons per acre, 90 acres are needed. Additional cropland must be provided if corn silage and feed grains are to be produced.

Beginning farmers who are fortunate enough to start out as a father-son team, as Henry Beland did, usually attain the needed size of business sooner than young men who start out directly as tenants or owners. Not all beginning farmers can get started as a father-son partnership. However, most beginning farmers, regardless of how they get started, will need to invest their limited capital wisely if they are to attain the needed volume of business.

Building Up a Run-Down Farm

The Beland farm was so badly depleted that it was difficult to grow wheat the first few years. Yields of other crops were also low. Use of a good rotation and heavy applications of fertilizer and manure have made this one of the most productive farms in the community. About 400 lb. of high-analysis fertilizer are applied per acre on the wheat and oats. A heavy application of barnyard manure and 150 lb. 4-16-16 fertilizer are applied to the corn. A heavy green-manure crop is usually plowed down before the corn is planted. Two hundred pounds of 0-20-20 are applied each year to all of the meadows.

Henry's crop rotation is wheat seeded to sweet clover; sweet clover is plowed down for corn; oats, which follows the corn, are seeded to alfalfa, brome grass, and ladino clover and left for about 3 years. This brings the rotation back to wheat with the third-year alfalfa sod plowed down early. Beland has followed this 6-year rotation since 1948, the year he started cooperating with the Ionia Soil Conservation District.

Crop yields per acre in 1954 were as follows: corn—86 bushels, wheat—45 bushels, oats—55 bushels, corn silage—15 tons, and alfalfa-brome hay—more than 4 tons. Beland expects to increase crop yields still more by putting in additional tile lines in some poorly drained areas.

Beland has achieved low feed costs by making liberal use of high-quality meadows. He makes it a year-to-year practice to harvest a ton of grass silage for each cow, to supplement pastures in late July and August. Two silos are filled with corn for winter feeding. All of the hay is baled. Investment in forage-harvesting equipment is kept at a minimum by custom-hiring the chopping of the grass and corn silage.

Building Up a High-Producing Herd

Henry Beland has been successful in improving the productive capacity of his cows along with the increases in crop yields. A production average of about 12,400 lb. per cow the last 2 years is evidence of good management. Henry attributes his high production average to the use of improved sires, a culling program based on D.H.I.A. records and study of individual cows, and use of management practices that lengthen the productive years of the better cows.

FIG. 1. High-producing cows and good quality roughage result in lower milk production costs on the Beland farm.

Herd sires have been purchased from pure-bred breeders that have achieved high production records under ordinary farm conditions. Beland likes to select herd sires from cow families with uniformly high production records. He has made only limited use of artificial breeding methods, although some of his higher producers are a result of this service.

All Phases of Production Must Be Efficient

Adoption of improved crop and pasture practices is basic to more efficient production in all phases of dairy farming. These practices include better drainage and the use of improved rotations, adequate quantities of lime and fertilizer, improved forage harvesting and storage methods, and adapted varieties. The total feed supply on an average dairy farm could be increased by as much as 40% by following all recommendations.

High-producing cows are a must from the viewpoint of both volume of business and net returns. A goal of at least 10,000 lb. of milk per cow can be attained over a period of time.

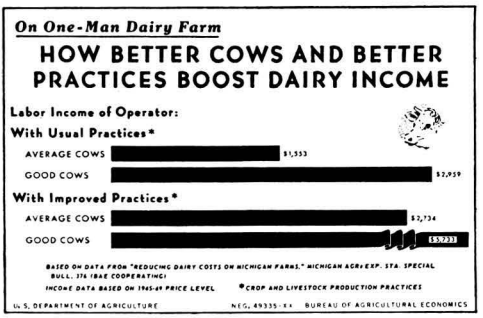

FIG. 2. Attainment of optimum efficiency and high labor incomes in dairy farming involves the use of improved practices in all phases of the farm business.

The 10 most progressive of the 40 dairy farmers studied averaged 10,650 lb. of milk per cow after they had been farming for about 10 years. One of these good cows will net as much as 3 or 4 average cows (7,000 lb. of milk).

Some dairymen fail to achieve efficiency because they fall short in one or more phases of production. A dairyman who has attained low-cost feed production may achieve only half of his potential net returns if he has low-producing cows to utilize this feed. Likewise, the farmer who has high-producing cows but who is using inefficient crop production and labor practices loses much of the advantage of good cows.

Henry Beland stresses the importance of keeping usable farm records as an important step in achieving success. These records have helped improve crop and livestock efficiency. An improved forage program resulted in the feeding of less grain and the elimination of expensive protein supplement. He is now using these records to help him decide whether he should install a gutter cleaner, remodel his barn into a pen-system of housing, or add a hired man and more cows.

FIG. 3. The Belands believe herd records are an essential phase of successful dairy farming.

Balancing Farm and Home Activities

Attaining a well-balanced dairy business and high earnings is not the only attribute of success. The business of dairying, like other professions, is not an end in itself. It is merely a means of attaining the worthwhile things in life—a better living, happiness, education for the children, opportunities to travel, and an interest in the finer things in life. These are the things that make success important to the whole farm family.

Farm families face the problem of allocating farm earnings between the farm business and the home. Careful planning on the part of all members of the farm family is essential if the farm business is to prosper and if the goals of the family are to be realized. The capital needs of both the farm business and the family change over a period of years. When the farm family is just getting started, there is usually a tremendous need for making investments in more and better livestock, improved machinery,

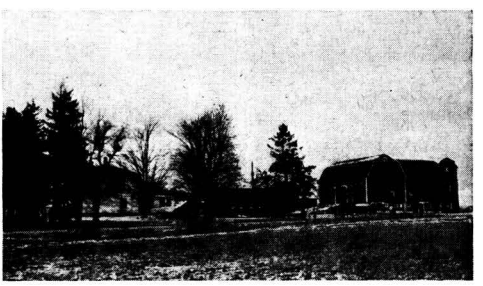

FIG. 4. The Beland family enjoys the fruits of successful management and good planning.

fertilizer, lime, and other production goods that increase farm output and earnings. A few years later there is need to increase the family budget as the children grow up. Insurance, education, food, clothing, and other expenditures become more important.

Farm families as well as farm businesses have often suffered in the past because of poor allocation of savings. The purchase of a new tractor or machine has often delayed the purchase of needed household equipment, vacations, and investments for insurance. Farmers often need to consider the possibility of using an old machine for another year or custom-hiring certain work so that the needs of the family can be taken care of. On the other hand, the family may have to forego a new car or television set or an extended vacation at a time when the budget calls for a large farm investment.

The Beland family has worked out a satisfactory allocation of funds for family and farm needs. During their first years on the farm, all available savings were invested in production goods that have made the farm more efficient. In recent years, the family has

benefited from these investments in the form of annual vacations, a modern, convenient home, musical activities for a daughter, and a farm free of debt. Every fall, Henry takes off for the north woods to hunt deer for a week. Mrs. Beland and his father do the chores when he is gone.

Working for a Better Community

Both Mr. and Mrs. Beland are community-minded. Mrs. Beland is president of the local Girl Scout group. She also participates in the local Grange and Farm Bureau. Henry is on the town board, is a member of the board of directors of the local elevator, and president of the County D.H.I.A. organization. He helped organize the new experimental township extension program in his area and is a member of the board of directors. He spends a good deal of time in promoting better farming practices. His crop rotation has led the way in his community toward more plow-down of green manure crops. He works closely with the county agricultural agent, the local soil conservation district and Michigan State College.

The Belands have two children, a daughter, Sandra, who is 10 years old, and a son, Dallas, who is seven. Sandra is a 4-H Club member in home economics and dairy work, is a Girl Scout, and is interested in piano and band. Dallas is too young to have become interested in many hobbies or community activities as yet. Will he also become a successful dairy farmer? Only time will tell, but it is certain that he and Sandra will be given the encouragement and opportunity to do an outstanding job in whatever they tackle.

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JOURNAL OF DAIRY SCIENCE

ABSTRACTS OF LITERATURE

W. O. Nelson, Abstract Editor

BOOK REVIEWS

283. Agricultural Process Engineering. S. M. HENDERSON and R. L. PERRY. John Wiley and Sons, Inc., New York. 402 pp. $8.50. 1955.

The preface states, "This textbook was designed primarily to assist in teaching the engineering elements of agricultural processing to advanced students in agricultural engineering." Among the many activities included in the definition of agricultural processing are those dealing with milk and milk products.

Our plants perform a number of operations which are common to the production of several dairy products as well as of other foods. These "unit operations" form the basis of this book. Those stressed are heat transfer, fluid-flow, drying, size reduction, cleaning and sorting, and materials handling. The book opens with a chapter on the engineering approach to the processing field. In addition, discussions on the principles of refrigeration, thermometers and controls, plant design and cost analysis would be of special interest to the dairy technologist.

A large number of excellent illustrations, either line drawings or phantom views whenever the internal mechanism is of interest, are used. A psychometric chart for air and Mollier diagrams for ammonia and freon are included. The tables and charts are easy to read and understand. The appendix includes an abridged steam table. The authors have included at the end of each chapter a list of key references for further study on the subject. Sample problems and their solutions, interspersed throughout the text, do a good job of integrating and using the factual matter presented.

This book is excellently written and composed. Of greatest interest to the reviewer was the chapter on heat transfer, especially the section on heat exchanger analysis.

As noted above, this work was intended as a textbook for agricultural engineers. An engineering background, including a knowledge of mathematics, thermodynamics, and mechanics, is expected for a comprehension of all the material. The subject matter is treated rigorously wherever possible. However, the plant production man should be able to understand most of the descriptive portions and some of the analytical sections. Therefore, we recommend that every library on the subject of dairy manufacturing should contain this book.

M. P. Steinberg

284. Air Conditioning Refrigerating Data Book. Applications Vol., Fifth Edition. American Society of Refrigerating Engineers. 1955.

The art and science of refrigeration and the recommended practice for their application to specific problems in the refrigeration and air conditioning fields are described in detail. This volume comprises eight main sections, I. Frozen Foods; II. Refrigeration in Food Industries; III. Refrigeration Warehouse Practice; IV. Refrigerated Food Distribution; V. Low Temperature Applications; VI. Industrial Applications of Refrigeration; VII. Comfort Air Conditioning; VIII. Industrial Air Conditioning. In these sections are found 61 chapters. Chapters of previous volumes which have become obsolete have been either omitted or rewritten. Three new chapters have been added, Chapter 26 on Precooling, Chapter 32 on Fishing Boats, and Chapter 50 on Passenger Automobiles. In addition to rewriting entirely several chapters most of the remaining chapters were revised, some of them extensively, to conform to current practices in their respective fields. Chapters of interest directly to members of the dairy industry are, 3. Ice Cream, C. J. Bell, reviser, 9. Milk Plants, C. A. Blanchard, author, 10. Butter Manufacture, M. E. Parker, author, and 11. Cheese Manufacture, E. Traisman, reviser. A number of other chapters are to be found with information bearing directly on storage, transportation and refrigerated display of dairy products.

A Refrigeration Classified Section in the rear of the volume is a supplement, listing manufacturers of components and assembled units of refrigerating equipment, which many readers will find helpful.

L. M. Dorsey

285. Standard Values in Nutrition and Metabolism. Edited by Errett C. Albritton, The George Washington University. Prepared under the direction of the Committee on the Handbook of Biological Data, American Insti-

tute of Biological Sciences, The National Research Council. W. B. Saunders Company, Philadelphia, 1954.

The 160 tables in this monograph are the contribution of 800 specialists in these fields in this country and abroad. Tables as originally compiled were subjected to intensive review by experts in the respective subjects. By this procedure the table were stripped of most of the controversial or questionable or borderline material, leaving for final presentation to the user only what is presently accepted as fact by those who are competent to judge. There are 223 pages of tables and 16 pages of diagrams containing many thousand items of authoritative data, much of it quantitative. Each table has its separate bibliography in the bibliography section of the handbook and each individual item of a table is keyed to its specific reference. This indicates a most meticulous treatment of reference material. The information covers the nutrition of man, beasts, birds, insects, fish, reptiles, plants, bacteria, yeasts and molds.

Sponsors of the work are the Air Force, Army, Navy, and Atomic Energy Commission, issued in August 1954 as Wright Air Development Center Technical Report 52-301.

L. M. Dorsey

286. Manual on Industrial Water. American Society for Testing Materials, 1954.

This manual contains new and revised methods for the examination and testing of industrial water. Industrial water is distinguished from sanitary water in that the latter is water used for human consumption or used directly in the processing of foodstuffs. Many of the same tests are used for complete examination of both kinds of water but industrial water, for instance, is not examined for pathogenic bacteria but only for bacteria associated with formation of mineral deposits in piping or heat exchange equipment. The manual covers, Uses of Industrial Water, Difficulties Caused by Water in Industry, Composition of Industrial Water and Water-Formed Deposits, Treatment of Industrial Water, Analysis of Industrial Water, Sampling and Identification of Water-Formed Deposits, and Analysis of Water-Formed Deposits. It is designed as a brief reference source for three types of users; executives and plant designers; individuals engaged in industrial operations involving the use of water; and analysts, operators of special instruments, engineers and consultants. Numerous footnotes provide references to original source material while at the end of the book section G of the appendix is a list of A S T M Symposiums and Technical Papers on Industrial Water. A number of diagrams and photomicrographs are found to illustrate textual matter. Sections A, B, C and D of the appendix present all of the A S T M industrial water standards and methods that have been adopted by the American Society for Testing Materials. A glossary of technical terms in common usage and words used in the Manual in a special sense is Section E. Section F is a tabular presentation of the amounts of water or steam required per unit in many industrial operations. While water requirements are given for milk products no steam amounts are given and it would seem that a more complete list could be set up to include milk condenseries and ice cream plants along with steam usage. L. M. Dorsey

BUTTER

287. Butter dispenser. K. Michaelis. U. S. Patent 2,702,942. 2 claims. Mar. 1, 1955. Offic. Gaz. U. S. Pat. Office, **692**, 1: 18. 1955.

A screw operated dispenser for extruding individual portions of butter. R. Whitaker

CHEESE

288. Device for curing and turning cheese. C. O. G. Persson. U. S. Patent 2,702,943. 2 claims. Mar. 1, 1955. Offic. Gaz. U. S. Pat. Office, **692**, 1: 19. 1955.

A rack of shelves for holding cheese is described. The rack is suspended from rails attached to the ceiling of the curing room which permits movement of the rack in the direction of the rails. Provision is also made for turning the entire rack upside down by means of a gear attached to the axis.

R. Whitaker

CONDENSED AND DRIED MILKS; BY-PRODUCTS

289. Milk evaporation apparatus. J. A. Cross (assignor to Mojonnier Bros., Inc.). U. S. Patent 2,703,610. 1 claim. Mar. 8, 1955. Offic. Gaz. U. S. Pat. Office, **692**, 2: 190. 1955.

A multiple effect tube chest type vacuum pan is described in detail. Vapor and liquid from the tube chests are separated in centrifugal vapor separation chambers, the liquid from the outside returning for recirculation in the same tube chest and the vapor from the center passing to the next effect. R. Whitaker

290. Method of making a dry and wet salad dressing. E. P. Haney. U. S. Patent 2,704,258. 2 claims. Mar. 15, 1955. Offic. Gaz. U. S. Pat. Offic., **692**, 3: 366. 1955.

Salad dressing is prepared by mixing 5 parts warm water and 1 part vinegar, and moistening a dry powdered mixture of 1 part of dextrin, 4 parts of powdered milk, and 1 part of a blend of sugar, salt, and other seasoning, until the desired consistency is obtained.

R. Whitaker

DAIRY CHEMISTRY

291. Structure of reducing disaccharides by lead tetraacetate oxidation. A. S. Perlin, Div.

of Appl. Biol., Natl. Research Lab., Ottawa, Can. Analyt. Chem., **27**, 3: 396. 1955.

The use of lead tetraacetate oxidation for determining the structure of reducing disaccharides is described. Measurements included the production of formic acid and formaldehyde and the consumption of lead tetraacetate. A wide variety of hexose and pentose disaccharides including lactose was examined. The oxidations required only a few milligrams of material and were both convenient and rapid. The rate of formic acid production was determined from the evolution of carbon dioxide in the Warburg respirometer. The reaction mixture was analyzed at chosen intervals for consumption of lead tetraacetate and for other products of the oxidation. B. H. Webb

292. Improvements in Karl Fischer method for determination of water. E. D. PETERS and J. L. JUNGNICKEL, Shell Development Co., Emeryville, Calif. Analyt. Chem., **27**, 3: 450. 1955.

A substantial gain in stability can be achieved by substituting methyl cellosolve for methanol in the formula for Karl Fischer reagent. In addition to its greater stability, the modified reagent extends the applicability of the method by permitting an appropriate choice of sample solvent. B. H. Webb

293. Enzymic inhibition of gelatin in frozen egg yolk. A. LOPEZ, C. R. FELLERS, and W. D. POWRIE, Univ. of Mass., Amherst. J. Milk and Food Technol., **18**, 3: 77. 1955.

In order to inhibit gelatinlike consistency in commercial egg yolk a concentration of 0.05 per cent papain was added to fresh yolk and incubated for 15 to 20 min. Trypsin and rhozyme were equally effective but off-flavors and off-odors developed in the yolk. Pancreatin, erepsin and lipase did not inhibit gelation.

Gelation in frozen and thawed yolk was not inhibited by the addition of fresh yolk of either acid or enzymatic hydrolysates of yolk.

A protein or protein complex is believed responsible for gelation in frozen and thawed yolk. H. H. Weiser

DAIRY ENGINEERING

294. Oil maintenance equipment. L. S. METCALFE. Ice Cream Rev., **38**, 8: 46. 1955.

Careful selection, use, and maintenance of lubricants is a means of reducing the amount of oil purchased and will also assist in maintaining production at a high level of efficiency by reducing equipment failures. It has been established that oil does not wear out if kept clean. The problem of oil maintenance is three fold. First, foreign material must be prevented from getting into the oil. Second, operating conditions must be controlled. Third, contaminants which may gain entrance to the lubricating system must be removed.

The author suggests that the services of a specialist in the lubrication field should be obtained to determine the type of oil which should be used and the type of equipment best adapted for oil maintenance.

W. J. Caulfield

DAIRY PLANT MANAGEMENT AND ECONOMICS

295. Application of machine accounting to milk and ice cream industries. N. V. BELLENOIT, H. P. Hood & Sons, Boston, Mass. Sou. Dairy Prod. J., **56**, 4: 140. 1954.

There is an increasing need to mechanize accounting in milk and ice cream plants of all sizes. The advantages of electronic devices is so great that it is not a question of "if" but of "when" mechanizing will occur. We should start a program which will progress to punched cards and which will include source recording, intermediate processing and end results. Our efforts for the present should be concentrated upon the first and last. Uniformity, accuracy and completeness are of paramount importance. When the information is entered properly on punched cards, electronic machines can be used effectively to obtain any needed end results, such as pay checks, operating reports, cost analyses and statistics. F. W. Bennett

296. Price supports and parity. A selected bibliography. O. CUMMINGS, Calif. Agr. Expt. Sta., Berkeley. 1954.

This publication is a listing of numerous articles pertaining to various price support programs which have been established in the United States. R. W. Hunt

297. Bottle holder. J. B. BIEDERMAN. U. S. Patent 2,703,253. 5 claims, Mar. 1, 1955. Offic. Gaz. U. S. Pat. Office, **692**, 1: 96. 1955.

A holding device for easily clamping around the lip of milk and other bottles to facilitate holding several bottles with one hand.

R. Whitaker

298. Carton carrier. H. A. TOMARIN (assignor to Loroco Industries, Inc.). U. S. Patent 2,704,222. 13 claims. Mar. 15, 1955. Offic. Gaz. U. S. Pat. Office, **692**, 3: 358. 1955.

A handle designed to carry two cartons having tapered tops, of the type employed in the Pure-Pak paper milk bottle. R. Whitaker

FEEDS AND FEEDING

299. Effects of feeding different grades of hay and cod-liver oil concentrate to dairy cattle. III. From 361 to 720 days of age. H. B. ELLENBERGER, J. A. NEWLANDER, and C. H. JONES, Vt. Agr. Expt. Sta., Burlington. Bull. 576. 1954.

These trials extended through 3 and 4 generations in order to evaluate the cumulative

effects from feeding hays of different quality, cod-liver oil concentrate, or from lack of calcium or phosphorus supplements. This report covers the second year in the life of the animals.

The grain fed consisted of a mixture of three parts corn meal, one ground oats, two wheat bran, one old process oil meal, one corn gluten feed, and two of 41% cottonseed meal with 1% salt. The consumption rate of this feed was about 1.6 lbs. Succulent feeds, grass clippings, and silage were eaten at a rate of about 20 lbs. per animal per day. Each animal was allowed to eat all the hay it desired, averaging about 6.5 lbs. per day.

The results of this experiment indicated that heifers fed simple rations such as used during this study do not need additional calcium and phosphorus. On the whole, heifers did well on the poorer quality hay, averaging slightly greater gains in weight and more economical gains as regard to T.D.N. than the heifers fed better quality hay. However, calves showed better gains in the good quality hay. The feeding of vitamin A & D supplements in conjunction with poor hay was advantageous. Vitamin A & D supplement with good hay showed no promise. Yearling heifers weighing from 500 lbs. and up are able to make normal gains on less T.D.N. than called for by the lower limit of the Morrison standard. R. W. Hunt

300. Some factors affecting the stability of carotene in mixed feeds. A. W. HALVORSON and C. M. HENDRICK, S. D. Agr. Expt. Sta., Brookings. Tech. Bull. 14. 1955.

The purpose of this study was to determine the effect of different feed ingredients upon the stability of different carotene supplements. Three carotene supplements were used: alfalfa meal, carotene feeding oil, and carotene-Wesson oil preparation. The carotene losses in the various diets following storage at 37° C. for 30 days were observed. The data show that alfalfa carotene losses were little affected by the diet modifications used, although the replacement of corn with oats tended to decrease the loss to some extent. Losses from all alfalfa diets were rather high, amounting to about 1/3 of the original carotene. Results of oil supplemented diets differed from those with alfalfa in several respects. Carotene losses from basal diets with oil supplements were less than half those observed for alfalfa supplemented basal. In some instances, the losses became nearly as great as alfalfa, when a high trace mineral level or fish meal or meat scraps were present. Oats and wheat bran-middling also increased, especially with carotene-Wesson oil, but all other substitutions or additions resulted in only small increases. Thus, it was shown that alfalfa and oil carotene supplements vary considerably in their sensitivity to diet modifications of the type used. However, oil supplements of different composition and carotene content appear quite similar. R. W. Hunt

301. Forsoeg med tilskud af et E-vitamin (d,l-α-tocoferol-acetat) til malkekvaegets foder [Experiments with addition of a vitamin E (d,l-α-tocopherol-acetate) to feed for dairy cows]. (English summary). A. H. PEDERSEN, A. N. FISKER, H. RAMBAEK, and E. O. PETERSEN, State Expt. Sta. Creamery, Hillerød, Denmark. Bull. 91. 1954.

A group of 9 dairy cows of Red Danish breed was given an addition of 2 g. of d,l-α-tocopherol-acetate per cow per day over a period of 63 days. Butter was made from the milk and compared to butter made during the pre-experimental and post-experimental periods as well as with butter made from the milk from a similar sized group of cows serving as control. The butter was examined for keeping quality, salt and moisture, loose moisture, acid number, pH, peroxide number and bacteriological quality after 14 and 28 days of storage at 13° C. and after 3 months at –12° C.

Contrary to expectations the experimental butter had a tendency to become more oxidized than the control as demonstrated by higher peroxide values, lower flavor scores and a more pronounced "oily" flavor.

The experiment was repeated. This time the butter was only examined after 3 months of storage at –12° C. In addition to the before mentioned test the butter was examined for its tocopherol content.

The previous results were confirmed. The experimental butter became more oxidized than the control. The addition of 2 gm. tocopherol to the daily rations of the cows increased the tocopherol content of the butter from 10-12 mcg./gm. to 23-26 mcg./gm.

T. Kristoffersen

302. Synthetic milk product. H. G. LUTHER, (assignor to Chas. Pfizer & Co., Inc.). U. S. Patent 2,703,285. 5 claims. Mar. 1, 1955. Offic. Gaz. U. S. Pat. Office, **692**, 1: 103. 1955.

A food for baby pigs suitable for feeding, starting about 48 hr. after birth. It consists of at least 10% edible fat, suitably emulsified together with a balanced vitamin content including vitamin B_{12} in an amount of about 1.5 to 6 mg/100 lb., trace food minerals and 20 p.p.m. of an antibiotic such as oxytetracycline and chlortetracycline. R. Whitaker

303. Grasslands improvement—a vast profit potential II. Part IV. Southwest by R. R. HUMPHREY. **Part V. Intermountain** by L. A. STODDART and A. D. SMITH. **Part VI. Pacific Coast** by R. M. LOVE. J. Agr. Food Chem., **3**: 300. 1955.

This is a continuation of an interpretive survey of grassland management (see also J.

Agr. Food Chem., 3: 23. 1955). Particular emphasis is placed on the problems, progress and potentialities in the western sections of the country. S. Patton

GENETICS AND BREEDING

304. Osmotic pressure of extended bovine semen during storage. J. T. SMITH, D. T. MAYER, and H. A. HERMAN, Mo. Agr. Expt. Sta., Columbia. Research Bull. 538. 1953.

Semen from dairy bulls was extended with media used in the practice of artificial insemination and with media especially designed to alter one or more of the physical and chemical properties. The extended semen was stored at 4 to 7° C. and evaluated daily for motility and osmotic pressure. Motility was rated by a standard method. Osmotic pressure was measured with a modification of the Hill-Baldes thermoelectric osmometer and expressed in terms of freezing point depression.

The data show that the diluting media which maintained osmotic pressure within the limits of −0.44 to −0.61° C. were superior in the maintenance of motility in extended semen during a 10-day storage period. Glucose aids in maintenance of osmotic pressure within narrow limits during storage. Addition of antibiotics increased osmotic pressure during the first 48 hr. of the storage period, but maintenance of osmotic pressure was at a slightly lower level than that obtained with the same diluent without antibiotics. If the diluting medium without antibiotics maintained osmotic pressure within the optimal range, addition of antibiotics did not change the osmotic pressure to the extent that it was no longer within the optimal range. Authors' Abstract. R. W. Hunt

HERD MANAGEMENT

305. Malkemaskinens konstruktion og hygiejnisk maelkeproduktion (The construction of the milking machine and the production of low count milk). (English summary). A. H. PEDERSEN and A. MOELLER-MADSEN. State Expt. Sta. Creamery, Hillerøed, Denmark. Bull. 91. 1954.

The authors discuss the milking machine and point out the requirements which should be met with regard to the construction of the individual parts and the role each of these play in the production of low count milk. T. Kristoffersen

306. Dehorning device. S. S. MIMS (assignor to Lykes M. Boykin). U. S. Patent Reissue 23,953. 8 claims. Feb. 22, 1955. Offic. Gaz. U. S. Pat. Office, **691**, 4: 458. 1955. U. S. Patent 2,582,450.

A modification of a device for dehorning cattle, described previously in U. S. Patent 2,582,450. See J. Dairy Sci., Abst. of Literature, **35**, A37, 1952. R. Whitaker

307. Collapsible valve for milking machines. A. G. PERKINS. U. S. Patent 2,703,100. 8 claims. Mar. 1, 1955. Offic. Gaz. U. S. Pat. Office, **692**, 1: 57. 1955.

A collapsible valve for including in the milk line from a vacuum operated type of milker to facilitate the proper action of the milker. R. Whitaker

308. Flushing arrangement for pipe line milking system. F. G. HODSDON (assignor to International Harvester Company). U. S. Patent 2,703,068. 8 claims. Mar. 1, 1955. Offic. Gaz. U. S. Pat. Office, **692**, 1: 50. 1955.

Details are given for an arrangement of pipes for cleaning and flushing the pipe lines of a vacuum type milking machine. R. Whitaker

309. Quarter milker. F. L. CARLSON. U. S. Patent 2,703,067. 2 claims. Mar. 1, 1955. Offic. Gaz. U. S. Pat. Office, **692**, 1: 49. 1955.

A vacuum type of milker with a valve in the milk line which permits either discharge of milk from separate teat cups into glass jars for inspection purposes or into the common milk reservoir. R. Whitaker

310. Calf weaner. W. ANDERSON. U. S. Patent 2,703,555. 3 claims. Mar. 8, 1955. Offic. Gaz. U. S. Pat. Office, **692**, 2: 177. 1955.

A wire device is described for preventing a calf from nursing. R. Whitaker

311. Surcingle for suspended milking machines. T. B. CRAWFORD and C. C. GORMAN. U. S. Patent 2,703,473. 2 claims. Mar. 8, 1955. Offic. Gaz. U. S. Pat. Office, **692**, 2: 157. 1955.

A description is given of a belt for supporting a milking machine. R. Whitaker

ICE CREAM

312. Foam studies of pasteurized ice cream mix. H. B. SIEGMUND, Hendler Creamery Co., Inc., Baltimore, Md. Ice Cream Rev., **38**, 8: 106. 1955.

The presence of foam in ice cream mix has been shown to be responsible for the survival of coliform organisms during pasteurization. The use of space heaters on batch pasteurizers did not eliminate coliform organisms from the mix. Entrainment of air in HTST system also resulted in the survival of coliform bacteria. In a HTST system where high pressures are necessary to force the mix through the system it was important that a constant pressure be maintained. If the pressure dropped, a condition similar to foam insulation occurred which prevented every particle of the mix from being properly heated.

In the light of these results, it is important that all foam be transformed to the fluid state prior to heating or that it be circulated in such a manner as to insure that every particle will

reach the proper temperature. The use of partial vacuum on batch pasteurizers to eliminate foam and to prevent burn on is suggested as one solution to the problem. W. J. Caulfield

313. Pasteurization of ice cream mix by HTST methods. K. R. Fowler, Franklin Ice Cream Co., Kansas City, Mo. Sou. Dairy Prod. J., **56**, 4: 148. 1954.

A comparison of HTST pasteurization with a conventional holder system for a capacity of 1,500 gal. of mix per hr. showed a disadvantage of about $4,000 in cost of equipment, a requirement of about 13% more floor space, about equal refrigeration requirements, a need for only 50% as much boiler capacity, a saving of 8% in labor for processing and clean-up and an equally satisfactory product. Altogether, there was a slight advantage in favor of the HTST system. The study was based upon the use of fluid dairy products. For operations of less than 1,000 gal. per hr., involving a large quantity of frozen or concentrated ingredients or for the making of a number of small mixes, the advantage was considered to be in favor of the holder method. F. W. Bennett

314. Coliforms in ice cream. J. M. Frayer, Univ. of Vt., Burlington. Sou. Dairy Prod. J., **56**, 3: 148. 1954.

A series of coliform line tests of ice cream from the pasteurizing vat to the consumer was made at the Vermont Station. Pasteurization at 160° F. for 30 min. resulted consistently in negative counts with only the merest trace of foam occurring. All equipment from the pasteurizer through the freezer was a potential source of contamination which could be detected in most cases only by laboratory tests. Unheated products added at the freezer and hand-formed containers required checking especially. The greatest numbers of coliforms were added by the dipping operation. Most methods of rinsing scoops in moving water were potentially satisfactory but still water receptacles were uniformly bad. F. W. Bennett

315. High-short pasteurization ups capacity, cuts labor. R. O. Tardiff, Breyers Ice Cream Co. Philadelphia. Food Eng., **27**, 3: 63. 1955.

A continuous system for processing ice cream mix is utilized by Breyer Ice Cream Company at its Newark, N. J. plant. The system involves HTST pasteurization with a Roswell pasteurizer. Chocolate and sugar are mixed in a Norman mixer and pumped to one compartment of a two compartment blending tank. Milk and cream are metered to this tank. One compartment is filled while the other is being emptied to the pasteurizing section of the system. Mix is heated to 250° F., immediately cooled to 207° F. and held for 25 sec. in 2-in. holding tubes. It then goes through the flow diversion valve to a second cooling tube where temperature is reduced to 172-175° F. From here the mix flows to the filter and homogenizer and is finally cooled to 32-36° F. over a surface cooler. The system has given increased capacity, improved bacterial destruction, and savings in steam and refrigeration. The recirculation system of cleaning used is simpler and more reliable than former procedures. T. J. Claydon

316. Freezing apparatus for production of ice cream. A. Mooser. U. S. Patent 2,702,992. 8 claims. Mar. 1, 1955. Offic. Gaz. U. S. Pat. Office, **692**, 1: 30. 1955.

An ice cream freezer consisting of a vertical rotating drum and a scraping device.

R. Whitaker

MILK AND CREAM

317. Dispensing valves for gas pressure containers. A. S. Lapin (assignor to Reddi-Wip, Inc.). U. S. Patent 2,704,172. 6 claims. Mar. 15, 1955. Offic. Gaz. U. S. Pat. Office, **692**, 3: 345. 1955.

Details are given for the construction of a valve suitable for dispensing whipped cream from a can containing fluid cream under high gas pressure. The valve is normally closed and is opened in proportion to the pressure applied by a finger. R. Whitaker

318. Marketing cream in Missouri through cooperative buying stations. C. C. Erwin and D. N. Harrington, Mo. Agr. Expt. Sta., Columbia. Research Bull. 539. 1953.

Sales of farm-separated cream in Missouri accounted for 1/6 of the total dairy income in 1950. Local cooperative buying stations play an important role in marketing cream in the state. As a means of studying some of the problems of marketing cream cooperatively, a random sample of 50 cream buying stations was selected from 178 cooperative stations. Much of the importance of marketing cream through local cooperative buying stations apparently is due to the small output per farm which warrants only occasional deliveries, and the convenience realized by farmers from marketing cream where supplies needed on the farm may be obtained. This study showed that the income from an equal amount of cream could be increased by as much as ¼ million dollars if buying stations, creamery managers, and producers agreed to produce Grade A cream under the Four-Day Grading Act, as established in the Missouri Dairy Law. The greatest problem is to get the producers to deliver their cream to the buying stations often enough to maintain its quality. Of all patrons delivering cream to a group of sample stations during a two-week period in Feb. 1947, only 7% delivered their cream as often as three times. During a similar period in 1950, the proportion had risen to 20%. Patrons tend to deliver their

cream more often in summer than winter, but much still remains to be desired. The size of the cream buying operation at any one station had a direct bearing on the handling practices. The smaller the cream buying operations within a given plant, the more neglect was given to the processing of the cream. Another important problem revealed by this study was that most cooperatives showed a loss for their cream buying operation. This would indicate that the cooperatives are performing a service only for farmers who are not able to switch to a more profitable business operation. R. W. Hunt

319. The new HTST and vacreator pasteurization standard provided for milk and its products. M. L. SPECK, N. C. State Coll., Raleigh. Sou. Dairy Prod. J., **56**, 3: 31. 1954.

Heating ice cream mix at 175° F. for 19.9 ± 0.7 sec. was equivalent to the standard of 155° F. for 30 min. recommended by the U. S. Public Health Service. Using a Stevac pasteurizer, 172.18 ± 0.48° F. for 25 sec. was comparable to 155° F. for 30 min.

Tests with a Vacreator have shown that 191.5° F. in the first chamber is equivalent to 155° F. for 30 min. A temperature of 194° F. in the Vacreator has been recognized as being equally efficient to those currently defined in the USPHS Ordinance, provided a constant steam supply is supplied, a substantially constant temperature is maintained in the pasteurizing chamber, the passage of unpasteurized product through the system is prevented and the entry of raw products into the first chamber is prevented when the steam supply falls below the necessary pressure. The same vacreation standard is applied tentatively to milk and cream.

The new standards are considered to be more effective than 155° F. for 30 min. which gives a six-fold margin of safety in time.

F. W. Bennett

320. Vacreation of fluid milk, W. M. ROBERTS, L. F. BLANTON, and F. G. WARREN, N. C. State Coll., Raleigh. Sou. Dairy Prod. J., **56**, 5: 36. 1954.

From 21 to 73% more time was required for the passage of skimmilk through a No. 3 Vacreator than for the passage of water under similar conditions. No definite comparative trend in the flow rates in the first chamber was indicated. Milk unsalable because of onion and feed flavors was rendered acceptable by vacreation. Flavor scores of the milk differed little as temperatures and intensities of treatments varied in the experiment. Vacreation of milk at 200° F. and 195° F. compared favorably in keeping quality with vat pasteurized milk up to 7 days. Phospatase was destroyed by vacreation at 185° F. Milk which was vacreated was preferred to milk which was commercially available. F. W. Bennett

321. Low fat multi-vitamin milk. C. E. HILDRETH, Vitex Labs., Inc., Harrison, N. J. Sou. Dairy Prod. J., **56**, 4: 43. 1954.

The formula for vitamin and mineral fortification of skimmilk has been usually the same as for whole milk; i.e., 4000 u. of Vitamin A, 400 u. of Vitamin D, 10 mg. of thiamine, 2 mg. of riboflavin, 10 mg. of niacin, 10 mg. of iron and 0.1 mg. of iodine (per quart). The National Research Council, American Medical Association and certain nutritionists recommend that nutritional needs be supplied as far as possible by ordinary foods but too many people eat only what they like. Fortification of milk or milk products tends to protect such consumers. There seems to be a permanent demand for low-fat milk and a multi-vitamin-mineral fortified skimmilk with 0.5% butterfat and 2% added serum solids seems most acceptable to the public. The use of the same formula by every dairy in a given market, will result in greater confidence of the consumer.

F. W. Bennett

322. Rapid melting, the key to good cream from frozen fat. F. L. BRYANT, Bryant Machinery Corp., Jersey City, N. J. Sou. Dairy Prod. J., **56**, 6: 50. 1954.

Slow melting of frozen cream usually results in oxidation, poor keeping quality, increased acidity, partial destabilization, poor housekeeping and excessive labor costs. By conveying all of the frozen products to one point, removing the containers without thawing, liquefying rapidly and processing the cream immediately, the disadvantages of slow thawing can be eliminated. F. W. Bennett

NUTRITIVE VALUE OF DAIRY PRODUCTS

323. Milk proteins. Nutritive value of whey powder protein. L. K. RIGGS, A. BEATY, and B. MALLON, Natl. Dairy Research Lab., Oakdale, N. Y. J. Agr. Food Chem., **3**: 333. 1955.

The nutritive value of the protein in several whey powders was determined by rat feeding tests. Spray-dried whey powder protein was higher in nutritive value and more digestible than roller-dried whey powder protein. Neither type was equal to lactalbumin. Roller-dried whey powder protein was improved by lactalbumin or lysine supplementation. Spray-dried whey powder protein was improved by lactalbumin supplementation, but not by lysine in the amounts which improved roller-dried whey powder. The nutritive value of protein concentrates prepared by heat coagulation or methanol extraction was higher for preparations made from spray-dried whey. S. Patton

324. Milk vitamins. Biological availability of vitamin B_6 of heated milk. R. M. TOMARELLI, E. R. SPENCE, and F. W. BERNHART, Wyeth

Laboratories, Inc., Mason, Mich. J. Agr. Food Chem., 3: 338. 1955.

Heat sterilization of liquid milk products results in a loss of vitamin B_6 as determined by a microbiological assay. A rat growth procedure was adapted for analysis of milk samples by the use of a semisynthetic basal diet with a composition simulating milk. The biological assay of heat-sterilized liquid milk yielded vitamin B_6 values that were lower than those obtained by the microbiological method. The bioassay and the microbiological method were in agreement in the assay of spray-dried milk.

S. Patton

PHYSIOLOGY AND ENDOCRINOLOGY

325. The role of folic acid in hormonally-induced tissue growth. M. Silver. J. Endocrinol., **10**: 95. 1954.

After weanling ovariectomized rats were made deficient in folic acid by the administration of 2.0 μg. aminopterin daily for 20 days (increased 1 μg. for each 5 g. gain in body weight), the response in growth of the mammary gland to a maximum stimulation by estradiol benzoate (0.25 μg./2 day period) was much less than the pan fed controls. The growth obtained, however, appeared to be normal. In similar rats 6-36 μg. aminopterin per day reduced the uterine weight increase induced by 0.2-10 μg. estradiol benzoate daily. However, even lethal doses of aminopterin did not completely inhibit the weight increase. The increased water uptake and the enlargement of the lining of the uterus, characteristic of estrogenic stimulation were not affected by aminopterin. One mg. aminopterin daily caused a highly significant reduction in the response of the pigeon to 0.4 mg. prolactin daily. The inhibition by aminopterin could be prevented by administering folic acid. The author suggests that folic acid is an essential metabolite for nucleic acid synthesis in these types of growth. R. L. Hays

326. The secretion of the adrenal cortex in the sheep. I. E. Bush and K. A. Ferguson. J. Endocrinol., **10**: 1. 1953.

The kinds and amounts of cortical hormones in the blood of the adrenal vein of 4 intact and 2 hypophysectomized sheep were determined after severe surgical operation, ACTH infusion and epinephrine injection. Three hormones were found: 17 α hydroxycorticosterone, corticosterone and 11 β hydroxyandrost 4-ene-3:17-dione, with the first being 5 to 15 times the concentration of either of the other two. The sheep adrenal is capable of secreting 1-3 mg./kg./24 hrs. of α β unsaturated ketosteroids. The adrenals of the two hypophysectomized sheep secreted at a fairly high rate before ACTH administration. R. L. Hays

327. The progesterone content of body fluids and tissues. D. G. Edgar. J. Endrocrinol., **10**: 54. 1953.

The amount of progesterone was determined spectrophotometrically after chromatographic separation. The method can detect as little as 0.1 μg./ml. with volumes of 40 ml. Progesterone was found in follicular fluid of the sow and cow, fluid of ovarian cysts of the sow, blood from the ovarian vein of the pregnant and non-pregnant ewe and the non-pregnant sow. It was not found in the blood of the ovarian vein of a ewe with inactive ovaries, in peripheral blood of ewe, mare, cow, sow or rabbit.

R. L. Hays

328. Effect of exercise on comb response of androgen-treated capons. H. Y. C. Wong, N. Lavenda, and E. W. Hawthorne, Howard Univ., Washington, D. C. Am. J. Physiol., **178**: 269. 1954.

Twenty-five capons were divided into 4 groups: groups I and II were controls with injections of cottonseed oil. Groups III and IV received 1 mg. testosterone propionate daily in cottonseed oil. Groups II and IV had 30 min. forced exercise on a treadmill daily. Comb growth was used as a measure of androgenic activity. Androgen caused a highly significant increase in comb size in groups III and IV. Exercise had no effect on comb size in the controls but caused a highly significant reduction in the response of the comb to androgen.

R. L. Hays

329. The production of coincident oestrus and ovulation in the anoestrous ewe with progesterone and pregnant mare serum. T. J. Robinson, Univ. of Melbourne, Victoria, Australia. J. Endocrinol., **10**: 117. 1954.

Of 54 ewes receiving 1000 i.u. of PMS, 50 ovulated. With PMS alone none showed estrus. When 75 mg. of progesterone was given in a single dose 33% showed estrus and this percentage increased to 100 when the progesterone was given in 6 injections in 3 days. Estrus began 24-36 hours after PMS injection and preceded ovulation by 10-20 hours.

R. L. Hays

330. Factors affecting the response of the uterus to serotonine. J. M. Robson, J. R. Trounce, and K. A. H. Didcock. J. Endocrinol., **10**: 129. 1954.

The uterus of the estrous rat is highly sensitive to serotonine (5-hydroxy-tryptamine) responding to concentrations as low as 1:500,000,000. Serotonine is similar to a substance liberated when blood clots which can cause contraction of smooth muscle. The response appears to diminish during diestrus. Ovariectomy decreases the uterine sensitivity to an extent that no motor response was found and an inhibitory response was noted in concentrations of 1:100,000. Estrogen restored the sensitivity of the uterus of the castrate to sero-

tonine but testosterone, progesterone, cortisone acetate or desoxycortisterone acetate did not.
R. L. Hays

SANITATION AND CLEANSING

331. Evaluation of a detergent-sanitizer for use on producer milking utensils. M. L. Speck, W. R. Murley, H. L. Lucas, and L. W. Aurand, N. C. State Coll., Raleigh. J. Milk and Food Technol., 18 3: 71. 1955.

This study, made on 26 grade A milk farms, compared a detergent-sanitizer with the ordinary method of cleaning and sanitizing milking equipment. The farms were divided into two groups and placed on a double-reversal trial with three 5 week periods. The detergent-sanitizer was superior to the regular procedure in removing milk-stone deposits and cleansing ability.

There were no statistical differences in the thermoduric and microbial counts of raw milk, and apparently no relationship existed between the effectiveness of the detergent-sanitizer and the hardness of the water. The activity of butter cultures indicated that appreciable amounts of quaternary ammonium compound were not present in any of the milk samples.
H. H. Weiser

332. Milk can rinsing apparatus. R. Wiskerchen. U. S. Patent 2,702,557. 3 claims. Feb. 22, 1955. Offic. Gaz. U. S. Pat. Office, **691**, 4: 503. 1955.

A simple milk can washing and cleansing device is described. A shallow cylindrical-shaped platform with a raised edge is supported on three legs. Cleaning liquid or rinse water is injected upward into an inverted can resting on the platform and tipped slightly to allow the can edge to depress a lever which releases the rinsing solution. R. Whitaker

333. Forskellige desinfektionsmidlers indflydelse paa Storchs proeve i lavpasteuriseret maelk (The effect of different type sanitizers on the Storch test on pasteurized milk). K. P. Andersen and K. Joergensen. State Expt. Sta. Creamery, Hillerød, Denmark. Bull. 92, 68. 1954.

A concentration of 20,000 p.p.m. active Cl_2 rendered milk heated to 82° C. in a HTST system Storch negative. 100,000 p.p.m. active Cl_2 had the same effect on milk heated only to 72° C. Different quarternary ammonium compounds had no effect on the Storch test even in very high concentrations. T. Kristoffersen

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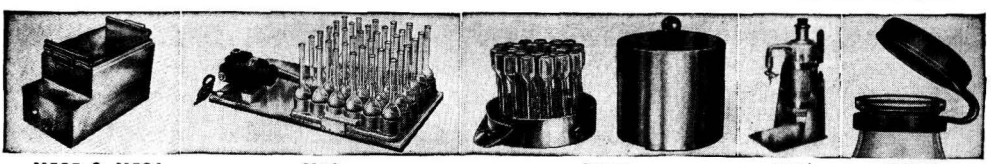

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