Journal of DAIRY SCIENCE

Vol. 43 July, 1960 Number 7

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JOURNAL OF DAIRY SCIENCE

OFFICIAL PUBLICATION OF THE
AMERICAN DAIRY SCIENCE ASSOCIATION

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Editorial Address: Station A, Box 718, Champaign, Illinois
Phone: FLeetwood 6-6323

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PEOPLE AND EVENTS

W. E. Petersen, Professor of Dairy Husbandry at University of Minnesota Retires

W. E. PETERSEN, internationally known professor of dairy husbandry, retired from the University of Minnesota in June.

In his 39 yr. at the University, the colorful Pine City, Minnesota, native has advanced a number of new findings and principles on milk and milk production—findings noted around the world. He has been a speaker on dairying and dairy research in all but two states and in more than a dozen other nations.

"Doc Pete," as he is known, has received several major awards, including knighthood from the King of Denmark in 1952.

W. E. Petersen

Petersen was born February 3, 1892, near Pine City, and grew up on a dairy farm there. He attended the University of Minnesota, where he earned his B.S. in 1916, his M.S. in 1917, and his Ph.D. degree in 1928—all in dairy fields.

He was a dairy extension specialist from 1917-20 at Kansas State college, was field secretary of the state Holstein-Friesian association in Minneapolis for a year, and in 1921 joined the University of Minnesota dairy husbandry department.

His research in dairy physiology—particularly in milk secretion—began when he did his graduate work on synthesis of milk fat. This led him to studies on how many different milk ingredients are formed. In recent years, for example, he used radioactive isotopes to study how the mammary gland produces milk sugar.

In 1929, Petersen produced the first experimental case of milk fever in cattle, and in the process found that a calcium deficiency is involved. This information led other research workers to development of effective milk fever treatments.

In 1935, he constructed his mechanical cow, a mass of tubes, beakers, and other devices which kept udders from freshly slaughtered cows functioning long enough to study several aspects of the milk secretion process. The device has been used in practically all of Petersen's research projects.

Petersen and his co-workers in 1937 found that oxytocin, the hormone which induces labor at calving time, also causes cows to let their milk down. He then formulated a rapid milking and cow-handling procedure which would stimulate secretion of this hormone and, thereby, aid milking. Many farmers now follow this procedure.

One of the basic findings from the oxytocin research was that no cow gives all her milk during a normal milking. Instead, Petersen found that all cows hold back a certain amount of complementary milk, which can be released with oxytocin injections and that the amount of complementary milk gives some indication of how long a cow's lactation period will be. And, since the complementary milk trait is hereditary, it may in the future be useful in selecting long-milking heifers.

Petersen was also one of the first scientists to experiment with ova transplant procedures—recovering a fertilized egg from the uterus of one cow and placing it in the uterus of another. He was able to make successful recoveries but was not able to transplant the eggs without use of surgical techniques.

In 1946, Petersen began work on antibodies in milk, and in 1950 he and Campbell produced the first report on this principle. It was known for a long time that colostrum, a cow's first milk after calving, contains many antibodies that protect newly born calves from a wide range of diseases. But the belief was that, a few days after calving, the cow lost the ability to produce milk antibodies.

However, Petersen and Campbell early concluded that antibody proteins can be produced in the udder at any time, and believed that antibodies specific to a number of diseases in humans can be produced as a result of injecting killed germs into the udder.

In 1955, they reported that adult humans and other creatures drinking antibody-containing milk receive temporary immunity to diseases which these antibodies counteract.

In 1952, he was made a knight by King Frederick IX of Denmark, in recognition of his contributions to scientific research. He also was the 1942 recipient of the Borden Award in dairy science and the 1956 recipient of both the Milk Industry Foundation teaching award and the F. B. Morrison award.

Petersen is listed in Who's Who and American Men of Science and was President of the American Dairy Science Association in 1949, and has been president of the Minnesota Society of Sigma Xi and chairman of the state section of the Society for Experimental Biology and Medicine. He has been a member and officer of several other professional and scientific organizations.

Dr. Petersen is the author and co-author of more than 500 technical and scientific papers. He has produced two motion pictures dealing with the function of the cow's udder. He is a member of nine scientific societies and has had 150 graduate students under his supervision.

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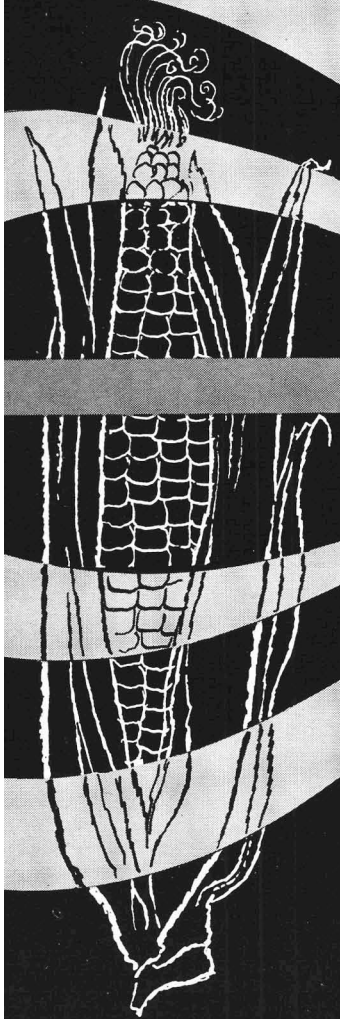

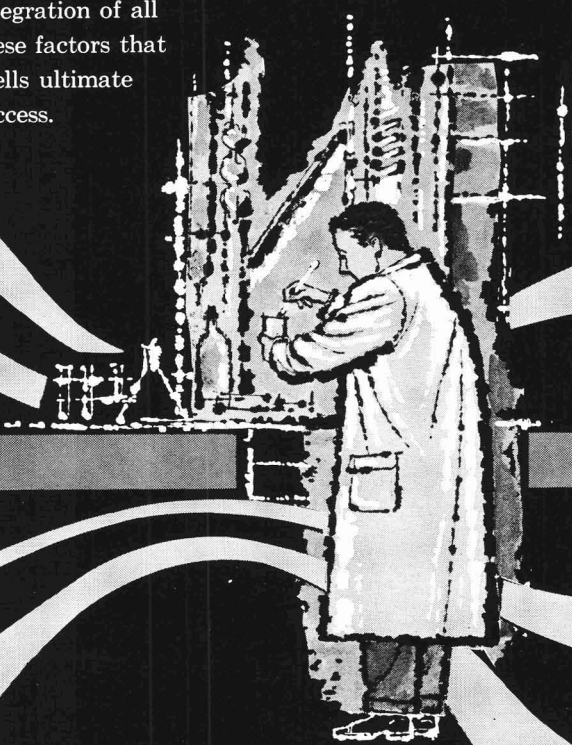

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He is author or co-author of seven college textbooks in dairy husbandry.

He was married in 1917 to the former Alma Linstrom, Rush City. They have five children: Dorothy (Mrs. John F. Grimmel), registrar, Macalester College, St. Paul; Joanne (Mrs. Robert Wilson), St. Louis, Missouri; William E. Jr., Minneapolis physician; Allan, Minneapolis dentist; and Raymond, dentist in Golden Valley, Minnesota.

He was honored on May 2 by the student Dairy Science club on the St. Paul campus. The club presented him with a genuine Swiss cow bell, on which is inscribed "Dr. Pete," the combination title and nickname by which Petersen has long been known among students and fellow staff members. Also engraved on the brass bell is a citation which recognizes him as a scientist, educator, and scholar.

Extension Specialist at Minnesota to Serve at Waterloo, Iowa

R. W. WAYNE, extension dairyman at the University of Minnesota, has been appointed superintendent of the cattle department at the National Dairy Cattle Congress to be held Oct. 1-8 at Waterloo, Iowa.

This is the major dairy cattle show in the United States and is the national show for five dairy breeds. Exhibits come from nearly every state.

The Congress is also the site of the National 4-H, FFA, and intercollegiate Dairy Cattle Judging contest. The world's largest farm machinery show is also held there.

Oregon State College News

T. D. CASE, Grants Pass high school senior, has been awarded the $1,000 P. M. Brandt Memorial scholarship given for study in dairy technology at Oregon State College.

The 4-yr. scholarship is given annually by Oregon Dairy Industries to an outstanding high school student interested in taking dairy technology work at OSC. Named for the late P. M. Brandt, long-time head of dairy work at the college, it is sponsored as a means of encouraging and developing leadership in the milk processing industry. Oregon Dairy Industries is the state organizations of milk processing plants.

Case, 19, has established an enviable record of scholarship and leadership at Grants Pass high school. He has been active in 4-H club work for the past 8 yr. and has received numerous county, district, and state awards.

Selection of Case for the $1,000 award was announced by P. V. SULLIVAN, Umpqua Dairy Products Company of Roseburg, who is chairman of the ODI education committee; J. O. YOUNG, OSC dairy technology professor and a member of the ODI scholarship committee;

and F. W. THOMAS, principal of the Grants Pass high school.

Selection of the winner is based on interest in the dairy industry, scholarship, need, and character. Case will receive one-twelfth of the $1,000 each term during his college career. Recipients must maintain specified grade averages to retain the study grant from year to year.

1960 Ohio State Fair

The 1960 Ohio State Fair Dairy Products Competition will feature two new events: a) Quality Milk Production, open to Future Farmers of America and b) Ice Cream Competition, open to ice cream manufacturers in Ohio. In addition, two events from previous years will be continued: a) Swiss Cheese Makers Competition, and b) Cottage Cheese Makers Competition. The individual competitive judging of dairy products have been eliminated from this year's fair.

For the Quality Milk Production contest the entries, which are composed of teams of three F.F.A. members, will submit samples of raw milk to represent the daily milk production of the family farm. The entries will be evaluated on the basis of (a) knowledge of milk production, and (b) composition and quality of the milk. The latter will involve flavor, raw and pasteurized bacterial contents, fat, and total solids.

Blue, red, and white ribbons will be awarded to the team entries based on the total scores. Special certificates will be given to individuals who achieve a blue-ribbon rating. The highest scoring team will be awarded a $250 cash award to be used for a trip to Chicago for the three team members and a supervisor. In Chicago, the team will visit various dairy enterprises, to become familiar with the processing, manufacturing, merchandising, and engineering aspects of the Dairy Industry.

The Ice Cream Competition is strictly a quality contest. Companies may submit entries in either one, or all, of the following three classes: vanilla, chocolate, and strawberry. Blue, red, and white ribbons will be awarded according to quality ratings of 93, 92-91, and 90, respectively. Blue-Ribbon ice creams will also be awarded special certificates and cash awards.

Official judges for the contests will be R. E. HARDELL, Monroe, Wisconsin, for Swiss Cheese, and J. M. JENSEN, Michigan State University, East Lansing, Michigan, for ice cream and Cottage Cheese. In addition to scoring the products, Professor Jensen will conduct special clinics on ice cream and Cottage Cheese.

The Cottage Cheese Competition will be similar to other years' contests, except that two classes have been established, one for large style curd and one for small style curd. Also, the awarding of prizes will be based on a system similar to that indicated for the ice cream competition.

Two classes are open in the Swiss Cheese Maker Competition: (a) Wheel cheese, and (b) rindless block cheese. No basic changes have been made in the judging and awarding of prizes in this competition.

Hustead and Delhey Promoted at Johnson & Johnson

G. L. HUSTEAD, Sandy, Utah, has been named territory sales manager for Filter Products Division of Johnson & Johnson in Utah, Colorado, Idaho, Wyoming, and Montana.

A native of Roosevelt, Utah, Hustead attended the University of Utah and served 4 yr. in the Air Force. He and his wife, the former Janice Joan Smith of Murray, Utah, have two children, Garth and Joni. He is an elder in the Church of Jesus Christ of Latter Day Saints.

R. W. DELHEY, Wausau, Wisconsin, has been named territory sales manager in the Upper Michigan Peninsula and Wisconsin regions by Filter Products Division of Johnson & Johnson. Filter Products is the world's largest producer of milk filters and also produces engineered filters for industrial use.

A native of Chicago, Delhey joined Johnson & Johnson in 1956 as a dairy technologist and served as liaison between the sales and research departments.

A graduate of the University of Illinois, Delhey is married to the former Jean Marie Horrigan of Chicago. He is a member of the Chicago Dairy Technology Society and the American Dairy Science Association.

Completed Theses

M. S. Theses:

ROBERT W. HAND—A pelleted complete ration for lactating dairy cows. Kansas State University, Manhattan.

JOHN A. KOBURGER—Identification of substances in milk cultures of *Pseudomonas fluorescens* which stimulate lactic starter cultures. Kansas State University, Manhattan.

ALI MAHMOUD—A study of the bovine vagina as related to reproductive performance. Kansas State University, Manhattan.

CHESTER N. PETERSON—Some factors associated with the estrus cycle of the dairy cow. Kansas State University, Manhattan.

CHARLES E. POLAN—The effect of continuous feeding of chlortetracycline on lactating dairy cows. West Virginia University, Morgantown.

RAY R. SCHOOLEY—The frequency and heritability of muzzle color and several coat and skin color characteristics in Milking Shorthorn cattle. Kansas State University, Manhattan.

JAMES A. TRAMMEL—A study of rancidity in milk. Clemson College, Clemson, South Carolina.

Dairy Technology Societies

Atlanta—The group's June Dairy Month Jamboree was held Friday, June 3 at Ansley Country Club. Dinner and Dancing and a Social Hour were enjoyed.

Detroit—The Annual Father-Daughter and Son meeting was held June 6 at Cregar's Pickwick House on Grand River. G. M. Trout, Department of Food Science at Michigan State University, provided the program, showing films on a recent trip to Europe.

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Ohio—Dairy Technology Societies' newly elected officers of the Four Ohio Dairy Technology Societies for 1960-61 include:

Maumee Valley: DON SHUNK, Cherry Grove Dairy, Toledo, president; PAUL LIND, Babcock Dairy, Toledo, vice-president; MARVIN GEORGE, Northwest Sales Association, secretary; ROBERT HEINDRICKS, Sterling Milk Co., Wauseon, treasurer; F. WILLINGER, Babcock Dairy, Toledo, sergeant-at-arms. Northeastern Ohio: JOHN PAXTON, Parson's Jersey Dairy, Oberlin, president; JOE SEIBERT, Old Meadow Dairy, Cleveland, vice-president; MIKE SWINEHART, Reiter and Harter Dairy, Barberton, secretary; ED WEBER, Wyandotte Chemical Company, Orville, treasurer; DON BARTHOLEMEW, Oberlin, sergeant-at-arms. Central Ohio: RONALD DOUGLAS, Beatrice Foods, Co., New Bremen, president; DICK STAFFORD, Diamond Milk Co., Columbus, vice-president; RICHARD SPIRES, Borden's Moores and Ross, Columbus, secretary; THAD HUNTERMARK, Bessire & Company, Columbus, treasurer; ROBERT ASHLEMAN, Borden's Moores & Ross, Columbus, sergeant-at-arms. Cincinnati: LOU TRAUTH, Trauth Dairy, Cincinnati, president; VERNON LEIN, Kroger Company, Cincinnati, vice-president; ELMER HANNEKEN, Hanneken Dairy, Cincinnati, secretary; ED MCGRATH, Glacier Orange Company, Cincinnati, treasurer; RALPH KAISER, America Dairy Supply, Cincinnati, sergeant-at-arms.

ADVERTISERS' INDEX

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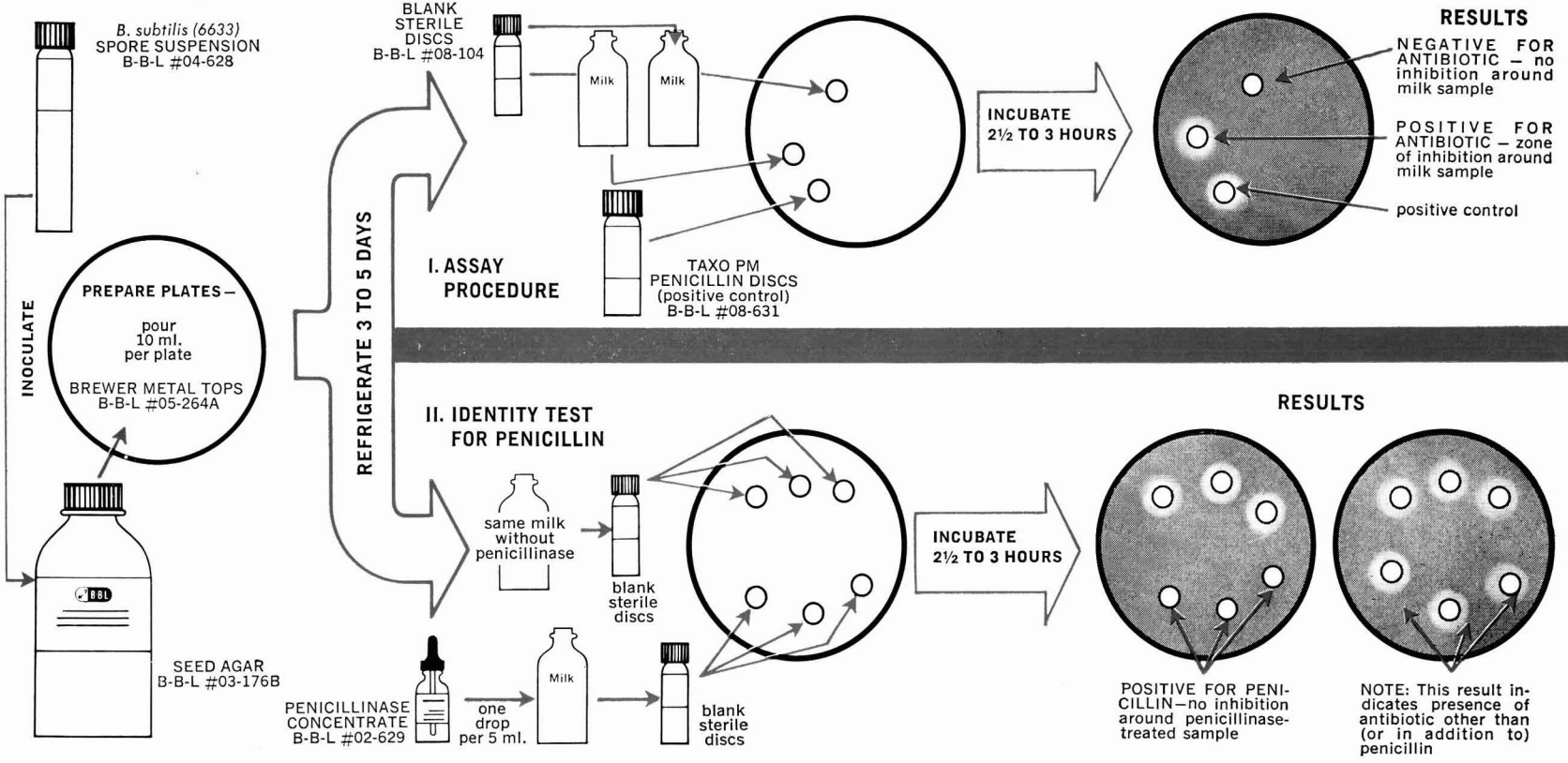
DETECTION OF PENICILLIN IN MILK



The presence of antibiotics in milk following mastitis therapy in cows has created serious public health problems and caused technical difficulties within the dairy industry. A rapid, practical laboratory procedure to assist regulatory agencies and the dairy industry in solving these problems was described by Arret and Kirshbaum.* This procedure employs rapid growth of a sensitive strain of *B. subtilis* in milk and for determining its identity with penicillin. Inhibition of growth by the presence of as little as 0.05 unit of penicillin per ml. of milk sample is detectable within 2½ hours.

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*Arret, B., and Kirshbaum, A.: J. Milk and Food Technol. 22:329, 1959.

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Prepared by

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THE AMERICAN DAIRY SCIENCE ASSOCIATION

H. B. HENDERSON, University of Georgia, Athens, Georgia, Chairman
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I. A. GOULD, The Ohio State University, Columbus, Ohio
FARNUM GRAY, Southern Dairies, Charlotte, North Carolina
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H. F. WILLIAMS, Carnation Company, Los Angeles, California

SUMMARY OF CONTENTS

Section I Objectives and Requirements of the Program

Section II Training Program Schedules for Plant Operation

1. Orientation
2. Plant operations
3. Laboratory
4. Engineering and maintenance
5. Procurement
6. Sales and distribution
7. Office work

Schedules providing for varying periods of time are provided for the above categories for the operation of

1. Milk plants
2. Ice cream plants
3. Condensed and dry milk plants
4. Cheese plants
5. Butter plants
6. Evaporated milk plants

Many questions are asked for the trainee to answer before passing from one phase of training to the next and progress reports and rating forms are provided.

Section III Management and Development

1. Developing skills in personnel management
2. Developing skills in quality control and cost

Many study projects are outlined which are not only essential to management development but may result in savings to the plant that will more than pay the cost of training.

Appendix

1. List of professional and trade organizations
2. List of reference books, booklets, and periodicals

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Plant Managers
Plant Supervisors
Plant Trainees
Teachers, for a reference text

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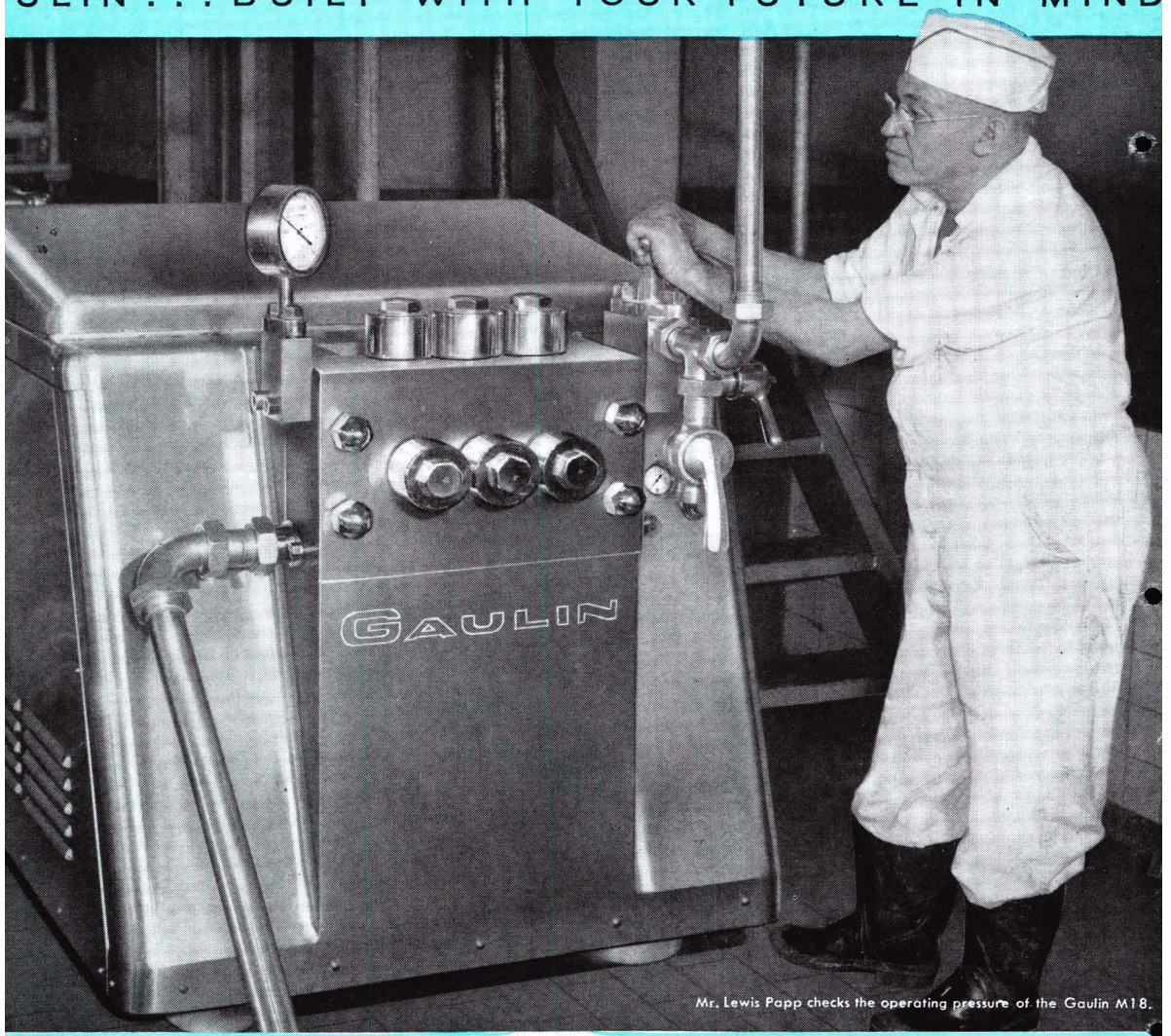

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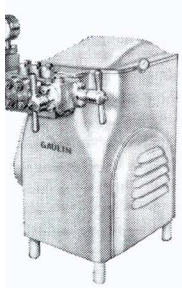

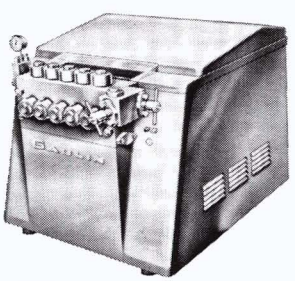

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NOMENCLATURE OF THE PROTEINS OF BOVINE MILK—FIRST REVISION

Report of the Committee on Milk Protein Nomenclature, Classification, and Methodology of the Manufacturing Section of A.D.S.A. for 1958-59

J. R. BRUNNER [1] (Chairman), C. A. ERNSTROM,[2] R. A. HOLLIS,[3] B. L. LARSON,[4] R. McL. WHITNEY,[5] AND C. A. ZITTLE [6]

SUMMARY

The α-casein component of the casein system of milk, which appears as a single, leading electrophoretic peak at pH 8.6, is heterogeneous and should be referred to as the α-casein fraction. The components comprising this fraction vary in distribution, dependent upon the experimental conditions. *Alpha*-casein has been separated into calcium-sensitive and calcium-insensitive fractions which have been designated by various symbols. These fractions usually are in the form of an α (Ca sensitive)—α (Ca insensitive) complex in equilibrium with its components. In view of the complexity of the α-casein fraction, this Committee feels that no recommendations on nomenclature should be made at this time. Cherbuliez's δ-casein, Hammersten's proteose, and the 2% and 12% TCA-soluble peptides of Alais and Nitschmann, materials apparently derived from α-casein by various procedures, are discussed in this report.

β-lactoglobulin, obtained from mixed milk, is composed of at least two forms of β-lactoglobulin which are genetically determined and referred to as β-lactoglobulins *A* and *B*, discernible by paper electrophoresis in veronal buffer at pH 8.6, where Type *A* constitutes the leading component. Further, Type *A* associates in acetate buffer between pH 3.7 and 5.2 and is essentially monomeric at pH values alkaline to its isoelectric point, whereas Type *B* exists in its monomeric form under similar conditions. These characteristics explain in part the electrophoretic and ultracentrifugal heterogeneity of normal (mixed) β-lactoglobulins *A* + *B* and β-lactoglobulin *A*.

A previous Committee report, Jenness *et al.* (22), published in 1956, contributed materially to the clarification of a rapidly expanding system of nomenclature for the proteins of bovine skimmilk. The electrophoretically discernible protein components were categorized in terms of their classical or more traditional nomenclature and in relationship to the contemporary nomenclature. Although electrophoretic resolution of the protein components in free-boundary electrophoresis was selected as a convenient criterion for classification, the possibility was recognized that many of these so-called individual proteins were actually complexes or heterogeneous mixtures of proteins possessing similar electrophoretic characteristics at pH 8.6.

Received for publication January 29, 1960.

[1] Department of Dairy, Michigan State University, East Lansing.

[2] Department of Dairy–Food Industries, University of Wisconsin, Madison.

[3] Research and Development Div., National Dairy Products Corporation, Oakdale, Long Island.

[4] Department of Dairy Science, University of Illinois, Urbana.

[5] Department of Food Technology, University of Illinois, Urbana.

[6] Eastern Utilization Research Branch, USDA, Philadelphia, Pa.

Recent investigations have demonstrated the heterogeneity of the α-casein and β-lactoglobuliln fractions. New, and at times confusing, terminology has been introduced by investigators to identify the proteins they have reported.

The present Committee, in an attempt to cope with the problem of expanding terminology, recognized that: Within reasonable limits, the prerogative of the investigator to assign an appropriate nomenclature to the proteins he has isolated and/or characterized should be preserved, provided that he shows conclusive evidence that his protein differs in fact, not purity, from any protein previously isolated and characterized; and the Committee's principal function was the resolution of newly introduced terminology in terms of contemporary nomenclature rather than recommending prematurely a rigid nomenclature system. Conceivably, newly reported protein fractions of similar characteristics could be classified tentatively pending: (a) the presentation of additional confirmatory evidence in support of the homogeneity of the protein, and (b) the development of a sound nomenclature system for milk proteins. An awareness of the progress being made in this field would indicate that a stabilized nomenclature system must await a more complete elucidation of the milk protein system.

Based upon the foregoing considerations, this Committee has considered the terminology introduced by investigators to designate the components of the α-casein fraction and β-lactoglobulin and has presented a compilation of this terminology in terms of contemporary usage. Also, certain additions and revisions have been made to the table of characteristics published in the 1956 report. In so doing, the Committee realized that further development will necessitate additional revisions, until a more complete elucidation of the milk protein system makes possible the establishment of a nomenclature and classification system which will facilitate research in the field of milk proteins.

Casein. The 1956 report recognized the individuality of the components α-, β-, and γ-casein in the protein fraction precipitated by acidifying raw skimmilk to pH 4.6. A fourth component, δ-casein, isolated by Cherbuliez and his coworkers (7, 8, 9) and characterized by its solubility in 10% TCA, was considered as a possible entity. The similarity between δ-casein and Hammersten's proteose, found as a product of the reaction of rennet on casein, was postulated (10). Alais (1) showed similarities between Hammersten's proteose, Cherbuliez's δ-casein, and a 2% TCA-soluble fraction obtained by the action of crystalline rennin on casein. The bulk of the 2% TCA-soluble fraction stems from the α-casein fraction (2) and more specifically from the calcium-insensitive fraction (κ-casein) (57, 59) by the primary action of rennin. The fact that Cherbuliez and Baudet (8) did not isolate δ-casein from paracasein suggests further that δ-casein and the rennin-liberated proteins may have similar origins. Further investigations are required to establish the classification of δ-casein.

Within recent years several papers have been published elucidating the α-casein fraction. Waugh and von Hippel (61) were the first to show that α-casein could be separated into calcium-sensitive [α_s-casein (59)] and calcium-insensitive [crude-κ-casein] fractions, based upon its dissociation and differential solubility in the presence of calcium ions. The fractions of casein not classified

as as α_s-, crude-κ-, or β-casein were designated *m* fraction (60). Long *et al.* (30), working along a similar approach, were able to isolate a slow sedimenting fraction from Waugh's second cycle, crude-κ-casein, characterized by its high phosphorus content (1.1%), which they called λ-casein. They suggested that the calcium-insensitive fraction of Waugh possessed properties similar to the ζ-casein fraction isolated by Linderstrøm-Lang (29). The calcium-sensitive fraction of the α-casein fraction was called α_R-(30) and, in a previous study, α_P-casein (44). Wake (57) has referred to the calcium-sensitive fraction as α-casein.

McMeekin *et al.* (33) reported the isolation of a fraction from α-casein possessing minimum solubility at pH 5.8-6.0, but soluble at pH 4.7 as well as pH 4.0 (32), which they called α_2-casein. This fraction was obtained from acid casein in yields approximating 1%, was low in phosphorus content (0.1%), not precipitated by calcium ions nor clotted by rennet, but was split by rennet at pH 7.3 into soluble and insoluble fractions. The remaining and larger fraction was called α_1-casein. Electrophoretic mobilities for the α_1- and α_2-casein fractions in veronal buffer at pH 8.4, $\Gamma/2 = 0.1$ were -6.7 and -5.0, respectively. McMeekin (31, 32) has since referred to the α_2-casein fraction as α_z-casein. Characteristics of α_z-casein suggest its similarity to Linderstrøm-Lang's Z-casein and Waugh's κ-casein. Further, McMeekin (33, 34) referred to the calcium-sensitive fraction of α-casein as α_1-casein. More recently, Hipp *et al.* (19) have reported the isolation of α_3-casein, characterized by a single ultracentrifugal component at pH 7.1 ($S_{20} = 23$ in phosphate buffer) and by its resemblance to κ-casein in physical properties.

Cherbuliez and Baudet (7) fractionated α-casein into two fractions by warming it to 40° C. in 5% $(NH_4)_2SO_4$ solution. The soluble (α_I) and insoluble (α_{II}) fractions were similar in composition and can not be considered as different protein entities.

Nitschmann and Lehmann (37) showed that rennin, when added to sodium α-caseinate, induced an electrophoretically discernible split in the alpha component; the two peaks were called α_1 and α_2 in descending order of mobility. Payens (40), studying the action of rennin on Waugh's first- and second-cycle casein fractions, concluded that Nitschmann's α_2-casein was quite similar, if not identical, to Waugh's *kappa*-rich fraction. The split in the electrophoretic peak of the α-casein fraction, which has been noted by many investigators, indicates the presence of more than one component and presents data sometimes difficult to interpret, since any one or a combination of factors, i.e., individual differences among cows, variations in ionic strength, freezing or dehydration of the casein preparation, and prolonged storage of casein solutions, could contribute to the occurrence of such an observation (12, 58).

Obviously, the α-casein fraction consists of a highly integrated system composed of calcium-sensitive and calcium-insensitive protein components. Actually, a precise interpretation of the data reported in support of the various casein fractions is most difficult because of the tendency of casein components to form aggregates under all except very drastic conditions (20, 36). For example, it is difficult to know whether a fraction with given properties is a pure component,

a mixture of components, or a fraction reflecting the specific conditions used in its isolation. Aggregation makes moleculer weight determinations especially troublesome and uncertain.

In view of these advancements in the knowledge of the casein system, the classical term calcium *para*-caseinate should be redefined to indicate the specificity of the action of rennin (14, 57, 59, 50). Waugh et al. (59, 60) have proposed a new terminology for the action of rennin on the α-casein fraction: *para*-κ-casein for the primary reaction product of rennin on κ-casein and α_s-*para*-κ-casein for the clots which are formed. The role of other α-casein components and of β- and/or γ-casein in this transformation has not been elucidated.

No recommendation relative to the nomenclature of the α-casein complex is suggested at the present time. As reviewed above and in Table 2, the α-casein complex is currently the subject of a great deal of research. It appears desirable to withhold a recommended nomenclature for the α-casein complex until the components and physical/chemical equilibria involved are more precisely and completely understood.

β-lactoglobulin. Pedersen's (41) electrophoretic and ultracentrifugal studies with Palmer's β-lactoglobulin led him to believe that the protein was homogeneous. Li (28), employing more sensitive electrophoretic techniques, observed that β-lactoglobulin showed a single electrophoretic peak in acetate buffer at pH 5.3 and 5.6, but showed three components when observed in buffer at pH 4.8 and 6.5. In both cases, the fastest-moving boundary constituted the major portion of the pattern and had an isoelectric point of 5.1. Polis *et al.* (43) found that β-lactoglobulin isolated by alcohol fractionation and differential solubility at pH 4.8 and 5.3 from a pooled milk supply showed a single electrophoretic peak in buffers alkaline to the isoelectric point and two maxima in buffers on the acid side. They isolated the slow-moving fraction (pH 4.8) in small yields, which they designated β_1-lactoglobulin. The fast-moving component, which was not purified, was termed β_2-lactoglobulin. Aschaffenburg and Drewry (4) prepared β-lactoglobulin-rich fractions from the milk of individual cows, which they studied by paper electrophoresis in veronal buffer at pH 8.6, $\Gamma/2 = 0.05$. They observed that individual animals gave milk containing one or the other or a mixture of both of two electrophoretically discernible β-lactoglobulins. They designated the faster of the two components β_1-lactoglobulin and the slower as β_2-lactoglobulin.

Ogston and Tombs (38), working with β-lactoglobulin crystallized from the milk of individual cows, found that β_1-lactoglobulin, the fastest-moving component on filter paper electrophoresis at pH 8.6, was also the fastest component observed during free-boundary electrophoresis in acetate buffer at pH 4.6. This observation caused them to regard the protein isolated by Polis as a subfraction, not related to the β_1-lactoglobulin observed by Aschaffenburg and Drewry (5). In addition, they observed that both β_1- and β_2-lactoglobulin were electrophoretically heterogeneous at pH 4.6. Klostergaard and Pasternak (23) observed that the mobility of Polis' β_1-lactoglobulin was slower than Aschaffenburg's β_1-lacto-

TABLE 1

Protein fractions of bovine skimmilk and some of their properties

Protein fraction									
Classical nomenclature[a]	Contemporary nomenclature	Occurrence in electrophoretic pattern[b] (Peak No.)	Reference to preparation	Approximate per cent of skimmilk protein[c]	Sedimentation constant[d] (S_{20})	Molecular weight[e]	PI[f]	Electrophoretic mobility at pH 8.6[g]	Other characteristics
Casein (precipitated from skimmilk by acid at pH 4.6)		In casein pattern (17)	18, 58	76-86	1.18 (20)[i]	15,000 (20) 33,600 (6)			
	α-casein	1	18, 58	45-63	3.99 (50)	27,000 (36)	4.1 (22)	−6.7 (22)	Contains 1% phosphorus. Consists of a mixture of interesting proteins (see Table 2). Formed in the udder[n]
	β-casein	2	18, 58	19-28	1.57 (50)	24,100 (50)	4.5 (22)	−3.1 (22)	0.6% Phosphorus. Formed in udder
	γ-casein	3	18	3-7	1.55 (35)	30,600 (35)	5.8-6.0 (22)	−2.0 (22)	0.1% Phosphorus. Preformed from blood
Noncasein proteins		In acid whey pattern (27)		14-24					
Lactalbumin (Soluble in saturated $MgSO_4$ soln.)	β-Lactoglobulin A	6	4		2.8 (53)[i] 5.3 (53)[j]	35,000 (38)		−5.3 (54)	Associates in pH range 3.7 to 5.3. Formed in udder
	β-Lactoglobulin B (Mixed A and B)	6 39	4	7-12[h]	2.7 (53)	35,000 (38)		−5.2 (54)	Exists principally in monomeric form. Formed in udder.
	α-Lactalbumin	4	15, 16	2-5	1.75 (15)	16,500 (15)	5.1 (24)[i]	−4.2 (15)[m]	7% tryptophane. Formed in udder
	Blood serum albumin	7	42	0.7-1.3	4.0 (11)	69,000 (42)	4.7 (42)	−6.7 (42)	Apparently identical to bovine serum albumin. Preformed from blood

Lactoglobulin (Insoluble in saturated $MgSO_4$ soln.)	Euglobulin	1	48	0.8-1.7	8.77 (35)[k]	252,000 (35)[k] 180,000 (48)[k]	6.0 (35)	−1.8 (35)	Fractions containing antibodies. Contain hexose and hexosamine. Electrophoretically and ultracentrifugally heterogeneous. Preformed from blood.
	Pseudoglobulin	2	48	0.6-1.4	8.07 (35)[k]	289,000 (35)[k] 180,000 (48)[k]	5.6 (35)	−2.0 (35)	
Proteose-Peptone Fraction (Not precipitated at pH 4.6 from skimmilk previously heated to 95–100° C., 30 min.)			3, 22, 27	2-6	0.96 (3)[k] 2.75 (3)	4,900 (3)[k] 24,000 (3)			Glycoprotein (51). Electrophoretically and ultracentrifugally heterogeneous. Poorly defined except for serum component 5 (21).
		3						−3.0 (27)	
		5			1.0 (22)			−4.6 (27)[k]	
		8						−7.9 (27)	

[a] Rowland fractions (22, 46, 47).

[b] Free-boundary electrophoresis in veronal buffer at pH 8.6, $\Gamma/2 = 0.1$. Casein components designated in descending order of mobility. Serum protein components designated in ascending order of mobility.

[c] Values compiled and/or calculated from Rowland nitrogen distribution data, relative areas of electrophoretic patterns, and protein yield studies (18, 22, 27, 45, 46, 47).

[d] s_{20} = sedimentation constant = $(dx/dt)\ (1/w^2x)$, in Svedberg units ($S = 1 \times 10^{-13}$) corrected to 20° C. See original literature for experimental conditions. Sedimentation characteristics are dependent upon ionic strength of solvent, temperature, pH, and concentration of solute. The sedimentation and molecular weight values reported are not necessarily the best values obtainable, nor do they constitute endorsement by the Committee.

[e] Refer to original literature for method and conditions of determination.

[f] Isoelectric point, i.e., pH of no electrophoretic movement.

[g] Electrophoretic mobility (u) = $\times 10^{-5}$, $cm.^2$, $volts^{-1}$, $sec.^{-1}$ obtained by the Tiselius moving boundary method at 2° C. in veronal buffer at pH 8.6, $\Gamma/2 = 0.1$. Measured from descending pattern.

[h] Distribution of β-lactoglobulin A and B are genetically determined (4, 5).

[i] Denotes the characteristic of the monomeric specie.

[j] Denotes the characteristic of the associated specie.

[k] Denotes the characteristics of the major component.

[l] Value replaces previously reported value of 4.1 to 4.8.

[m] Mobility reported at −3.6 in milk serum protein mixture (26).

[n] Source of information pertaining to the origin of the milk proteins (25).

TABLE 2

Reported fractions or components of α-casein and some of their properties[a]

Contemporary nomenclature	Occurrence in electrophoretic pattern (Peak No.)	Reference to preparation	Approximate per cent of skimmilk protein	Sedimentation constant (S_{20})	Molecular weight	PI	Electrophoretic mobility at pH 8.6	Other characteristics
α-casein[b]								
Ca-sensitive component(s)	(1)							
α_s-casein		61[c]	37-54 (61)	1.59(61)[g]	23,300(59)			1.10% phosphorus
α_1-casein		33[c]		3.0 (34)		4.3-4.7 (34)	−6.7(33)	0.85% phosphorus
α_B-casein		30[c]		4.55(30)				1.16% phosphorus
Ca-insensitive component(s)	(1)		(49, 57, 61)					
κ-casein		13, 61[d,e]	11-13	1.4 (61)[g] 13.5 (61)[h]	16,300(59)			0.19 to 0.33% phosphorus Stabilizes α_s-casein to Ca ions
α_2-or α_z-casein		33[e]					−5.0(33)	0.1 to 0.15% phosphorus. Isolated in small yields. Soluble at pH 4.7. Resembles κ-casein.
α_3-casein		19[e]		23.0 (19)				Stabilizes α_1-casein to Ca ion.
λ-casein		30[f]	1.2 (30)	1.1 (30)				1.18% phosphorus, not stabilizing.
m casein		59, 60[f]						

[a] Units and experimental conditions similar to those described for Table 1.
[b] Method of isolation from whole casein can determine content of calcium-sensitive components (62).
[c] Similar characteristics suggest that proteins are similar.
[d] Gross calcium-insensitive fraction.
[e] Similar characteristics suggest that proteins are similar.
[f] Similar characteristics suggest that proteins are similar.
[g] Denotes characteristics of monomeric form.
[h] Denotes characteristics of associated form.

globulin on paper electrophoresis at pH 8.6, indicating that Polis' β_1-lactoglobulin corresponded to Aschaffenburg's β_2. This observation has since been confirmed in free-boundary electrophoresis by Timasheff and Townend (54).

Aschaffenburg and Drewry (5) discovered that the secretion of β_1-lactoglobulin and β_2-lactoglobulin was under genetic control. This induced them to abandon the old nomenclature in favor of the β-lactoglobulin-*A* and *B* nomenclature which is more acceptable from a genetic standpoint. β-lactoglobulin *A* and *B* are discernible in descending order of electrophoretic mobility in veronal buffer at pH 8.6.

Recent work of Timasheff and Townend (52, 53, 54, 55) contributed significantly to the characterization of β-lactoglobulins *A* and *B*. The electrophoretic mobility at pH 4.65 of β-lactoglobulin *B* was slower than the polymer Type A, but slightly faster than the monomer. They concluded, from supporting ultracentrifugal data, that the observed association of β-lactoglobulin in the pH range of 3.7 to 5.2 was due to β-lactoglobulin *A*. Type *B* sedimented as a single molecular specie at concentrations up to 7%. The conclusions supported the work of Ogston and Tombs (38, 56), who suggested that the association of β-lactoglobulin was due to the aggregation or association of Type *A*.

The following recommendations relative to the nomenclature of the β-lactoglobulins are suggested: β-lactoglobulin exists in two forms which are genetically defined and discernible by paper electrophoresis at pH 8.6. These were designated by Aschaffenburg and Drewry (5) as β-lactoglobulin *A* (faster-moving component) and *B* which appears to be the accepted designation for reasons mentioned earlier. β-lactoglobulin *A* associates in the range of pH 3.7 to 5.2, while β-lactoglobulin *B* exists principally in a monomeric state. This particular characteristic of the β-lactoglobulins manifests itself in the heterogeneity observed in the electrophoretic and ultracentrifugal patterns of β-lactoglobulin *A* and *B* (mixed) and β-lactoglobulin *A*, when studied within this pH range. The β-lactoglobulins are essentially monomeric in alkaline media.

The foregoing discussions serve as background material upon which the deliberations of this Committee were based. The present status of the available knowledge relating to the characteristics of the protein fractions of milk presently known as α-casein and β-lactoglobulin has induced us to suggest revisions to the report of the 1956 Committee (see Tables 1 and 2). Obviously, the voids in data within these tables demonstrate the paucity of our information relating to physical and chemical properties of skimmilk proteins. Also, the Committee wishes to point out a certain lack of evidence in support of the homogeneity of fractions or components listed under α-casein, and that inclusion in the table does not constitute unmitigated acceptance of the component as a protein entity, but, rather, a compilation of the current information relating to the characteristics and nomenclature of the α-casein fraction. In some instances, apparently, similar components have been designated differently by the various authors. The Committee hopes that by compiling these data, it willl, to some degree, stimulate continued studies toward the elucidation of the milk protein system

Free-boundary electrophoresis continues to be recognized as a primary standard for classification in the nomenclature scheme, but it is apparent that other physical and chemical criteria are required to elucidate the complexity of electrophoretically homogeneous components.

REFERENCES

(1) ALAIS, C. Étude des Substances azotees non-proteigues (NPN) separees de la Caséine du Lait de Vache sous de l'Action de la Presure. 14th Intern. Dairy Congr., (Rome), 2: 823. 1956.

(2) ALAIS, C., MOCQUOT, G., NITSCHMANN, H. S., AND ZAHLER, P. Das Lab und seine Wirkung auf das Casein der Milch. VII. Über die Abspaltung von Nicht-Protein-Stickstoff (NPN) aus Casein durch Lab und ihre Beziehung zur Primärreaktion der Labgerinnung der Milch. Helv. Chim. Acta, 36: 1955. 1953.

(3) ASCHAFFENBURG, R. Surface Activity and Proteins of Milk. J. Dairy Research, 14: 316. 1946.

(4) ASCHAFFENBURG, R., AND DREWRY, J. Occurrence of Different beta-Lactoglobulins in Cow's Milk. Nature, 176: 218. 1955.

(5) ASCHAFFENBURG, R., AND DREWRY, J. Genetics of the β-Lactoglobulins of Cow's Milk. Nature, 180: 376. 1957.

(6) BURK, N. F., AND GREENBERG, D. M. Physical Chemistry of the Proteins in Non-aqueous and Mixed Solvents. J. Biol. Chem., 87: 197. 1930.

(7) CHERBULIEZ, E., AND BAUDET, P. Recherches sur la Caséine. V. Sur les Constituants de la Caséine. Helv. Chim. Acta, 33: 398. 1950.

(8) CHERBULIEZ, E., AND BAUDET, P. Recherches sur la Caséine. VI. Sur la Transformation de la Caséine en Paracaséine. Helv. Chim. Acta, 33: 1673. 1950.

(9) CHERBULIEZ, E., AND JEANNERAT, JEAN. Recherches sur la Caséine. III. Sur le Fractionnement de la Caséine et de la Paracaséine au Chlorure d'Ammonium. Helv. Chim. Acta, 22: 952. 1939.

(10) CHERBULIEZ, E., AND JEANNERAT, JEAN. La Protéose de Hammarsten n'est pas un Produit de Degradation de la Caséine. Helv. Chim. Acta, 22: 959. 1939.

(11) EHRENPREIS, S., MAURER, P. H., AND RAM, J. S. Modified Bovine Serum Albumin. I. Preparation and Physicochemical Studies of Some Derivatives. Arch. Biochem. and Biophys., 67: 178. 1957.

(12) FITZPATRICK, MARGARET M., AND SULLIVAN, R. A. Effect of Processing and Storage on Electrophoretic Patterns of Skimmilk Proteins. J. Dairy Sci., 40: 1262. 1957.

(13) FOX, K. K. Separation of a Calcium-Soluble Fraction of Casein from Isoelectric Casein. J. Dairy Sci., 41: 715. 1958.

(14) GARNIER, J. Fraction Proteique dégradée Spécifiguement par la Présure dans la Caséine. Proc. Intern. Symposium on Enzyme Chem., Tokyo and Kyoto, 1957, 1: 524. 1958.

(15) GORDON, W. G., AND SEMMETT, W. F. Isolation of Crystalline α-Lactalbumin from Milk. J. Am. Chem. Soc., 75: 328. 1953.

(16) GORDON, W. G., SEMMETT, W. E., AND ZIEGLER, J. Crystalline α-Lactalbumin. An Improved Method for Its Isolation. Sulfur Distribution. J. Am. Chem. Soc., 76: 287. 1954.

(17) HIPP, N. J., GROVES, M. L., CUSTER, J. H., AND MCMEEKIN, T. L. Separation of γ-Casein. J. Am. Chem. Soc., 72: 4928. 1950.

(18) HIPP, N. J., GROVES, M. L., CUSTER, J. H., AND MCMEEKIN, T. L. Separation of α-, β-, and γ-Casein. J. Dairy Sci., 35: 272. 1952.

(19) HIPP, N. J., GROVES, M. L., AND MCMEEKIN, T. L. Separation of the Components of α-Casein. The Preparation of α_s-Casein. Abstr. No. 12. 136th meeting Am. Chem. Soc. September, 1959.

(20) VON HIPPEL, P. H., AND WAUGH, D. F. Casein: Monomers and Polymers. J. Am. Chem. Soc., 77: 4311. 1955.

(21) JENNESS, R. Characterization of Milk Serum Components. J. Dairy Sci., 42: 895. 1959.
(22) JENNESS, R., LARSON, B. L., MCMEEKIN, T. L., SWANSON, A. M., WHITNAH, C. H., AND WHITNEY, R. MCL. Nomenclature of the Proteins of Bovine Milk. J. Dairy Sci., 39: 536. 1956.
(23) KLOSTERGAARD, H., AND PASTERNAK, R. A. Electrophoresis and Ultracentrifuge Studies of Milk Proteins. II. α-Lactalbumin. J. Am. Chem. Soc., 79: 5674. 1957.
(24) KLOSTERGAARD, H., AND PASTERNAK, R. A. Electrophoresis and Ultracentrifuge Studies of Milk Proteins. II. α-Lactalbumin. J. Am. Chem. Soc., 79: 5674. 1957.
(25) LARSON, B. L., AND GILLESPIE, D. C. Origin of the Major Specific Proteins in Milk. J. Biol. Chem., 227: 565. 1957.
(26) LARSON, B. L., AND JENNESS, R. Identification of α-Lactalbumin in the Electrophoretic Pattern of Milk Serum Proteins. J. Dairy Sci., 38: 313. 1955.
(27) LARSON, B. L., AND ROLLERI, G. D. Heat Denaturation of the Specific Serum Proteins in Milk. J. Dairy Sci., 38: 351. 1955.
(28) LI, C. H. Electrophoretic Inhomogeneity of Crystalline Beta-Lactoglobulin. J. Am. Chem. Soc., 68: 2746. 1946.
(29) LINDERSTRØM-LANG, K. Studies on Casein. II. Is Casein a Homogeneous Substance? Compt. rend. trav. lab. Carlsberg, 16: 48. 1925.
(30) LONG, J., VANWINKLE, Q., AND GOULD, I. R. Isolation and Identification of λ-Casein. J. Dairy Sci., 41: 317. 1958.
(31) MCMEEKIN, T. L. The Separation and Characterization of the Components of α-Casein. (Invited paper) 53rd Annual Meeting, Am. Dairy Sci. Assoc., Raleigh, North Carolina. June, 1958.
(32) MCMEEKIN, T. L. Personal communication. July, 1959.
(33) MCMEEKIN, T. L. GROVES, M. L., AND HIPP, N. J. The Separation of a New Component of Casein. Abstr. No. 143. 131st Meeting Am. Chem. Soc. April, 1957.
(34) MCMEEKIN, T. L., HIPP, N. J., AND GROVES, M. L. The Separation of the Components of α-Casein. The Preparation of α_1-Casein. Arch. Biochem. and Biophys., 83: 35. 1959.
(35) MURTHY, G. K., AND WHITNEY, R. MCL. A Comparison of Some of the Chemical and Physical Properties of γ-Casein and Immune Globulins of Milk. J. Dairy Sci., 41: 1. 1958.
(36) NIELSEN, H. C. Molecular Weight Studies on Acid-Precipitated, Calcium-Precipitated, Alpha, and Beta Caseins by Osmotic Pressure Measurements in 6.66 M Urea. Ph.D. thesis. Michigan State University. 1957.
(37) NITSCHMANN, H. S., AND LEHMANN, W. Elektrophoretische differenzierung von Saure-und Lab Casein. Experimentia, 3: 153. 1947.
(38) OGSTON, A. G., AND TOMBS, M. P. The Heterogeneity of Bovine β-Lactoglobulin. Biochem. J., 66: 399. 1957.
(39) PALMER, A. H. The Preparation of a Crystalline Globulin from the Albumin Fraction of Cow's Milk. J. Biol. Chem., 104: 359. 1934.
(40) PAYENS, T. A. J. First and Second Cycle Casein in Milk. Nature, 181: 114. 1958.
(41) PEDERSEN, K. O. Ultracentrifugal and Electrophoretic Studies on Milk Proteins. I. Introduction and Preliminary Studies with Fractions from Skimmilk. Biochem. J., 30: 948. 1936.
(42) POLIS, B. D., SHMUKLER, H. W., AND CUSTER, J. H. Isolation of a Crystalline Albumin from Milk. J. Biol. Chem., 187: 349. 1950.
(43) POLIS, B D., SHMUKLER, H. W., CUSTER, J. H., AND MCMEEKIN, T. L. Isolation of an Electrophoretically Homogeneous Crystalline Component of β-Lactoglobulin. J. Am. Chem. Soc., 72: 4965. 1950.
(44) REISFELD, R. A. A Study of the Calcium-Binding Properties of Whole Casein, α-Casein and β-Casein. Ph.D. thesis, The Ohio State University. 1957.
(45) ROLLERI, G. D., LARSON, B. L., AND TOUCHBERRY, R. W. Protein Production in the Bovine, Breed and Individual Variation in the Specific Protein Constituents of Milk. J. Dairy Sci., 39: 1683. 1956.

(46) ROWLAND, S. J. The Determination of the Nitrogen Distribution in Milk. J. Dairy Research, 9: 42. 1938.
(47) SHAHANI, K. M., AND SOMMER, H. H. The Protein and Nonprotein Nitrogen Fraction in Milk. II. Their Content in Fresh Raw Skimmilk. J. Dairy Sci., 34: 1010. 1951.
(48) SMITH, E. L. The Isolation and Properties of the Immune Proteins of Bovine Milk and Colostrums and Their Role in Immunity: A Review. J. Dairy Sci., 31: 127. 1948.
(49) SULLIVAN, R. A., FITZPATRICK, MARGARET, M., AND STANTON, ELIZABETH K. Distribution of Kappa-Casein in Skimmilk. Nature, 183: 616. 1959.
(50) SULLIVAN, R. A., FITZPATRICK, MARGARET M., STANTON, ELIZABETH K., ANNINO, R., KISSEL, G., AND PALERMITI, F. The Influence of Temperature and Electrolytes upon the Apparent Size and Shape of α- and β-Casein. Arch. Biochem. and Biophys., 55: 455. 1955.
(51) THOMPSON, M. P., AND BRUNNER, J. R. The Carbohydrates of Some Glycoproteins of Bovine Milk. J. Dairy Sci., 42: 369. 1959.
(52) TIMASHEFF, S. N. The Stoichiometry of β-Lactoglobulin Association. Abstr. No. 34. 135th Meeting, Am. Chem. Soc., April, 1959.
(53) TIMASHEFF, S. N., AND TOWNEND, R. The Association Behaviour of β-Lactoglobulins A and B. J. Am. Chem. Soc., 80: 4433. 1958.
(54) TIMASHEFF, S. N., AND TOWNEND, R. (Unpublished data). Personal communication from C. A. Zittle. 1958.
(55) TIMASHEFF, S. N., AND TOWNEND, R. Heterogeneity of the Genetically Different β-Lactoglobulins. Abstr. No. 33. 135th Meeting, Am. Chem. Soc. April, 1959.
(56) TOMBS, M. P. The Heterogeneity of β_2-Lactoglobulin. Biochem. J., 69: 491. 1958.
(57) WAKE, R. G. The Action of Rennin on Casein. Australian J. Sci., 20: 147. 1957.
(58) WARNER, R. C. Separation of α- and β-Casein. J. Am. Chem. Soc., 66: 1725. 1944.
(59) WAUGH, D. F. The Interaction of α_s-, β- and κ-Caseins in Micelle Formation. Discussions Faraday Soc., No. 25: 186. 1958.
(60) WAUGH, D. F., AND GILLESPIE, J. M. Structure of the Stoichiometric Complex of α_s- and κ-Caseins. Abstr. No. 123. 134th Meeting, Am. Chem. Soc. September, 1958.
(61) WAUGH, D. F., AND VON HIPPEL, P. H. κ-Casein and the Stabilization of Casein Micelles. J. Am. Chem. Soc., 78: 4576. 1956.
(62) ZITTLE, C. A., CERBULIS, J., PEPPER, L., AND DELLAMONICA, E. S. Preparation of Calcium-Sensitive α-Casein. J. Dairy Sci., 42: 1897. 1959.

CHARACTERISTICS OF PROTEIN FRACTIONS ISOLATED FROM THE FAT/PLASMA INTERFACE OF HOMOGENIZED MILK[1]

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SUMMARY

The technique employed to isolate the proteins of the fat-globule membrane of homogenized milk was based on a procedure described by Herald and Brunner (4) for isolating the membrane proteins of nonhomogenized milk. Since homogenization results in the alteration of the components constituting the fat/plasma interface in milk, their methods were modified and extended to include a fractionation for milk proteins other than the normal membrane-proteins.

The insoluble membrane-protein, constituting a portion of the normal membrane, was isolated from the homogenized fat-globule membrane. This reddish-brown fraction remained, at least in part, with the fat globule following homogenization.

The soluble membrane-protein of the nonhomogenized fat globule was recovered from preparations of the membrane of homogenized milk. A portion of this protein fraction appeared to be associated electrophoretically with the α-casein oriented at the fat/plasma interface. The remaining portion was recovered from the membrane as an unassociated protein fraction.

An atypical casein fraction, in which the alpha component appeared to be associated with the normally occurring soluble membrane-protein (glycoprotein) was isolated from the resurfaced fat-globule. The association occurred when milk was homogenized or when artificial systems containing whole casein, or α-casein, soluble membrane-protein, and milk fat were homogenized. This phenomenon was characterized by a trailing peak on the α-casein peak in the electrophoretic patterns. When considered as a part of the alpha peak, unusually high α-β-casein ratios were obtained. An accompanying decrease in the electrophoretic mobility of α-casein was noted.

The presence of a heat-coagulable protein fraction, presumed to be whey proteins but not identified conclusively, was also noted.

Commercial homogenization of fluid milk changes many of its characteristics. The milk fat-globules are reduced in size from an average diameter of 5 or 6 μ to less than 2 μ, increasing the fat surface by a factor of from five to six (7). Conceivably, this transformation affects the orientation and/or the composition of the materials most closely associated with the fat globule at the fat plasma interface. In addition to the components comprising the normal fat-globule membrane of nonhomogenized milk, casein and other plasma proteins have been suggested as components of the newly created fat surface in homogenized milk (1, 2, 3, 7). Changes in the susceptibility of homogenized milk to copper and sunlight-induced flavor deterioration further accentuate this concept (7, 9).

To better understand the changes in the physical and chemical properties of milk resulting from homogenization, information relating to the constituent components of the fat/plasma interface would be desirable. For this purpose, the present study was pursued.

EXPERIMENTAL PROCEDURE

Pooled herd milk, obtained from the Michigan State University Dairy Plant,

Received for publication January 22, 1960.

[1] Journal Article 2575, Michigan Agricultural Experiment Station, East Lansing.

was used in these experiments. Milk was heated to temperatures ranging from 168° F. for 18 sec. to 190° F. for 20 min., cooled to 140° F., and homogenized in a two-stage Manton-Gaulin homogenizer at 2,000 and 500 p.s.i., respectively. Artificial emulsions of selected milk constituents were prepared by a similar procedure.

Separation of fat-membrane proteins. Fat-membrane proteins were isolated from the homogenized milk by a method similar to that described by Herald and Brunner (4) but modified as follows: A 3% sucrose solution was employed for the first three washings to increase the differential in specific gravity between the fat and the aqueous phase, thus increasing the recovery of fat from the homogenized milk. Three additional washings with three volumes of tap water removed all but a trace of the sugar.

Because milk-plasma proteins were suspected of orienting at the fat/plasma interface in homogenized milk, the isolation procedure was extended to provide for the separation of casein, heat-labile protein, and heat-stable protein fractions (Figure 1). The membrane proteins obtained from homogenized milk and

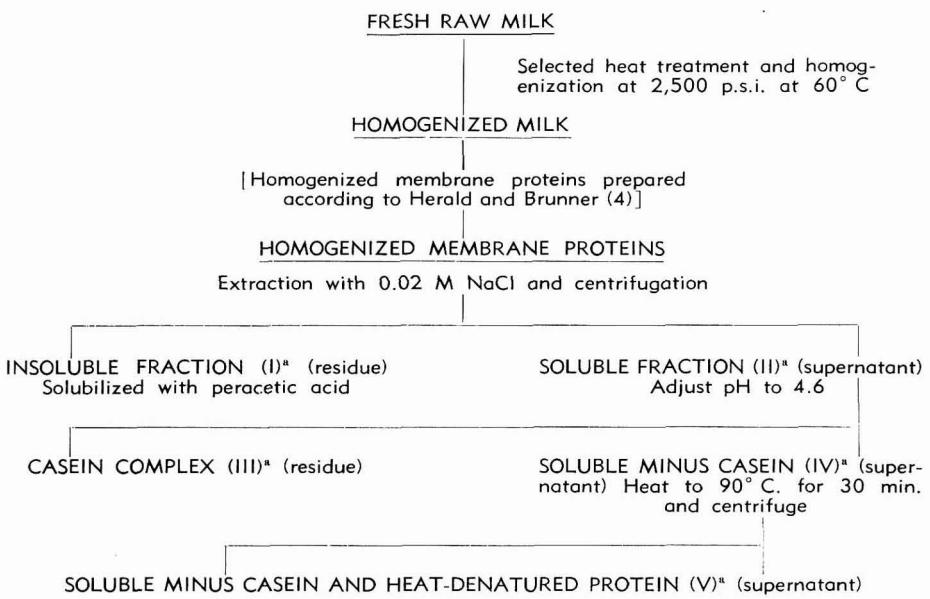

FIG. 1. Procedure for isolating the proteins associated with the fat globule in homogenized milk.

[a] See Figure 2 for electrophoretic patterns of isolated fractions.

related artificial systems were extracted with 0.02 *M* NaCl solution and centrifugally separated for 30 min. at 25,000 × G. This operation was repeated four times. The reddish-brown pellet was designated as the Insoluble Fraction (I) and the proteins in the supernatant as the Soluble Fraction (II). The latter

solution was adjusted to pH 4.6 and centrifuged for 30 min. at 25,000 G. The resulting pellet was dispersed by the addition of dilute NaOH solution—never exceeding pH 9.0—reprecipitated and centrifuged. This operation was repeated four times, the final precipitate being designated as the Casein-Complex Fraction (III) and the combined supernatants as Soluble Minus Casein Fraction (IV). The heat-labile proteins were cleared from Fraction IV by heating to 90° C. for 30 min., followed by centrifugation at 25,000 × G for 30 min. The resulting supernatant was designated as the Soluble Minus Casein and Heat Coagulated Protein Fraction (V).

Analytical methods. Electrophoretic analyses of the protein fractions were performed with a Perkin-Elmer Model 38-A electrophoresis apparatus, using the 6-ml. cell at approximately 1° C. Veronal buffer was made to pH 8.6 at 22° C. and ionic strength of 0.1. The migration of moving boundaries was measured from the initial boundary position on the descending pattern.

Sedimentation-velocity studies were performed in the Spinco Model E ultracentrifuge, employing the analytical accessories at approximately room temperature (22-25° C.). Proteins were carried in the same veronal buffer at three levels of concentration, ranging upward to 2%. Sedimentation values were corrected to 20° C. and zero concentration, but were uncorrected for the buffer viscosity.

Ultraviolet spectrophotometric scanning of the protein solution was accomplished with a Beckman Model DK-2 ratio recording spectrophotometer.

RESULTS AND DISCUSSION

Yield of membrane protein. Previous studies have reported that the fat membrane from nonhomogenized milk consisted of from 0.46 to 0.86 g. protein/100 g. fat (4, 5). The yield data from this study indicated a recovery of approximately 2.27 g. protein/100 g. fat, a value four to six times greater than previously reported. This observation is noteworthy when one considers that homogenization increases the fat surface by a factor of five to six, suggesting that the surface of fat, whether homogenized or nonhomogenized, is covered with approximately the same quantity of protein.

Characteristics of the isolated protein fractions. Characterization of the isolated protein fractions was based principally on the techniques of free-boundary electrophoresis, sedimentation-velocity ultracentrifugation, ultraviolet absorption spectrophotometry, and reaction to heat-treatment at 90° C. for 30 min.

Electrophoretic patterns representing the protein fractions are shown in Figure 2.

Fraction (I) appeared to be identical to the insoluble membrane reported by Herald and Brunner (4). Its reddish-brown color, mucoidial appearance, and solubility characteristics served as identifying traits. Since this protein possesses properties similar to those for a pseudo-keratin, treatment with peracetic acid or sodium sulfide was used to solubilize it in preparation for electrophoresis. Such solubilizing treatments have questionable applicability to the

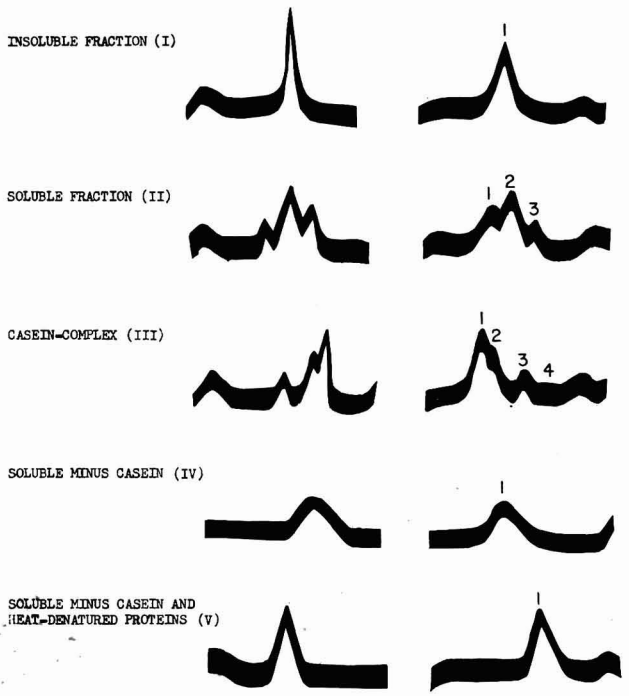

FIG. 2. Electrophoretic patterns representative of the various protein fractions isolated from the fat globules of homogenized milk.

characterization of proteins as they exist in nature, but do provide for comparison of the insoluble protein fractions of nonhomogenized and homogenized milk membranes, and indicated in this study that the fraction was not completely dissociated from the fat/plasma interface by homogenization.

Fraction (II), so designated because of its solubility in water or dilute salt solution, showed an electrophoretic pattern too complex to be of value in the identification of the constituent components. Because of the suspected presence of casein in this fraction, it was adjusted to pH 4.6 and centrifuged to remove the casein. The 4×-extracted casein, Fraction (III), showed an electrophoretic pattern similar to that which is generally attributed to casein, the major difference being the presence of an electrophoretic discernible peak which appeared to migrate with the leading α-casein peak during the electrophoretic run. Other noticeable differences were the abnormally high ratio of α-β-casein and the low mobility of α-casein. These characteristics of the complex were not affected by repeated isoelectric reprecipitations of the casein. A precursory examination of the electrophoretic pattern suggests the occurrence of a heat-induced β-lactoglobulin–α-casein interaction (6, 8). Although this possibility has not been entirely eliminated by the experimental evidence presented herein, the likelihood of such a material constituting the complex seemed remote, since the com-

plex was observed in all preparations of Fraction III following heat treatments of the fluid milk ranging from 165° F. for 30 sec. to 190° F. for 20 min. Treating the complex (Fraction III) with ethyl ether at room temperature resulted in a reduction of approximately 23% in the electrophoretic area of the peak associated with that attributed to α-casein, suggesting that a lipid-protein association was involved. A determination for total lipids in Fraction III showed a value of 8.5%. Heating a solution containing the fraction to 90° C. for 30 min. or treating it with rennet or urea had no effect on its electrophoretic characteristics.

Artificial emulsions were prepared from model systems containing casein, soluble membrane-protein, butteroil, and/or washed membranes in various combinations in an effort to simulate a typical Fraction (III) similar to the complex isolated from homogenized milk fat–membrane. Electrophoretic patterns of these Casein-Complex Fractions are shown in Figure 3.

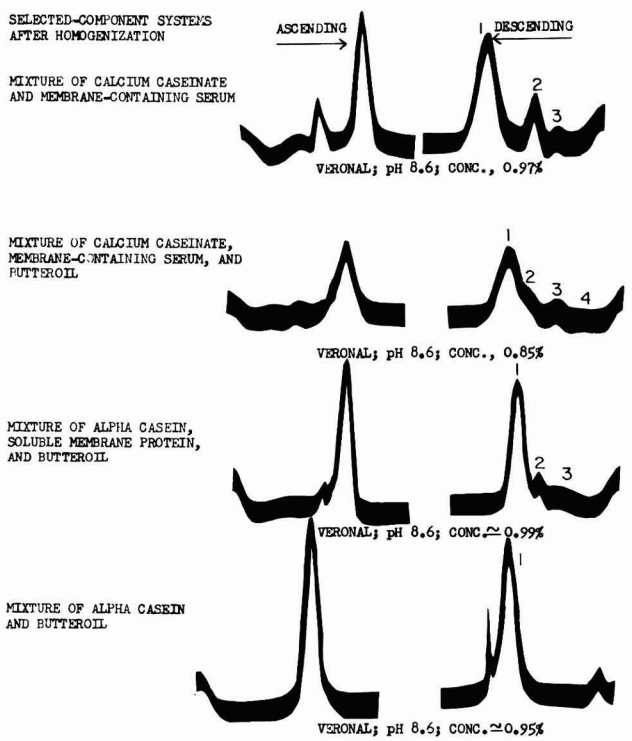

FIG. 3. Electrophoretic patterns of homogenized casein model systems.

Homogenization of a mixture of dispersed calcium caseinate and washed membranes from nonhomogenized milk failed to produce a typical Casein-Complex. However, when butteroil was incorporated in the above mixture prior to homogenization, a casein fraction approximating Fraction (III) was

observed. Homogenization of a mixture of α-casein and butteroil failed to produce the complex, but when soluble membrane-protein was incorporated a typical electrophoretic Casein-Complex was observed. Interestingly, the added soluble membrane-protein could not be quantitatively accounted for in the electrophoretic pattern, nor was it recovered quantitatively from the supernatant of the protein solution following adjustment to pH 4.6. An increase in the electrophoretic area and a decrease in the mobility of the α-casein peak were noted. Apparently, the soluble membrane-protein interacts with the α-casein in the presence of butteroil, forming a complex composed of casein, soluble membrane-protein, and fat.

Fraction IV consisted of the proteins in the supernatant of the pH 4.6 solution following removal of Fraction IV by centrifugation. Its broad electrophoretic peak offered little opportunity for characterization but was believed to contain whey proteins adsorbed at the fat/plasma interface of homogenized milk. Fifty-two per cent of the protein materials herein was precipitated by heating to 90° C. for 30 min. A sample of the whole fraction, isolated from homogenized milk previously pasteurized at 60° F. for 30 min., was submitted to ultracentrifugal analyses and showed three molecular species, Line 1, Figure 4; sedimentation constants ($S^{c=0}_{20°\,C.}$) were 7.3, 4.5, and 2.5.

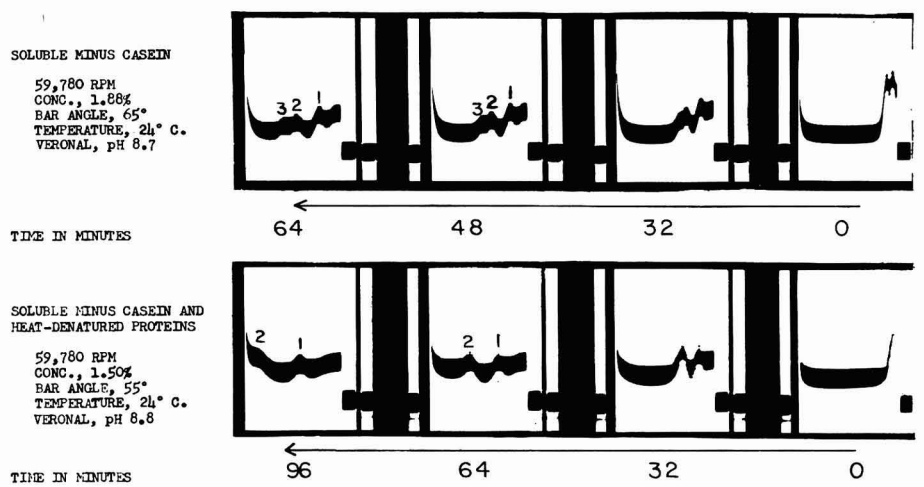

FIG. 4. Sedimentation diagrams of the noncasein fractions isolated from the fat globules of homogenized milk.

Fraction V consisted principally, if not entirely, of the soluble membrane-protein. This fraction was heat-stable, as is the soluble membrane-protein, and showed two molecular species in the ultracentrifuge (Line 2, Figure 4), with sedimentation constants ($S^{c=0}_{20°\,C.}$) of 6.0 and 2.7. A comparison of the ultraviolet-scan absorption patterns of this fraction and a sample of the soluble membrane-protein fraction of nonhomogenized milk indicated dissimilarities between the

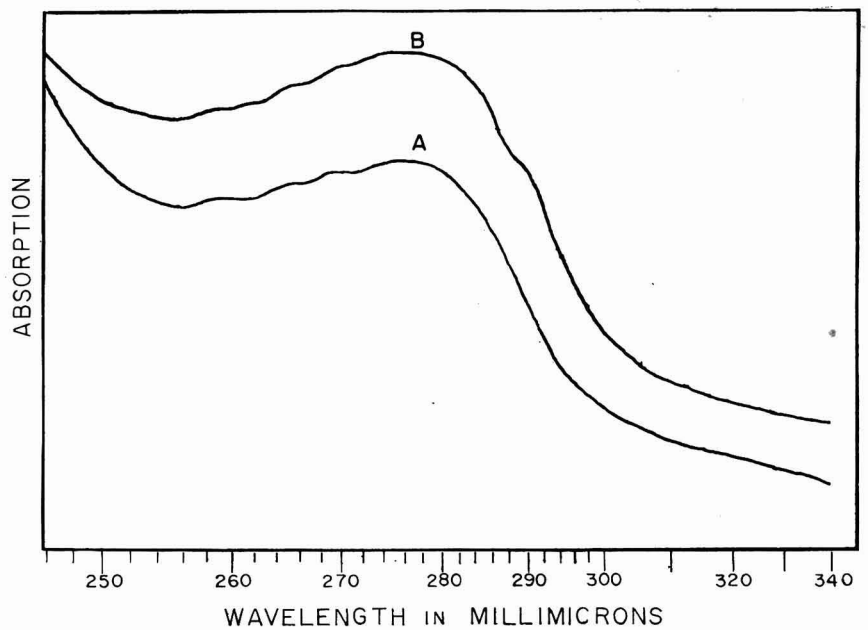

FIG. 5. Ultraviolet spectrograms of (A) soluble-membrane protein of nonhomogenized milk following heat treatment and (B) the heat-stable soluble protein fraction from the membrane of homogenized milk.

two fractions. However, after subjecting the normal soluble membrane-protein to the same heat treatment (90° C. for 30 min.) as employed in the preparation of Fraction V, its absorption characteristics were altered and became quite similar to those of Fraction V, Figure 5.

CONCLUSION

As was previously postulated (1, 2, 3), the fat globule membrane-proteins did not completely dissociate from the fat/plasma interface as a result of homog-

TABLE 1

Mobilities and areas of the electrophoretic peaks shown in Figures 2 and 3

Electrophoretic patterns		Mobilities[a] (μ = cm^2, volt^{-1}, sec.$^{-1}$ × 10^{-5}) Peak No.				Relative areas[a] (%) Peak No.			
Figure	Row	1	2	3	4	1	2	3	4
2	1	4.61	.....	.....	.....	100.0	.....	.....	.....
	2	4.47	3.74	2.52	.....	31.8	56.1	12.1	.....
	3	5.10	4.49	3.23	1.74	63.5	23.5	12.0	1.0
	4	3.55	.....	.....	.....	100.0	.....	.....	.....
	5	4.02	.....	.....	.....	100.0	.....	.....	.....
3	1	6.00	3.28	2.08	.....	80.0	15.8	4.2	.....
	2	5.93	4.75	3.13	1.71	61.1	27.8	10.0	1.1
	3	5.85	4.16	2.99	.....	83.7	11.5	4.8	.....
	4	6.38	.....	.....	.....	100.0	.....	.....	.....

[a] Measured from the descending patterns.

enization. The increased fat surface created by homogenization served as an adsorbing site for casein, complexed with a portion of the milk lipids and the normally occurring membrane materials, and whey proteins of undetermined nature. The occurrence of a casein complex involving milk lipids and normal membrane-proteins offers opportunity for speculation relative to its contributions to the characteristics of homogenized milk.

REFERENCES

(1) BRUNNER, J. R., DUNCAN, C. W., AND TROUT, G. M. The Fat-Globule Membrane of Nonhomogenized and Homogenized Milk. I. The Isolation and Amino Acid Composition of the Fat-Membrane Proteins. Food Research, 18: 454. 1953.

(2) BRUNNER, J. R., DUNCAN, C. W., TROUT, G. M., AND MACKENZIE, M. The Fat-Globule Membrane of Nonhomogenized and Homogenized Milk. III. Differences in the Sedimentation Diagrams of the Fat-Membrane Proteins. Food Research, 18: 1. 1953.

(3) BRUNNER, J. R., LILLEVIK, H. A., TROUT, G. M., AND DUNCAN, C. W. The Fat-Globule Membrane of Nonhomogenized and Homogenized Milk. II. Differences in the Electrophoretic Patterns of the Fat-Membrane Proteins. Food Research, 18: 463. 1953.

(4) HERALD, C. T., AND BRUNNER, J. R. The Fat-Globule Membrane of Normal Cow's Milk. I. The Isolation and Characteristics of Two Membrane-Protein Fractions. J. Dairy Sci., 40: 948. 1957.

(5) JENNESS, R., AND PALMER, L. S. Substances Adsorbed on the Fat-Globule in Cream and Their Relation to Churning. V. Consumption of the Membrane and Distribution of the Adsorbed Substances in Churning. J. Dairy Sci., 28: 611. 1945.

(6) TRAUTMAN, J. C., AND SWANSON, A. M. Protein Complex Formation in Evaporated Milk. J. Dairy Sci., 42: 895. 1959.

(7) TROUT, G. M. Homogenized Milk, A Review and Guide. Michigan State College Press, East Lansing, Michigan. 1950.

(8) VANWINKLE, Q. The Effect of Temperature on Protein-Interactions in Milk and Milk Protein Systems. Paper presented at the 54th Annual Meeting, Am. Dairy Sci. Assoc. Urbana. June, 1959.

(9) WEINSTEIN, B. R., AND TROUT, G. M. The Solar-Activated Flavor of Homogenized Milk. I. Susceptibility of Individual Cow's Milk. J. Dairy Sci., 34: 554. 1951.

SPECTROPHOTOMETRIC DETERMINATION OF FLURORAL AND URANINE IN MILK

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SUMMARY

To render antibiotic-containing milk easily recognizable, it has been suggested that a dye marker be added to veterinary antibiotic preparations which are to be infused into the udder. Ideally, the dye would be eliminated from the udder at the same rate as the antibiotic, and the milk should be discarded as long as a trace of dye was detected. A mixture of two fluorescein dyes, (Fluroral and Uranine), has shown promise for this purpose. To properly evaluate such a procedure, quantitative methods for measuring the marker content of milk were needed. Therefore, simple and rapid spectrophotometric methods have been devised for the quantitative determination of fat-soluble fluorescein (fluroral) and water-soluble fluorescein (uranine) in milk. Reproducibility values ranged from 92 to 100%, and recovery values from 94 to 103%.

Recent work by Hargrove, Lehman, and Matthews (1) and Hargrove, Plowman, and Wright (2) has established the feasibility of detecting antibiotics in milk by incorporating fluorescent materials in antibiotic preparations intended for intramammary infusion. Best results were obtained by adding a mixture of fluroral (Fluroral 7GA,[1, 2] oil-soluble fluorescein) and uranine (disodium fluorescein) to the antibiotic preparations. These authors established the presence of the dyes by examining the milk under ultraviolet illumination and obtained a semiquantitative estimate of their concentration by diluting the milk until the fluorescence could no longer be seen. For laboratory and statistical purposes a precise numerical measurement of the concentration of each dye in a sample of milk was desired. The following simple and rapid methods were evolved to fill this need.

The use of dye markers to detect antibiotics in milk has not been approved by regulatory agencies. It would be advantageous, however, to have suitable procedures and analytical methods available, in the event that they are approved and used in veterinary antibiotic preparations. The principles developed in this study may be useful, even though a different marker is eventually employed.

APPARATUS AND REAGENTS

Spectrophotometer, Beckman Model B or similar instrument.
Shaking machine, reciprocating type, capable of agitating extraction flasks about 400 times per minute.
Sodium hydroxide solution, about 1 *N*.
Ethyl ether, U.S.P. or better.

Received for publication January 8, 1960.

[1] E. H. Sargent and Company, Chicago, Illinois.

[2] The use of trade names is for the purpose of identification only, and does not imply endorsement of the product or its manufacturer by the U. S. Department of Agriculture.

Sodium tungstate solution, dissolve 1 lb. C.P. $Na_2WO_4 \cdot 2H_2O$ in one liter of water.

Sulfuric acid solution, 50% by volume.

Standard dye solutions, 50 mg. of uranine are weighed into a 500-ml. volumetric flask and made up to volume with 1 *N* NaOH. Fifty milligrams of **fluroral** are weighed into a 500-ml. volumetric flask and made up to volume with ethyl ether. Each solution contains 100 μg. of dye per milliliter.

EXPERIMENTAL PROCEDURE

Preparation of standard curves. Using the standard dye solutions, dilutions are prepared so that 1 ml. of solution will contain 0.5, 1.0, 2.0, 5.0, and 10.0 μg. of dye. The color of the uranine dilutions is measured at the wave length of maximum absorption (480 mμ), using 1 *N* NaOH as the reference solution. The color of the fluroral dilutions is measured at the wave length of maximum absorption (420 mμ), using ethyl ether as the reference solution. Both curves follow Beer's law over the range of concentrations used here. Micrograms of dye per milliliter of solution are plotted against the absorbance readings.

Presence of fluroral and uranine. The presence of fluorescent dyes is ascertained by examining the milk under ultraviolet illumination.

Blank value. A sample of milk known to be free of the dyes is treated in the same maner as the regular samples, so that an appropriate blank correction can be made.

Preparation of milk sample. A representative portion of milk (about 50 ml.) is warmed in a 45–50° C. water bath with occasional shaking for 15 min., or until sample is uniform in appearance.

Determination of uranine. Fifty-five milliliters of 1 *N* NaOH are placed in a 125-ml. Erlenmeyer flask. Exactly 5.0 ml. of the warmed milk sample are pipetted into the NaOH, and mixed thoroughly by quickly rotating the flask 40–50 times. The mixture is allowed to stand for 5 min., until the precipitate has clumped into large particles. The solution is filtered through a lint-free fluted filter paper and the filtrate is collected in a 100-ml. volumetric flask. The Erlenmeyer flask is rinsed with 40 ml. of 1 *N* NaOH and the washings poured through the filter. The filter paper is washed with 1 *N* NaOH from a wash bottle until the filtrate is exactly 100 ml. The volumetric flask is stoppered and the contents mixed thoroughly. Since the color will darken on standing, the color is measured in the spectrophotometer within 0.5 hr. at 480 mμ, using 1 *N* NaOH as the reference solution. The absorbance reading is corrected for the effect of the blank and the micrograms of uranine per milliliter of solution are obtained from the standard curve. This value is then converted into micrograms of uranine per milliliter of original milk.

Determination of fluroral. Five milliliters of the well-mixed milk sample are placed in a 125-ml. Erlenmeyer flask (or suitable shaking flask). Then 0.5-ml. sodium tungstate solution and 0.5-ml. sulfuric acid solution are added and all mixed thoroughly. Forty milliliters of ether are added and the flask is tightly stoppered with a clean rubber stopper. The flask is placed in the

shaker and shaken for 3 min. A 100-mm. funnel is prepared for filtering the ether solution by tamping a small plug of pyrex wool into the upper end of the funnel stem. Filter paper is not used, since it absorbs too much of the dye. A 100-ml. volumetric flask is placed under the funnel. The pressure which builds up in the extraction flask is released cautiously and away from the operator, as the curd is dangerously acidic. The ether is decanted into the funnel, leaving all curd in the flask. As much ether as possible is expressed by carefully jarring the curd to the bottom of the flask. This ether is decanted into the funnel also. The shaking-extracting-filtering procedure is repeated three more times, using 30 ml. of ether each time. The last 30-ml. portion of ether, after extraction, is usually colorless to the eye. If color is apparent it indicates that the sample size was too large. In this event the determination is repeated, using a 2.50-ml. sample of milk, 0.25 ml. sodium tungstate solution, and 0.25 ml. sulfuric acid solution. Less ether will also be needed with a smaller sample, 40 ml. for the first extraction and 25 ml. for each of the three remaining extractions. Regardless of the sample size, exactly 100 ml. of ether filtrate is collected, the volumetric flask is stoppered, and the contents mixed thoroughly. The color is measured in the spectrophotometer at 420 mμ, using ether as the reference solution. The absorbance reading is corrected for the effect of the blank, and the micrograms of fluroral per milliliter of solution are obtained from the standard curve. This value is then converted into micrograms of fluroral per milliliter of original milk.

DISCUSSION

The problem involved in this study was one of separating quantitatively from milk a water-soluble compound (uranine) and a fat-soluble compound (fluroral). Although chromatographic and fluorometric methods were tried, the simple colorimetric method described here proved to be the most rapid and satisfactory.

Uranine determination. To separate the uranine from the insoluble constituents of milk, sodium hydroxide was chosen as the precipitating agent because the yellow-green color of uranine is most intense in an alkaline solution; however, the strength of the alkaline solution is not critical. To show this, equal amounts of uranine were dissolved in equal amounts of 0.5 *N*, 1.0 *N*, and 2.0 *N* NaOH. The absorbance readings of these solutions were the same, .136 in 0.5 *N*, .135 in 1.0 *N*, and .136 in the 2.0 *N* solution. Also, the precipitation of the milk proteins and the liberation of the uranine from milk is not dependent upon a specific normality of the NaOH. Milk samples containing 250 μg. of uranine were analyzed, using 0.5 *N*, 1.0 *N*, and 2.0 *N* NaOH as the precipitating agent. In each case, quantitative recovery of the 250 μg. of uranine was obtained.

It is important to allow enough time for the precipitate to form into large aggregates; otherwise, some cloudiness may pass through the filter paper. Fresh milk samples are more likely to give a cloudy filtrate than milk samples stored for some time. If a cloudy filtrate is encountered, it can be satisfactorily clarified by centrifuging.

Throughout the uranine determination the fat-soluble fluroral is trapped,

along with the fat, in the precipitate and does not pass through the filter paper.

The increase in color intensity obtained by allowing the alkaline solution of uranine to stand is not the result of any change in the uranine but is due to the action of the alkali on the lactose present. This color, which is also yellow-green, can be easily demonstrated by placing a few crystals of lactose in some 1 *N* or 2 *N* NaOH and allowing it to stand for some time. Although the blank correction should allow for this change in color, it is best to measure the uranine color as soon as possible.

Duplicate uranine determinations usually agree within 5% of each other, except in samples of very low concentration, and the method can detect 1 μg. of uranine per milliliter of milk with ease. Recovery data for the uranine determination are given in Table 1.

TABLE 1
Recovery of uranine and fluroral added to milk

Uranine *(μg.)*		
Added	Recovered	% Recovered
41	42	102
100	97	97
150	141	94
250	257	103
400	380	95
	Fluroral *(μg.)*	
200	200	100
250	240	96
300	310	103
400	390	97
500	480	96

Fluroral determination. In analyzing for fluroral, a curd was desired which would bind up all the water and uranine present but allow the fat-soluble fluroral to be extracted with ether. The sodium tungstate–sulfuric acid curd is a gelatinous curd having these properties. Shaking must be vigorous, however, to allow the ether to penetrate into the curd and extract the fluroral. This curd also has no tendency to form an emulsion, which is always a problem when extracting milk with ether. The four portions of ether used to extract the fluroral (40-30-30-30) make a total of 130 ml., but only 100 ml. of ether are desired in the filtrate. Normal evaporation which will occur during the filtration process usually reduces the 130 ml. to the desired 100 ml.

The preparation of the milk before pipetting the fluroral sample should be thorough, since fluroral has a tendency to clump with the fat and cling to the sides of the container. Warming the milk and mixing completely is a necessity.

Duplicate fluroral determinations usually agree within 8% of each other, except in samples of very low concentration, and the method can detect 2 μg. of fluroral per milliliter of milk without difficulty. Recovery data for the fluroral determination are given in Table 1.

APPLICATION OF THE METHODS

Analyses were made on milk obtained from udder quarters which had

received infusions of antibiotic preparations containing 125 mg. of each dye. Infusions were made after milking and the collection of milk samples for analysis was started the next milking. Details of the procedure are covered in the article by Hargrove, Plowman, and Wright (2). Table 2 shows the decrease

TABLE 2
Amount of fluroral and uranine in successive milkings after infusion

Milking	Fluroral		Uranine	
	Absorbance	Micrograms per milliliter of milk	Absorbance	Micrograms per milliliter of milk
1	1.066	510.0	1.162	144.4
	1.020	490.0	1.178	146.0
2	.222	106.0	.147	18.0
	.215	102.6	.160	19.6
3	.040	17.8	.050	6.2
	.041	18.2	.048	6.0
4	.032	14.2	.049	6.0
	.030	13.4	.053	6.6
5	.018	8.0	.037	4.6
	.016	7.2	.040	5.0
6	.008	3.6	.045	5.6
	.006	2.6	.034	4.2
7	.004	1.8	.026	3.2
	.004	1.8	.030	3.8

in dye content for seven milkings after infusion, the range of concentrations which were encountered, and the agreement between duplicate determinations.

REFERENCES

(1) Hargrove, R. E., Lehman, R. J., and Matthews, C. A. The Use of Fluorescent Materials for the Indirect Detection of Antibiotics. J. Dairy Sci., 41: 617. 1958.

(2) Hargrove, R. E., Plowman, R. D., and Wright, W. W. Use of Markers in Veterinary Preparations for the Detection of Antibiotics in Milk. J. Dairy Sci., 42: 202. 1959.

STRONTIUM IN MILK. III. DISTRIBUTION IN CREAM, SKIMMILK, CHEDDAR CHEESE, AND WHEY[1]

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SUMMARY

Cream of approximately 40% fat from milk containing Sr^{89} and Ca^{45} has been found to have concentrations of these nuclides equal to about one-half those found in the milk. The skimmilk has slightly higher concentrations than the milk. No difference is apparent in the Sr^{89}/Ca^{45} ratio between the skimmilk and cream after the first milking following dosing of the cow. Cream containing Sr^{89} was washed by reseparation, resulting in a product essentially free of the nuclide.

Cheddar Cheese made from milk containing Sr^{89} and Ca^{45} showed a slightly larger ratio of Sr^{89}/Ca^{45} than did the milk from which it was made. Fourteen vats of cheese made from dosed milk had a $\frac{Sr^{89}/Ca^{45}\text{ cheese}}{Sr^{89}/Ca^{45}\text{ milk}}$ ratio of 1.23 ± 0.28. Four vats of cheese made from milk of dosed cows showed a ratio of 1.06 ± 0.12. The Sr^{89}/Ca^{45} ratio in whey was less than in the milk.

This study was undertaken to obtain information on the distribution of radioactive strontium in skimmilk, cream, Cheddar Cheese, and whey from milk obtained at various times following ingestion of the nuclide by the cow, and possible means of treatment of the cream to obtain a usable product of lower strontium content.

Strontium is known to react chemically in a manner similar to calcium in lactating animals (1, 4) and milk (2, 3).

EXPERIMENTAL PROCEDURE

Four Jersey cows were each given oral doses of 2.5 mc. of $Sr^{89}Cl_2$ and 3.0 mc. of $Ca^{45}Cl_2$. The composite from each milking was separated at 35° C. into skimmilk and cream, using a De Laval Junior Model separator. Composites from 18 milkings were obtained for study. Duplicate samples of whole milk, cream, and skimmilk from each milking were analyzed for Sr^{89} and Ca^{45} by the method described by Easterly *et al.* (2).

Received for publication December 24, 1959.

[1] This manuscript is published with the permission of the Director of the University of Tennessee Agricultural Experiment Station, Knoxville, Tennessee. The radioactive materials used in this work were obtained from the Oak Ridge National Laboratory on allocation from the United States Atomic Energy Commission. The work was completed under Contract No. AT-40-1-GEN-242 between the University of Tennessee College of Agriculture and the Atomic Energy Commission.

Experiments designed to remove radioactive strontium from cream were conducted, using both milk to which approximately 2.0 μc/liter of $Sr^{89}Cl_2$ was added 16 hr. before treatment and milk from cows to which Sr^{89} had been given orally. The separations were made at 35° C.; the resulting cream was made up to the original milk volume with distilled water at 35° C. and reseparated. Liquid counting of 10-ml. samples was used in those experiments where Sr^{89} was the only radioactive isotope present.

Milk obtained 30, 45, 54, and 69 hr. after oral administration of 4.0 mc. Sr^{89} to each of four Jersey cows was made into Cheddar Cheese, using two 9.46-liter (10-qt.) lots of milk from each milking period. One per cent starter and 0.02% rennet were used, and samples of curd and whey were taken at cutting and draining times and of the curd at milling time. The radioactivity of 10-g. samples of milk and whey was determined in the fluid state without further treatment; the curd samples were ashed at 600° C., dissolved in HCl, and the solution analyzed for radioactivity.

To determine if a retention difference existed between strontium and calcium in cheese making, raw mixed herd milk was dosed with approximately

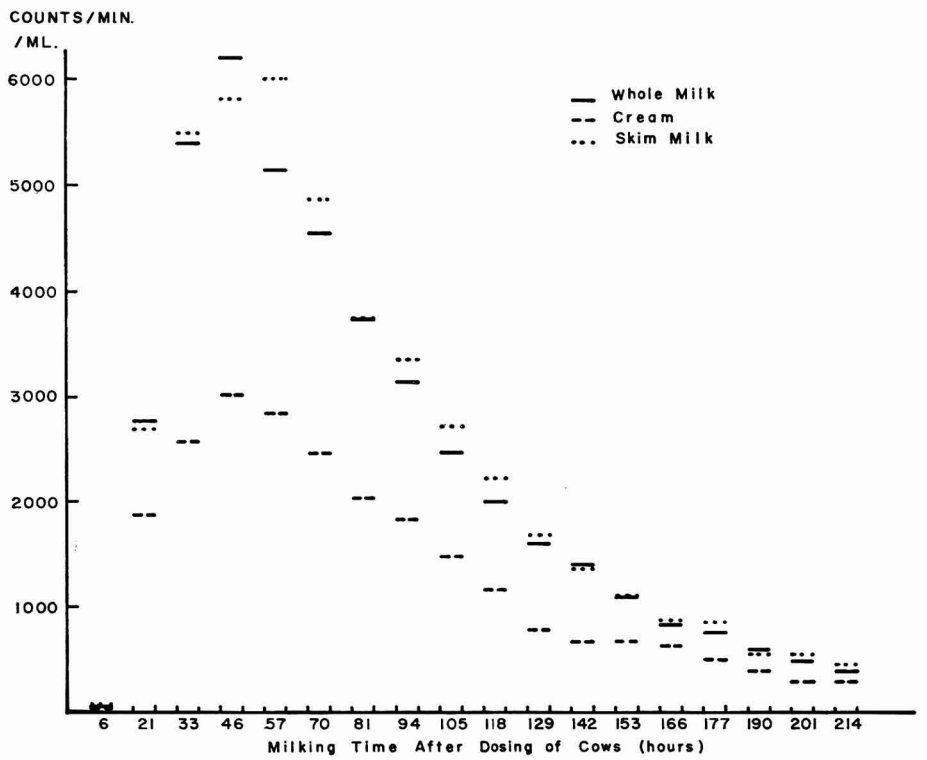

FIG. 1. Concentration of Ca^{45} in milk, cream, and skimmilk at various times following dosing of the cows.

1.0 μc/liter of both $Sr^{89}Cl_2$ and $Ca^{45}Cl_2$ and made into Cheddar Cheese. These nuclides in samples of milk, curd, and whey were counted as the oxalate precipitates (2). Because of the high concentration of Sr^{89} and Ca^{45} in the curd samples, only 1/25th of the ashed sample was precipitated as the oxalate.

To check the validity of the dosed milk technique, four vats of cheese were made with milk from three Jersey cows given an oral dose of 4.0 mc. Sr^{89} and 5.0 mc. Ca^{45}. Pressing was accomplished by the use of weights and a modified hoop, and samples were counted as the oxalate precipitates (2), using modifications for curd as described above.

RESULTS AND DISCUSSION

The distribution of Sr^{89} and Ca^{45} in milk, cream, and skimmilk obtained from each of the 18 milkings following dosing of the cows with the isotopes is shown in Figures 1 and 2. The peak of both Sr^{89} and Ca^{45} secretion occurred

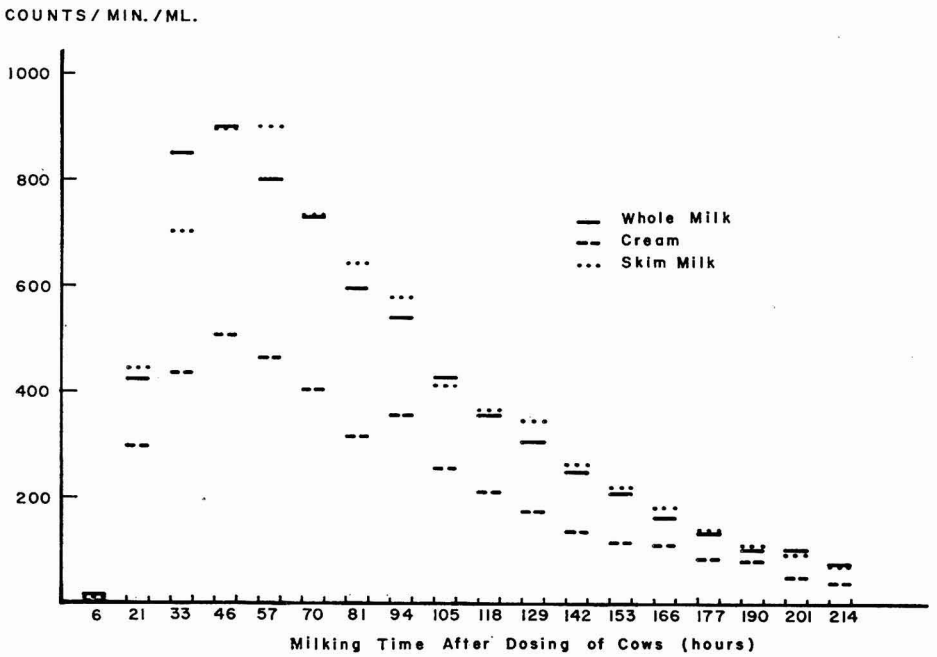

FIG. 2. Concentration of Sr^{89} in milk, cream, and skimmilk at various times following dosing of the cows.

in the fourth milking after dosing. The average Sr^{89}/Ca^{45} ratios of 17 samples collected between 21 and 214 hr. following dosing are given in Table 1. The Sr^{89}/Ca^{45} ratio in the milk obtained 6 hr. after dosing of the animals was 0.32, considerably higher than milk obtained in subsequent milkings, indicating a faster movement of Sr^{89}, when compared to Ca^{45}, from the feed into the milk. After the first milking, however, the ratios in milk, cream, and skimmilk were

TABLE 1

Sr^{89}/Ca^{45} ratios in milk, cream, and skimmilk, and cream/whole milk and skimmilk/whole milk ratios for Sr^{89} and Ca^{45}
(Average ± standard deviation for 17 samples obtained from 21 to 214 hr. after dosing the cows)

Sr^{89}/Ca^{45}	Whole	0.18 ± 0.02
	Cream	0.18 ± 0.02
	Skim	0.17 ± 0.02
Cream/Whole	Sr^{89}	0.60 ± 0.02
	Ca^{45}	0.59 ± 0.09
Skim/Whole	Sr^{89}	1.02 ± 0.08
	Ca^{45}	1.05 ± 0.08

very similar. The average of the 17 milkings (excluding the 6-hr. sample) showed the cream to have about 0.60 as much Sr^{89} per milliliter as did the milk from which it came, while the skimmilk showed about the same concentration as the milk (1.02). Comparable figures for Ca^{45} were 0.59 in cream and in skimmilk, 1.05. Random samples of the cream showed it to contain approximately 40% fat as measured by the Babcock method. The volume of cream obtained in all cases was approximately 10% of the milk volume.

Data obtained in preliminary experiments by liquid counting of 10-ml. samples of milk drawn from cows receiving oral doses of Sr^{89} also showed that the peak count of Sr^{89} was contained in the fourth milking after dosing. Again disregarding the first milking, the Sr^{89} skimmilk/Sr^{89} whole milk ratio was 1.14 ± 0.08, and that of cream was 0.69 ± 0.19. These ratios are similar to those obtained by counting the precipitates.

Raw milk dosed with $Sr^{89}Cl_2$ was separated 16 hr. later; the cream was diluted to the original milk volume with distilled water and reseparated. The data on the percentage of Sr^{89} remaining in the washed cream after successive separations are recorded in Table 2, and are based on radioactivity per milliliter

TABLE 2

Concentration of Sr^{89} in cream as a percentage of that in milk

Dosed milk				Dosed cows		
	Separation			Days after dosing	Separation	
Trial	1st	2nd	3rd		1st	2nd
1	52	5.1	0.6	1	58	7.0
2	50	11.6	1.1	2	63	2.4
3	56	5.1	0.6	3	54	3.8
4	58	5.2	0.9	4	68	11.8
5	57	5.3	0.8			

without regard to the fraction of the original milk going into the fat and skimmilk portions.

Cream obtained from the first separation had about half the concentration of Sr^{89} found in the milk from which it came. The washed cream samples from the second separation, except in one case, had concentrations equal to about one-tenth the first cream. Washed cream from the third separation showed about one-tenth the concentration observed in the second separation.

Data obtained by reseparation of cream from milk of cows given a single

oral dose of Sr^{89} also are recorded in Table 2. Variations are greater than with dosed milk, which might be partially explained on the basis of the time lapse between dosing of the cow and the separation process. The first milk obtained probably contains more Sr^{89} in the ionic state than in later milkings. Variations in fat content of the cream might also be a factor.

In an effort to obtain a product which had radioactivity equal to or less than that occuring by natural radiation, cream from dosed milk and cream from milk from dosed cows were washed by dilution and reseparation. Usually, after four separations, the washed cream was at this level. As was expected, the number of separations necessary was greater with milk of high activity than with milk of low activity.

As the milk solids are concentrated in the curd during the cheese making process, the concentration of Sr^{89} in the curd also became greater, as shown in Table 3. The ratio between the Sr^{89} concentration in curd to that in whey

TABLE 3

Sr^{89} activity in milk, curd, and whey at various times following administration of the isotope to the cows

Time of milking following dosing	Milk before adding rennet	At cutting		At draining		At milling
		curd	whey	curd	whey	curd
(hr.)	*(counts/min/10 g.)*					
30	128	121	26	392	23	731
45	153	173	26	332	32	879
54	145	174	31	448	42	500
69	137	162	25	560	28	905
Av.	141	158	27	433	31	754

was much greater at the time of draining than at the time of cutting. The concentration of Sr^{89} in the whey at cutting and draining times was relatively constant, but some data indicated that the concentration in the whey at milling and during pressing was several times the concentration at the time of draining. Due to the small amount of whey obtained at this point, data were not sufficient for conclusions. Small differences in the acidity of the whey of the various trials might account for variations in the Sr^{89} level in the final curd.

Fourteen vats of cheese were made from milk to which Sr^{89} and Ca^{45} were added 16 hr. prior to setting. The concentration of both isotopes in the curd became greater as the whey was expelled from the curd. The concentration in the whey at the time of draining and cutting showed a much lower concentration than the curd. Sr^{89} appeared to concentrate slightly in the curd when compared to Ca^{45}. To obtain definite numerical relationships, the Sr^{89}/Ca^{45} ratio in a sample of curd or whey may be divided by the Sr^{89}/Ca^{45} ratio in the milk at the time of setting. Quotients greater than one indicate a higher concentration of Sr^{89} in relation to Ca^{45} in the curd or whey than that found in the milk used. Data in Table 4 show that Sr^{89} concentrated slightly in the curd and Ca^{45} concentrated slightly in the whey. The same general pattern was evident both in the dosed milk and in milk from dosed cows. The ratios on the pressed cheese

TABLE 4
Ratio of Sr^a to Ca^a in curd and whey as compared to milk

Point in process	Nature of sample	Sr^a/Ca^a Sample / Sr^a/Ca^a Milk	
		Dosed milk	Dosed cows
Setting	Milk	1.00	1.00
Cutting	Curd	1.18 ± 0.21[c]	1.00 ± 0.15
	Whey	0.86 ± 0.16	0.63 ± 0.03
Draining	Curd	1.25 ± 0.21	1.06 ± 0.03
	Whey	0.85 ± 0.10	0.62 ± 0.07
Milling	Curd	1.18 ± 0.19	1.07 ± 0.10
After pressing	Curd	1.23 ± 0.28	1.06 ± 0.12[b]

[a] Radioactive.
[b] 1.75% salt added.
[c] Mean ± standard deviation.

from the two milk sources were shown to be different at the probability level of $P < 0.2$.

These results indicate that cheese contained more strontium per gram of calcium than did the milk from which it was made.

The protein contents of neither the milk nor the cheese were determined in this investigation. However, since Cheddar Cheese is used as a protein source in human nutrition, and without implying a physical relationship between strontium and protein, it is of interest to calculate the number of strontium units per gram of protein in milk and cheese. Such calculations indicate that cheese has a lower Sr/protein ratio than does the milk from which it was made.

REFERENCES

(1) CRAGLE, R. G., AND DEMOTT, B. J. Strontium and Calcium Uptake and Excretion in Lactating Dairy Cows. J. Dairy Sci., 42: 1367. 1959.
(2) EASTERLY, D. G., DEMOTT, B. J., AND CRAGLE, R. G. Strontium in Milk. I. Removal by Means of Reverse-Flow Ion Exchange Columns. J. Dairy Sci., 43: 137. 1960.
(3) EASTERLY, D. G., DEMOTT, B. J., AND CRAGLE, R. G. Strontium in Milk. II. Removal by Batch Ion Exchange Methods. J. Dairy Sci., 43: 146. 1960.
(4) WASSERMAN, R. H., LENGEMANN, F. W., AND COMAR, C. L. Comparative Metabolism of Calcium and Strontium in Lactation. J. Dairy Sci., 41: 812. 1958.

OBSERVATIONS ON CREAMING OF COTTAGE CHEESE[1]

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SUMMARY

A method of measuring the amount of free dressing or free liquid in a carton of creamed Cottage Cheese was devised by standardizing size of draining surface, time, temperature, and other conditions having possible effects on such an empirical test.

Increases in the following factors increased the amount of dressing retained per 100 g. of curd and decreased curd firmness: holding time after creaming, curd breakage, pH, and fat in the finished cheese—providing the increase in fat was attained by adding more dressing with the same fat content. Higher temperature of holding creamed cheese decreased firmness of curd but had little effect on amount of dressing retained. Adding stabilizers to thicken the dressing or increasing pressures used for homogenizing dressing caused more retention of dressing. Increasing salt from 0 to 2% increased the amount of dressing retained and decreased firmness of curd, but adding higher percentages reversed these effects.

This study indicated the probable functioning of two separate mechanisms affecting the retention of dressing by curd: one was the absorption of serum from the dressing by the curd, and the other was adsorption of dressing on the surface of the curd.

Consumers judge the quality of Cottage Cheese by appearance and body characteristics as much as by flavor. This is recognized by the definitions of the American Dairy Science Association Scoring Guide for Cottage Cheese. Over-creaming, lack of cream, and appearance of free whey are factors which detrimentally affect appearance, body, and texture characteristics. The scoring guide lists various degrees of firmness and consistency which can be judged by sensory observations.

In a recent survey in the state of Michigan, Harmon, Trout and Bonner (2) observed wide variations in the loss of weight by 48 lots of creamed Cottage Cheese drained for 20 hr. through cheese cloth. Factors which contributed to the release of liquid included curd of large size, low pH, low total solids in the curd, and low fat content in the dressing. The addition of gelatin to the Cottage Cheese dressing reduced drainage from curd with high solids, but had little effect on curd with low total solids. Storage for five days decreased drainage in both types when gelatin was used.

Manus (3) stated that increased viscosity produced by homogenizing dressing for Cottage Cheese at lower temperatures and higher pressures made the cream cling to the curd particles and decreased the amount of free liquid.

It is the purpose of this paper to describe a draining test to measure free liquid in creamed Cottage Cheese and to report observations of effects of certain variables on firmness of curd and on retention of dressing by creamed Cottage Cheese.

Received for publication December 12, 1959.

[1] Approved for publication by the Director of the Experiment Station, University of Wisconsin, Madison.

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In this paper the term dressing refers to the mixture of milk and cream containing 12-18% fat that is added to curd to form creamed Cottage Cheese. The dressing retained by the curd represents the difference in weight between the dressing added to the curd in creaming and the liquid removed from the curd in the draining test. This liquid consisting of dressing, water, whey, or a combination of them is called free dressing. Although the amount of free dressing is important to the processor and the consumer, observations on the amount of cream retained by a given amount of curd would seem to be of more fundamental importance, since the ratio of curd to dressing can be varied by the manufacturer. The term dressing retained refers to the grams of dressing retained by 100 g. of uncreamed curd during the drainage test.

EXPERIMENTAL METHODS

The apparatus shown in Figure 1 was used to measure free dressing in

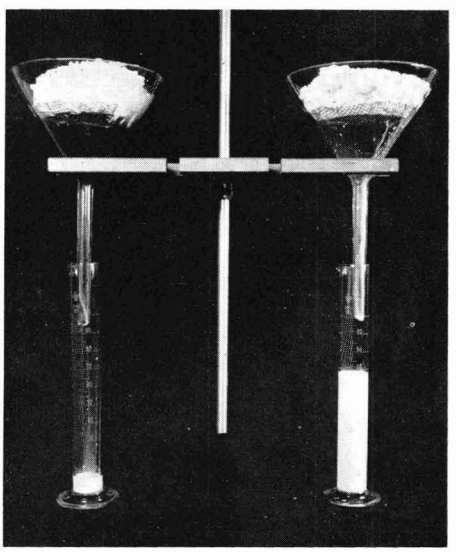

FIG. 1. Apparatus used to measure free dressing in creamed Cottage Cheese.

creamed Cottage Cheese. The apparatus consists of a 6-in. glass funnel and a circular 8-mesh wire screen placed horizontally in the funnel to hold the cheese. The flat bottom of the screen is 3.75 in. in diameter. A sloping edge, 0.5 in. in width, surrounds the flat bottom of the screen and fits the sides of the funnel to provide stability. Sheets of aluminum foil are used to cover the cheese and funnel to minimize drying while the free dressing drains and is collected for weighing.

A standard procedure was used for creaming the curd and for measuring the free dressing. All creaming tests were made at 50° F. The curd used in this study had been cut during manufacture with 0.5-in. knives. Dressing for

the Cottage Cheese was made by standardizing 18% pasteurized, homogenized cream to 12% fat with pasteurized, homogenized milk. The curd was weighed into 12-oz. Cottage Cheese cartons and sufficient dressing was added to give 341 g. of creamed Cottage Cheese with 4.5 or 6% fat. Contents of cartons were not mixed or stirred. The creamed cheese was held at 50° F. for 24 hr. before measuring the amount of free dressing. Free dressing was determined by emptying the entire contents of each carton on to the drainage device and weighing the liquid which separated from the curd during the next 30 min.

Identical samples of creamed curd were also prepared and held at 50° F. for the curd firmness test (1). These samples were also held 24 hr. before testing. Cheese was prepared for the firmness test by emptying the carton of creamed curd on to a 9-in., flat-bottomed strainer pail to remove excess dressing.

Unless otherwise stated, each value for curd firmness and free dressing in this paper is the average of quadruplicate determinations, each made on a different sample.

All pH measurements were made at approximately 22° C. (71.6° F.) with the quinhydrone gold electrode, saturated calomel half-cell, and a Leeds-Northrup portable potentiometer (Catalog No. 7655).

RESULTS

Drainage tests were made on 103 lots of creamed curd: ten lots were tested in replicates of six, one lot in triplicate, and 92 lots in quadruplicate. The mean squares for variation between replicate tests within each of the 103 lots were combined to calculate a pooled standard deviation of 3.04 g. of free dressing. The mean values of free dressing per carton for each lot of creamed curd varied from 6.6 to 102.5 g. in the 103 lots.

Effect of interval and duration of draining on free dressing. Twenty-four cartons were taken from one batch of freshly creamed Cottage Cheese and were divided into two groups. Draining was started either 6 hr. after creaming (Group A_1) or 24 hr. after creaming (Group A_2). The amount and fat content of the liquid drained from the cheese were noted at the end of 30 min., 8 hr., and 24 hr. The data are recorded in Table 1.

Nearly all of the fat which drained from the cheese was obtained in the first 30 min. The fluid which drained after 30 min. progressively changed in appearance from skimmilk to a watery whey. There had been no indication of any free, whey-like liquid in the creamed cheese before draining.

When the compacted creamed cheese was sliced with a sharp knife after draining, the color of its fat revealed dressing clinging or trapped in the interstices of the curd particles. Since relatively little fat left the cheese after the 30-min. draining period, it must be assumed that at least a large portion of the liquid draining thereafter came from the interior of the curd rather than from free dressing remaining on the surfaces of the creamed curd. This whey-like liquid resembled that which sometimes comes from uncreamed Cottage Cheese held under similar conditions. The amount of free dressing appearing during the first 30-min. interval of draining is most significant, because it represents

TABLE 1

Amount and fat content of free liquid drained from identical samples of creamed Cottage Cheese

Group	Interval of draining					
	0 to 30 min.		30 min. to 8 hr.		8 hr. to 24 hr.	
	Liquid drained[a]	Fat[b]	Liquid drained[a]	Fat[b]	Liquid drained[a]	Fat[b]
	(g.)	*(%)*	*(g.)*	*(%)*	*(g.)*	*(%)*
A_1	30.7	9.8	28.4	0.8	11.7	< 0.5
A_2	20.6	10.2	25.4	1.0	14.9	< 0.5

[a] Average amount of liquid drained from 12 samples of creamed Cottage Cheese weighing 341 g. at start of draining.
[b] Fat content of free liquid drained from the creamed curd.
A_1 = Drainage tests started on 12 samples 6 hr. after creaming.
A_2 = Drainage tests started on 12 samples 24 hr. after creaming.

free liquid which the housewife sees after removing the creamed cheese from the carton. This is illustrated in Figure 2.

Effects of time of holding after creaming. Figure 3 shows the effect of holding creamed curd after adding the dressing on curd firmness and amount of dressing retained. Observations were made on samples of dry curd, and on samples of creamed curd at 0, 4, 8, 24, 48, and 96 hr. after adding the dressing. Observations at 0 hr. were made less than 5 min. after adding the dressing,

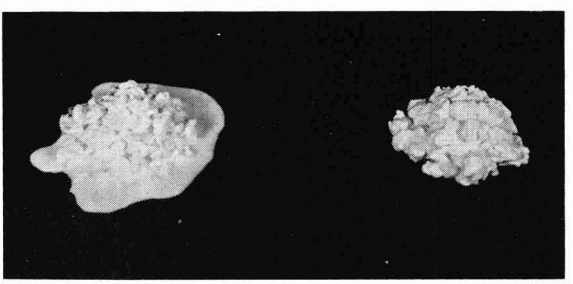

FIG. 2. The free liquid which the housewife sees after taking the creamed cheese from the carton. The dressing clings to the curd on the left and separates from the curd on the right. Consumer preferences for free liquid are not alike.

but measurable changes in curd firmness and in dressing retained occurred even in that short time interval. Changes in amount of dressing retained continued until the observations ended at 96 hr. Decreases in curd firmness after 24 hr. were relatively small.

Relationship between firmness of uncreamed and creamed curd. Relationships betweeen curd firmness of creamed and uncreamed curd in 31 different lots are shown in Figure 4. Firmness of both creamed and uncreamed curd was measured 24 hr. after adding dressing to the curd. The dressing contained 12% fat, and the creamed curd had 4.5% fat in all lots. A very high correlation coefficient was obtained, $r = 0.992$. As firmness of the uncreamed curd increased, so did firmness of the creamed Cottage Cheese.

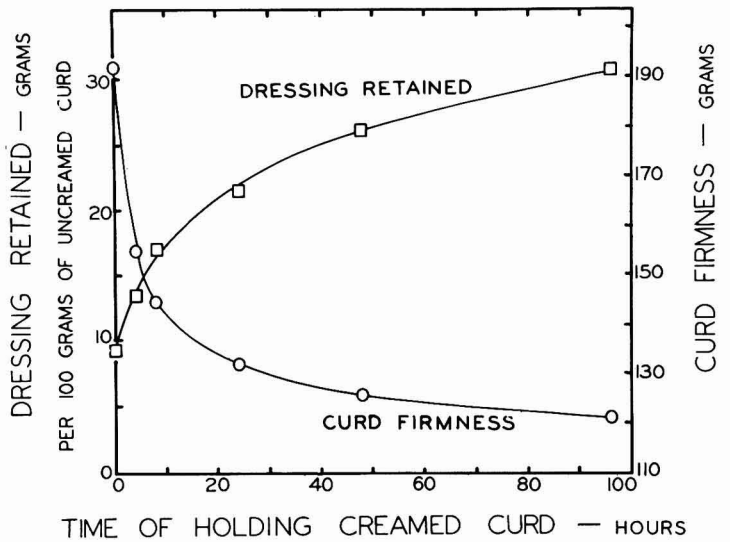

FIG. 3. Effect of time between creaming and draining test on curd firmness and dressing retained. The firmness of the uncreamed curd was 210 g.

Total solids and curd firmness of uncreamed curd. The effect of total solids and curd firmness of uncreamed curd, on dressing retained 24 hr. after creaming, was measured on 27 lots of curd. Dressing of 12% fat was added to all

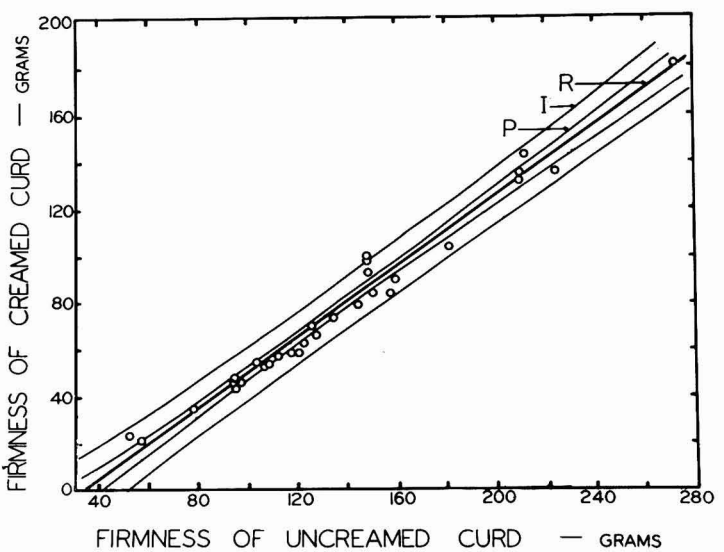

FIG. 4. The relation between the curd firmness of uncreamed curd *(X)* and creamed cheese *(Y)* in 31 different lots of curd, as shown by regression line *(R)* and 95% confidence limits of individual *(I)* and population *(P)* values. $Y = -25.15 + 0.750\,X$; $r = 0.992$.

lots to give a fat content of 4.5%. Results showed that larger amounts of dressing were retained when softer curd or curd with lower total solids content was used.

The regression equations of dressing retained (Y) on total solids (X_a) and on curd firmness (X_b) and their associated coefficients of correlation (r) were calculated as follows:

$$Y = 62.9 - 1.48X_a, \quad (r = -0.405, \text{ significant at } 5\% \text{ level})$$
and $$Y = 44.7 - 0.084X_b, \quad (r = -0.649, \text{ significant at } 1\% \text{ level})$$

Breakage of the softer curd during creaming may partially account for the dependence of dressing retention on curd firmness and total solids. This was shown in principle by another experiment, in which one lot of normal large curd was divided into two portions. One portion was creamed in the usual fashion, and the other portion was pressed through a 6-mesh wire screen prior to creaming. Data in Table 2 indicate a considerable increase in dressing-holding capacity of broken curd.

TABLE 2

Effect of broken curd on curd firmness, drainage of free dressing, and dressing retained

Type of curd	pH	Curd firmness	Free dressing drained from 341 g. of creamed cheese	Dressing retained per 100 g. uncreamed curd
			(g.)	
Whole curd:				
Creamed	5.00	182	68.6	27.8
Uncreamed	4.78	272	.....	.....
Broken curd:				
Creamed	5.02	71	6.6	55.2
Uncreamed	4.76	225	.....	.....

Firmness of curd also influences the creaming of Cottage Cheese by its effect upon packing characteristics. Soft curd is more pliable and settles or flows into a smaller volume than an equal weight of firm curd. The amount of dressing required to fill the spaces between curd particles is much greater with firm particles than with soft curd particles. This suggests that curd firmness must be uniform for optimum control of weight and fat content of creamed Cottage Cheese in mechanized creaming and packaging operations; otherwise, fat content of dressing, viscosity of dressing, or the curd mixing and handling procedures must be changed to attain optimum distribution and retention of dressing.

Adjustment of the pH of the creamed cheese. The pH of creamed cheese was varied experimentally by the addition of acid or base to the dressing before combining the dressing with the curd.

Each of several lots of curd was divided into six portions. One portion (213 g.) was creamed in the normal fashion with 128 g. of 12% dressing to which 3 ml. of distilled water had been added. Acid or base was added to the dressing for the five other portions of each lot as follows: To 128 g. of dressing

was added either 1 or 2 ml. of 3 *N* NaOH or 1, 2, or 3 ml. of 3 *N* lactic acid. Appropriate amounts of distilled water were added so that 3 ml. of fluid was mixed with the dressing for each carton. After holding the creamed curd 24 hr. at 50° F., tests were made for curd firmness, dressing retained, and pH.

Figure 5 shows the differences in the amount of dressing retained by a

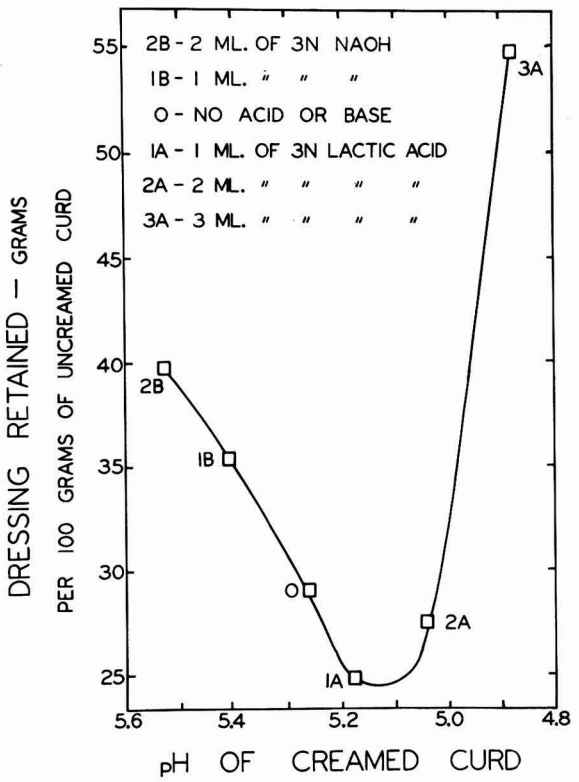

FIG. 5. Effect on changes in the amount of dressing retained when pH of creamed curd was adjusted by adding base or acid to the dressing.

typical lot of curd when the pH was adjusted by adding acid or base to the dressing. Minimum retention occurred when 1 ml. of lactic acid was added to the dressing. Further additions of acid caused increases in the amount of dressing retained. The addition of 2 and 3 ml. of acid increased viscosity immediately, which undoubtedly explains the increased retention of dressing. Increases in pH attained by the addition of base also increased the amount of dressing retained, but did so without any apparent increases in viscosity of the dressing. The explanation of this apparent anomaly was suggested by the data of Figure 6, which relates the adjusted pH of the creamed Cottage Cheese to the firmness of the cheese. The curves (Figure 6) show that lower values for curd firmness were associated with higher pH values in the creamed Cot-

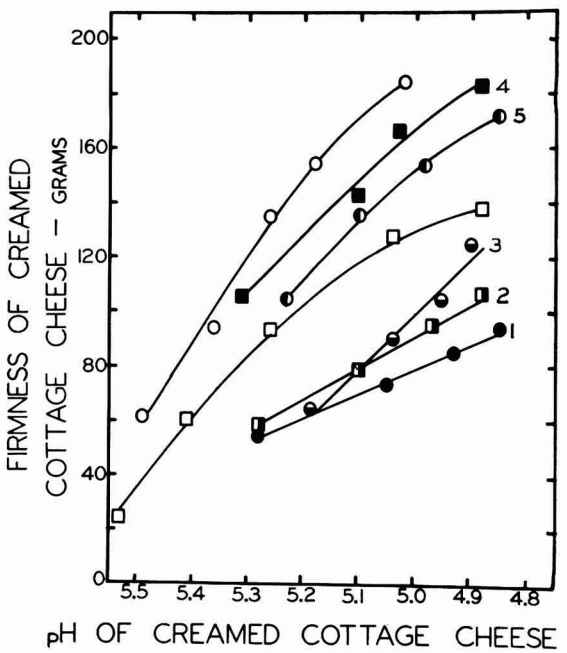

FIG. 6. Relation betwen the adjusted pH of the creamed Cottage Cheese and the firmness of seven lots of cheese. Curves 1-5 identify lots of the same numbers used in Table 3.

tage Cheese. A similar but less marked change occurs in curd firmness during the normal creaming of Cottage Cheese.

The data of Table 3 and the five numbered curves in Figure 6 are based upon the same five lots of curd. Table 3 shows selected data for pH and firmness of Cottage Cheese before creaming, after normal creaming, and after creaming with acidified cream. Curd firmness of acidified creamed Cottage Cheese was estimated at the pH of the uncreamed curd from the curves of Figure 6. Average curd firmness values before and after acidified creaming

TABLE 3

Relationship between pH and curd firmness of Cottage Cheese before and after creaming with normal and acidified dressing

	Uncreamed		Dressing acidified		Normal dressing not acidified	
Lot No.	pH	Curd firmness	pH	Curd firmness[a]	pH	Curd firmness
1	4.84	134	4.84	95	5.05	73
2	4.88	144	4.88	105	5.10	79
3	4.85	159	4.85	130	5.04	90
4	4.88	212	4.88	195	5.10	143
5	4.85	225	4.85	176	5.10	136
Mean	4.86	175	4.86	140	5.08	104

[a] Estimated from Curves 1-5 of Figure 6 at pH of uncreamed curd.

were 175 and 140 g., and after normal creaming, 104 g. This would indicate that approximately half of the softening effect of dressing on curd, as measured by the firmness test, is due to the increase in pH of the curd that occurs when normal dressing and curd are mixed.

An estimate of the buffer capacity of creamed Cottage Cheese curd can be obtained from the data of this series of experiments. The pH of the 341 g. creamed curd was changed an average of 0.15 pH units by the addition of 1 ml. of 3 *N* NaOH; the pH was changed an average of 0.11 pH units by the addition of 1 ml. of 3 *N* lactic acid.

Adjustments of pH affected glossiness of curd, smoothness of body, curd firmness, free dressing, and the appearance of the creamed cheese after dressing had been removed during the drainage tests. Adjustment to a higher pH made the curd particles more glossy. Judges observed that the higher pH values were associated with the softer and smoother curd. Presence of 2 ml. of the base in a carton of 341 g. made the product objectionably smooth and slippery, often producing a gelatinous, translucent curd. Additions of 1 ml. of base sometimes made the curd smoother, more meaty, and less gritty, but at other times it injured the quality by producing slippery curd. When the pH of the creamed curd was lowered by adding acid to the dressing, the curd was criticized for increased roughness or grittiness.

Judges' observations of amount of free dressing followed closely those made by the objective measurements shown in Figure 5. The curd with lower pH values, probably because of its greater firmness, tended to remain as discrete, pebble-like particles, and when a carton of such curd was inverted on a plate, the dressing flowed freely from it. The softer curd at the higher pH values tended to flow slowly, to flatten out, and to stick together.

The flavor of the creamed curd became more bland when base was added to it. Sour flavors became definite with 2-ml. additions of acid and were highly objectionable with additions of 3 ml.

Added salt. The effect of salt on curd firmness and dressing retention was investigated by adding 128 g. of 12% dressing to 213 g. of unsalted curd to each 12-oz. carton. Salt was then added to each of several cartons at the rates of 0, 0.5, 1.0, 2.0, 4.0, 6.0, and 10.0% of the 341 g. of creamed cheese. The salt was carefully mixed with the creamed cheese at frequent intervals for a period of 30 min. Each level of salt was added to six cartons of creamed cheese, two of which were used for firmness tests and four for measuring free dressing. Results are shown in Figure 7. In calculating percentage of dressing retained, it was assumed that the salt was uniformly distributed in the dressing and the curd.

Maximum amount of dressing retained and minimum curd firmness occurred with 1 and 2% of added salt. Unsalted cheese and cheese with salt additions of 6 and 10% showed higher levels of curd firmness and lower levels of retained dressing. Under practical commercial conditions, the effect of normal variations in salt would probably be unimportant.

Temperature of holding creamed curd. Identical cartons of creamed cheese were prepared at 50° F. and immediately stored at 71, 60, 50, 37, and 33° F.

Measurements of curd firmness and dressing retention were made 24 hr. later.

The data in Figure 8 indicate that temperature had little effect on amount of dressing retained, but that high temperatures of storage, as would be expected, decreased curd firmness. Within the temperature range of 30-50° F. temperature had little measurable effect on curd firmness.

Fat content of cream and creamed cheese. Raw cream was divided into three lots and standardized to 12, 15, and 18% fat. Each lot was heated to

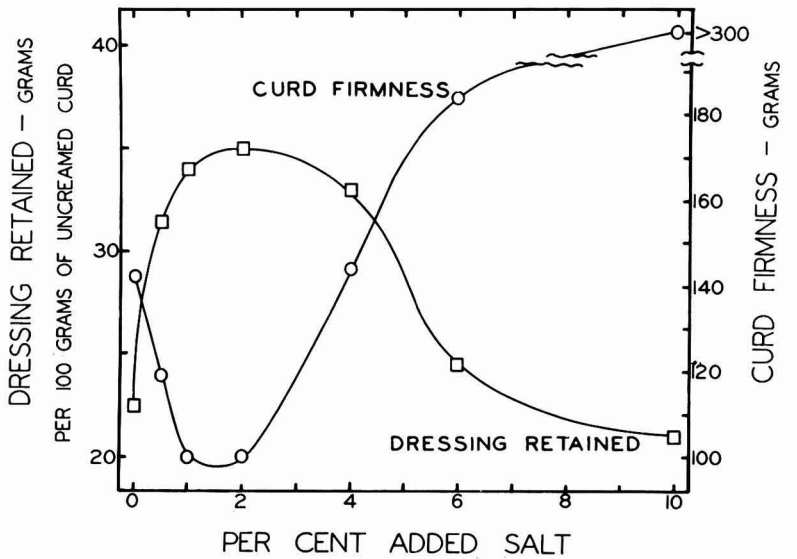

FIG. 7. Effect of added salt on dressing retained on curd firmness of creamed Cottage Cheese.

170° F. for 20 min., homogenized at 2,500 lb. at 130° F., cooled over a surface cooler, and used immediately for creaming. Identical curd was creamed with each dressing to give creamed Cottage Cheese with 4.5 and 6% fat. Measurements of free dressing and curd firmness were made on all lots. The results are summarized in Table 4.

The data of Table 4 show that as the percentage of fat in the dressings increased, the percentage of dressing retained by the curd increased, and the grams of free dressing per carton decreased. This was apparent when the curd was creamed to either 4.5 or 6% fat. The amount of dressing retained per 100 g. of uncreamed curd showed no continuous trend with changes in fat in the dressing at either level of fat in the creamed cheese. The creamed Cottage Cheese with 6% fat retained more dressing than that containing only 4.5%, but smaller percentage of the added dressing was retained by the curd, even though there were more grams of free dressing per carton.

There appeared to be no constant relation between the fat content of the dressing and the grams of dressing retained per 100 g. of uncreamed curd.

It should be noted, however, that at the 6% level of fat in the creamed cheese, the maximum amount of dressing retained per 100 g. of curd was achieved with dressing containing 15% fat. The fact that there was no constant relationship between fat in dressing and dressing retained may be explained by the combined influences of increasing the percentage of fat in the dressing and reducing the amount of dressing thus required in the carton; the grams of dressing retained at the 6% fat level increased with the fat content of the dressing until the reduced amount of dressing required became the limiting factor.

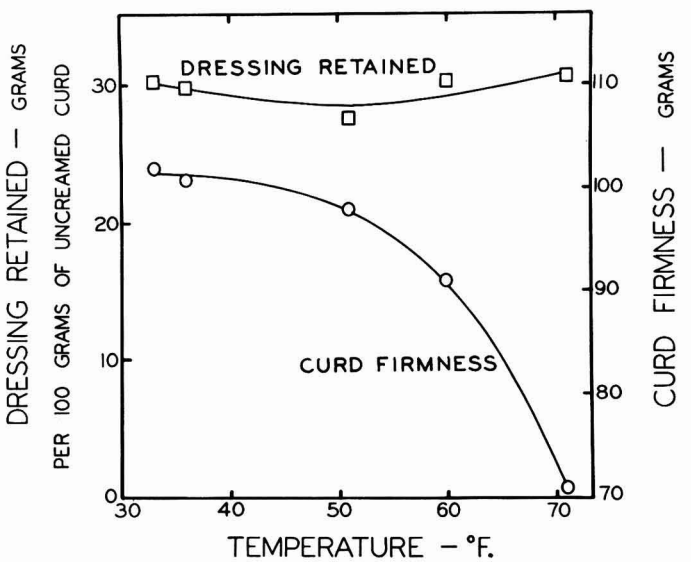

FIG. 8. Effect of temperature of holding creamed cheese on dressing retained on curd firmness.

The use of more dressing in creamed cheese with 6% fat not only increased the amount of dressing retained by the cheese, but also raised the pH of the creamed Cottage Cheese. This association of increased pH and more dressing retained suggests the effect observed when the pH of Cottage Cheese was raised by using dressing to which base had been added.

The data in Table 4 show that with either 4.5 or 6% fat in the creamed cheese there was an increase in curd firmness as the fat in the dressing increased. The data also show that with any one of the three percentages of fat in the dressing the curd firmness was always less in the creamed cheese with 6% fat. These trends can be explained in part by differences in pH of the curd when the ratio of curd to dressing was changed to achieve the two levels of fat. The higher ratios of dressing to curd caused greater increases in the pH of the mixture of curd and dressing. Again, this recalls the effect of added base on softening of curd.

TABLE 4

Effects of fat in dressing on dressing retained, curd firmness, and free dressing per carton of creamed Cottage Cheese

Fat in dressing	Dressing added per 100 g. of curd	Dressing retained per 100 g. of curd	Per cent dressing retained	Free dressing per carton	Curd firmness
(g.)		*(g.)*	*(%)*	*(g.)*	*(g.)*
		4.5% fat in creamed Cottage Cheese			
12	60	36.6	61	50	73
15	43	36.5	85	15	75
18	33	30.3	92	8	82
		6.0% fat in creamed Cottage Cheese			
12	100	39.9	40	103	64
15	67	50.1	75	33	70
18	50	44.7	89	13	73

Homogenization pressures for processing dressing. Raw cream was standardized to 12% fat and divided into four lots. Each lot was heated to 170° F. for 20 min. and cooled to 130° F. One lot was not homogenized, the others were homogenized at pressures of 1,500, 2,500 or 4,500 lb. and cooled immediately over a surface cooler. Each lot of dressing was mixed with identical curd to give 4.5% fat, held for 24 hr. at 50° F., and tested for dressing retention and curd firmness. Higher homogenization pressures increased the viscosity and clinging properties of dressing. The direct relation between dressing retained and homogenization pressures is shown in Table 5. Slightly lower values of curd firmness seemed to be associated with greater amounts of dressing retained.

Stabilizers added to dressing. Further trials were made to observe the effects on dressing retained by using stabilizers as thickening agents in the dressing. These were varied in type and concentrations. The results were quite predictable in terms of thickening and could be easily regulated. As an example, gelatin (250 Bloom), pectin, and a guar gum were each added at the rate of 0.4% of the dressing. The gelatin, at this concentration, formed a jelly-like mass of curd and dressing in the cartons; the liquid draining from these cartons resembled whey. Pectin thickened the dressing slightly, but the guar gum had no effect at this concentration. Without stabilizer added, 37 g. of dressing was retained per 100 g. of curd. Dressing containing the gelatin, pectin, and guar gum retained 55, 46, and 36 g. of dressing per 100 g. of curd,

TABLE 5

Effect of homogenizing pressures on dressing retained, free dressing, and firmness of curd

Homogenizing pressure	Free dressing per carton	Dressing retained per 100 g. curd	Firmness of curd
(lb.)		*(g.)*	
0	74	25.8	90
1,500	65	29.5	85
2,500	50	36.6	73
4,500	26	47.9	76

respectively. In this same manner, other concentrations were used to aid the study of the effect of dressing retained on curd firmness.

Effect of dressing retained on curd firmness. A series of 22 lots of creamed Cottage Cheese, prepared from the same lot of uncreamed curd, provided data on the relationship between dressing retained and firmness of the creamed curd. Differences in dressing retention were obtained by varying the viscosity of the dressing by altering homogenizing treatments and by adding different levels of stabilizers. Values for pH, salt concentration, time and temperature of holding the creamed curd, and the ratio of dressing to uncreamed curd were alike in all 22 lots. The data on comparable measurements of curd firmness *(Y)* and dressing retained per 100 g. of curd *(X)* were analyzed to determine the degree of relationship. The regression equation disclosed was:

$$Y = 94.2 - 0.467\,X$$

The correlation coefficient of $-.533$ for the 22 lots of creamed curd was significant at the 5% level. This lowering of curd firmness by increased dressing retention would be expected and may be explained, at least in part, by the extent of dilution of the relatively firm curd particles by the viscous dressing. Creamed cheese was always prepared for the curd firmness test by draining free liquid from the sample; the more viscous dressing was always retained to a greater extent. This dilution effect of the more viscous dressing on curd firmness is believed to be unlike the physical effects on the curd itself attained by altering temperature of curd, pH, or salt concentration.

The results of these studies of the effects of the various factors upon dressing retention and curd firmness are summarized in Table 6.

TABLE 6

Summary of factors affecting curd firmness and cream retained per 100 g. of curd

Factor increased:	Effect on dressing retained per 100 g. of curd	Effect on curd firmness
Time after creaming	Increase	Decrease
Curd breakage	Increase	Decrease
Salt from 0 to 2%	Increase	Decrease
Salt from 2 to 10%	Decrease	Increase
Temperature of holding creamed curd	Little change	Decrease
pH	Increase	Decrease
Fat in creamed curd, constant fat in cream	Increase	Decrease
Fat in dressing, constant fat in curd	Increase	Increase
Homogenizing pressures	Increase	Slight decrease
Use of stabilizers	Increase	Slight decrease

DISCUSSION

Certain general observations can be made from this survey of various factors affecting curd firmness and dressing retained in creamed Cottage Cheese: (a) The addition of dressing to curd lowered curd firmness and increased pH; (b) marked decreases in curd firmness were often accompanied by increases in the amount of dressing retained; and (c) when the dressing had been treated

in some way to increase its viscosity, there was a marked increase in cream retained and usually some decrease in curd firmness.

Decreases in curd firmness associated with increases in dressing retention suggest that liquid or serum from the dressing is absorbed by the curd particles to make the curd softer. Changes in pH and salt content which affect curd firmness appear to support this supposition.

The data indicate that two separate phenomena are involved in explaining the retention of dressing by curd: (1) absorption of serum from the dressing by the curd, and (2) physical clinging of dressing to curd, an action which appears to be influenced primarily by viscosity of the dressing.

The data do not provide clear-cut evidence on the mechanisms by which the firmness of Cottage Cheese curd is affected by creaming. It seems logical to postulate that one mechanism involves serum absorption by the curd, accompanied by a simple swelling of the curd and reduction of its strength. Another possible mechanism seems to be independent of serum absorption and appears to involve changes in the structure of the curd itself as it might be influenced by pH, ionic atmosphere, or temperature. Most of the factors studied seemed to influence both of these hypothetical mechanisms, excepting the storage temperature of the curd. Storage temperature seemed to affect only the physical strength of the curd, with no apparent effect on seedum absorption.

In these experiments many factors affected these several mechanisms in a quantitative manner; among these were the breakage of curd, pH of the creamed curd, salt concentration, changes in temperature of storage, viscosity of dressing, amount of available dressing, and time of holding after creaming.

Even though these observations of effects of different factors on creaming of Cottage Cheese have a practical application in controlling the operation, they are at best only qualitative because of the interactions involved in these experiments. Eventually, these factors must be studied to develop the quantitative values necessary for the most accurate control of the quality of the finished product.

REFERENCES

(1) EMMONS, D. B., AND PRICE, W. V. A Curd Firmness Test for Cottage Cheese. J. Dairy Sci., 42: 553. 1959.

(2) HARMON, L. G., TROUT, G. M., AND BONNER, M. D. A Market Survey of Cottage Cheese. I. Some Characteristics Influencing Consumer Acceptance and Shelf-Life. Mich. Agr. Expt. Sta., Quart. Bull., 38 (1): 146. 1955.

(3) MANUS, L. J. High Viscosity Cottage Cheese Dressing. Milk Products J., 48 (10): 56. 1957.

RELATION BETWEEN COMPOSITION AND CONSUMER ACCEPTANCE OF MILK BEVERAGES[1, 2]

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SUMMARY

Consumer acceptance observations were made on milk beverages of varying fat and solids-not-fat (SNF) content. Threshold taste tests indicated that many people can differentiate between milk beverages with variations in fat and SNF of 0.5 and 1.0%, respectively. The addition of 1.0% SNF to whole, low-fat, or nonfat milk beverages caused a highly significant increase in consumer acceptance of each type of beverage. A slight but significant preference was shown for a low-fat beverage with 1.0% added SNF when compared with whole milk of normal composition. Equal preference was indicated for a low-fat beverage without added SNF when compared with a nonfat product fortified with 1.0% SNF. A slight but significantly greater preference was evident for a regular whole milk with 1.0% added SNF, when compared to a higher fat (4.0%) product without added SNF.

Standards for minimum fat and solids-not-fat (SNF) content of regular whole milk are usually set by law. Irregular, unavoidable variations in one or both constituents may occur, however, in any given market. In most judicatorial areas, composition standards for nonfat and low-fat beverages have not been adopted. Furthermore, the practice of standardizing the SNF content of whole milk beverages is not provided for. There is a general awareness (2-5) that the addition of from 0.5 to 2.0% nonfat milk solids to either whole milk, low-fat, or nonfat beverages improves the palatability, flavor, and food value of the beverages. Of equal importance is the fact that the utilization of nonfat solids could be significantly increased by a wider application of the SNF standardization or fortification principle in milk beverages.

This controlled consumer preference study was conducted to provide data on which standards and practices relating to the optimum composition of milk beverages can be based.

EXPERIMENTAL PROCEDURE

Materials used in the beverages. Excellent quality raw milk from the University dairy herd of Guernsey, Holstein, and Jersey animals was used for the tests. When it was necessary to increase the fat content, cream separated at 90-95° F. from the same lot of milk was used. In cases where the SNF content was increased, good-flavored low-heat nonfat dry milk of high solubility was used. It was dispersed in the milk prior to pasteurization and homogenization. To prevent development of oxidized flavors during storage, 0.005% nordihydroguaiaretic acid (NDGA) and 0.01% citric acid (on the fat basis) were added prior to pasteurization.

Received for publication November 6, 1959.

[1] This research was supported by a grant from the American Dairy Association.
[2] Contribution from the Arizona Agricultural Experiment Station, Journal Paper No. 556.

Processing procedure. Analyses for fat and total solids by the Babcock and Mojonnier methods, respectively, were used. The batches were standardized, heated to 135° F., then homogenized with a double-stage machine at 2,500-500 p.s.i. After homogenization, the products were pasteurized at 143° F. for 30 min., then cooled to 35-40° F.

Selection of the taste panel. Threshold values for fat and SNF were determined by the use of a taste panel. The taste acuity of potential judges was determined for varying levels of fat and SNF. For the panel, a group of 20 men and eight women were selected from volunteers on the faculty and staff of the College of Agriculture, The University of Arizona. Some of their characteristics are shown in Table 1. These 28 individuals were subjected to threshold

TABLE 1
Taste panel characteristics

	Age *(yr.)*					Sex		Use of tobacco		Use of milk as beverage	
Taste acuity level	20-30	30-40	40-50	50-60	Above 60	Male	Female	Yes	No	Regularly	Occasionally
Acceptable (No.)	5	7	1	0	1	10	4	4	10	13	1
Not acceptable (No.)	3	6	4	1	0	10	4	5	9	8	6

tests by the use of paired and triangulation techniques as suggested by Clements (1). Fourteen of the original group were accepted as members of a more select panel, based on their ability to discriminate differences in milk samples. It is of interest to note that six of the 14 rejected members were occasional milk drinkers, while only one of the accepted panel members was in this category.

Consumer populations used. Milk beverage samples were submitted to people for preference observations. These preference samplings were made in food markets, schools, and public gatherings. Thus, the sampled population included a wide range of economic, social, and educational levels.

A. Retail food markets. All retail markets used in the experiment were located in Tucson, Arizona, a city with 250,000 inhabitants in its metropolitan area. Some areas in the city are populated largely by a laboring, multi-racial class. Population in other areas is largely Caucasian and of varying employment or economic status.

B. Public schools. Since schools in Tucson have racial integration, the population in several is multi-racial. For purposes of this experiment, students in the fourth through the eighth grades were used.

C. Public gatherings. In this series, observations were obtained from special groups of consumers: (1) the Arizona State Fair, Phoenix, (2) the dedication ceremonies of the new Dairy Science Center at The University of Arizona, (3) the Annual Arizona Dairy Industry Conference at the University, and (4) the annual meeting of the American Dairy Association of Arizona, Phoenix.

Tasting procedure and presentation of samples.

A. Taste panel. Members of the panel were seated in individual booths which prevented cross conversation during the tasting periods. The milk

samples were tempered to a temperature of 65-70° F. before serving. Twenty-five to 30 ml. of the samples were dispensed into nontransparent 1¾-oz. sham glasses for tasting in the various comparisons. The tasters were instructed to taste a portion of the sample without swallowing. After disposing of the sample, they rinsed their mouths with tap water at room temperature. There was a 4- to 5-min. interval between each pair or series.

B. Others. Some variation in tasting procedure was necessary in retail markets and public gatherings as compared to the schools. In the markets and at the public gatherings, milk was dispensed from an attractive portable booth erected at a suitable site. Location of the booth in markets was influenced primarily by the flow of traffic within the store and the discretion of the store manager. Samples of milk were dispensed from 5-gal. stainless steel cans placed in a refrigerated (38 ± 2° F.) milk dispenser. In markets and gatherings, as well as in schools, only one pair of samples was tested at any one place in any one time period (day, etc.). The 14,294 observations (Table 4) were, therefore,

TABLE 2
Taste panel discrimination tests

Taste acuity level	Decisions			
	Correct	Total	Average correct	Range
	(*No.*)		(*%*)	
Acceptable	774	1,025	76	67-92
Not acceptable	315	616	51	41-60

TABLE 3
Taste panel preference for beverages of 12.0% total solids and varying fat contents

Preference ranking				1st	2nd	3rd	4th	
Sample No.	Product			Total choices				Weighted total
	Fat (%)	*SNF (%)*	*Total solids (%)*					
1	3.5	8.5	12.0	41	42	24	33	329
2	2.5	9.5	12.0	45	58	32	5	277
3	1.5	10.5	12.0	33	31	67	9	332
4	0.5	11.5	12.0	21	9	17	93	462

from essentially that number of individuals. It was apparent that only in extremely rare instances did an individual make more than one observation during the course of the study. Actual tasting was done when a 1- to 1½-oz. portion of each sample in the pair was dispensed into a paper cup and then put on a tray with two countersunk positions, which were labeled A and B. In schools, the booth was not used, for space as well as flexibility reasons. Children were brought in groups to either the school cafeteria or auditorium for the test. Milk was dispensed and containers placed on a sheet of paper with large letters marked A and B prior to the students' entry. After brief directions were given, students all participated in the experiment simultaneously.

TABLE 4
Composition of milk beverages and preference observations

Pair No.		Fat	SNF	Total solids	No. preferring	Per cent of total
		(%)	(%)	(%)		
I	A	3.5	8.5	12.0	1,087	40[a]
	B	3.5	9.5	13.0	1,643	60
II	A	2.0	9.0	11.0	1,448	46[a]
	B	2.0	10.0	12.0	1,683	54
III	A	0.1	9.0	9.1	882	41[a]
	B	0.1	10.0	10.1	1,254	59
IV	A	3.5	8.5	12.0	1,011	47[a]
	B	2.0	10.0	12.0	1,112	53
V	A	1.5	8.5	10.0	1,058	51 ns
	B	0.1	10.0	10.1	1,012	49
VI	A	4.0	8.5	12.5	986	48[b]
	B	3.5	9.0	12.5	1,078	52
				Total	14,294	

[a] Significant at the 1% level of probability.
[b] Significant at the 5% level of probability.
ns—Not significant.

The tests at schools were closely proctored by teachers and research personnel, to minimize conversation or other detracting elements.

Questionnaires. The questionnaires used in food markets and public gatherings and in schools are shown in Figure 1.

RESULTS AND DISCUSSION

Taste panel threshold tests. Table 2 gives a summation of the threshold tests. To be considered as an acceptable panel member, the individual had to make two out of three (67%) correct decisions in products which varied 0.5 or 1.0% in fat or SNF, respectively. In the acceptable group, the individual correct decisions ranged from 67 to 92%, with an average of 76%. In the group that was considered not acceptable, the individual correct decisions ranged from 41 to 60%, with an average of 51%. The results showed that many people can differentiate between milk beverages with variations in fat and SNF of 0.5 and 1.0%, respectively.

Taste panel preference tests. The taste panel was subjected to a series of preference observations. The composition of the beverages used and preference choices are given in Table 3. The samples were presented four at a time in random arrangements. Of particular interest is the weighted total. This value is the sum of the total choices of each ranking, times their respective numerical ranking. The lower the weighted total, therefore, the greater the preference for the beverage. The beverages with 3.5, 2.5, and 1.5% fat had weighted totals very comparable to one another. This indicates that the low-fat beverages with added SNF have an acceptability generally as high as that of whole milk. The 0.5% fat product was the least preferred, which was to be expected with the rather high SNF content and its resultant slightly salty flavor.

PLEASE MARK THE APPROPRIATE ITEM

1. PREFER SAMPLE IN: CONTAINER A ☐ ; CONTAINER B ☐.
2. AGE: UNDER 20 ☐; 20 TO 40 ☐; 40 TO 60 ☐; ABOVE 60 ☐.
3. SEX: MALE ☐; FEMALE ☐.
4. DO YOU USE TOBACCO? YES ☐; NO ☐.
5. DO YOU DRINK MILK? REGULARLY ☐; OCCASIONALLY ☐; NEVER ☐
6. OCCUPATION: _____ (IF HOUSEWIFE, INDICATE HUSBAND'S OCCUPATION.)
7. KIND OF MILK USED IN YOUR HOME. HOMOGENIZED PASTEURIZED OR WHOLE MILK ☐; LOW FAT ☐; SKIM ☐; POWDER ☐.

PLEASE DO NOT SIGN YOUR NAME

PLEASE MARK THE APPROPRIATE ITEM

1. PREFER SAMPLE IN: CONTAINER A ☐; CONTAINER B ☐.
2. SEX: BOY ☐; GIRL ☐
3. DO YOU DRINK MILK WITH MEALS EVERY DAY ☐; NOW AND THEN ☐; NEVER ☐.
4. PARENT'S OCCUPATION: _____

PLEASE DO NOT SIGN YOUR NAME

FIG. 1. Questionnaires used in food markets and public gatherings and in public schools.

Consumer preference observations. On the basis of the results secured with the taste panel, beverages of the three classes—whole, low-fat, and nonfat milk—were subjected to preference observations in larger groups of people, as described in the Experimental Procedure. The composition of the beverages and the over-all results are shown in Table 4. Comparisons obtained by cross-classifying the preference observations with the variables shown in the Questionnaires (Figure 1) are the subject of a more detailed report being prepared.

The data in Table 4 show that the addition of 1.0% SNF caused a highly significant improvement in the consumer acceptance of whole, low-fat, or nonfat milk beverages (Pairs I, II, and III). There was a slight but significant preference for a fortified low-fat beverage compared with a nonfortified whole milk (Pair IV). No significant difference was indicated in the preference for a nonfortified low-fat beverage compared with a fortified nonfat product (Pair V). A slight but significantly greater preference was shown for a regular fortified whole milk compared with a nonfortified higher fat milk (Pair VI). For the

purposes of this experiment, it should be noted that the SNF content of the 4.0% fat product was standardized to a value which is slightly less than some reported normal values.

ACKNOWLEDGMENT

The authors acknowledge the assistance of Dr. Henry Tucker, Statistician, in the design of the experiment and analysis of the data.

REFERENCES

(1) CLEMENTS, FORREST E. Psychophysical Methods in Market Research. Florida State Hort. Soc., Proc., 64: 148. 1951.

(2) CUSTER, E. W., HERZER, F. H., AND CARDWELL, J. T. The Effect of Various Levels of Solids Not Fat on the Flavor Acceptability of Fluid Milk. Mississippi Agr. Expt. Sta., Bull. 561. 1958.

(3) HOLLAND, B. K., AND WINDER, W. C. Preparation of Modified Skim Milk. Milk Dealer, 40: 43. 1951.

(4) MAYNARD, L. A. The Fortification of Whole Milk and Skimmilk. J. Dairy Sci., 37: 624. 1954.

(5) WECKEL, K. G. Flavor and Keeping Quality of Vitaminized Fortified Skim Milk. Milk Dealer, 41: 56. 1952.

ERRORS IN ESTIMATION OF LACTATIONAL YIELDS OF MILK, FAT, AND SOLIDS-NOT-FAT FROM INDIVIDUAL COWS

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SUMMARY

Individual milkings from 12 Holstein-Friesian cows were weighed and sampled daily through entire lactation periods. Samples from single milkings were combined to make one-day composite samples, which were analyzed for fat and solids-not-fat. Both milk yield and composition varied with stage of lactation. Percentages of fat and total solids in the composite samples were affected significantly by failure to take aliquot volumes, but percentage of solids-not-fat was not changed. Standard errors of estimate for the Babcock test and Watson lactometric procedure were ± 0.05% fat, ± 0.07% total solids, and ± 0.04% solids-not-fat. Bias for the Watson lactometric procedure, over whole lactations, was −0.02% total solids. This bias changed with stage of lactation. The large and small model Watson lactometers gave results that did not differ significantly.

Sampling errors for lactational yields of milk, fat, and solids-not-fat were 273, 13, and 26 lb., respectively. When expressed as coefficients of variability, the sampling errors for fat and solids-not-fat yields did not differ significantly. The monthly sampling procedure used in Dairy Herd Improvement Associations gave unbiased estimates for yields of milk, fat, and solids-not-fat. The final or total error of estimate consisted principally of sampling error.

Errors in estimated lactational yields of dairy cows may arise from several sources. These sources include day-to-day variations in yield and composition of the milk, errors in weighing and taking of samples, errors in chemical analyses, and those arising from periodic (rather than continuous) weighing and sampling. The purpose of this investigation was to secure estimates of these errors, particularly for percentages and yields of solids-not-fat.

METHODS AND MATERIALS

The experimental animals were Holstein-Friesian cows in the Virginia Agricultural Experiment Station herd. All cows were milked twice a day throughout the lactation period. The weight of each individual milking was recorded in whole pounds and tenths, and a sample of approximately 200 ml. taken for analysis. One-day composite samples were prepared by mixing aliquot portions (to the nearest 5 ml.) of the morning samples with those obtained the preceding evening. Each composite sample was analyzed for fat by the Babcock procedure and for specific gravity with the large model Watson lactometer (15).

In 1957, milk from each of 12 cows that calved between January 7 and February 17 was weighed and sampled at every milking during their lactation periods. Altogether, 6,766 milkings of individual cows were weighed and sampled, and 3,383 one-day composite samples were prepared and analyzed.

In 1958, eight cows were sampled one day each month during the lactation

Received for publication June 27, 1959.

period. The resulting 84 one-day composite samples were analyzed for fat and specific gravity, and also for total solids by the A.O.A.C. procedure (1).

Percentages of total solids were calculated by Watson's formula (15). Percentages of solids-not-fat were obtained by subtracting the percentages of fat from the corresponding percentages of total solids. Daily yields of fat and solids-not-fat were calculated from daily milk yields and the appropriate percentages.

RESULTS AND DISCUSSION

Variations in daily production. The lactational curve for milk yield was that usually observed for dairy cows, except that maximum yield was delayed until the cows were transferred from barn feeding to pasture. Mean daily percentages of fat and solids-not-fat, for the 12 cows sampled in 1957, are shown in Figure 1. Two of the 12 cows were removed from the herd at 230 and 233

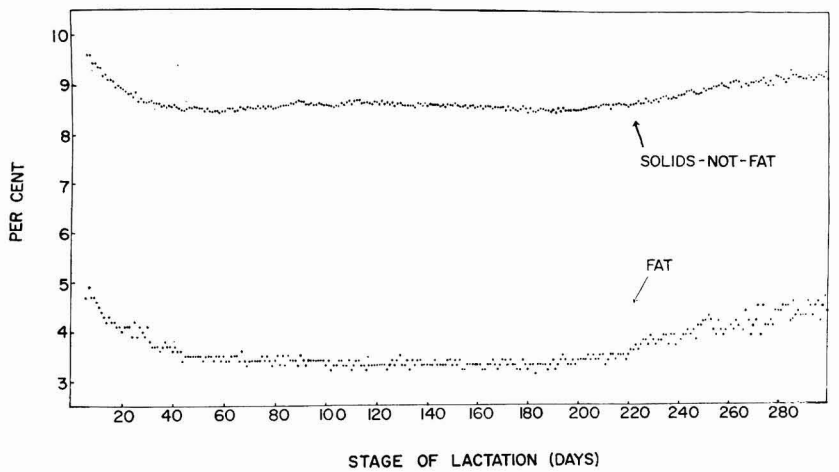

FIG. 1. Average daily percentages of fat and solids-not-fat for 12 cows, by stage of lactation.

days after calving and, thus, the curves beyond the 233rd day represent the remaining ten cows. The trends in percentages of fat and solids-not-fat are similar to those reported by Waite *et al.* (14).

Preparation of samples. The effect of improperly composited samples on percentages of fat, total solids, and solids-not-fat was investigated. The 12 experimental cows were milked at 11- and 13-hr. intervals, respectively, between morning to evening and evening to morning milkings. Evening samples from these 12 cows were combined with morning samples from the same cows, both by aliquot and by equal parts. Thus, one set of the resulting composite samples contained proportionate volumes of the evening and morning milkings, the other set contained equal volumes. Average milk yield per cow, on the chosen day, was 20.7 lb. in the evening and 25.7 lb. in the morning.

All samples were analyzed for fat content by the Babcock procedure and

for total solids by the Watson lactometric procedure (15). Analyses of variance of the resulting values showed that percentages of fat were affected significantly ($P < 0.025$) by the method of sample preparation, as were also percentages of total solids ($P < 0.005$). However, the method of sample preparation did not affect percentages of solids-not-fat in a significant manner.

Nicholson *et al.* (12) found significant differences between morning and evening milk with respect to percentages of fat and total solids, but not for solids-not-fat. Obviously, if yields at the individual milkings were equal, the taking of composite samples would reduce to that of equal volumes.

Comparison of methods for total solids. The 84 one-day samples obtained in 1958 were taken at various stages of the lactation period. The average percentage values for total solids, as determined by lactometric procedure, were higher in early months of lactation than were the corresponding values by the A.O.A.C. gravimetric method (Table 1). However, in later months of lactation

TABLE 1

Per cent total solids in whole milk from Holstein-Friesian cows, as estimated by two methods

Date of sampling[a]	No. cows sampled	Per cent total solids				
		Lactometric[b]		Gravimetric[c]		Difference[d]
		(%)	*(S.E.)*[e]	*(%)*	*(S.E.)*[e]	*(%)*
Jan. 13	8	14.33	.....	14.22	0.03	+0.11
Jan. 19	8	14.29	0.06	14.12	0.16	+0.17
Feb. 10	8	12.76	0.08	12.67	0.08	+0.09
March 17	8	12.30	0.08	12.12	0.06	+0.18
April 14	8	12.52	0.05	12.50	0.05	+0.02
May 12	8	12.65	0.05	12.59	0.13	+0.06
June 16	7	12.30	0.06	12.27	0.12	+0.03
July 14	7	11.82	0.09	12.01	0.12	−0.19
Aug. 11	7	12.19	0.06	12.35	0.02	−0.16
Sept. 15	7	12.90	0.10	13.20	0.13	−0.30
Sept. 29	5	13.09	0.05	13.47	0.04	−0.38
Oct. 13	3	12.86	0.11	12.90	0.01	−0.04
Av.		12.85	0.07	12.87	0.09	−0.02

[a] Year 1958.
[b] Watson's lactometric procedure.
[c] A.O.A.C. gravimetric procedure.
[d] Lactometric per cent minus gravimetric per cent.
[e] Standard error of estimate.

the values by lactometric procedure were lower than those by gravimetric. These data demonstrate that the lactometric procedure gives results which are biased upward in early lactation and downward in the later months.

Jarrige and Rossetti (8) found that the relative proportions of fat, protein, lactose, and minerals in milk will change with advance in lactation. The fat, protein, lactose, and minerals differ from one another in specific gravity. The lactometric formulas assume that the nonfat constituents, when considered as a unit, have a constant specific gravity. To the extent that the relative proportions of nonfat constituents do change, the lactometric formulas do not give precise estimates of either total solids or solids-not-fat. This reduction in precision, due to changes in relative proportions of nonfat constituents, appears not to have been noticed in the various investigations of mixed herd milk.

Presumably, the errors on individual cows and stages of lactation tend to cancel one another.

A comparison of the large and small model Watson lactometers showed that their use did not have significantly different effects on the resulting values for total solids and solids-not-fat. Brunner (3) concluded that "the small Watson-pattern lactometer offers a method sufficiently accurate and simple to use in large-scale testing programs, either in a laboratory or in field application." A possible source of error, in the use of a particular lactometer, may come from errors in calibration.

Errors from monthly sampling. If lactational yields of dairy cows were obtained by summation of daily yields, the resulting values would be affected only by experimental errors. Further, if the procedures for weighing, taking samples, and analysis were unbiased, the errors would be of a random type and would tend to cancel one another over whole lactation periods. Thus, lactational records obtained by summation of daily yields should be of high precision and, if all procedures are unbiased, of high accuracy.

In practice, the summation of daily yields is rather costly. Instead, a periodic sampling procedure is used on the herd as a whole, and one-day samples are taken from individual cows on or near median days at monthly intervals (9). This procedure was applied to data on the 12 cows sampled in 1957, and sampling errors were calculated for the resulting estimates (Table 2).

TABLE 2

Average sampling errors for milk composition and yield during whole lactations[a]

Constituent	Av. yield or value	Sampling error	Coefficient of variability[b, c]
Milk *(lb.)*	11,546	273 {298 / 253}	2.4 {2.6 / 2.2}
Fat *(%)*	3.6	0.10 {0.11 / 0.09}	2.8 {3.1 / 2.5}
Fat *(lb.)*	409	13 {14 / 12}	3.2 {3.4 / 3.0}
Solids-not-fat *(%)*	8.69	0.05 {0.055 / 0.046}	0.6 {0.7 / 0.5}
Solids-not-fat *(lb.)*	999	26 {28 / 24}	2.6 {2.8 / 2.4}

[a] Based on 12 cows sampled daily in 1957.
[b] Expressed as per cent.
[c] Values in brackets are 95% confidence limits.

The average sampling errors in Table 2 are similar to those reported in the literature (2, 5, 6, 11), for milk and fat yields. No reports on sampling errors for yields or percentages of total solids or solids-not-fat were found. The error for percentage of fat was about twice the size of the error for percentage of solids-not-fat. When expressed as coefficients of variability, only the error for percentage of solids-not-fat differed greatly from the others.

Varying the sample day. In Dairy Herd Improvement Associations, a

recommended practice with regard to monthly sampling is to vary the sample day from month to month (9). The recommended range of variation is ± 3 days from the median (or centering) day. This practice was applied to the data secured in this investigation, and the estimated yield from use of each of the seven recommended sampling days was compared with the actual yield (Table 3). An analysis of the percentage deviations showed no significant time trends

TABLE 3
Percentage deviations of sample estimates from actual yields, when sampling day is varied

Day of sampling	Percentage deviation from actual		
	Milk yield	Fat yield	Solids-not-fat yield
	(%)	*(%)*	*(%)*
−3	−0.3	−1.7	−0.1
−2	−2.3	−2.5	−2.6
−1	−3.8	−1.9	−4.0
Centering day	−2.4	−2.0	−2.5
+1	−2.4	−0.7	−2.5
+2	−2.9	−1.7	−3.2
+3	−3.2	−3.0	−3.7

and, therefore, no bias in estimated yields due to day of sampling.

Bias in lactational yields. Deviations of sample estimates from actual yields may be used to detect bias in these estimates (7). The expectation of deviations of sample estimates from the true value is zero, and the mean of such deviations may be tested against its standard error for significance. The estimated yields that were obtained by monthly sampling appear to be unbiased (Table 4).

TABLE 4
Deviations of estimated from actual yields[a]

Cow	Milk yield	Fat yield	Solids-not-fat yield
	(lb.)	*(lb.)*	*(lb.)*
1	+150.0	+5.4	+16.3
2	−275.0	−2.9	−26.1
3	−176.1	−9.9	−23.1
4	−345.2	−8.5	−22.8
5	−149.4	+19.4	−11.0
6	−445.7	−1.9	−33.0
7	−286.5	−12.4	−25.4
8	− 99.6	−4.6	−10.8
9	−247.4	−14.2	−19.9
10	−255.9	−24.2	−19.8
11	−575.5	−6.4	−50.4
12	−373.4	−15.0	−39.7
Av.	−256.6	−6.3	−22.1
Standard error	183.0	10.8	16.5
t-ratio	1.40[b]	.58[b]	1.34[b]

[a] Based on monthly samples taken on centering day.
[b] Not significant statistically.

The numerous negative deviations in Table 4 may lead one who is not familiar with the data to contend that bias, nevertheless, did exist. The fact is that the negative deviations are a result of the particular choice of sampling day. Estimates based on other sampling days would have differed. If, for

example, the tenth day of each month had been taken as the sampling day, the average deviation per cow would have been +589 lb. of milk.

Over-all error of estimate. Thus far, we have considered the various errors separately. The over-all or final error of estimate will be some linear combination of the various experimental and sampling errors. If the various errors are not correlated with one another, this linear combination reduces to the root-sum-square of these errors. A convenient formula for calculation of the final error, in this instance, is

$$E_F = \sqrt{\sum_i (N/n_i) E_i^2},$$

in which E_F is the final error, N is the total number of items in the population, n_i is the number of each kind of weighings or analyses performed, and E_i is the standard error of estimate for a single weighing or analysis.

In addition to the sampling errors of Table 2, the standard error of estimate obtained for the Babcock test for percentage of fat was ± 0.05, and for the percentage of solids-not-fat by lactometric procedure ± 0.04. No estimate of error in the weighing of milk was obtained in this investigation. Mackay *et al.* (10) found the average error of milk scales in use in Dairy Herd Improvement Associations to be 0.40%. This would be equivalent to a standard error of estimate of ± 0.59% of the true milk weight.

In addition to no correlation among errors, let us assume that:

1. Each cow was sampled ten times or days during the lactation period, and milked 282 days.
2. The yield of each cow was 15 lb. per milking, and each cow was milked twice a day.
3. There was no error in mixing of milk preparatory to removing a sample portion, and no error in compositing of samples.

Then the final errors of estimate may be calculated as indicated in the foregoing formula. Numerical values for these errors are shown in Table 5, together with the variances which contribute to them.

The results in Table 5 show that the final error of estimate, for these data,

TABLE 5

Contributing variances and final errors of estimates for yields and percentages during whole lactations

Yield or percentage	Source of variance				Final error of estimate[a]
	Weighing milk	Babcock test	Lactometric procedure	Monthly sampling	
Milk *(lb.)*	0.11	None	[b]	74,529	273
Fat *(%)*		0.00025	[b]	0.01	0.10
Fat *(lb.)*	0.11	0.0016	[b]	169	13
Solids-not-fat *(%)*		[b, c]	0.00016	0.0025	0.052
Solids-not-fat *(lb.)*	0.11	[b, c]	0.0010	676	26

[a] Square root of sum of pertinent variances.
[b] No effect on final error from this source.
[c] Variance for lactometric procedure includes effect from Babcock test.

consists principally of the sampling error. The sampling errors for fat and solids-not-fat yields, when expressed as coefficients of variability, are not significantly different (Table 2). It may be concluded, therefore, that sampling at monthly intervals for yields of solids-not-fat is quite as accurate as for fat yields.

The relative error for percentage of solids-not-fat, as shown by the coefficients of variability and confidence limits, is significantly lower than the error for percentage of fat. Therefore, it may be permissible to sample cows less frequently for percentage of solids-not-fat than for percentage of fat. However, this result has no application to sampling for yields of milk, fat, or solids-not-fat.

ACKNOWLEDGMENTS

The authors wish to thank J. F. Yates, M. R. Dickerson, W. E. Shepherd, and G. T. Carr for their help and cooperation in obtaining the samples of milk, and Miss Lucy L. Towe for assisting with the laboratory analyses.

REFERENCES

(1) Association of Official Agricultural Chemists. Official and Tentative Methods of Analysis. 8th ed. Washington, D.C. 1955.
(2) Bayley, N. D., Liss, R. M., and Stallard, J. E. A Comparison of Bimonthly and Quarterly Testing with Monthly Testing for Estimating Dairy Cattle Production. J. Dairy Sci., 35: 350. 1952.
(3) Brunner, J. R. Assay for the Solids-not-fat of Cow's Milk. J. Dairy Sci., 41: 444. 1958.
(4) Cochran, W. G. Sampling Techniques. John Wiley and Sons, Inc., New York. 1953.
(5) Dick, I. D. Some Results on the Accuracy of Milk Sampling Over a Complete Lactation and the Daily Variability in Milk Yields. New Zealand J. Sci. Technol., Agr. (A), 32: 25. 1950. (Animal Breeding Abstr., 19: 1622. 1951.)
(6) Erb, R. E., Goodwin, Mary M., Morrison, R. A., and Shaw, A. O. Lactation Studies. IV. Accuracy of Different Methods of Estimating Lactation Yields. J. Dairy Sci., 35: 977. 1952.
(7) Jardim, W. R., Peixoto, A. M., Filho, S. Silveira, and Gomes, F. Pimental. Study on the Precision and Accuracy of a Few Practical Methods of Estimating Milk Production of Dairy Cows. (Abstr.) Biometrics, 12: 231. 1956.
(8) Jarrige, R., and Rossetti, C. Études sur les Variations de la Richesse en Constituants Azotés des Laits de Vache. Évolution au Cours de la Lactation et de la Gestation. Ann. inst. nat. recherche Agron. Sér. D. Ann. Zootech., 6: 41. 1957.
(9) Kendrick, J. F. The D.H.I.A. Supervisor's Manual. USDA, Agriculture Handbook 96. 1956.
(10) Mackay, D. R., Hartsock, J. G., and Book, J. H. Preliminary Report on Tests of the Accuracy of Milk Scales. J. Dairy Sci., 41: 316. 1958.
(11) McKellip, Ivan, and Seath, Dwight. A Comparison of the Different Methods of Calculating Yearly Milk and Butterfat Records. J. Dairy Sci., 24: 181. 1941.
(12) Nicholson, W. S., Jr., Willard, H. S., Thomas, W. R., and Brown, D. C. Effect of Milking Time on Solids in Milk from Holsteins. J. Dairy Sci., 40: 1480. 1957.
(13) Thompson, N. R., and Graf, G. C. Error in Estimation of Lactational Yields of Solids-not-fat in Milk from Sampling One Day a Month. J. Dairy Sci., 41: 746. 1958.
(14) Waite, R., White, J. C. D., and Robertson, Alan. Variations in the Chemical Composition of Milk with Particular Reference to the Solids-not-fat. I. The Effect of Stage of Lactation, Season of Year and Age of Cow. J. Dairy Research, 23: 65. 1956.
(15) Watson, Paul D. Determination of the Solids in Milk by a Lactometric Method at 102° F. J. Dairy Sci., 40: 394. 1957.

FACTORS AFFECTING THE CONSUMPTION OF SUDAN GRASS BY DAIRY COWS[1]

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SUMMARY

Investigations of factors affecting the consumption of Sudan grass were made over a 6-yr. period. In each of the first 2 yr. three cows were hand-fed cut forage; whereas, in each of the remaining 4 yr. three cows were hand-fed cut forage and three cows simultaneously allowed to graze. A combination of internal and external indicators was used to determine the digestibility and intake of the grazed forage. The daily intake of green forage, by years, ranged from 100 lb. in 1957 to 228 lb. in 1953 for the hand-fed cows, and ranged from 119 lb. in 1955 to 251 lb. in 1953 for the grazing cows. The daily intake of dry matter by hand-fed cows ranged from 23.1 lb. in 1957 to 35.1 lb. in 1952. Each year the grazing cows consumed more forage dry matter than the hand-fed cows. The range of intake was from 25.2 lb. in 1955 to 34.2 lb. in 1957. This extreme variation in intake indicates rather great variation in the quantity of nutrients available for milk production. Dry matter digestibility had a range from 63.6% in 1955 to 71.3% in 1951 with hand-fed cows. The digestibility of dry matter in grazing trials, which were run simultaneously with the hand-fed trials, was always about four percentage points higher than in the hand-fed trials. A very high correlation was noted between dry matter intake and the total rainfall of the previous month ($r = .985$). The intake of dry matter was not significantly correlated with the dry matter content of the forage ($r = .317$); however, the intake of green forage was significantly correlated with forage dry matter content ($r = -.855$).

Sudan grass is now recognized as one of the most valuable annual pasture crops in the United States. It produces a heavy yield in a very short time and makes a luxuriant growth in July and August, when permanent pastures are short. Furthermore, it has a continuous growth habit and makes a good comeback after severe grazing. Land seeded in early June is usually ready for grazing by the middle of July. There is always the possibility of using the same land for cereal pasture from late fall until spring.

While Sudan grass has been used as pasture for many years, very little experimental work has been done to determine its feeding value. Average chemical analyses and digestible nutrients are given in tables compiled by Morrison (5) and Snyder (8). These averages are based on very few proximate analyses, and estimates of digestibility have apparently been based on foreign trials with sheep. The usual dry matter content of the immature grass is indicated to be between 19 and 21.6%. Newlander (6) has reported a digestibility of 70% for the dry matter of Sudan grass, whereas Gaessler and McCandlish (2) reported 61%.

No definite information has been published concerning the amount of digestible nutrients that a cow can obtain from a day's grazing on Sudan grass. Two questions are frequently raised—Does the milking cow on Sudan grass pasture need supplemental feeds? If so, what kind and how much?

Received for publication October 12, 1959.

[1] Delaware Agricultural Experiment Station Miscellaneous Publication No. 349.

This report covers a series of trials, which were inaugurated in 1950 at the Delaware Agricultural Experiment Station, to determine the feeding value of Sudan grass for dairy cows. The relationship of rainfall and temperature to the intake of green forage and dry matter, and their effect upon dry matter digestibility, also have been investigated and are reported herein. These trials were conducted in each of 6 yr.

EXPERIMENTAL PROCEDURE

At the time this project was initiated, there was no satisfactory method available for measuring the feed intake of grazing cows or the digestibility of the forage consumed. Keeping cows in the stable and bringing fresh-cut grass to them seemed to be the most practical approach. During the feeding periods the daily intake was determined by offering weighed fresh-cut green forage in excess, then subtracting the weight of feed refused. The digestibility of the forage dry matter was determined in the conventional way.

Three cows were fed individually in each of the trials. During the first 2 yr. each trial was continued for three days with a preliminary period of four days; in later trials the preliminary periods were increased to seven days.

In 1953, the fecal chromogens method of Reid *et al.* (7) for the determination of the digestible dry matter of grazed forage and the chromic oxide indicator method for the determination of total daily feces of the grazing animals were introduced. With this combination of indicators, total intake could be calculated. This method was utilized in 1953 and in subsequent years. In each of these years, three grazing cows were used and three similar cows were hand-fed forage cut from the same field.

In 1957 the intake of dry matter by the hand-fed cows was determined early in the preliminary period, then the dry matter intake per day was held constant throughout the remainder of the trial. This resulted in a slightly restricted intake for these cows. In all other years the cows were fed ad libitum.

RESULTS AND DISCUSSION

A summary of the data from each of the years in which trials were run is given in Tables 1 and 2.

The total green forage consumed varied considerably from year to year. With hand-fed animals the range was from 100 lb. daily in 1957 to 222 lb. daily in 1953, whereas the grazing animals' intake varied from 119 lb. in 1955 to 252 lb. in 1953. Individual cow variations also were quite great, as indicated by the size of the standard errors of the means (Table 1).

The calculation of green forage intake for the grazing animals assumes that the dry matter content of the grazed forage was the same as that of cut forage. This assumption is not likely to lead to large errors.

If the forage intake and dry matter content of the forage for 1951 and 1952 only are considered, it would appear that feeding the more watery forage (14.2% dry matter versus 28.0%) resulted in a reduced nutrient (dry matter) intake (27.7 lb. dry matter versus 35.1 lb.). The work of later years (1953-1957), however, does not support this proposition. When all trials are con-

TABLE 1

Composition, digestibility, and intake of Sudan grass by Holstein cows

Year	Dry matter in forage	Crude protein dry basis	Dry matter digestibility		Intake			
					Green forage		Dry matter	
			H. F.[a]	G.[b]	H. F.	G.	H. F.	G.
	(%)				(lb.)			
1951	14.2	17.0	71.3 ± 1.32[c]		195 ± 6.4		27.7 ± 0.89	
1952	28.0	5.5	65.4[d]		126 ± 12.0		35.1 ± 3.37	
1953	12.8	13.9	68.0 ± 1.26	72.5 ± 0.30	222 ± 11.6	252 ± 17.1	28.4 ± 1.48	32.2 ± 2.20
1954	14.5	18.5	67.4 ± 0.55	71.5 ± 1.24	177 ± 18.8	224 ± 34.1	25.7 ± 2.71	32.5 ± 4.94
1955	21.2	11.2	63.6 ± 0.44	67.9 ± 0.45	117 ± 10.7	119 ± 2.86	24.7 ± 2.21	25.2 ± 0.61
1957	23.0	11.3	66.2 ± 0.33	72.6 ± 0.14	100 ± 6.34	146 ± 4.16	23.1 ± 1.47	33.8 ± 1.41

[a] Hand-fed.

[b] Grazing.

[c] Standard error of the mean.

[d] In 1952 the fecal samples were pooled; therefore, individual cow data are not available.

TABLE 2

Rainfall and temperature data from Sudan grass feeding trials

	1951	1952	1953	1954	1955	1957
			Rainfall *(in.)*			
Week of trial[a]	0.30	0.26	0.23	0.88	0.00	0.54
Month of trial[b]	4.74	7.75	3.69	2.34	1.71	1.28
			Temperature[c]			
Week of trial	73.4	83.3	72.0	65.7	83.4	75.8
Month of trial	72.6	78.7	74.5	71.4	78.1	76.9

[a] The week ending the last day of the feeding trial.
[b] The month ending the last day of the feeding trial.
[c] The mean temperature of each day determined by maximum temperature plus minimum temperature divided by two.

sidered, the dry matter content of the forage did not significantly affect the dry matter intake of the cows (Table 3). This is in agreement with the findings of Balch (1), that a factor or factors other than the degree of fill may be involved in limiting intake.

Each year the Sudan grass was grazed or cut at approximately the same stage of plant maturity (30-36 in. high, with a very few heads showing). The variations in dry matter content of the forage from year to year, therefore, can not be due to differences in plant maturity. The correlations between the per cent dry matter in the forage and total rainfall during the week, and between per cent dry matter and the average temperature of the month, ending the last day of the trial, are shown in Table 3. The correlations with rainfall were not statistically significant. The correlation with the average temperature during the last week approached significance, whereas that with the average temperature for the month was significant. The regression line and data scatter are shown in Figure 1A. The standard error of regression is 3.403 percentage units. In spite of the significant correlation, the fit of data to the regression line is not very good.

TABLE 3

Correlation coefficients relating various factors from the Sudan grass trials

		Rainfall		Temperature		Intake	
	Digestible dry matter	Week of trial	Month of trial	Week of trial	Month of trial	Green forage	Dry matter
Percentage dry matter in forage	−.573	−.241	+.322	.788	.865*	−.855*	.317
Digestible dry matter		.127	.304	−.468	−.635	.708	.152
Rainfall							
Week of trial[a]						.073	−.265
Month of trial[b]						.173	.985**
Temperature							
Week of trial						−.627	.141
Month of trial						−.718	.251
Green forage intake							.206

* Significant at 5% level.
** Significant at 1% level.
[a] The week ending the last day of the trial.
[b] The month ending the last day of the trial.

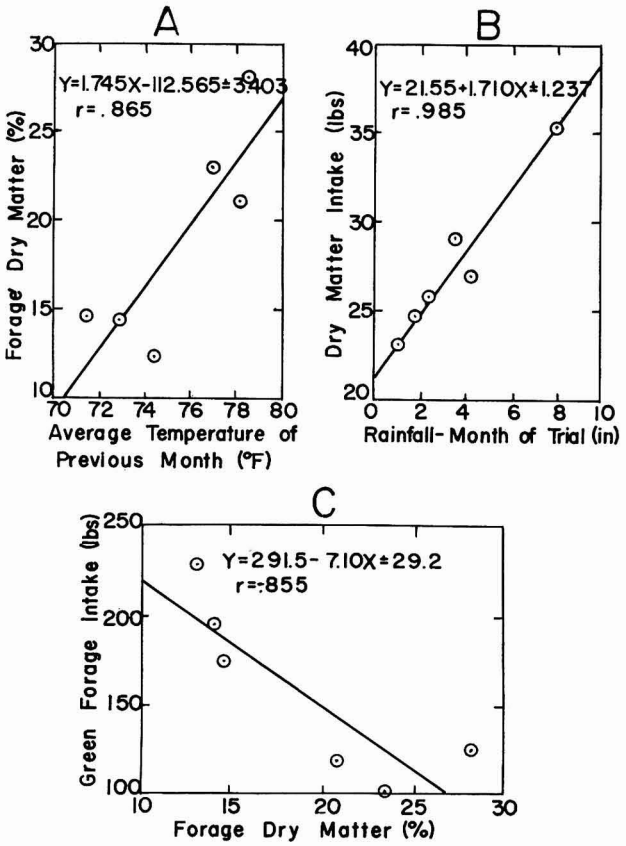

FIG. 1. Regression diagrams of factors in Sudan grass trials which had significant correlation coefficients.

The intake of green forage (using only data from hand-fed trials, so that the data from year to year are comparable) is also significantly correlated with the forage dry matter content (Table 3). The regression equation, regression line, and scatter of data are shown in Figure 1C. The intake in 1952 appears to be somewhat different and does not follow the same trend as that in other years. However, there appears to be no justification, based on other relationships, for discarding the 1952 data.

A somewhat surprising relationship was found between dry matter intake and the inches of rainfall during the month prior to the last day of the trial (correlation coefficient +.985), even though only a low nonsignificant correlation (−.265) was observed between current rainfall (the week ending the last day of the trial) and dry matter intake. The regression equation, regression line, and scatter of data are shown in Figure 1B. Even the 1952 data, which appear to be inconsistent with those of other years in regard to intake of green

forage, show this relationship with the rainfall of the previous month. Both rainfall and dry matter intake were unusually high in 1952 (Tables 1 and 2). Apparently, increasing the amount of rainfall has an effect upon plant composition, which renders the Sudan grass more acceptable to dairy cattle. This effect upon acceptability appears to be independent of dry matter content or dry matter digestibility.

Each year in which both hand feeding and grazing trials were conducted, the grazing cows consumed more forage than those hand-fed and they selected the more digestible plant parts, as indicated by higher dry matter digestion coefficients (Table 1). The average digestible dry matter intake by grazing cows was 5.1 lb. per day greater than those hand-fed. This amount of dry matter would supply nutrients for approximately 16 additional pounds of 4% fat-corrected milk (4% FCM) per cow per day (total production 37.6 lb.).

Some of the additional feed intake by the grazing cows will be required for maintenance and will not be available for milk production, because the energy expended by a cow while grazing is greater than that expended when fed cut forage. It is unlikely, however, that the increased requirement was as great as was the increased intake of digestible dry matter by the grazing cows in this study. Huffman (4) stated that grazing cows require 1 to 3 lb. more of TDN per day than do barn-fed cows. It appears to be safe to conclude that the grazing cows consumed significantly more nutrients, above maintenance requirements, than did the hand-fed groups.

The intake of digestible dry matter and the estimated intake of digestible protein, along with the theoretical quantities of 4% FCM which they would support, are shown in Table 4. The digestibility of crude protein was estimated at 70%. The crude protein content of the grazed forage was assumed to be the same as that of the cut forage. This is a conservative estimate, because the grazed forage most likely contained a higher level of crude protein.

TABLE 4

Digestible nutrient intake of cows fed Sudan grass and the amount of 4% fat-corrected milk it will theoretically support

Year	Digestible dry matter		Digestible crude protein[a]		Theoretical[c] 4% FCM[b]		Crude protein[d] adequate to balance:	
	H. F.[e]	G.[f]	H. F.	G.	H. F.	G.	H. F.	G.
1951	19.75		3.30		30.5		Ample	
1952	22.96		1.35		40.5		Very low	
1953	19.31	23.34	2.76	3.14	29.1	41.7	Ample	Ample
1954	17.32	23.24	3.32	4.21	22.9	41.4	Ample	Ample
1955	15.71	17.11	1.94	1.97	17.8	22.2	Ample	Yes
1957	15.29	24.54	1.83	2.67	16.5	45.4	Ample	Barley[g]

[a] Digestibility estimated at 70%.
[b] Four per cent fat-corrected milk.
[c] Assuming digestible dry matter to be similar to TDN; maintenance requirement (1,300 lb. body weight)—10 lb.; milk production—.32 lb. per pound of milk (5).
[d] Maintenance—.71 lb.; milk production—.049 lb. per pound milk (5).
[e] Hand-fed cows.
[f] Grazing cows.
[g] Meets the minimum of Morrison's range.

The crude protein content and intake were very variable from year to year (Tables 1 and 4). Gaessler and McCandlish (2) also mention wide variations in protein content. In 1951, and again in 1954, the crude protein content was high enough that no protein supplement would be required in the concentrate mix used. In 1952, the Sudan grass was quite inadequate in protein content. To balance this, a concentrate mix with a relatively high protein level would be required. In the three other years the digestible crude protein content balanced the digestible dry matter of the forage, but for high-producing cows a concentrate mixture containing about 16% crude protein would be required.

When the average intake of digestible dry matter by the hand-fed cows, over the 6 yr. of study, is used for comparison, the crude protein content required to meet the needs of the production of 4% FCM which the ingested nutrients could theoretically support would be about 10%. Only in 1952 was the forage content below this amount.

Although no direct measurement of the crude protein content of the forage consumed by the grazing cows was possible, it would be reasonable to assume that, due to selective grazing, the level would be above that in the cut forage. In general, then, the Sudan grass was adequate in crude protein content.

CONCLUSIONS

1. The intake of dry matter from Sudan grass pastures was not significantly correlated with the dry matter content of the pasture.

2. The average temperature immediately prior to a trial had a significant effect upon the dry matter content of the grass. Increased temperatures tended to decrease the dry matter digestibility; this, however, was not statistically significant.

3. There was a highly significant correlation between the rainfall of the previous month and the intake of dry matter.

4. Grazing cows consumed significantly more forage than did hand-fed stable-mates.

5. The forage consumed by grazing cows was more digestible than that consumed by hand-fed cows. This confirms previous reports (3) that cattle selectively graze.

6. The intake of nutrients from Sudan grass by grazing cows is quite variable, but is usually sufficient to support a level of milk production of about 35 lb. of 4% FCM without supplementation with concentrates.

7. The crude protein content was very variable, but in five of the six years the Sudan grass provided adequate protein for lactating cows.

ACKNOWLEDGMENTS

The assistance of the following in various phases of these trials is appreciated: D. L. MacFadden, R. J. Rifkin, J. D. Connolly, J. L. Fleeger, G. W. Waller, Jr., E. Shull, and W. Halsey.

REFERENCES

(1) BALCH, C. C. Observations on the Act of Eating in Cattle. Brit. J. Nutrition, 12: 330. 1958.

(2) GAESSLER, W. G., AND MCCANDLISH, A. C. Composition and Digestibility of Sudan Grass Hay. Iowa Agr. Expt. Sta., Bull. 46. 1918.
(3) HARDISON, W. A., REID, J. T., MARTIN, C. M., AND WOOLFOLK, P. G. Degree of Herbage Selection by Grazing Cattle. J. Dairy Sci., 37: 89. 1954.
(4) HUFFMAN, C. F. Summer Feeding of Dairy Cattle. A Review. J. Dairy Sci., 42: 1495. 1959.
(5) MORRISON, F. B. Feeds and Feeding. 22nd ed., p. 1042. The Morrison Publishing Co., Ithaca, N. Y. 1956.
(6) NEWLANDER, J. A. Digestibility of Artificially Dried Sudan Grass. Vermont Agr. Expt. Sta., Bull. 386. 1935.
(7) REID, J. T., WOOLFOLK, P. G., HARDISON, W. A., MARTIN, G. M., BRUNDAGE, A. L., AND KAUFMANN, R. W. A Procedure for Measuring the Digestibility of Pasture Forage Under Grazing Conditions. J. Nutrition, 46: 255. 1952.
(8) SNYDER, B. H. Feeds of the World. Their Digestibility and Composition. pp. 192, 268. Agricultural Experiment Station, West Virginia University. 1947.

JUDGING THE EFFECTIVENESS OF AGE-CORRECTION FACTORS[1]

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SUMMARY

Several criteria for comparing the effectiveness of age-correction factors are discussed. Their usage in comparing multiplicative, herd-level, and additive correction factors is considered in detail, based on analyzing 5,577 Holstein records which had been age-corrected by the three sets of factors. No analysis showed any difference between the herd-level and multiplicative factors.

Several methods of age-correcting dairy records are known and in use (1, 2, 7). The purpose of such factors is to correct a record for age alone, assuming that all other environmental factors remain the same. The corrected record is then an estimate of what the cow would have produced under exactly the same conditions had she been older. Consideration of the effectiveness of age-correction factors should, therefore, entail a comparison of the corrected records with those made by the same cow at a later age but under exactly the same environment. No absolute knowledge of this situation is ever available, because no young cow can suddenly become mature and produce again under the identical conditions of its early lactation. Other methods of judging the effectiveness of the correction factors must, therefore, be used. Most workers, when faced with the necessity of doing this, have made little use of analytical methods of evaluation, the need for which is important when any new set of age-correction factors is developed and one wishes to compare these new factors with those already in use. The problem of judging the effectiveness of a set of age-correction factors then becomes one of judging between two different sets of factors, and any analytical criteria which could assist in doing this would be most valuable. Some of the criteria that have been used from time to time are discussed in this paper, and others are considered.

Origin of the problem. The present authors have reported (9) a system of within-herd age-correction factors based on the level of herd production. These were developed from some 20,000 records made in 1950-52, and direct comparison was made of the herd-level factors with the New York multiplicative factors. This showed that on the average the two sets of factors were very much the same, indicating that the multiplicative factors take account of herd differences in age corrections. This comparison was for averages only and gave no indication of the effects on individual records, which would be reflected in the relative importance of different sources of variation among corrected records. Therefore, analyses were made to study the effects of the new age-correction factors when applied to a

Received for publication August 10, 1959.

[1] Some of the results in this paper are taken from a thesis submitted by the senior author in partial fulfillment of the requirements of the Ph.D. degree.

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set of records (other than those from which they had been developed) in comparison to the effects of additive and multiplicative factors applied to the same records. This paper discusses several criteria in terms of the results obtained from these analyses which, with one exception, were based on 5,577 Holstein records made in 688 New York herds in 1953.

In these analyses the butter fat yields were age-corrected by three different methods, using: (i) New York multiplicative factors, (ii) herd-level factors as in (9), (iii) additive factors. In (ii) and (iii), cows aged 5–9.5 yr. were taken as mature, and younger cows divided into four age groups: 19–27, 28–34, 35–44, and 45–59 mo. The age-correction factors for these groups are shown in Table 1 taken from (9). Analyses were also made on the actual butterfat records,

TABLE 1

Age-correction factors for cows younger than 5 yr.
Taken from Searle and Henderson (9)

Age group	Age-correction factors	
	Additive[a] herd-level factors	Absolute additive factors
(mo.)		
19–27	$8 + .24h$	113
28–34	$-19 + .25h$	90
35–44	$-18 + .19h$	65
45–59	$-8 + .07h$	23

[a] h is the age-corrected herd average.

simultaneously with the age-corrected records, hence giving results in terms of four variables, actual production, and production that had been age-corrected by multiplicative, herd-level, and additive factors.

Repeatability.

The effect of different age-correction factors on repeatability was studied in an analysis of a smaller amount of data than that mentioned above. This involved 1,431 Holstein records in 11 herds in 1950-54. Estimates of repeatability

TABLE 2

Repeatability estimates from 1,431 records in 11 herds in 1950–4

Actual records	Records age-corrected by 3 methods		
	Multiplicative	Herd-level	Additive
.54	.46	.45	.44

repeatability estimates shown in Table 2 are at first surprising, in that the were obtained by using Method I of Henderson (4) for estimating components of variance in the nested classification, records-within-cows-within-herds. The

highest value is for the uncorrected records, while for the age-corrected records repeatability is considerably lower. This situation is probably caused by the distribution of ages among the cows in the sample, because within the 5-yr. period of the study there will be some older cows having their last lactations during the first years of the study, and there will also be some young cows having their first lactations in the last years of the study. These young cows will, on average, have lower yields than the older cows having their last lactations in the first part of the 5-yr. period, so that in the uncorrected records these cow differences (which are really age differences) will tend to inflate the cow variance component. This means that repeatability, defined as $\frac{\sigma^2 \text{ cow}}{\sigma^2 \text{ cow} + \sigma^2 \text{ error}}$, will be inflated. In the corrected records these cow differences due to age have been removed, thereby reducing repeatability. It would seem, then, that a desirable criterion for good age-correction factors would be to make repeatability as small as possible! This is unreasonable and throws question on the whole concept of using repeatability as a criterion for assessing the value of age-correction factors. Repeatability is a measurement of the relationship of a cow's production from one year to the next. This is unconnected with the true purpose of age-correction factors, which is concerned with estimating what a young cow would have produced had she been mature, and not with what a young cow will produce when eventually she is mature. Thus, repeatability does not seem appropriate as a criterion for judging between different kinds of age-correction factors. Judging the effectiveness of age-correction factors by comparing age-corrected records made in one year, with mature records of the same cows made in another year, is invalid for the same reasons.

TABLE 3

Summary of data used for comparing different age-correction factors (5,577 Holstein records in 688 herds in the spring and fall of 1953)

Age group	No. of records	Mean record *(lb. fat)*			
		Actual records	Records age corrected by 3 methods		
			Multiplicative	Herd-level	Additive
(mo.)					
19– 27	626	326	430	437	439
28– 34	753	339	422	426	429
35– 44	1,088	365	427	428	430
45– 59	1,113	403	431	425	426
60–114	1,997	423	427	423	423

Age group means. Table 3 presents age group means used in subsequent analyses. The age group means are all very similar for the young cow age groups for each of the three sets of age-corrected records, there being a slight tendency for the multiplicative factors to give lower means in Age Groups 1, 2, and 3, and a higher mean in Age Group 4, than those given by the other factors. These tendencies are small and would need to be considered at each year of age before any conclusions could be drawn. The mature cow mean for the records corrected

by the multiplicative factors is a little higher than those corrected by the other factors, because the multiplicative factors include corrections for the cows older than 5 yr., whereas the others do not.

Coefficient of variation. Gowen (3) showed that over all ages, the coefficient of variation of dairy records is approximately constant. One of the advantages put forward for multiplicative age-correction factors is that records so corrected retain this constant coefficient of variation, whereas it is not retained when additive factors are used. To investigate this effect with the herd-level factors, which are additive on a within-herd basis, coefficients of variation were estimated for each age group, in each kind of a record, and are shown in Table 4.

TABLE 4
Coefficient of variation within age groups

Age group	Actual records	Records age-corrected by 3 methods		
		Multiplicative	Herd-level	Additive
(mo.)				
19–27	.20	.20	.17	.15
28–34	.21	.21	.20	.17
35–44	.22	.22	.21	.19
45–59	.23	.23	.23	.22

The records corrected by the multiplicative factors show the same coefficient of variation as do the actual records, as expected. There is a slight deviation from this in the first age group when the herd-level factors are used, while correcting by the additive factors does involve some departure from the coefficients found in the actual records. These figures might show greater differences if the coefficients of variation were estimated for each year of age rather than by the age groups of this analysis. Nevertheless, they indicate that in this respect the herd-level factors are not greatly different from the multiplicative ones.

The use of the coefficient of variation as a criterion for judging age-correction factors can be questioned. It is a useful adjunct for any set of age-correction factors, but if it is to be a criterion of judgment then the question "Is retaining the constant coefficient of variation one of the purposes of age-correction factors?" must be answered "Yes." If so, the same criterion should apply to other types of corrections, for example, those for environmental trends [*vide,* Henderson (5)], and this seems unreasonable.

Herd-by-age variance components. It has been pointed out (9) that a correction for age made to a young cow's record based on that record takes no direct account of any interaction that may exist between herd environment and the effect of age on production. Age corrections based on the level of herd production should, it would seem, take account of any such interaction. An analysis was made to investigate this effect by estimating the age-group by herd interaction variance component for the four sets of records. Components of variance were estimated by applying Henderson's Method I (4) to the two-way classification, herds by age group, as shown in Searle (8). The effects due to the five

TABLE 5
Variance component estimates when age effects are considered random

Variance component	Actual records	Records age corrected by 3 methods		
		Multiplicative	Herd-level	Additive
(mo.)				
Age	1,761	2	14	20
Herd–year–season	2,804	3,500	3,845	2,951
Interaction: Age × herd − Y − S	223	258	25	76
Error	4,460	5,194	4,460	4,460

age groups (four young cow groups and the mature cows) were assumed random, as were the herd effects. The results are shown in Table 5.

Two conclusions can be drawn from these results. First, that all three methods of age-correcting the records greatly reduce the variance component due to age; this is most desirable, and in some instances [Lush and Shrode (7)] it has been used as the main criterion for judging the value of age-correction factors. But it is not sufficient for, if it were, the easiest way to reduce the variance between ages would be to age-correct records so that all the corrected records were identical. The between-age variance would then be zero, but the resulting corrections would be valueless for the purpose for which they were really intended. Here again, the suggested criterion, while being a desirable adjunct for any set of age-correction factors, is not sufficient as a sole method of judging between sets of factors.

From Table 5 it is seen that the age-by-herd interaction variance component in the uncorrected records is perhaps smaller than might be expected, especially as the regressions in the original analysis of developing the herd-level factors were significantly different from zero. It is noticeable, too, that when the multiplicative factors were used this variance was larger; and its smallest value was when the herd-level factors were used. To a certain extent, this latter result is a natural consequence of the way in which the variance components have been estimated, using the same age groups as were used in developing the herd-level factors.

Expressed as percentages of the total variance in each case, the results indicate that the herd-level factors are little better than the multiplicative factors in reducing the herd-by-age interaction variance. One also notes that when the herd-level factors are used the variance component due to herd-year-seasons is larger than in the other age corrections. This is due to the manner of basing the age corrections on the herd level, thus increasing the difference between herds.

Exclusion of mature cows. The results shown in Table 5 include mature cows which, in the case of the herd-level and additive correction factors, have had no corrections made to their records. It was thought that if they were excluded from such an analysis a better comparison of the different factors might be obtained. The analysis was, therefore, repeated, excluding mature cows. The results are shown in Table 6, and are seen to be essentially the same as those in Table 5. The

TABLE 6
Variance component estimates when age effects are considered random
(Mature cows excluded)

Variance components	Actual records	Records age corrected by 3 methods		
		Multiplicative	Herd-level	Additive
Age	867	−13	−6	132
Herd–year–season	2,280	3,420	3,940	2,570
Interaction: Age × herd − Y − S	578	41	247	284
Error	3,813	5,018	3,813	3,813

error variances are smaller, as would be expected, but the herd-by-age-group variances show greater differences. The similarity between the multiplicatively corrected records and the herd-level-corrected records is still retained. That some of these variances component estimates are negative is perhaps a result of the small number of age groups, four, being used. Although differences in these variances have been noted, no tests of hypothesis are available to test the significance of such differences, because the distribution of these estimators is unknown (4).

A second estimate of these components was made, using the more reasonable assumption that the effects due to the age groups were fixed and not random; i.e., that these age groups are not a random sample of age groups from a large population, but that they are five predetermined age groups, which indeed they are. The herd and herd-by-age effects were still considered random. Henderson's Method III (4) analysis was used to estimate variance components from expressions which are free of the age effects. In this analysis there is no component due to age and that for error is estimated exactly as in Method 1, by the within-subclasses mean square. The only changes are in the herd and herd-by-age variances, and these results are shown in Table 7.

Here again the distribution of the estimators is unknown, but their similarity with those in Table 5 is some assurance that their sampling variances are probably not very large, thus strengthening the tentative conclusion that the herd-level factors take account of age-by-herd interactions only slightly better than do the multiplicative factors. If this is so, it means that the multiplicative factors as being used at present do take care of herd differences in age effects, and in stage of maturity as measured by age, and that these differences can be neglected when using the multiplicative age-correction factors.

TABLE 7
Herd and interaction variance components when age effects are considered fixed

Variance component	Actual records	Records age corrected by 3 methods		
		Multiplicative	Herd-level	Additive
Herd–year–season	2,953	3,581	3,848	2,971
Interaction: Age × herd − Y − S	75	157	22	57

Regressions on age. The criteria discussed above do not reveal important differences among the factors; despite this, one might expect age conversion factors based on herd-level to remove age-by-herd interactions and, if so, one would expect the individual within-herd regressions of production on age to vary little about their mean, for the records so corrected. Therefore, an analysis was made to consider the hypothesis, for the actual records and for the records corrected by the three different sets of factors. The within-herd-year-seasons (hereinafter referred to as within-herd) regressions of record on age in months were estimated for each set of records, and tests made by the usual analyses of variance procedures (6) for: 1. Significance of the mean regression for all herds. 2. Significance of the deviations of the individual within-herd regressions from their mean. The analyses are summarized in Table 8.

TABLE 8
Summary of analyses of variance for fitting within-herd regressions of record on age in months

	Actual records	Records age corrected by 3 methods		
		Multiplicative	Herd-level	Additive
Estimate of mean regression (weighted)	1.59	.19	.08	.06
Mean regression significantly different from zero? ($P \leqslant .05$)	Yes	Yes	No	No
Deviations from mean regression significant? ($P \leqslant .05$)	Yes	No	No	No
Standard error of individual regression estimates	5.2	6.1	5.2	5.1

The mean weighted regression is significantly different from zero for the actual records and for the multiplicatively corrected records, although this estimate is less than that for the actual records. With the herd-level and the additive factors the mean regressions do not differ significantly from zero. The deviations of the individual regressions from their mean are significant in the actual records, but not in the corrected records. This suggests that with each correction factor the within-herd regressions of production on age do not deviate greatly from their mean. This is perhaps unexpected in the additive factors, which are independent of a cow's own production and of her herd's production. Perhaps the reason that the within-herd regressions do not vary significantly about their mean is indicated by the magnitude of the standard error of these regression estimates, shown in the last line of Table 8. These values are at first surprising, for as estimates of standard errors of regression coefficients they are large; i.e., if b is an estimated regression, these standard errors are, for H herds,

$$\sqrt{\frac{b^2 - (\Sigma b)^2/H}{H-1}}$$

and the number of herds is high, namely 607. Tabulating some of the individual herd results indicated why these standard errors were so high. Some herds consisted of just two or three cows, and in such cases slopes of -20, $+17$, $+32$ . . .

appeared; this was particularly so where these small herds were mostly mature cows. Therefore, the analyses were repeated excluding mature cows, giving the results shown in Table 9.

TABLE 9

Summary of analysis of variance for fitting within-herd regressions of record on age in months (Mature cows excluded)

	Actual records	Records age corrected by 3 methods		
		Multiplicative	Herd-level	Additive
Estimate of mean regression (weighted)	3.05	.28	.01	.02
Mean regression significantly different from zero? ($P \leqslant .05$)	Yes	No	No	No
Deviations from mean regression significant? ($P \leqslant .05$)	Yes	No	No	Yes
Standard error of individual regression estimates	11.3	13.6	11.1	11.1

For all three age-correction factors the mean within-herd regression is not significantly different from zero, and the individual regressions do not differ significantly from their mean for the multiplicative and herd-level factors. In this case, however, the standard errors of the individual regression estimates are even larger than in the analysis which included mature cows. Again, small herds are probably the cause since, with the mature cows excluded, herds will be comprised of just young cows. At younger ages the gradient of the production-on-age curve is much steeper than with mature cows; consequently, some of the values of the regression slopes in the small herds are likely to be even higher than before. For example, in a herd of just two cows, one 20 mo. old producing 230 lb. of fat, and another 30 mo. old producing 430 lb. of fat, the estimated regression would be +20.

This criterion would perhaps be more sensitive if the age groups for the herd-level and additive factors had been on a 1-mo. basis, as they were for the multiplicative factors. The problem of small herds would still remain, however, and reliance would have to be put upon the analysis of variance procedure which takes account of these numbers, as in the first three lines of Tables 8 and 9. These show that even when mature cows are excluded, the mean regression is not significantly different from zero for any of the correction methods; and for the multiplicative and herd-level factors the deviations of the individual regressions from their mean are not significant. These results strengthen the conclusion that these two sets of factors do not differ and, hence, that the multiplicative factors take account of herd differences in the effect of age on production.

REFERENCES

(1) Beardsley, J. P. A Report of a Study of Age Conversion Factors to the Research Committee of the American Jersey Cattle Club. (Mimeo.) 1952.

(2) Castle, O. M. Memo on Age Correction in Sire Survey Work in New Zealand. (Mimeo.) 1953.

(3) GOWEN, J. W. Studies in Milk Secretion. V. On the Variations and Correlation of Milk Secretion with Age. Genetics, 5: 11. 1920.
(4) HENDERSON, C. R. Estimation of Variance and Covariance Components. Biometrics, 9: 226. 1953.
(5) HENDERSON, C. R. Estimation of Environmental Trends and Biases Resulting from Errors in Age Factors and Repeatability. J. Dairy Sci., 41: 741. 1958.
(6) KENDALL, M. G. The Advanced Theory of Statistics. Vol. II. Charles Griffin and Co., Ltd., London. 1948.
(7) LUSH, J. L., AND SHRODE, R. R. Changes in Milk Production with Age and Milking Frequency. J. Dairy Sci., 33: 338. 1950.
(8) SEARLE, S. R. Sampling Variances of Estimates of Components of Variance. Ann. Math. Stat., 29: 167. 1958.
(9) SEARLE, S. R., AND HENDERSON, C. R. Establishing Age-Correction Factors Related to the Level of Herd Production. J. Dairy Sci., 42: 824. 1959.

HERITABILITY, GENETIC AND PHENOTYPIC CORRELATIONS OF TYPE, CERTAIN COMPONENTS OF TYPE, AND PRODUCTION OF BROWN SWISS CATTLE[1]

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SUMMARY

The heritabilities of final type and eight components of type were estimated by doubling the intra-sire regression of daughter's single type score on dam's single type score from 3,161 daughter-dam comparisons by 294 sires. The heritability estimates were: General appearance .33, dairy character .30, body capacity .23, rump .36, feet and legs .19, mammary system .23, fore udder .28, rear udder .35, and final type .35.

The average within-sire phenotypic and genetic correlations between final type, all combinations of the components of type, and butterfat production were estimated from single type and butterfat records. Records on 3,161 daughter-dam pairs were used to estimate genetic correlations, and records on the 3,161 daughters were used to estimate phenotypic correlations.

The various genetic correlations between the components of type were positive and relatively large, indicating that positive selection can be made for these components of type simultaneously. The genetic correlation between final type and butterfat production was .24, indicating that selection on the basis of type alone should automatically bring about some genetic improvement in production.

Two major methods are used to predict the probable milk secretion of a dairy animal. These are the animal's conformation or physical appearance and her heredity as indicated by the ancestral records of milk secretion contained in her pedigree. Both methods are applicable to heifers which are too young to yield milk, but both methods are subject to great variability and are often disappointing to the breeders.

If specific physical characteristics were found with a high correlation to production, they could be used along with pedigrees as a basis for selection. Selection for production would then be more accurate than if pedigrees alone were used. The over-all type or conformation of an animal is given a great deal of emphasis in the show ring and the various herd classification programs. It is, therefore, desirable to determine if over-all type or some components of type are correlated closely enough with production to serve as such an indicator. It would be essential for such characteristics to have a high genetic correlation and to have heritabilities at least as high as the heritability for production. The progress made when selecting for two or more characteristics depends primarily on the heritabilities of these characteristics, the genetic correlation between them in the same individual, and the actual intensity of selection. This study was an attempt to estimate the heritability, genetic and phenotypic correlations of butterfat, type, and some of the components of type.

Received for publication December 14, 1959.

[1] Published with the approval of the Director of the Idaho Agricultural Experiment Station as Research Paper No. 480.

Hazel (8), by correlating the phenotypic expressions of one character in one animal with the phenotypic expression of another character in a closely related animal, obtained estimates of genetic correlations. Touchberry (11), using Hazel's method, found a genetic correlation of .71 between milk and butterfat production, which gives evidence that there are single genes affecting both characteristics. The correlation between transmitting ability for type and transmitting ability for production was estimated to be .18 by Harvey and Lush (7).

Freeman and Dunbar (3) found a phenotypic correlation of .08 between final type rating and butterfat production and a corresponding genetic correlation of −.52. They state that in selecting for butterfat production alone nothing can be gained by giving positive emphasis to final type score.

Gowen (5), in a study of Registry of Merit Jersey cows, found no correlation of practical significance between the body type of the sire or the dam and the production capacity of the daughters. Gowen (6) also found the average correlation coefficient between the daughters and sires on like body measurements to be .39 and on unlike body measurements .27. For the dams and daughters, the correlation was .45 on like body measurements and .30 on unlike body measurements. Although these correlations are not significantly different, they indicate a slight influence of the more common environment on the dams and daughters. Gowen (4) farther pointed out that there are some points of conformation that are valuable to the good judge for indicating milk yield. Some of these indications are general appearance, with a relationship of .88 to milk production, body shape .85, foreudder .73, udder levelness .66, rear udder .66, neck .49, udder size .36, thigh .30, and milk veins .23.

Copeland (1, 2) reported that 58% of the Jersey cows classified as Excellent had higher production than those classified Very Good. Sixty-three per cent of the Excellent cows had higher production records than Good cows. He found a correlation coefficient of .254 between type and milk production.

SOURCE OF DATA

Records of production and type classification from January 1, 1950, through December 31, 1953, were obtained from the Brown Swiss Breed Association. The production records were made either on Herd Improvement Registry or on Register of Production testing programs. All records of butterfat production were adjusted to a twice-a-day milking, mature-equivalent basis. If the actual record was for more than 305 days, but was recorded as a complete lactation for that cow, no adjustment for length was made. No record of less than 270 days' duration was used in this study.

Under the rules of the classification program a cow is placed into one of six grades: Excellent, very good, good plus, good, fair, and poor. For the analysis, the grades were given consecutive numerical scores beginning with one for a poor type cow through six for an excellent type cow. The cows were scored for over-all type and eight components of type on the basis of these grades. The components of type were: (1) general appearance, (2) dairy

character, (3) body capacity, (4) rump, (5) feet and legs, (6) mammary system, (7) fore udder, and (8) rear udder. Only daughter-dam pairs in which the sire of the daughter had at least five daughter-dam pairs with type and production records were used in this study. A total of 3161 daughter-dam pairs by 294 sires was used.

ANALYSIS OF DATA

Intra-sire phenotypic correlations between type and the various components of type and butterfat production were computed, to see which of these components were associated with butterfat production. The phenotypic correlations between the various components of type were next computed, to determine which of these constituents were phenotypically related. The intra-sire correlations between the ten variables in the dams and the ten variables in the daughters were next computed. Intra-sire correlations were used so as to avoid environmental trends that may have occurred over the period studied. From these data, heritability estimates and genetic correlations were determined.

Hazel (8) has suggested the use of close relatives in determining genetic correlations, to avoid the effects of the common environment on characteristics of the same animal. Touchberry (11) used a modification of Hazel's formula in reporting genetic correlations between the various characteristics of type in dairy cattle. The genetic correlations reported in this study were determined by means of Touchberry's formula. The correlations between the ten variables in the dams and the ten variables in the daughters, required when this formula is used, were computed on an intra-sire basis as described by Lush (9).

Using the appropriate correlations from Table 1 in this formula, estimates of the genetic correlations were made and these estimates are given in Table 4. As an example of the calculations, the genetic correlation between type and general appearance is derived by substituting the appropriate figures from Table 1 in the formula, thus:

$$\sqrt{\frac{(0.158)\ (0.147)}{(0.187)\ (0.175)}} = 0.841$$

TABLE 1

The intra-sire correlations between the ten characteristics on different but related (daughter-dam) animals

Dam		Daughter									
Characteristics	Symbol	A	B	C	D	E	F	G	H	I	J
Type	A	.187	.158	.129	.148	.132	.171	.150	.137	.128	.070
General appearance	B	.147	.175	.121	.110	.138	.113	.137	.124	.147	.070
Dairy character	C	.133	.210	.158	.140	.117	.081	.146	.115	.159	.129
Body capacity	D	.110	.175	.112	.124	.113	.101	.106	.090	.134	.038
Rump	E	.129	.130	.150	.139	.197	.059	.148	.098	.073	.014
Feet and legs	F	.088	.078	.090	.092	.101	.102	.095	.072	.081	.032
Mammary system	G	.149	.144	.130	.112	.142	.091	.122	.128	.159	.095
Fore udder	H	.146	.159	.130	.117	.130	.067	.120	.146	.115	.044
Rear udder	I	.137	.133	.144	.123	.140	.076	.118	.123	.179	.126
Butterfat	J	.018	.058	.023	.052	.071	.020	.024	.081	.099	.115

3,161 daughter-dam pairs by 294 sires.
Correlations $\geqq$ .062 are significant at the .05 level of probability.
Correlations $\geqq$ .081 are significant at the .01 level of probability.

RESULTS AND DISCUSSION

The intra-sire correlations and regressions of dam and daughter for the ten characteristics are shown in Table 1. These data were determined from 3,161 daughter-dam pairs by 294 sires.

Estimates of heritability for the ten characteristics can be made by doubling the ten correlations and regressions. The estimates of heritability and the 95% confidence limits are reported in Table 2.

TABLE 2

Heritabilities of type and the components of type with their respective 95% confidence limits of 3,161 daughter-dam pairs

Characteristic	Intra-sire correlation	Heritability	Intra-sire regression	Heritability	95% Confidence limits
Type	.187	.37	.176	.35	± .11
General appearance	.175	.35	.167	.33	± .13
Dairy character	.158	.31	.151	.30	± .16
Body capacity	.124	.24	.116	.23	± .15
Rump	.197	.39	.183	.36	± .16
Feet and legs	.102	.20	.095	.19	± .14
Mammary system	.122	.24	.116	.23	± .15
Fore udder	.146	.29	.141	.28	± .14
Rear udder	.179	.35	.175	.35	± .13
Butterfat	.115	.23	.142	.28	± .15

Heritability is often defined as the ratio of additive genetic variance to total variance. The estimates of heritability obtained from these data would include a fraction of the epistatic effects. However, since they are based on daughter-dam comparisons they would not be biased by dominance deviations. Similarly, the genetic correlations would include additive genetic variance, plus a small part of the epistatic variance and would be unaffected by dominance deviations.

The estimates of heritability for this study compared favorably with those reported by other workers for the characteristics that could be compared. It is difficult to compare heritability estimates, made of different samples, particularly when the samples are of different breeds, since a given breed may be either more homozygous or heterozygous than others for any given characteristic. The heritability of final type rating was estimated to be .31 by Freeman and Dunbar (3), .29 by Tabler and Touchberry (10), and .30 by Taylor (12), compared to .35 for this study. The slightly higher heritability estimate for this study may be the effects of artificial breeding. Since the data used were from cows that had production records and type scores from 1950 through 1953, some of the cows may have been sired artificially. Some sires may have had daughters in several different herds and the intra-sire analysis would not remove all of the herd contribution to the correlations and regressions for which heritability estimates were determined. Freeman and Dunbar (3) also estimated the heritability of feet and legs as .18, compared to .19 for this study. The heritability of butterfat was estimated as .28; most reports list it as .25 to .35. The other characteristics studied could not be compared as such.

Phenotypic correlations. The phenotypic correlations between butterfat production, final type, and the components of type were estimated to measure the degree of association of these traits in the same animal and are presented in Table 3. The phenotypic correlations between butterfat production and final

TABLE 3

Phenotypic correlations between the components of type, final type, and butterfat production

Characteristic	General appearance	Dairy character	Body capacity	Rump	Feet legs	Mammary system	Fore udder	Rear udder	Butterfat production
Final type	.794	.577	.560	.537	.330	.759	.638	.583	.259
General appearance		.464	.523	.616	.349	.584	.502	.460	.174
Dairy character			.321	.309	.174	.501	.388	.419	.319
Body capacity				.336	.245	.382	.358	.324	.174
Rump					.239	.366	.317	.527	.003
Feet and legs						.219	.202	.236	.088
Mammary system							.746	.667	.276
Fore udder								.435	.203
Rear udder									.233

Correlations $\geq$ .062 are significant at the .05 level of probability.
Correlations $\geq$ .081 are significant at the .01 level of probability.

type and all components of type, except rump, were significant at the .01 level of probability. However, all were of a relatively low order, the highest being .319 between dairy character and butterfat production and .276 between mammary system and butterfat production. This might be expected, since the high-producing cows would tend to carry less flesh, which would result in a higher score for dairy character. The higher-producing cows would also probably excel in some of the points considered under mammary system, such as udder size, shape, and quality. Relatively high phenotypic correlations existed between final type and most components of type. This was to be expected, since final type contains all the components and is an average of the component scores. The correlations between the various components were all significant at the .01 level of probability, but were quite variable, ranging from .174 between dairy character and feet and legs to .746 between mammary system and fore udder, and .667 between mammary system and rear udder. The correlation between mammary system and fore and rear udder would be expected to be high, since the type score for mammary system would be greatly influenced by the conformation of both the fore and rear udder. On the other hand, the type scores for dairy character and feet and legs would be independent of each other; therefore, the lower correlation between these characteristics is not unusual.

The phenotypic correlation of .259 between final type and butterfat production compared favorably with the .254 reported by Copeland (2) and the .26 by Touchberry (11).

General appearance and mammary system had the highest phenotypic correlation to final type of any of the components. This would be expected, since these two characteristics, more than any of the others, influence the final type grade given an animal.

TABLE 4
Genetic correlations between the components of type, final type, and butterfat production

Characteristic	General appearance	Dairy character	Body capacity	Rump	Feet legs	Mammary system	Fore udder	Rear udder	Butterfat production
Final type	.841	.762	.839	.680	.890	.987	.853	.723	.242
General appearance		.957	.942	.722	.704	.961	.819	.554	.450
Dairy character			.895	.752	.675	.991	.806	.897	.410
Body capacity				.804	.858	.885	.763	.864	.377
Rump					.544	.934	.667	.540	.210
Feet and legs						.831	.571	.578	.237
Mammary system							.901	.925	.410
Fore udder								.736	.461
Rear udder									.480

Genetic correlations. Genetic correlations were estimated from these data by the formula described by Touchberry (11) and are reported in Table 4. The genetic correlations were generally higher than the phenotypic correlations. The genetic correlations for the components of type that could be compared were very similar to those reported by other workers. Freeman and Dunbar (3) reported genetic correlations of .65 between rump and final type, .55 between feet and legs and final type, and 1.07 between udder size and shape and final type, as compared to genetic correlations of .68, .89, and .98, respectively, for this study. The genetic correlations between final type and butterfat production and the components of type and butterfat production were all positive for this study. This differs from the report by Freeman and Dunbar (3), in which they found that most of the characteristics studied had rather large negative genetic correlations with butterfat production. Touchberry (11) found some characteristics to be positive and some negative with butterfat production. He reported genetic correlations of .235 between body weight and butterfat production and .498 between paunch girth and butterfat production. The genetic correlation of .377 between body capacity and butterfat production for this study compares favorably with his results. Since both of these studies reported on different components of type and on different breeds of cattle from each other and from this study, it is difficult to make comparisons.

The estimate of the genetic correlation between final type and butterfat production obtained from these data is .24. This is higher than the −.52 obtained by Freeman and Dunbar(3) from 729 Ayrshire daughter-dam pairs, and the zero obtained by Touchberry (11) from 187 Holstein daughter-dam pairs. However, it compares very favorably with the estimate of .18 obtained by Harvey and Lush (7) from the larger sample of 2,786 Jersey daughter-dam pairs. The slightly higher genetic correlation of .24, compared to .18 obtained by Harvey and Lush, may be partly due to the effects of artificial insemination, whereby sires would have daughters in several herds and the intra-sire analysis would not remove all of the herd contributions to the correlations. There may also be an actual breed difference which could account for part of the difference in results.

The positive genetic correlation found between type and butterfat production in this study gives evidence that there are single genes affecting both type

and butterfat production. The breeder by selecting for type would automatically be selecting for butterfat production, but with less pressure. However, selection on type alone would require approximately four generations to obtain the genetic improvement in butterfat production that could be expected in one generation of selection on butterfat production alone.

REFERENCES

(1) COPELAND, LYNN. The Old Story of Type and Production. J. Dairy Sci., 21: 295. 1938.
(2) COPELAND, LYNN. The Relationship Between Type and Production. J. Dairy Sci., 24: 297. 1941.
(3) FREEMAN, A. E., AND DUNBAR, R. S. Genetic Analysis of the Components of Type Conformation and Production in Ayrshire Cows. J. Dairy Sci., 38: 428. 1955.
(4) GOWEN, J. W. Conformation and Milk Yield in the Light of the Personal Equation of the Dairy Cattle Judge. Maine Agr. Expt. Sta., Bull. 314. 1923.
(5) GOWEN, J. W. The Conformation of the Parent as Related to the Milk Secretion of the Daughters. J. Agr. Sci., 23: 514. 1933.
(6) GOWEN, J. W. On the Genetic Constitution of Jersey Cattle, as Influenced by Inheritance and Environment. J. Genetics, 18: 415. 1938.
(7) HARVEY, W. R., AND LUSH, J. L. Genetic Correlations Between Type and Production in Jersey Cattle. J. Dairy Sci., 35: 199. 1952.
(8) HAZEL, L. N. The Genetic Basis for Constructing Selection Indexes. Genetics, 28: 476. 1943.
(9) LUSH, J. L. Intra-sire Correlations or Regressions of Offspring on Dam as a Method of Estimating Heritability of Characteristics. Proc. Am. Soc. Animal Production, 33: 293. 1940.
(10) TABLER, K. A., AND TOUCHBERRY, R. W. Estimating the Heritability of Type Classification of Dairy Cattle. J. Dairy Sci., 39: 1550. 1956.
(11) TOUCHBERRY, R. W. Genetic Correlations Between Five Body Measurements, Weight, Type, and Production in the Same Individual Among Holstein Cows. J. Dairy Sci., 34: 242. 1951.
(12) TYLER, W. J. The Heritability of Official Type Ratings and the Correlation Between Type Ratings and Butterfat Production of Ayrshire Cows. J. Dairy Sci., 31: 63. 1948.

GENETIC IMPROVEMENT IN PRODUCTION ATTRIBUTABLE TO SIRES USED IN ARTIFICIAL INSEMINATION IN NORTH CAROLINA[1]

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SUMMARY

The genetic advantage attributable to the sires used in artificial insemination in North Carolina was evaluated. The effect of season of calving on production was pronounced. Cows freshening during the months of December through May produced 21 lb. of fat and 595 lb. of milk more than cows freshening in June through November. The performance of artificially sired animals was evaluated by using the first-lactation contemporary comparison, and also by using all lactations of the naturally sired progeny as contemporaries. The 305-day, 2 ×, M.E. lactations of the artificially sired progeny were 15.7 lb. of fat and 366 lb. of milk above those of the naturally sired progeny. The use of the records of all contemporaries imposed a negative bias on the comparisons, presumably due to the selection of the older animals on the basis of previous performance.

Comparisons of the production of artificially sired daughters and their dams in North Carolina and throughout the United States generally have shown artificially sired daughters to be superior. However, the value of daughter-dam comparisons for assessing the impact of artificial insemination is questionable, because environmental conditions may differ markedly for the daughters and dams. Studies to date (9, 11) suggest that the first-lactation contemporary comparison offers much promise as a method for evaluating the effects of artificial insemination. In most cases where this method is employed, the contemporaries being compared have been contemporary since calfhood. The production differences of these two groups should be as free of environmental influences as it is reasonably possible to make them.

This study (12) was concerned with the evaluation of the genetic improvement in production attributable to sires used in artificial insemination in North Carolina, and with an examination of the magnitude of the bias introduced when other methods of evaluation are used.

EXPERIMENTAL PROCEDURE

Data. All available North Carolina DHIA and HIR records initiated between January, 1948, and January, 1955, were collected for herds using the statewide artificial insemination program initiated in 1948. Only normal lactation records of from 150 to 305 days duration were used; hence, no correction was made for lactations which were short of the usual 305 days. All records were converted to a 2 ×, mature equivalent basis. A total of 6,888 usable records for the Guernsey, Holstein, and Jersey breeds was available.

Received for publication March 19, 1960.

[1] Contribution from the North Carolina Agricultural Experiment Station and published with the approval of the Director of Research as Paper No. 1136 of the Journal Series.

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Methods. Previous studies (1-3, 5-8) have indicated that the effects of season of freshening on the animal's production should be removed to provide reliable comparisons between the production of different animals. In this study, the year was divided on the basis of high and low consecutive 6-mo. groupings in an attempt to eliminate as much of the seasonal influence on production as possible.

The first-lactation contemporary herd-mate comparison (11) within seasons was used to evaluate the influence of artificial insemination. Contemporary herd-mates are those cows which begin lactations in the same herd, year, and season as the artificially sired animals. The first record available on an individual was considered to be her first lactation, provided it was initiated before the animal was 35 mo. of age. Since the numbers of artificially sired and naturally sired progenies within each herd comparison varied, each comparison was weighted in accordance with the number of individuals in it. The following procedure was used to evaluate the various contemporary comparisons:

Y_{1i} = mean performance of artificially sired daughters in the i^{th} contemporary group

Y_{2i} = mean performance of naturally sired daughters in the i^{th} contemporary group

$d_i = Y_{1i} - Y_{2i}$

a_{1i} = number of individuals in Y_{1i}

a_{2i} = number of individuals in Y_{2i}

$w_i = \dfrac{a_{1i} \cdot a_{2i}}{a_{1i} + a_{2i}}$ = the weighting factor for each contemporary group

N = number of contemporary comparisons

$D = \dfrac{\Sigma\, w_i d_i}{\Sigma\, w_i} = \dfrac{WD}{W}$ = the mean weighted difference and as indicated $W = \Sigma w_i$

$\sigma^2_{d_i} = \dfrac{\sigma^2(a_1 + a_2)}{a_1\, a_2} = \dfrac{\sigma^2}{w_i}$

where σ^2 is the variance of individual lactation records

Therefore, the variance of

$$\frac{w_i\, d_i}{W} = \frac{w_i^2}{W^2} \cdot \frac{\sigma^2}{w_i} = \frac{w_i}{W^2}\, \sigma^2.$$

Then the variance of

$$D = \frac{W\, \sigma^2}{W^2} = \frac{\sigma^2}{W}$$

An estimate (s^2) of the variance of individual records can be obtained as follows:

$$s^2 = \frac{N}{(N-1)\, W} \left[\Sigma (w_i d_i)^2 - \frac{(WD)^2}{N} \right]$$

Thus, the estimated variance of D is

$$\frac{s^2}{W} = \frac{N}{(N-1)\, W^2} \left[\Sigma (w_i d_i)^2 - \frac{(WD)^2}{N} \right]$$

Differences in milk production, fat production, and fat test were determined, and the significance of the weighted differences were tested by the "t" test.

More contemporary comparisons can be made if the records of all contemporary naturally sired animals are used to compare with the first lactations of the artificially sired ones. However, this would mean that the artificially sired animals, presumed to be unselected, would be compared with naturally sired animals, part of which had survived culling on the basis of their past performance. A comparison was made in this way to examine the effect which culling might have had on the results obtained from these data.

RESULTS AND DISCUSSION

As is shown in Table 1, the average lactation production of animals of all of the three breeds, Guernsey, Jersey, and Holstein, varies depending on the month of calving. Production for milk and fat was highest for cows freshening in April and May and lowest for cows freshening in July and August.

TABLE 1

Average 305-day, 2 ×, M.E. production of Guernsey, Holstein, and Jersey cows according to month of calving[a]

Month of calving	No. of records	Av. milk yield	Av. fat yield
January	500	10,106	404
February	438	9,817	398
March	343	9,789	401
April	273	10,026	414
May	317	10,220	415
June	382	9,865	386
July	524	9,596	379
August	834	9,500	375
September	920	9,715	388
October	892	9,802	393
November	778	9,992	401
December	687	9,996	400
Total	6,888	9,833	394

[a] These averages include records on 1,304 Guernsey, 4,056 Holstein, and 1,528 Jersey lactations.

The breakdown which appeared to remove most of the seasonal differences, when compared on the basis of all possible consecutive 6-mo. intervals, was the interval of June through November, as compared with December through May. There was an average difference of 595 lb. of milk and 21 lb. of fat between the high and low consecutive 6-mo. intervals. Climatic and nutritional factors are probably responsible for most of the observed seasonal differences. However, a tendency to freshen a larger proportion of heifers in the fall months could be responsible for some genetic differences among the cows freshening in each season. Seasonal effects can be expected to vary for different populations, depending on the location of the herds and the feeding and management practices followed. Hence, the relationships found in these data can not be taken to represent a general pattern for the United States.

TABLE 2

Differences between artificially and naturally sired contemporary first-lactation herd-mates

Breed	Comparisons	No. natural progeny	No. artificial progeny	Mean weighted differences[a]		
				Milk	Per cent	Fat
Jersey	36	96	52	396 ± 221	−0.06 ± 0.09	14.7 ± 11.8
Holstein	103	249	179	355 ± 217	0.04 ± 0.03	17.8 ± 7.8
Guernsey	28	76	39	371 ± 298	−0.11 ± 0.09	8.8 ± 15.2
Combined	167	421	270	366 ± 152	0.00 ± 0.03	15.7 ± 6.0

[a] Positive differences are in favor of the artificially sired animals.

The results of the contemporary comparison computed for artificially and naturally sired first-lactation herd-mates are shown in Table 2 for milk, fat, and test. The animals resulting from artificial insemination appear to have been genetically superior, as was indicated by an increase in production of 366 lb. of milk and 15.7 lb. of fat per lactation over that of their naturally sired contemporaries. There was no over-all change in the fat test of the milk produced, although test has declined slightly for the Jerseys and Guernseys and increased for Holsteins.

A contemporary comparison of the dams of the artificially and naturally sired progenies gave a weighted difference of 149 lb. of milk and 11.7 lb. of fat in favor of the naturally sired dams. These differences, however, were not statistically significant. The superiority of the artificially sired progenies is presumably due to the genetic superiority of their sires.

The results of the comparison of the first lactations of artificially sired animals used in the original study and of all the available contemporary records on their naturally sired herd-mates, are shown in Table 3 for milk, fat, and test

TABLE 3

Comparisons of first lactations of artificially sired animals and all available contemporary records of naturally sired herd-mates where at least one first-lactation contemporary animal is in each comparison

Breed	No. of Comparisons	No. natural progeny	No. artificial progeny	Mean weighted differences[a]		
				Milk	Per cent	Fat
Jersey	36	270	52	179 ± 136	−.10 ± .08	0.5 ± 11.1
Holstein	103	720	179	322 ± 194	.07 ± .03	18.9 ± 7.0
Guernsey	28	325	39	191 ± 248	−.01 ± .09	6.4 ± 11.8
Combined	167	1,315	270	276 ± 136	.03 ± .03	13.3 ± 5.3

[a] Positive differences are in favor of the artificially sired animals.

differences. This comparison would be representative of the situation where a young sire is evaluated for continued use when only first-lactation records are available on his progeny. These results indicate that there is a significant difference in favor of the artificially sired progeny of 13.3 lb. of fat and 276 lb. of milk, there being no apparent change in fat test. These differences are 90 lb. less milk and 2.4 lb. less fat than those obtained by the first-lactation contemporary comparison. The addition of second and later lactation records of naturally sired progeny reduced the apparent superiority of the first-lactation

artificially sired progeny, presumably because the records of the older animals in the natural group were selected on the basis of past performance.

The importance of the selection bias, imposed by using second and later lactation records of naturally sired progeny, becomes even more evident when all possible contemporary comparisons are made using the first lactations of artificially sired animals with all available contemporary records on their naturally sired herd-mates. First lactations of artificially sired animals that had no contemporary herd-mates with first lactations are available for comparisons with this procedure. Over twice as many comparisons can be made with this procedure, as can be seen from Table 4, but the apparent bias increased from 90 to 120 lb. for milk and from 2.4 to 3.1 lb. for fat. The increased difference in fat test resulted because the Holsteins represented a larger proportion of the additional cows included in the comparisons than they did in the previous comparisons.

TABLE 4

Comparisons of first lactations of artificially sired animals and all available contemporary records of naturally sired herd-mates, where comparisons which do not include first-lactation contemporary animals have been added

Breed	No. of Comparisons	No. natural progeny	No. artificial progeny	Mean weighted differences[a]		
				Milk	Per cent	Fat
Jersey	51	362	82	233 ± 167	0.02 ± 0.06	12.1 ± 8.9
Holstein	253	1,483	451	121 ± 122	0.09 ± 0.02	13.9 ± 4.4
Guernsey	52	445	76	191 ± 177	−0.02 ± 0.06	6.0 ± 8.4
Combined	356	2,290	609	146 ± 112	0.06 ± 0.02	12.6 ± 4.2

[a] Positive differences are in favor of the artificially sired animals.

The difference in the results for the comparisons given in Tables 3 and 4 is due to the absence of first lactation naturally sired contemporary herd-mates in 189 of the comparisons presented in Table 4. In these cases, the unselected artificially sired animals are compared with naturally sired animals, all of which have been selected to remain in the herd on the basis of one or more previous records. An analysis of data from North Carolina Institutional Herds also revealed a significant selection bias, which varied in magnitude from herd to herd. This same type of bias, and possibly even more pronounced, would be encountered in making a contemporary comparison on a sire with progeny in a single herd. Most of the contemporary animals not by that sire would be in their second or later lactations. Under these conditions, a sire whose daughters even equal their contemporaries actually may be a desirable sire.

The superiority of the artificially sired progeny in this study exceeded that reported in previous studies (4, 10, 11, 13). Robertson and Rendel (11) did not find any significant difference in milk yield, although the differences in favor of the artificially sired progeny for test were significant. Hahn *et al.* (4) did not find any appreciable difference between the artificially and naturally sired animals, although many of the same sires whose daughters were included as artificially sired daughters in their data were included in this study also. The New Zealand Dairy Board (10) has reported that in compari-

sons of 1,127 artificially sired progeny with mature cows after age correction, their average superiority was 19 lb. fat. However, 549 of these were by only two sires with an average superiority of 30 lb.

The results reported have not generally reflected the anticipated increase in production from the use of artificial insemination that was predicted initially. If this program is to become of real value in herd improvement, more effective methods of proving and selecting sires must be put into practice.

REFERENCES

(1) Arnold, P. T. D., and Becker, R. B. The Effect of Season of the Year and Advancing Lactation upon Milk Yield of Jersey Cows. J. Dairy Sci., 18: 621. 1935.

(2) Cannon, C. Y. Seasonal Effect on Yield of Dairy Cows. J. Dairy Sci., 16: 11. 1933.

(3) Frick, G. E., Mann, A. I., and Johnson, S. The Relation of Season of Freshening to Milk Production. J. Dairy Sci., 30: 631. 1947.

(4) Hahn, E. W., Carmon, J. L., and Miller, W. J. An Intra-herd Contemporary Comparison of the Production of Artificially and Naturally Sired Dairy Cows in Georgia. J. Dairy Sci., 41: 1061. 1958.

(5) Hammond, J., and Sanders, H. G. Some Factors Affecting Milk Yield. J. Agr. Sci., 13: 74. 1923.

(6) Henderson, C. R. Selecting and Sampling Young Bulls. Proc. 7th Ann. Conv. Nat'l Assoc. Artificial Breeders. p. 93. 1954.

(7) Hofmeyr, Jan. A Study of Danish and Swedish Progeny Testing Methods for Dairy Bulls. Kgl. Lantbruks-Hogskol. Ann., 22: 425. 1955.

(8) Lewis, C., Johnston, J. E., Branton, C., and Stone, E. J. Influence of Season of Freshening on Milk Production Records of Jersey and Holstein Cows. (Abstr.) Proc. Assoc. Southern Agr. Workers, 53: 124. 1956.

(9) Mason, I. L., and Robertson, A. The Progeny Testing of Dairy Bulls at Different Levels of Production. J. Agr. Sci., 47: 367. 1956.

(10) New Zealand Dairy Board. Ann. Rept. 1951, 1952.

(11) Robertson, A.,and Rendel, J. M. The Performance of Heifers Got by Artificial Insemination. J. Agr. Sci., 44: 184. 1954.

(12) Tucker, W. L., and Farthing, B. R. Genetic Improvement of Dairy Cattle Attributable to Sires Used in Artificial Insemination in North Carolina. J. Dairy Sci., 41: 740. 1958.

(13) Wadell, L. H., and McGilliard, L. E. Influence of Artificial Breeding on Production in Michigan Dairy Herds. J. Dairy Sci., 42: 1079. 1959.

COMPARISON OF A PROTEIN SUPPLEMENT AND SHELLED CORN FOR DAIRY COWS ON GOOD PASTURE

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SUMMARY

Ground shelled corn and a simple grain mixture containing 20% total crude protein were compared as supplements for cows grazing good-quality pasturage during three seasons.

Daily FCM averaged 31.3 and 30.3 lb. for the corn and for the 20% protein groups, respectively. This difference was not statistically significant. Mean weekly persistency of production was 97.3% for the cows fed corn and 98.1% for the cows supplemented with the 20% protein mixture.

The average daily gain for the cows fed corn was 0.37 lb. compared with 0.12 lb. for the protein-supplemented cows.

Average daily concentrate consumption was slightly higher for the 20% mixture than for the ground corn. The intake of both supplements was higher during the first part of the grazing season than during the later part of the season.

Proper supplementation of the lactating animal on pasture is difficult because the quantity and quality of the herbage available for grazing are constantly changing. Periodic chemical analysis of plucked or clipped herbage samples does not provide accurate information as to the adequacy of the pasture for meeting the animal's needs, since experiments (4, 7) have shown that grazing cattle select herbage higher in crude protein, lower in crude fiber, and higher in energy than the whole plant which is available to the animal.

In grazing trials with first-growth forages, Reid and coworkers (12) found that a deficiency of energy occurred earlier in the season and was more critical for growing cattle than a deficiency of protein. With aftermath herbages, the intake of digestible protein was always in access of the minimum allowances set forth by Morrison (8); however, in several instances the energy intake of the animals was borderline or below the recommended allowances. These experiments indicate that energy is the first limiting factor in the diet of the grazing animal.

From a theoretical study, Crampton (1) concluded that energy is the primary limiting factor in the nutritive value of forage and that the chances of encountering pasture herbage which, relative to energy, is grossly deficient in protein is somewhat remote.

Investigations (3, 5, 6, 9, 10, 11) with dairy cattle in which grain mixtures of high and low protein content have been fed as supplements to pasture, support the hypothesis that protein is usually not the nutrient limiting production of the grazing animal. Further evidence is provided by the results of balance trials (2), in which it was shown that grass alone may provide two or more

Received for publication September 8, 1959.

times the amount of protein required by an 1,100-lb. cow producing 30 lb. of 4% milk daily.

It was the object of the experiments reported here to compare ground shelled corn and a simple grain mixture containing approximately 20% total crude protein as supplements for dairy cows on good pasture.

EXPERIMENTAL PROCEDURES

Twenty-six Holstein cows were used in three trials carried out during the 1955, 1956, and 1957 pasture seasons. Since two treatments were compared during each season, the cows were selected in pairs with the members of each pair being as nearly alike with respect to milk production, body weight, age, and stage of lactation as possible. Eight cows were used during the 1955 and 1956 trials, and ten were used in 1957.

Each trial consisted of a 28-day standardizing period and a comparison period of varying lengths (1955—16 wk.; 1956—24 wk.; 1957—21 wk.). During the standardization period in 1955, the cows were grazed rotationally among several grass-legume pastures. In 1956 and 1957 the cows were full-fed alfalfa–orchardgrass hay and silage. During each standardization period all cows were individually fed a grain mixture averaging 20% total crude protein and composed of 641 lb. of ground shelled corn and 359 lb. of cottonseed meal (41% total crude protein). The feeding rate was 1 lb. of grain to each 8 lb. of milk produced in 1955 and 1:4 and 1:6 in 1956 and 1957, respectively.

Immediately after the standardization period, one cow of each pair was shifted to the ground corn supplement. The other member of each pair remained on the 20% protein mixture (control group). The rate of supplement feeding during the comparison period was based on milk production during the standardization period and the amount fed remained constant throughout the period. One pound of supplement was fed to each 8 lb. of milk produced daily in 1955 and 1956, and 1 lb. of supplement to each 8 lb. of 4% fat-corrected milk (FCM) in 1957. The amounts fed and refused were recorded daily.

Each year the cows were grazed rotationally among the following mixtures: orchardgrass–Ladino clover and orchardgrass–Kentucky bluegrass–bird's-foot trefoil–white clover. Alfalfa–orchardgrass, as well as millet and Sudan, were used for supplementary grazing. The quality and quantity of forage grazed were judged as excellent on the basis of visual observations.

The cows were milked twice daily. The milk weights were recorded and one-day composite samples were obtained monthly for fat test by the standard Babcock procedure.

The cows were weighed for two consecutive days at the beginning and end of each comparison period. In addition, weights were taken once weekly throughout the 1955 and 1957 comparison periods and once every 28 days in 1956.

EXPERIMENTAL RESULTS

The average daily production of FCM by weeks for the 3 yr. is presented (Table 1). The average daily milk production of the corn group was 1.4, 3.0,

TABLE 1

Average daily production of Fat-Corrected-Milk (by weeks)

Expt. week	1955			1956			1957		
	Control group	Corn group		Control group	Corn group		Control group	Corn group	
	(lb.)	*(lb.)*	*% (control)*	*(lb.)*	*(lb.)*	*% (control)*	*(lb.)*	*(lb.)*	*% (control)*
−1[a]	34.4	37.6	109.3	39.7	39.8	100.2	33.5	33.3	99.4
1	35.4	35.6	100.6	44.2	51.6	116.7	38.6	40.0	103.6
2	31.2	31.0	99.4	45.4	53.3	117.4	40.3	45.9	113.9
3	33.8	36.0	106.5	44.6	49.0	109.9	37.8	43.4	114.8
4	30.7	34.0	110.7	44.5	44.9	100.9	35.8	39.4	110.0
5	30.7	33.6	109.4	42.0	47.2	112.4	33.1	35.4	106.9
6	30.4	33.1	108.9	42.8	43.6	101.9	34.3	33.6	98.0
7	30.3	31.3	103.3	39.3	43.8	111.4	31.4	32.1	102.2
8	29.0	29.8	102.8	39.1	42.1	107.7	25.5	26.7	104.7
9	29.9	31.1	104.0	38.3	41.4	108.1	25.7	26.7	103.9
10	27.6	28.0	101.4	34.9	37.0	106.0	24.5	25.0	102.0
11	30.0	29.0	96.7	30.8	33.2	107.8	23.6	24.5	103.8
12	29.5	28.0	94.9	31.9	32.6	102.2	25.0	25.5	102.0
13	24.6	24.7	100.4	34.4	37.0	107.6	23.2	23.6	101.7
14	25.0	23.1	92.4	34.9	37.1	106.3	24.8	27.0	108.9
15	20.9	19.5	93.3	31.7	32.2	101.6	25.5	27.5	107.8
16	22.1	21.8	98.6	32.2	34.4	106.8	21.6	21.4	99.1
17				29.8	30.7	103.0	26.2	26.4	100.8
18				28.0	29.0	103.6	21.7	19.7	90.8
19				28.9	29.2	101.0	19.3	19.7	102.1
20				27.5	27.0	98.2	21.9	21.1	96.3
21				25.5	23.4	91.8	22.2	16.4	73.9
22				25.7	22.0	85.6			
23				22.3	18.5	83.0			
24				24.3	19.6	80.6			
Av.	28.8	29.4	101.4	34.3	35.8	103.0	27.7	28.6	102.2

[a] Average milk production for the last week of the pre-experimental feeding period.

and 2.2% higher than that of the control group for 1955, 1956, and 1957, respectively. When treated statistically, these differences were found to be non-significant.

During the last week of the standardization period, the cows assigned to the shelled corn gave on the average 3.3% more milk than the control cows. However, the control group produced an average of 18.6% more milk during the last week of the comparison period. The cows fed corn produced more milk for 10 of the 16 wk. in 1955, 19 of the 24 wk. in 1956, and 16 of the 21 wk. in 1957, than the control group. The mean weekly persistency of milk production for the control group was 97.5, 98.2, and 98.6%, as compared to 97.0, 97.5, and 97.5% for the corn group, for the years 1955, 1956, and 1957, respectively.

The cows fed corn made an average daily gain for the three trials of 0.37 lb. compared with 0.12 lb. for the protein-supplemented cows. This difference was not statistically significant.

The average amounts of supplement refused by the two groups of cows are shown (Figure 1). Although the amount of supplement consumed was very similar for the two groups, the interesting thing is that consumption declined with advancing season. It should be pointed out, however, that only 1 yr.'s data are available for the later part of the grazing season.

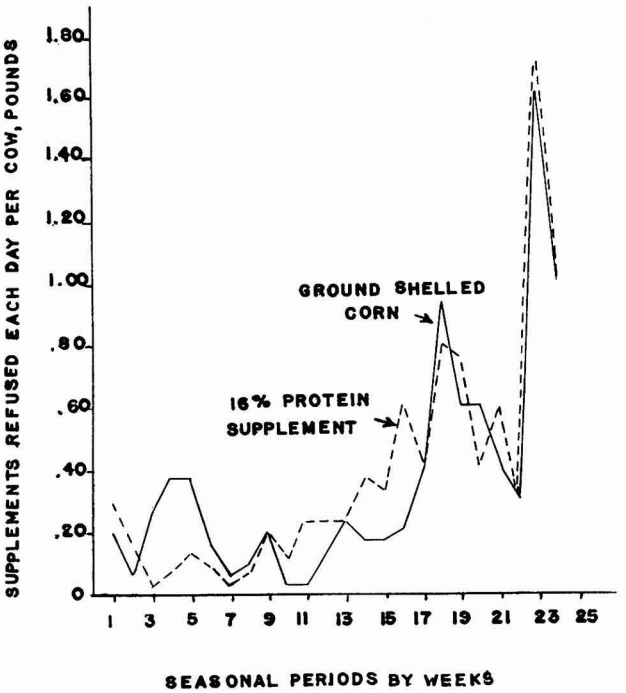

FIG. 1. Pounds of feed supplements refused daily per cow: first to 17th wk., average of 3 yr.; 17th to 21st wk., average of 1956 and 1957; 21st through 24th wk., 1957.

DISCUSSION

The results of this study are in general agreement with those of similar studies (3, 5, 6, 9, 10, 11). Published results show that when cows have access to good-quality legume-grass pasturage a concentrate mixture containing about 10% total crude protein will support milk production at a level equal to that which is obtained when a 15 to 20% total crude protein mixture is fed. In two out of three grazing trials, the Ohio workers (9, 10) obtained slightly higher production from a 20% total crude protein concentrate than from one containing 12% total crude protein. However, a monetary loss was incurred in feeding the higher protein mixture.

The high mean weekly persistency of production obtained in the present study (98.1 and 97.3%, respectively, for the control and the corn group), coupled with the fact that consumption of the supplements declined as season advanced, indicates that ample herbage of good quality was provided throughout most of the grazing season. The variation in milk production from week to week, which may be noted in Table 1, was due in part at least to rotational grazing of different crops and environmental conditions which influenced the growth of the herbage and the grazing habits of the animals.

Low protein intake may have limited the production of the cows fed shelled

corn during the last 3 or 4 wk. of the 1956 season and during the last week of the 1957 season. Apparently, this reflects the advanced stage of maturity of the herbage grazed during the last weeks of these 2 yr. A similar trend was not noted in the 1955 trial.

The supplement refusal data indicate that the herbage available during the later part of the grazing season was more abundant and/or more palatable than that available during the first part of the season. On the other hand, the lower consumption of supplement during the last part of the season could be partially explained by the drop in milk production. Since milk production was lower during the later part of the season, and since the supplement was not decreased accordingly, the reduced intake may simply reflect a reduction in nutritive needs of the animals.

The observation that the cows fed the 20% total crude protein supplement ate slightly more than those fed corn confirms the findings of Pratt and Davis (11), that a concentrate containing one of the oil meals is usually higher in palatability than one consisting only of cereal grains.

REFERENCES

(1) Crampton, E. W. Interrelationships Between Digestible Nutrient and Energy Content, Voluntary Dry Matter Intake, and the Over-all Feeding Value of Forages. J. Animal Sci., 16: 546. 1957.

(2) Eheart, J. F., and Pratt, A. D. The Digestibility and Utilization by Dairy Cows of Nutrients from Fertilized and Unfertilized Bluegrass Pasture. Virginia Agr. Expt. Sta., Tech. Bull. 81. 1942.

(3) Hardison, W. A., and Graf, G. C. Unpublished data. Virginia Agr. Expt. Sta. 1957.

(4) Hardison, W. A., Reid, J. T., Martin, C. M., and Woolfolk, P. G. Degree of Herbage Selection by Grazing Cattle. J. Dairy Sci., 37: 89. 1954.

(5) Hayden, C. C., and Perkins, A. E. High Protein Grains as a Supplement to Pasture for Dairy Cows. Ohio Bimonthly Bull., 13: 99. 1928.

(6) Horwood, R. E., and Putnam, G. W. High vs. Low Protein Grain with Pasture. Michigan Agr. Expt. Sta., Quart. Bull., 16: 11. 1933.

(7) Meyer, J. H., Lofgreen, G. P., and Hull, J. L. Selective Grazing by Sheep and Cattle. J. Animal Sci., 16: 766. 1957.

(8) Morrison, F. B. *Feeds and Feeding.* 21st ed. Morrison Publ. Co., Ithaca, New York. 1948.

(9) Perkins, A. E. Proportion of Protein Needed in the Grain Mixture. Ohio Bimonthly Bull., 167: 44. 1934.

(10) Perkins, A. E., and Hayden, C. C. High Protein Grains. Are They Needed as a Supplement to Pasture for Dairy Cows? Ohio Bimonthly Bull., 14: 89. 1929.

(11) Pratt, A. D., and Davis, R. R. High vs. Low Protein Grain Mixtures as Supplements to Legume-Grass Pastures. J. Dairy Sci., 39: 1304. 1956.

(12) Reid, J. T., Turk, K. L., Hardison, W. A., Martin, C. M., and Woolfolk, P. G. The Adequacy of Some Pastures as the Sole Source of Nutrients for Growing Cattle. J. Dairy Sci., 38: 20. 1955.

IMMATURE FORAGE MIXTURES WITH CITRUS PULP VERSUS MORE MATURE FORAGE WITHOUT ADDITIVE FOR SILAGE[1]

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SUMMARY

Two forage mixtures, each ensiled at two stages of maturity, were compared using lactating dairy cows in a switch-back feeding trial. Citrus pulp was added to the early-cut forage but not to the more mature forage. The mixture containing rye, rye grass, and crimson clover gave a higher yield of dry matter per acre at both dates of harvest than did a similar mixture which contained no rye. Likewise, the later stage of maturity resulted in a higher yield of each of the mixtures. Dry matter losses during the ensiling period were considerably higher in the early-cut material than those in the more mature forages. However, milk production was higher when the non-rye mixture was fed, and was higher at the early-cut stage of both forage mixtures.

In the southeastern section of the United States large acreages of winter forages are grown on dairy farms. However, there are wide differences of opinion, and a dearth of research data, concerning the place of rye as compared to oats in mixtures with crimson clover and rye grass. It has been shown with other forages that in silage-making the stage of maturity may affect yield of forage per acre, nutrient losses in the silages, and animal response (4, 5, 8). Since these effects usually are not optimum at the same time, determining the most practical stage for harvest is dependent on a knowledge of each.

In the experiments herein reported the effects of two stages of maturity of two important mixtures on the yield of forage per acre, nutrient losses in the ensiling process, and the response of lactating dairy cows when fed the silages were compared. The use of conditioners, such as citrus pulp, is a frequently recommended practice when high-moisture, immature forages are being ensiled, but not when higher dry matter forage is being made into silage. For this reason, citrus pulp was added to the younger forage and not to the more mature material. Another purpose of this study was to determine the efficiency of the experimental design, which was a switch-back with four treatments (3).

EXPERIMENTAL PROCEDURE

A uniform field was equally divided into two areas. One was seeded with 84 lb. of Wrens Abruzzi rye, 40 lb. of Italian rye grass, and 20 lb. of crimson clover per acre; whereas, in the other area 96 lb. of Arlington oats replaced the rye. Both areas were treated alike in all other respects. The seeding was made October 9-11, 1956. As often occurs in the Athens area, a majority of the oats was winter-killed. The species composition of the forage from the two areas is shown in Table 1. Hereafter, the two mixtures will be referred to as the rye mixture and the non-rye mixture.

Received for publication November 13, 1959.

[1] Journal Paper No. 115 of the College Experiment Station, University of Georgia, College of Agriculture Experiment Stations.

TABLE 1

Species composition and yields of forages ensiled and silage removed
(Dry matter basis)

	Early-cut		Late-cut	
	Rye	Non-rye	Rye	Non-rye
Rye *(%)*	68	....	71	....
Oats *(%)*	....	5	....	12
Rye grass *(%)*	10	26	11	41
Crimson clover *(%)*	22	67	17	44
Other *(%)*	0	1	1	3
Forage *(tons/acre)*	1.46	1.30	1.82	1.69
Silage *(tons/acre)*	1.07[a]	0.83[a]	1.60	1.46

[a] Calculated assuming no loss of citrus pulp.

Four tower silos measuring 8 by 24 ft. were used to ensile the two forage mixtures at each of two stages of maturity. During the period April 15-17, each of the mixtures was used to fill one silo. At this time the stages of maturity of the various species were estimated to be as follows: rye, less than 1% flower; rye grass, 100% vegetative; and crimson clover, less than 0.1% flower. The other two silos were filled during May 7 and 8, when the stages of maturity were estimated as follows: rye, soft dough; rye grass, 50% head; and crimson clover, ten days past full bloom.

The forage was chopped with a conventional cutter-bar type forage chopper, using a 0.25-in. theoretical cut. Citrus pulp was added to the early-cut forages at the rates of 163 and 162 lb. per ton of fresh forage for the rye and non-rye mixtures, respectively. Before the citrus pulp was added, each load of forage was sampled for chemical analyses by an accepted procedure (1). At each of the silo-filling periods, the forage of each area was sampled at a dozen or more randomly selected points to determine the species composition. The yield per acre was determined by weighing the forage put into the silos and measuring the land area from which it came. These composition and yield values are presented in Table 1.

The silos were opened June 11, thus giving a minimum storage period of 5 and 8 wk. Silage samples were taken five days each week during the feeding period, and composited. Moisture determinations on the silages and forages were made by the toluene distillation method (2). Protein, crude fiber, ether extract, and ash were determined by A.O.A.C. methods (2). Seepage samples were analyzed for dry matter, using an oven at 70-80° C., and for total nitrogen by the Kjeldahl Method (2). The pH determinations were made with a Beckman pH meter, using glass electrodes.

Twelve lactating dairy cows were used to compare the silages in a switch-back trial for four treatments (3). Silage was fed *ad libitum* as the only source of roughage, in each of the three periods which were 21 days in length. The amount of concentrates fed was based on the quantity of 4% FCM produced during the preliminary period. As citrus pulp is regarded as a grain equivalent, the cows fed the silages containing it were intentionally fed somewhat less concentrate. It was not possible to determine the amount of silage which

would be eaten or the proportion of the actual silage dry matter which originated from the citrus pulp. Thus, it was necessary arbitrarily to choose the relative amounts of concentrates for the cows fed the silages containing citrus pulp, versus those fed the silages which were made without the conditioner. The average quantities of concentrates fed the cows receiving the various silages are shown in Table 4. The mixture consisted of the following: ground snapped corn, 400 lb.; citrus pulp, 400 lb.; cottonseed meal, 200 lb.; and salt, 15 lb.

Milk weights were taken and samples analyzed for butterfat four days near the end of each of the first two periods and two days at the end of the third period. Three-day average weights were taken before the experiment began, on the fifth, sixth, and seventh days, and at the end of each experimental period.

RESULTS AND DISCUSSION

The per-acre yields of the two forage mixtures at the two stages of maturity are shown in Table 1. The mixture containing rye gave a higher yield of dry matter at both dates of harvest. Likewise, the later stage of maturity resulted in a higher yield for each of the mixtures. Since citrus pulp was added to only the immature forages, the effects of stage of maturity and citrus pulp were confounded. However, this was purposely done, as it was intended to make each silage by what was believed to be the most practical method. With forages excessively high in moisture, it is usually considered that a conditioner such as citrus pulp will result in better silage and reduce seepage. Since the more mature forages had a considerably higher dry matter content, it was not considered practical to add citrus pulp to them.

The dry matter losses during the ensiling period are presented in Table 2. These losses in the early-cut material were considerably higher than those of the more mature forage, with the difference being larger in the non-rye mixture. Total dry matter losses from the early-cut material plus citrus pulp silage were calculated by two methods (Table 2). If it had not been put in the silo, all the citrus pulp could have been available for feeding. Thus, it appears more

TABLE 2
Ensiling losses as measured by input-output data

	Early-cut		Late-cut	
	Rye	Non-rye	Rye	Non-rye
Total losses—fresh *(%)*	22.2	30.1	5.7	14.8
Dry matter losses *(% of total material)*	18.8	24.5	12.0	13.7
Dry matter losses *(% of forage)*	27.0[a]	36.5[a]	12.0	13.7
Dry matter losses by top spoilage *(%)*	0.8	1.1	0.6	0.6
Protein losses *(%)*	14.1	11.5	25.5	9.2
Crude fiber losses *(%)*	+8.7	3.1	5.3	2.5
N.F.E. losses *(%)*	34.2	39.9	16.5	27.7
Ash losses *(%)*	36.4	33.3	25.4	13.8
Ether extract losses *(%)*	+51.5	+66.1	+120.7	+72.6

[a] Calculated assuming no loss of citrus pulp.

TABLE 3
Chemical composition of the silages and the fresh material

	Early-cut		Late-cut	
	Rye	Non-rye	Rye	Non-rye
Dry matter[a, b]	23.2	22.2	29.2	24.4
(forage)	(16.7)	(14.9)	(31.3)	(24.1)
(forage + citrus)[c]	(22.2)	(20.6)	....	....
Protein	12.8	15.5	11.1	14.0
(forage + citrus)[c]	(12.1)	(13.1)	(13.0)	(13.3)
Ether extract	5.0	7.6	3.5	4.4
(forage + citrus)[c]	(2.7)	(3.4)	(1.4)	(2.2)
Crude fiber	34.0	25.5	41.0	36.5
(forage + citrus)[c]	(25.4)	(19.9)	(38.1)	(32.3)
N. F. E.	41.1	43.2	38.8	36.9
(forage + citrus)[c]	(50.7)	(54.3)	(41.4)	(44.0)
Ash	7.1	8.2	5.6	8.2
(forage + citrus)[c]	(9.1)	(9.3)	(6.6)	(8.2)
pH	4.2	4.0	4.6	4.4
T.D.N.[d]	56.8	66.5	48.2	53.7

[a] Expressed as per cent of total. All measures except dry matter are on a dry matter basis.
[b] Values in parentheses are for the forages. Others are for silages.
[c] Calculated from forage and citrus pulp analyses. No citrus pulp in late-cut material.
[d] Calculated by formula of Schneider *et al.* (7).

practical to charge the entire dry matter loss to the forage. However, this should not be interpreted to mean that all the material which was lost originated from the forage. Dry matter, pH, and total nitrogen (expressed as NH_3 equivalent on a dry matter basis) values of the seepage averaged 9.8, 4.9, and 2.5; 7.2, 4.5, and 3.9; and 9.4, 4.8, and 2.9, respectively, for the early- and late-cut non-rye and for the early-cut rye. There was essentially no seepage from the late-cut rye. Thus, the seepage from the immature forage plus citrus pulp contained more dry matter but less nitrogen than that from the late-cut material.

The chemical composition of the four forages and silages is presented in Table 3. The higher protein, lower crude fiber, lower pH, and higher calculated TDN values would suggest that the early-cut silages were of better quality than the late-cut material. Also, these analyses would suggest that the non-rye mixtures were superior to the rye combinations.

The results from the feeding experiment are presented in Table 4. Milk and FCM yields were significantly higher for the cows fed each of the early-cut materials than for those fed the same mixture cut at the more mature stage. Likewise, cows fed the non-rye materials at each cutting date produced more than those fed rye silages. Thus, the milk yields were in opposite directions to the dry matter yields per acre (Table 1). Milk production from cows fed the mature non-rye mixture was approximately equal to that of those fed the early-cut rye mixture plus citrus pulp. However, a higher dry matter yield per acre and lower ensiling losses were obtained from this non-rye silage. Thus, the late-cut non-rye combination was more practical than the early-cut rye mixture plus citrus pulp.

There were no significant differences in the total dry matter or silage intakes of the cows fed the various silages. In a study such as this, the method of

TABLE 4
Milk production, feed consumption, and weight changes of cows fed the various silages

	Early-cut		Late-cut		Coefficient of variation
	Rye	Non-rye	Rye	Non-rye	
					(%)
Milk *(lb/day)*	29.0	31.2	26.1	29.1	4.5
Fat test *(%)*	3.7	3.8	3.6	3.7	9.1
Fat *(lb/day)*	1.07	1.15	0.92	1.04	13.5
FCM *(lb/day)*	27.6	29.7	24.3	27.2	9.3
Dry matter intake					
a. Total	24.0	25.7	26.3	28.2	8.6
b. Concentrate	6.9	7.4	10.1	10.4	....
c. Silage (with citrus)	17.1	18.3	16.2	17.8	10.0
d. Silage (less citrus)	11.9	12.3	16.2	17.8	11.1
e. Concentrate + citrus	12.1	13.4	10.1	10.4	....
Weight gains *(lb/day)*					
a. Between periods	−3.51	−4.61	2.73	−0.76	126
b. Within periods	0.72	0.31	−0.19	0.24	412

separating concentrate consumption from forage intake presents a problem. The silage, less citrus pulp, was calculated assuming that the dry matter losses of citrus pulp and forages were in proportion to the amounts ensiled (Table 4). However, it is not suggested that this is necessarily what occurred. By this method of calculation, the amount of dry matter consumed from forage by the cows fed early-cut silage was highly significantly less than that of the other cows. When the concentrates plus citrus pulp and silage from forage were calculated assuming no loss of citrus pulp, the relative amounts of total concentrates were even higher. These values were 13.3 and 15.4 lb. of dry matter per cow per day for the groups fed the early-cut rye and non-rye silages, respectively. When the differences in concentrates required, and the yields per acre, are considered, it is doubtful if the additional amounts of milk produced would have made the early-cut method as practical under most conditions.

Weight changes of the cows were calculated by two methods and the results presented in Table 4. The between-periods method represents the change when cows were switched from another silage to the one in question. In the within-period method, weight changes are for the time from the fifth, sixth, and seventh days after treatment was started to the end of the period. The weight changes between periods of the cows fed the various silages were highly significantly different. Most of these were probably due to changes in fill. Therefore, it is not proper to consider such changes as being true weight gains or losses. The within-period method is considered to be the best method of studying differences in true gains which are due to experimental treatments. By this method, differences among cows fed the various silages were not significant, statistically. However, it is interesting to note that the differences among the treatments as calculated by the within-periods method were in opposite direction to the changes occurring between periods. Accordingly, it is suggested that initial

weights should be taken after the animals have become adjusted to the feeds under study.

The coefficients of variation associated with the various measurements are shown in Table 4. For weight gains, they are very large. However, this is to be expected and is discussed in a previous publication (6). The relatively small coefficients of variation for milk production demonstrate satisfactory precision in this measure for the design.

ACKNOWLEDGMENTS

The authors thank Mr. B. O. Bohannon and Miss Geraldine Speering for chemical analyses, Dr. T. H. Rogers and Dr. A. R. Brown for estimating the stages of forage maturity, Dr. J. L. Carmon for advice with statistical analyses, and Professor H. B. Henderson and Dr. R. E. Proctor for assistance in the preparation of the manuscript.

REFERENCES

(1) ANON. Summary of a Conference Held at Beltsville, February 20-21, 1956, on Procedures and Technics Used in Silage Studies. 1956.

(2) ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS. Official Methods of Analysis. 8th ed. Washington, D. C. 1955.

(3) LUCAS, H. L. Switch-Back Trials for More than Two Treatments. J. Dairy Sci., 39: 146. 1956.

(4) MARTZ, F. A., NOLLER, C. H., HILL, D. L., AND CARTER, M. W. Feeding Value for Milking Cows of Oat Silages Cut at Three Stages of Maturity. J. Dairy Sci., 41: 735. 1958.

(5) MCCULLOUGH, M. E., SISK, L. R., AND SELL, O. E. Influence of Stage of Maturity and of Ground Snap Corn or Sodium Meta Bisulfite as Preservatives on the Feeding Value of Oat Silage. J. Dairy Sci., 41: 796. 1958.

(6) MILLER, W. J., BEATY, E. R., AND UNDERWOOD, L. M. A Comparison of Tift Sudan and Starr Millet for Dairy Cows by Several Techniques. Georgia Agr. Expt. Sta., Tech. Bull. N.S. 16. 1958.

(7) SCHNEIDER, B. H., LUCAS, H. L., CIPOLLONI, M. A., AND PAVLECH, H. M. The Prediction of Digestibility for Feeds for Which There Are Only Proximate Composition Data. J. Animal Sci., 11: 77. 1952.

(8) THURMAN, R. L., STALLCUP, O. T., STEPHENS, J. L., AND JUSTUS, N. E. When to Harvest Oats for Hay and Silage. Arkansas Agr. Expt. Sta., Bull. 586. 1957.

TECHNICAL NOTES

AN APPROACH TO A RAPID TEST FOR ANTIBIOTICS IN MILK[1]

The introduction of antibiotics as therapeutic agents for mastitis, more than a decade ago, created a need for a simple and rapid method for their detection in milk. Several tests have been developed for this purpose, but the more rapid ones (1, 3, 5, 6) require from 2.5 to 3.5 hr. for completion, and are either insensitive to low concentrations or demand specialized equipment and trained personnel. Recent attention to antibiotics in milk and dairy products has indicated the inadequacy of present testing methods and has emphasized further need for a rapid but simple assay procedure. This note is a preliminary report of a method which appears to meet these requirements. Concentrations of 0.1 μg. or more of terramycin per milliliter of milk can be detected in 20-30 min.

The test is based on the observation that a given number of bacteria reduce 2,3,5-triphenyl tetrazolium chloride (TTC) at a greater rate when the bacterial cells are packed together than when they are suspended in a liquid medium (2, 8). Six to eight 0.5-in. filter paper antibiotic assay discs are placed in a petri dish and each is inoculated with exactly 0.1 ml. of a fresh 5-hr. growth of *Streptococcus cremoris* (Strain 806), cultured in Sobol's broth[2] (7). Then a dry disc is placed on to each of the inoculated discs and 0.15 ml. of the milk sample is added for complete saturation of the two discs. The petri dishes containing the discs are incubated for 10 min. at 30° C., whereupon the top discs are removed, one drop of TTC (0.25% aqueous) is added, and the top discs are replaced. Incubation is continued until reduction is indicated by the appearance of a pink color on the bottom discs.

This test has shown that positive results due to terramycin in milk can be obtained in less than 30 min., including total performance and reaction times. Representative results of studies made with milk containing from 0 to 10 μg/ml of added terramycin are presented in Table 1. A pink color appeared on the control discs about 10 min. after the addition of TTC. However, from 15 to 25 min. were required before color appeared on discs saturated with milk containing 0.1 to 1.0 μg/ml of terramycin. The TTC on the discs soaked with milk containing 10 μg/ml of terramycin was not reduced. The intensity of color and the size of the colored areas were inversely proportional to the terramycin concentration in the milk. The average time required for visual detection of color development was directly related to terramycin concentration.

TABLE 1

Effect of terramycin in milk on TTC reduction by *S. cremoris* (806)

Terramycin per milliliter of milk	Relative color developed[a] Incubation time after TTC additions		Time required for first detectable pink color	
	10 min.	25 min.	After adding TTC	Total incubation time[b]
(μg.)			*(min.)*	
0	+	+++	10	20
0.1	−	++	15	25
0.5	−	+	18	28
1.0	−	−	25	35
10.0	−	−	−	−

[a] Code: − = no color, + = definite small pink area, ++ or +++ = deeper pink color over larger area.

[b] Preliminary incubation period of 10 min. before TTC addition.

Some observations regarding the test indicated that 0.5-in. filter paper assay discs are superior to both Seitz filter pads and Millipore filter discs as holders for the bacteria. Also, application of the culture with a pipette, rather than by direct dipping of the disc into the broth culture or the use of a vacuum filter assembly, provides a more uniform distribution of cells, and thus a more uniform color development. *S. cremoris* (806) was selected for this purpose because it is very susceptible to terramycin and because it reduces TTC more rapidly than other cultures tested, including representatives of the genera *Escherichia, Bacillus, Staphylococcus, Lactobacillus,* and *Sarcina.* For optimum results, strict adherence to the described procedure is imperative, as the rapidity and reproducibility of the test are affected by (a) age of culture, (b) growth medium, (c) lot and concentration of TTC, (d) rate of diffusion of TTC on the discs, (e) method of application of culture to discs, and (f) number of cells.

The results suggest that terramycin inhibition of TTC reduction by bacteria under the stated conditions may be due to interference with normal metabolic processes by either (a) physical-chemical-cellular binding of terramycin (4), which prevents penetration of TTC through the cell wall, or (b) blockage of certain enzymatic processes. The mixing of TTC

[1] Article No. 4: 60 of the Department of Dairy Technology, The Ohio State University. Supported by a grant from the United States Public Health Service.

[2] Composition of Sobol's broth—per liter: 4 g. yeast extract, 4 g. K_2HPO_4, 2 g. neopeptone, 4 g. lactose, 2 g. peptonized milk, 4 g. milk protein hydrolysate, pH 6.8.

with terramycin prior to application to bacterial cells does not prevent color development, indicating that a reaction between TTC and the antibiotic, per se, is not involved.

Studies are in progress to determine the applicability of the test to the detection of antibiotics other than terramycin in milk, and to investigate the factors involved in the antibiotic inhibition of bacterial reduction of TTC.

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REFERENCES

(1) ARET, B., AND KIRSHBAUM, A. A Rapid Disc Assay Method for Detecting Penicillin in Milk. J. Milk and Food Technol., 22: 329. 1959.
(2) KENNEDY, H. E. Unpublished data. 1958.
(3) MATTICK, L. R., ANDERSON, E. O., AND WILDASIN, H. E. A Quantitative Procedure for the Detection of an Inhibitory Substance in Milk-Penicillin. J. Dairy Sci., 38: 829. 1955.
(4) MIKOLAJCIK, E. M. Physiology of Oxytetracycline-Resistant and -Sensitive *Streptococcus lactis*. Ph.D. dissertation, The Ohio State University. 1959.
(5) NEAL, C. E., AND CALBERT, H. E. The Use of 2,3,5-Triphenyltetrazolium Chloride as a Test for Antibiotic Substances in Milk. J. Dairy Sci., 38: 629. 1955.
(6) SHAHANI, K. M., AND BADAMI, M. C. A Resazurin Disk Assay Method for Detecting Antibiotics and Natural Starter Inhibitory Activity in Milk. J. Dairy Sci., 41: 1510. 1958.
(7) SOBOL, F. S. A Comparative Study of the Amino Acid Requirements of Terramycin-Resistant and Terramycin-Sensitive Strains of *Streptococcus lactis*. M.S. thesis, The Ohio State University. 1957.
(8) VANDIVIERE, H. M., GENTRY, W. H., AND WILLIS, H. S. A Rapid Chemical Test of Total Viability for Suspensions of Tubercle Bacilli. Am. Rev. Tubere., 66: 95. 1952.

A PLASTIC REPLICA–EMBEDDING AND STAINING TECHNIQUE FOR STUDYING THE BEHAVIOR OF MICROORGANISMS ON FOOD-CONTACT SURFACES

In some recent studies conducted on the interactions of *Staphylococcus aureus* ATCC-6538;FDA209 with food-contact surfaces, a technique was employed which greatly facilitated the evaluation of both the removal and growth of this organism under a variety of conditions. The technique consists essentially of embedding the bacteria, or other surface residues, in a plastic matrix while they are on the surface in question, removing the hardened plastic matrix layer (which is an exact negative replica of the surface) with the embedded bacteria, etc., mounting the plastic replica layer on a standard glass microscope slide, and staining the embedded bacteria by the Gram-staining procedure described by Hucker and Conn (1).

This stained preparation can then be conveniently examined under the microscope for such things as the relationship of bacteria to different kinds of surface features, the growth patterns of the bacteria over the various surface formations, and the relation of the bacteria to the residual organic and inorganic residues which might serve as sources of nutrients for the growth of the bacteria. These preparations can be advantageously viewed with many of the standard bright field, dark field, and phase contrast optical systems available in laboratory microscopes used for routine bacteriological determinations. The plastic preparations can also be embedded in such materials as Tissuemat,[1] and cross sections cut on a microtome of any desired angle or thickness for the examination of residual bacteria and other materials in relation to the surface features. The plastic preparations are stable to the effects of aging, and may be conveniently stored as a permanent record for future reference.

The plastic-embedding technique was carried out with equal ease on flat and curved surfaces (pipe sections); however, the procedure will be illustrated in this note with the flat 1-in.-diameter test discs utilized in some of the studies referred to previously. These were the steps followed in the preparation of the plastic replicas:

(1) Three drops of a 3:1, 40% Celloidin (C.P.)-nitrocellulose mixture,[2] diluted 1:15 with acetone (C.P.), were deposited and quickly and relatively evenly spread over the test disc in a leveled glass enclosure kept at a low relative humidity with anhydrous silica gel (Figure 1—Step 1).
(2) The specimens were permitted to dry overnight at room temperature. A layer of about 0.5 μ of hardened plastic was formed upon drying.

[1] Tissuemat—a product of the Fisher Scientific Company.
[2] Revlon Company, Product No. 61.

FIG. 1. Steps in the preparation of a plastic replica of a food-contact surface.

(3) To add strength to the 0.5-μ layer, and facilitate its removal and subsequent handling, ten drops of the same plastic mixture, diluted 1:3 with acetone (C.P.), were applied directly over the first dry plastic layer, and once again quickly and evenly spread over the disc (Figure 1—Step 2).

(4) Upon drying at room temperature, a layer about 5 μ thick was formed, which included the first layer fused to the second heavier layer.

(5) The specimen with this dried plastic matrix on its surface was then submerged in distilled water and permitted to soak for 1 hr. (Figure 1—Step 3).

(6) Next, the specimen was withdrawn from the distilled water bath, carefully dried, and the plastic layer peeled off with the aid of a razor blade. Removal was usually easy, even from the roughest surfaces studied (Figure 1—Step 4).

(7) The detached plastic film was next flipped over carefully on to a drop of 30% Bioloid[3] (toluene solution) mounting medium placed on a chemically clean microscope slide, allowing it to gradually flatten out on the drop from one edge (Figure 1—Step 5).

(8) This preparation was transferred to a leveled, dust-free enclosure and permitted to dry at room temperature overnight.

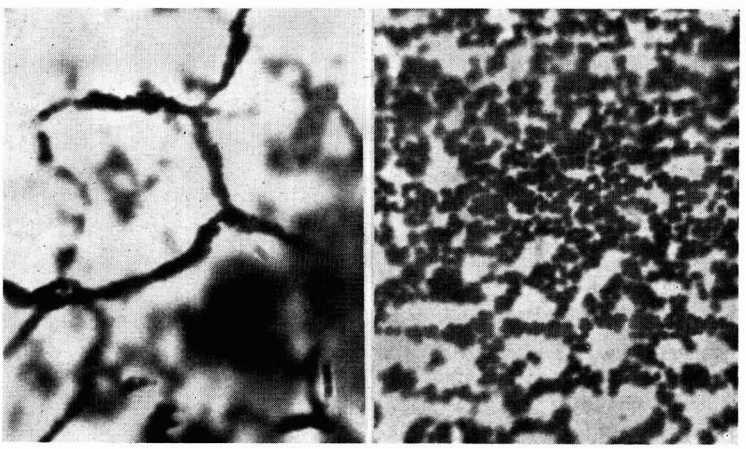
(9) The preparation was Gram-stained on a staining rack in the manner previously referred to (1), using Crystal Violet[4] (Cert. No. NS 31), Gram's Iodine, 95% ethyl alcohol, Safranin[5] O (Cert. No. NS 24), and distilled water (Figure 1—Step 6).

(10) For embedding and sectioning in Tissuemat, the stained preparations were removed from the glass slide with toluene, placed in a paper embedding box in the desired position, and submerged in melted Tissuemat. After solidification of the Tissuemat, the sectioning block was cut on the rotary microtome in the desired manner.

Figure 2 is a series of photomicrographs

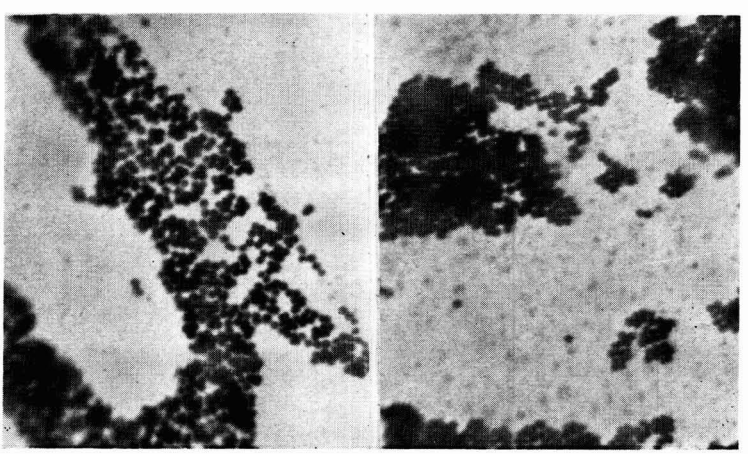
[3] Bioloid—a product of the Will Corporation.

[4, 5] Products of the American Analine Company.



2D Finish on stainles steel

No. 3 Finish on stainles steel



Optically polished pyrex glass

High density polyethylene

FIG. 2. Photomicrographs of Gram-stained plastic surface replicas containing embedded *Staphylococcus aureus*.

of Gram-stained plastic replicas containing embedded *Staphylococcus aureus* organisms which have grown on several types of materials with different surface finishes. Notice the alignment of the microbes in the grain boundaries of the 2D stainless steel finish, and in the grinding marks of the No. 3 stainless steel finish. Some surface detail and surface residues are noticeable on the Pyrex glass and polyethylene surfaces.

SUMMARY

A simple, versatile technique of embedding and staining microorganisms which are present on food-contact surfaces has been developed which can be performed with equipment and materials readily available from scientific supply houses. A Gram-staining procedure can be advantageously employed to differentiate bacteria and other microbes from other surface residues on the plastic-embedding matrix utilized in this technique. The stained preparations may be embedded in material such as Tissuemat and conveniently sectioned and examined from many angles. The plastic replicas are quite stable to the effects of aging; thus, can be stored for future reference.

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REFERENCE

(1) HUCKER, G. J., AND CONN, H. J. Further Studies on the Methods of Gram Staining. New York State Agr. Expt. Sta., Bull., 128. 1924.

RELATIVE VALUE OF CAROTENE AND VITAMIN A FED AT MEDIUM LEVELS IN A MILK REPLACER[1]

Several studies (5, 7, 9, 14) have shown that emulsification of the oil carrier of vitamin A or carotene by use of dispersing agents or homogenization and aqueous dispersions or colloidal suspensions resulted in increased utilization of carotene or of vitamin A. Little information, however, existed on the relative value of carotene and of vitamin A when fed over a range of intakes in a milk replacer diet. The following study presents a comparison of the relative values of carotene to vitamin A, both in water-dispersible forms and vitamin A from a feeding oil when incorporated into a milk replacer.

Twenty-four one-day-old Holstein male calves obtained from various Connecticut State institution herds during the period November, 1958, to January, 1959, were brought to the research barn and placed in individual tie stalls. Upon arrival, each calf received one 500-mg. oblet of chlortetracycline. Calves nursed their dams, or were given 8.0 lb. of colostrum during their first day, 8.0 lb. of whole milk on their second and third day, 8.0 lb. of two-thirds whole milk and one-third liquid milk replacer, fourth day, 8.0 lb. of one-third whole milk and two-thirds liquid milk replacer, fifth day; and 8.0 lb. of liquid milk replacer, sixth and seventh day. Based on the seventh day live weight, each calf received the liquid milk replacer at the rate of 10% of live weight and this allotment was increased on successive seven-day periods by a factor of 1.07, 1.14, and 1.21. The milk replacer was mixed with warm water, 0.15 lb. of milk replacer to 0.85 lb. of water. Both milk and milk replacer were fed in open pails. The composition of the milk replacer, per ton, was: 100 lb. brewer's dried yeast, 200 lb. corn distiller's dried solubles, 154 lb. dextrose, 1,000 lb. dried skimmilk, 300 lb. dried whey, 200 lb. oat flour, 35 lb. dicalcium phosphate, 0.4 lb. ferrous sulfate, 0.2 lb. copper sulfate, 0.2 lb. manganese sulfate, 10 lb. Lederle's Aurofac D containing 5 g. chlortetracycline per pound, 7.0 g. cobalt sulfate, 50.0 g. D-activated sterol equivalent to 100,000 I.U. vitamin D per gram. The proximate constituents of the milk replacer as determined by A.O.A.C. methods (1) were, with their standard errors, as follows: dry matter (%), 93.5 ± 0.4; crude protein, ash, fat, fiber, and N.F.E. as per cent of dry matter were, respectively, 28.0 ± 0.5, 8.5 ± 0.2, 1.6 ± 0.3, 0.8 ± 0.1, and 61.1 ± 0.4.

Beginning the eighth day and according to previous random allotment, each calf received for three successive seven-day periods either β-carotene beadlets (Hoffman-LaRoche, dry β-carotene beadlet, Lot No. 018, containing approximately 2.4% β-carotene, dissolved

[1] This study was supported in part from funds provided by Wirthmore Feeds, Inc., Waltham, Mass. The authors are grateful to B. A. Donohue and T. Watts for care of the animals, to Mrs. Mae Miller for technical assistance, to Dr. R. H. Bunnell, Hoffman-LaRoche, Nutley, N.J., for supplying the vitamin supplements, and to Dr. H. J. Fisher, Connecticut Agricultural Experiment Station, New Haven, for proximate analysis of the milk replacer. A preliminary report of this study was presented at the North Atlantic Section meeting of the American Society for Animal Production, August 25-26, 1959, Storrs, Conn.

in a vegetable oil and dispersed in a gelatin-sugar-starch matrix with added food antioxidants) at one of three levels per pound of live weight per day, 40, 80, or 160 γ, or vitamin A-beadlets (Hoffman-LaRoche, Rovimix A-325, Lot No. 366, synthetic vitamin A palmitate in a gelatin-sugar-starch matrix with added food grade antioxidants and with a claimed potency of 325,000 U.S.P. units of vitamin A per gram), 9, 12, or 15 γ or vitamin A–oil (E. F. Drew and Co., vitamin A feeding oil, Lot No. 2270, and containing approximately 10,000 U.S.P. units of vitamin A per gram), 9 or 15 γ. Milk replacer was weighed to the nearest 10 g., and water (100° F.) was weighed to the nearest 0.1 lb. The carotene and vitamin A beadlets were weighed to the nearest 0.5 mg. and the vitamin A feeding oil was measured to the nearest 0.01 cc., using a 1-cc. tuberculin syringe, and incorporated in the A.M. milk replacer (dry) allowance for each calf the afternoon prior to its feeding.

Calculation of the amount of carotene or of vitamin A to be fed to furnish the indicated intake and treatment for scours was identical to previous studies conducted at this station (11). Average daily minimum and maximum barn temperatures during the vitamin supplementation period with their standard errors were, respectively, 55.0 $\pm$ 7.3 and 62.9 $\pm$ 2.8° F. Only artificial light was provided from 6 A.M. to 6 P.M. daily, and averaged 7.0 $\pm$ 3.0 foot-candles.

Live weights to the nearest 1 lb. were recorded for each calf upon arrival at the research barn and, thereafter, at successive seven-day periods. Linear growth measurements, height at withers, heart girth, and girth of paunch were taken to the nearest one-quarter inch on the seventh and 28th days of age, respectively. Venous blood samples were drawn by jugular puncture on the seventh day of age, the day prior to vitamin supplementation, and thereafter, at seven-day periods for carotenoid and vitamin A analyses by the Kimble procedure (8). The carotene content of the beadlets was analyzed by dispersing a 100-mg. sample in 20 ml. of warm water (40° C.), adding 20 ml. of 95% ethanol, extracting the resulting mixture with 20 ml. of purified Skellysolve-B by vigorous shaking for 3 min., centrifuging, evaporating a 2-ml. aliquot of the extract under nitrogen, and taking up the residue in 100 ml. of cyclohexane (spectro grade). The absorbancy was read in a Beckman DU spectrophotometer at 454 mμ and the carotene concentration was obtained by comparison with an iodine isomerized synthetic β-carotene standard, Bunnell *et al.* (3). The vitamin A content of the beadlets and feeding oil were analyzed by the procedure described in the U.S. Pharmacopeia (4), except that 23 ml. of alcohol and 7 ml. of glycerine were used in saponification instead of 30 ml. of 95% alcohol. The carotene content of the milk replacer was analyzed by A.O.A.C. procedures (1). The average concentrations of these vitamins with their standard errors were as follows: carotene-beadlets 26,175 $\pm$263 γ/g carotene; vitamin A–beadlets, 105,925 $\pm$ 3734 γ/g vitamin A; vitamin A–oil 2557 $\pm$ 42 γ/g vitamin A; milk replacer, 0.14 $\pm$ 0.02 mg/lb, carotene. Statistical procedures used in the analysis of these data were from Bliss (2) and Snedecor (13).

No milk replacer weighbacks were observed in this study. The daily incidence of scours averaged per calf 4.6% of the 21 days of supplementation. Statistical analyses of the percentage values in the arcsin transformation indicated no differences due to source or level of the vitamin. Treatment for scours when accompanied by rectal temperature equal to or greater than 103° F. averaged per calf 0.1 and were found, upon transformation of the incidence to the square root of number plus one-half, not to be affected by treatment.

The average live weight with its standard deviation at the beginning of supplementation was 98 $\pm$ 12 lb.; height at withers 29.5 $\pm$ 1.4 in.; heart girth 29.8 $\pm$ 1.7 in.; and circumference of barrel 29.5 $\pm$ 1.9 in. During the 3-wk. comparison period, the average increase in live weight was 15 $\pm$ 4 lb.; in height at withers 1.6 $\pm$ 0.7 in.; in heart girth 1.7 $\pm$ 0.5 in.; and in circumference of barrel 2.3 $\pm$ 1.0 in. None of these increases in growths was affected by source or level of intake of the vitamin.

Average plasma carotenoids and vitamin A values obtained the day prior to vitamin supplementation and during the comparison period, and average plasma vitamin A values adjusted by covariance for values obtained on the day prior to vitamin supplementation, are presented in Table 1. The regressions of the change of the adjusted plasma vitamin A concentrations, in γ per 100 ml., y, on the logarithm (base 10) of carotene or vitamin A intake, x_i, in γ per pound of live weight per day, resulted in the following linear functions: (a) for carotene-beadlets, x_1, $y = 2.02 + 4.98\ x_1$, (b) for vitamin A–beadlets, x_2, $y = 2.31 + 10.74\ x_2$, and (c) for vitamin A–oil, x_3, $y = -1.74 + 10.74\ x_3$. Since the slopes of the linear regressions of the two sources of vitamin A did not differ significantly from each other, a common slope was calculated for both.

The amounts of vitamin A, from beadlets or oil, necessary to obtain an equivalent response to the 40, 80, and 160 γ carotene intake from carotene-beadlets based on plasma vitamin A concentrations were calculated (10, 11) from the regressions of plasma vitamin A on log intakes of carotene-beadlets, or on log intakes of vitamin A–beadlets or of vitamin A–oil, and presented in Table 2. Since the linear responses of plasma vitamin A to the two

TABLE 1

Effect of carotene and of vitamin A on plasma carotenoids and plasma vitamin A of Holstein calves fed a milk replacer[a]

Vitamin intake	Plasma carotenoids		Plasma vitamin A		
	Initial	Comparison period average	Initial	Comparison period average	Adjusted[b] comparison period average
	(γ/100 ml.)				
Carotene-beadlets (γ)					
40[c]	9	25	11.1	11.0	9.9
80	2	46	3.7	10.1	11.7
160	10	81	8.2	12.9	12.9
Vitamin A–beadlets (γ)					
9[c]	6	6	8.0	12.2	12.3
12	10	7	7.8	14.3	14.4
15	4	5	9.0	15.0	14.6
Oil (γ)					
9[c]	12	9	11.6	9.8	8.5
15	6	6	5.2	9.9	10.9
Standard deviation per calf	....	....	4.1	2.6	2.2

[a] Represents data from three calves per treatment group.
[b] Adjusted by covariance for initial plasma vitamin A values.
[c] Per pound of live weight per day.

sources of vitamin A, beadlets, and oil, were parallel, the average relative value of one form to the other was calculated by parallel line biological assay procedures (2). It was found

TABLE 2

Relative value of carotene-beadlets to vitamin A–beadlets and to vitamin A–oil

Source of vitamin A	Amount of carotene fed	Amount of vitamin A necessary to obtain response equivalent to carotene	Ratio of carotene to vitamin A
	(γ/lb live weight/day)		(γ carotene/γ vitamin A)
Vitamin A–beadlets	40	5.2	7.7
	80	7.2	11.1
	160	9.9	16.2
Vitamin A–oil	40	12.4	3.2
	160	23.6	6.8

that vitamin A–oil was 42% as effective for the maintenance of blood plasma vitamin A concentrations as vitamin A–beadlets.

These data with respect to the widening ratio of carotene to vitamin A, as the intakes are increased, are in general agreement with previous work as to trend, as reported in the early studies of Guilbert and associates (6), as well as with recent work from this station (10, 11), all dealing with nonnursing or non-milk-fed ruminants. The data contained herein may serve as approximations of the relative value of carotene to vitamin A in milk replacer fed calves, but not as exact estimates, due to the numerous factors influencing the utilization of carotene or vitamin A as recently discussed by Wise *et al.* (14). Also, the superiority of a water-dispersible form of vitamin A over that of the vitamin A–oil confirms studies with the milk fed calf (5, 7, 9, 14), as well as recent data with steers (12).

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REFERENCES

(1) Association of Official Agricultural Chemists. Official Methods of Analysis. 8th ed. Assoc. Offic. Agr. Chemists, Washington, D. C. 1955.
(2) Bliss, C. I. The Statistics of Bioassay. Academic Press, Inc., New York, New York. 1952.
(3) Bunnell, R. H., Driscoll, W., and Bauernfeind, J. C. Coloring Water-Base Foods with β-Carotene. Food Technol., 12: 536. 1958.
(4) Committee of Revision of the U. S. Pharmacopeial Convention, Inc. The United States Pharmacopeia. 15th rev. Mack Printing Co., Easton, Pa. 1955.
(5) Crowley, J. W., and Allen, N. N. The Effect of Kind of Carrier and Method of Dispersion on the Absorption of Carotene by Young Dairy Calves. J. Dairy Sci., 33: 380. 1950.

(6) GUILBERT, H. R., HOWELL, C. E., AND HART, G. H. Minimum Vitamin A and Carotene Requirements of Mammalian Species. J. Nutrition, 19: 91. 1940.
(7) JACOBSON, N. L., ALLEN, R. S., BLAKE, J. T., AND HOMEYER, P. G. The Effect of Method of Administration on the Absorption and Storage of Vitamin A by Dairy Calves. J. Nutrition, 54: 143. 1954.
(8) KIMBLE, MARIAN S. The Photocolorimetric Determination of Vitamin A and Carotene in Human Plasma. J. Lab. Clin. Med., 24: 1055. 1939.
(9) KON, S. K., MCGILLIVRAY, W. A., AND THOMPSON, S. Y. Metabolism of Carotene and Vitamin A Given by Mouth or Vein in Oily Solution or Aqueous Dispersion to Calves, Rabbits and Rats. Brit. J. Nutrition, 9: 244. 1955.
(10) MYERS, G. S., JR., EATON, H. D., AND ROUSSEAU, J. E., JR. Relative Value of Carotene from Alfalfa and Vitamin A from a Dry Carrier Fed to Lambs and Pigs. J. Animal Sci., 18: 288. 1959.
(11) ROUSSEAU, J. E., JR., EATON, H. D., HELMBOLDT, C. F., JUNGHERR, E. L., ROBRISH, S. A., BEALL, GEOFFREY, AND MOORE, L. A. Relative Value of Carotene from Alfalfa and Vitamin A from a Dry Carrier Fed at Minimum Levels to Holstein Calves. J. Dairy Sci., 37: 889. 1954.
(12) SHERMAN, W. C., KUHN, G. O., WHITE, E. A., LEWIS, W. D., REYNOLDS, W. M., AND LUTHER, H. G. Utilization of Vitamin A in Different Carriers by Beef Cattle. J. Animal Sci., 17: 586. 1958.
(13) SNEDECOR, G. W. Statistical Methods. The Iowa State College Press, Ames. 1956.
(14) WISE, G. H., JACOBSON, N. L., ALLEN, R. S., AND YANG, S. P. Rate of Absorption of Carotene and of Vitamin A from the Alimentary Tract of Dairy Calves. II. Effects of Methods of Dispersion and of Administration. J. Dairy Sci., 41: 143. 1958.

CHANGES IN SERUM, GLYCO-, AND LIPOPROTEINS IN NORMAL AND COLOSTRUM-DEPRIVED CALVES[1]

Serum proteins play important roles in various metabolic processes including nutrient transport (5) as well as in the maintenance of osmotic pressures (1, 2). Recently, Japanese workers (14) have studied the affinities of serum protein fractions to steroid hormones. Certain fractions of the serum proteins undergo characteristic changes in specific disease conditions (15) and, thus, the changes in serum protein observed by electrophoretic studies would be a valuable clinical aid in diagnosis.

Filter paper electrophoresis has been shown to be a valuable research technique, especially in the study of blood proteins (15, 16). In serum proteins, the results of paper electrophoresis agree closely with those of free electrophoresis (16). Although quantitative results may not be expected for glyco- and lipoproteins (16), paper electrophoresis is, nevertheless, a valuable aid in the study of the changes which these proteins undergo in the serum.

Several groups of workers (4, 6, 8, 11, 12) have studied the development of serum protein fractions in lambs (4), calves (8, 11, 12), and goats (6). Montemagno *et al.* (10) have reported the percentage values of serum, glyco-, and lipoproteins in the mature bovine and equine species. However, no references could be found to date on the changes of glyco- and lipoproteins in the young ruminant. This work was initiated to study the fluctuations in the electrophoretic pattern of serum, glyco-, and lipoproteins in normal and colostrum-deprived calves.

One Jersey and one Holstein female calf and two male Holstein calves were used as experimental animals in this study, which was conducted from February to June of 1959. The male calves were removed from the cow immediately after birth and deprived of colostrum. They were maintained on mature milk which was fed at the rate of 1 lb. of milk per day per 10 lb. of body weight. Hay and concentrate were provided. The female calves served as normal controls. They were allowed to remain with their dams for the first three days after birth. Thereafter, they were managed the same as those deprived of colostrum. Treatments were randomly assigned to the calves before birth. Blood samples were withdrawn at 1, 3, 7, 14, 21, 28, 42, 56, and 70 days of age and serum was frozen at $-30°$ C. until fractionated electrophoretically. Serum samples (.01 ml. for serum proteins and .04 ml. for glyco- and lipoproteins) were electrophoretically fractionated in a Spinco Model R electrophoresis apparatus on paper strips in sodium veronal buffer (pH = 8.6; $\mu = .075$; 2.5 ma.) for 16 hr. Serum proteins were dyed with alcoholic bromphenol blue and glyco- and lipoproteins were stained with a Schiff base reagent and oil red "O" dye, respectively (16). After the staining process, the strips were scanned immediately in a Spinco Model RB analytrol and the percentages of each protein fraction were calculated (16).

Typical changes in the electrophoretic patterns of serum, glyco-, and lipoproteins of normal (C) calves and a colostrum-deprived (O) calf from one to 56 days of age are shown in Figure 1. Table 1 shows the fluctuations in the average per cent of serum, glyco-, and lipoprotein fractions of the (C) and (O) calves from one to 70 days of age.

[1] Contribution from Agricultural Experiment Station, Technical Paper No. 551.

The average per cent of the serum albumin fraction of the (C) sera was markedly lower at one day after birth than that of the (O) sera (Table 1). Albumin per cent increased steadily in the (C) sera until the age of 70 days, when it reached a per cent above that of the (O) sera. The average per cent of the (C) alpha and beta fractions was also lower at one day post-partum than the alpha and beta of the (O) sera. The alpha fraction of the (C) calf sera decreased from one to three days, after which it remained fairly constant. On the other hand, the alpha fraction of the (O) calves increased slightly from one to three days and then decreased steadily until 42 days of age to a relatively constant level. The beta fraction of both (C) and (O) calves followed a similar pattern in regard to fluctuations. The (C) beta fraction increased for 21 days and then dropped until an age of 28 days and increased slightly thereafter. The beta fraction of the (O) calves rose sharply

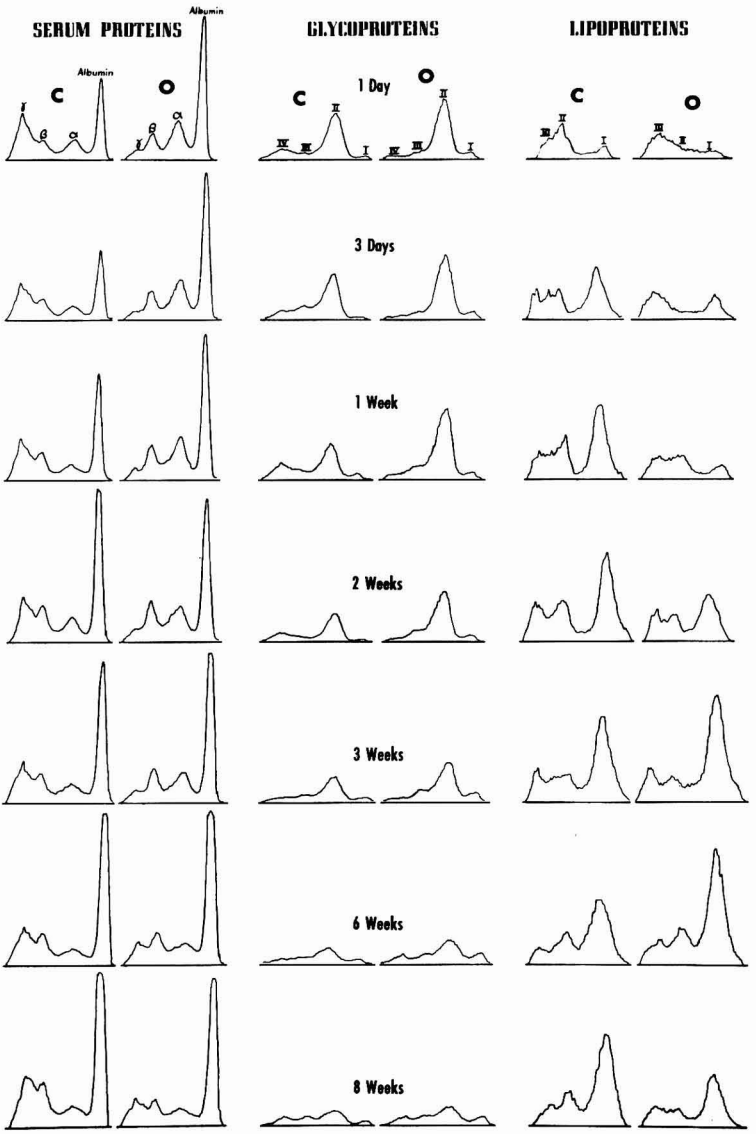

FIG. 1. Changes of serum, glyco-, and lipoprotein fractions of normal (C) and colostrum-deprived (O) calves with age.

TABLE 1
Changes of serum, glyco-, and lipoproteins in normal and colostrum-deprived dairy calves

	Calf treatment	Days of age								
		1	3	7	14	21	28	42	56	70
Serum proteins										
Albumin	C	29.1	33.2	35.8	44.4	49.3	48.9	50.3	49.5	51.5
	O	53.2	49.2	48.8	49.0	52.6	57.0	57.3	52.3	44.7
Alpha globulin *(%)*	C	18.5	15.3	14.5	14.3	14.0	14.7	13.8	14.0	13.9
	O	28.6	29.1	27.4	23.7	22.8	19.3	17.0	17.7	16.6
Beta globulin *(%)*	C	11.1	15.5	16.1	15.7	17.0	14.0	16.6	16.2	15.6
	O	15.4	19.0	21.1	22.1	18.5	14.8	14.8	14.5	14.5
Gamma globulin *(%)*	C	41.3	36.0	33.6	25.6	19.7	22.4	19.3	20.3	19.0
	O	2.8	2.7	2.7	5.2	6.1	8.9	10.9	15.5	24.2
Glycoproteins										
Total[a]	C	128	110	109	86	77	69	111	89	86
	O	136	149	144	89	82	97	128	94	135
Fraction I *(%)*	C	2.0	1.8	5.1	2.3	5.4	9.9	12.8	12.9	12.9
	O	9.6	9.2	8.5	6.5	11.2	9.5	16.4	15.2	17.4
Fraction II *(%)*	C	70.3	69.8	61.5	63.7	69.4	61.6	47.8	48.1	51.5
	O	76.1	70.4	69.8	68.9	58.7	67.8	46.6	46.5	44.3
Fraction III *(%)*	C	6.5	9.0	12.9	10.9	15.3	11.1	17.6	20.2	14.4
	O	8.7	12.0	14.4	14.8	15.1	14.0	17.6	17.9	12.7
Fraction IV *(%)*	C	21.2	19.4	20.5	23.1	13.0	17.4	21.8	18.8	21.2
	O	8.7	8.4	7.3	9.8	15.0	8.7	19.4	20.4	25.6
Lipoproteins										
Total[a]	C	71	156	184	196	239	234	218	231	245
	O	68	133	188	283	329	308	252	162	196
Fraction I *(%)*	C	26.0	51.5	57.2	67.7	71.2	61.8	66.5	61.9	59.7
	O	12.2	34.9	37.6	56.3	75.7	62.0	57.5	55.2	54.9
Fraction II *(%)*	C	38.3	24.4	16.5	16.6	17.3	24.3	20.1	25.5	25.7
	O	27.9	21.7	34.8	26.9	12.2	14.3	25.9	25.6	27.5
Fraction III *(%)*	C	35.7	24.1	26.3	15.7	11.5	13.9	13.4	12.6	14.6
	O	59.9	43.4	27.6	16.8	12.1	23.7	16.6	19.2	17.6

[a] Based on analytrol counts—indicative of total dye binding.

until the age of 2 wk., then decreased to an average per cent similar to that of the (C) calves at 28 days of age.

The large percentage of gamma globulin in the (C) calf sera at birth largely accounted for the differences between albumin per cent in the (C) and (O) calves. Gamma globulin per cent in (C) and (O) serum decreased and increased, respectively, in a linear fashion. After 42 days (Table 1), gamma globulin per cent in (O) calves approximated the percentage in (C) calf sera.

Four different glycoprotein fractions were observed by this method of electrophoretic separation and were designated as I, II, III, and IV, in respect to electrophoretic mobility. These results are in agreement with those reported by Montemagno *et al.* (10). Fraction I moves in an electric field at a rate similar to serum albumin, whereas Fractions II, III, and IV move as alpha, beta, and gamma globulin, respectively. Total glycoproteins (total analytrol counts) of the (C) calves remained below those of the (O) calves during the study (Table 1). However, both underwent similar fluctuations. The total counts decreased for a period of 21 days (O) to 28 days (C), then increased until an age of 42 days of age, and then leveled off. The average percentage of Fraction I in the (C) calves remained below that of the (O) calves during the study. Fraction II of the glycoproteins also underwent similar changes in the (C) and (O) calves and showed no marked difference in fluctuations. Fraction II decreased in both (C) and (O) calves between one and 42 days and remained constant thereafter. There was also little difference in the average per cent of Fraction III between the normal and colostrum-deprived calves during the study. The average per cent of Fraction III in both (C) and (O) calves increased with minor fluctuations until 56 days of age, when a drop in the average percentage occurred. There was a distinct difference in the concentration of Fraction IV in the (C) and (O) calves from one to 21 days of age. During this period, the average per cent of Fraction IV in the sera of the normal calves was much higher than in that of the colostrum-deprived animals. At 21 days of age the average per cent (C) decreased sharply and increased gradually thereafter for the remainder

of the study. The concentration of Fraction IV (O) remained at a low level until an age of 21 days, after which the average percentage increased for the remainder of the study.

Five lipoprotein fractions are generally found upon analysis of human sera (16). Montemagno *et al.* (10) report the results of lipoprotein analysis of bovine sera as two general protein fractions. Three distinct lipoprotein fractions were separated by this method of electrophoresis and were designated in the same manner as the glycoprotein fractions with regard to electrophoretic mobility. Total lipoprotein (total analytrol counts) of the normal sera increased sharply after birth to an age of 21 days, decreased to 42 days, and increased thereafter. Total liproprotein of the colostrum-deprived sera increased sharply to 21 days of age, decreased thereafter (Table 1). Fraction I of both normal and colostrum-deprived calves followed the same general trend throughout the experiment. An increase in average per cent was noted for 21 days, after which a slight decrease to a more constant level occurred. Fraction II (C) showed a decrease for 14 days corresponding to the increase in Fraction I (C). A rise in average percentage then occurred to an age of 42 days. On the other hand, Fraction II (O) dropped slightly from one to three days, increased sharply at seven days, dropped again to 21 days of age, and increased thereafter to a constant level at 42 days of age. Fraction III of the lipoproteins followed the same general pattern of fluctuations in the sera of the normal and colostrum-deprived calves. A sharp decrease in average per cent was noted from one to 21 days, after which Fraction III (C) increased slightly to a constant level. Fraction III (O) rose sharply at this time to 28 days of age and then decreased to 42 days of age, after which a slight increase in average per cent occurred.

The changes in the electrophoretic patterns of serum, glyco-, and lipoproteins reflect the rapid physiological development of the young calf. The drop in percentage of serum albumin in the (O) calves at an age of 70 days appeared to be somewhat proportional to the rapid rise of the autogenous globulin reported by Pierce (11, 12). Conversely, the serum albumin of the (C) calves underwent an increase in albumin percentage which perhaps could be explained by the proportional drop in the gamma globulin per cent to an age of 21 days. The appearance of small amounts of a fraction which migrated electrophoretically as gamma globulin also coincided with the results of Pierce (12) and could be either autogenous globulin or gamma globulin acquired in utero. On the other hand, Jameson *et al.* (8) found no gamma globulin present in the sera of newborn calves at birth. However, this is presumably a factor which could vary considerably between various animals. The results are similar to those reported by Jameson *et al.* (8), in that alpha globulin is high at birth in both (C) and (O) calves and decreased thereafter, and the percentage of beta globulin undergoes an increase for a short period and drops. The changes in the per cent of serum albumin observed in this study showed a marked difference between the (C) and (O) calves. This was not in accord with the results of Dunlap *et al.* (4), who reported no significant changes in the albumin fraction of newborn lambs. Possibly, there could be a species difference involved or environmental factors that were not controlled in these studies.

Since the exact functions of lipo- and glycoproteins remain obscure, the explanations of the significance of the developmental fluctuations can not be offered. The higher concentration of Fraction IV in the glycoproteins in the (C) calves and the reverse picture in the (O) calves indicates a relationship of this fraction to gamma globulin. More study is needed to determine the exact roles of glyco- and lipoproteins in regard to physiology and nutrition, in order that the significance of developmental changes can be understood.

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REFERENCES

(1) AUTIO, L., KARKAINEN, V. J., AND WARTIOVARRA, V. Connection Between Blood Protein and Colloidal Osomotic Pressure. Ann. Med. Exptl. et Biol. Fenniae (Helsinki), 35: 409. 1957. (Chem. Abstr., 52: 17351. 1958.)

(2) CHAKRAVARTI, H. S. Plasma Proteins in Malnutrition with Special Reference to Nutritional Edema. Nutrition Abstr. & Rev., 23: 105. 1953.

(3) DEUTSCH, H. F., AND SMITH, V. R. Intestinal Permeability to Proteins in the Newborn Herbivore. Am. J. Physiol., 191: 271. 1957.

(4) DUNLAP, J. S., AND DICKSON, W. M. The Effect of Age and Pregnancy on Ovine Blood Protein Fractions. Am. J. Vet. Research, 16: 91. 1957.

(5) ERWIN, E. S., VARNELL, T. R., AND PAGE, H. M. Relationship of Vitamin A and Carotene to Bovine Serum Proteins. Proc. Soc. Exptl. Biol. Med., 100: 373. 1958.

(6) FALASCHINI, A., BIONDO, G., AND LEONTI, F. Modifications of the Blood Proteins in Kids in Relation to Their Age and Alimentary Regime. Boll. soc. ital. biol. sper., 31: 306. 1955. (Chem. Abstr., 49: 14981d. 1955.)

(7) HILL, K. J. Gastric Development and Antibody Transference in the Lamb, with Some Observations on the Rat and Guinea Pig. Quart. J. Exptl. Physiol., 41: 421. 1956

(8) JAMESON, E., ALVAREZ-TOSTADO, D., AND SORTOR, H. H. Electrophoretic Studies on Newborn Calf Serum. Proc. Soc. Exptl. Biol. Med., 51: 163. 1942.
(9) MASON, J. H., DALLING, T., AND GORDON, W. S. Transmission of Maternal Immunity. J. Pathol. Bacteriol., 33: 783. 1930.
(10) MONTEMAGNO, F., AND AGRESTI, A. Blood Serum Electrophoretic Picture (Serum, Glyco-, and Lipoprotein) in Bovine and Equine Animals. Acta Med. Vet., 3: 35. 1957. (Chem. Abstr., 52: 18760d. 1958.)
(11) PIERCE, A. E. Electrophoretic and Immunological Studies on Sera from Calves from Birth to Weaning. I. Electrophoretic Studies. J. Hyg., 53: 247. 1955.
(12) PIERCE, A. E. Electrophoretic and Immunological Studies on Sera from Calves from Birth to Weaning. II. Electrophoretic and Serological Studies with Special Reference to the Normal and Induced Agglutinins to *Trichomonas foetus*. J. Hyg., 53: 261. 1955.
(13) SMITH, VEARL R., AND ERWIN, E. S. Absorption of Colostrum Globulins Introduced Directly into the Duodenum. J. Dairy Sci., 42: 364. 1959.
(14) TAKIKAWA, H. Interaction of Steroid Hormones with Serum Protein. Yakugaku Zasshi, 78: 1269. 1958. (Chem. Abstr., 53: 6313. 1959.).
(15) VESSELINOVITCH, S. D. The Analysis of Serum Proteins of Domestic Animals by Filter Paper Electrophoresis. A Review. Cornell Vet., 49: 82. 1959.
(16) WUNDERLY, C. H. Paper Electrophoresis. Electrophoresis; M. Bier, ed. Academic Press, New York, New York. 1959.

METABOLISM OF VOLATILE FATTY ACIDS BY THE PERFUSED LIVER OF COWS WITH KETOSIS[1]

A preliminary study of the biochemical aspects of bovine ketosis was conducted by perfusing with heparinized blood the livers from two mature cows afflicted with the disorder. Two lactating cows, each weighing approximately 1,200 lb., were selected for the study. These animals had a history of susceptibility to ketosis, and at the time of sacrifice exhibited the clinical and biochemical symptoms and signs of the disease. It was hoped that a comparison of results obtained in this investigation with those of previous perfusion studies of normal livers would give new insights into the metabolic aspects of bovine ketosis.

Experiments with both ketotic livers were essentially similar, except in regard to the C^{14}-labeled acids which were added to the perfusate. In Perfusion A, 1.0 mc. of acetate-1-C^{14} Na was added to the blood, whereas 0.5 mc. of *n*-butyrate-1-C^{14} Na was added in Perfusion B. The perfusion procedure and methods of blood and tissue analysis were as previously reported in studies with goat livers (4). Six liters of heparinized blood were used as the perfusate in each perfusion. The rate of blood flow through the organ was maintained at six to eight liters per minute for a total period of 30 min. A mixture of C_2 to C_5 volatile fatty acids (VFA) was introduced into the blood perfusate at the initiation of each perfusion. As the experiment progressed, more VFA (C_3 to C_5) were added to the perfusate because the previous work had demonstrated their rapid utilization by the liver (4). The ketotic livers exhibited fatty deposits and were greatly enlarged, each weighing approximately 10 kg.

Quantitative results for the two perfusions are reported in Table 1. The per cent glycogen in the liver at the initiation of perfusion was very low. This is a characteristic of bovine ketosis (10). Nevertheless, the quantity of glycogen did decrease, as usual with liver perfusions. The decrease in glycogen was accompanied by an increase in blood glucose and lactic acid. This was observed previously with normal goat livers (4).

The increase in blood levels of formic acid agrees with earlier results (4, 5), showing that the liver is the source of this normal blood metabolite in ruminants. However, the rate of formation of formic acid was markedly less than that observed with normal goat livers (4). In four perfusions of normal livers, the average production of formic acid was 685 mg. per hour per kilogram of tissue. During the perfusion of the ketotic cow livers the rate of production was 100 mg. per hour per kilogram of tissue.

From the studies of normal livers (4), it was concluded that the liver is the source of blood ketone bodies in ruminants and that these metabolites arise from hepatic lipid metabolism. It was, therefore, surprising to find in the case of ketosis, which is characterized by high levels of ketone bodies in the blood, a greatly reduced production of ketones by the liver. In Experiment A there was no increase in perfusate levels of ketone bodies, whereas in Experiment B the rate of production of these metabolites was 71% less than what had been previously noted in normal animals.

As in the normal liver (4), the contribution of acetate from the blood to hepatic metabolism was small. It will be noted from Table 2 that 90% of the recovered C^{14} from labeled acetate still remained in blood acetate. The quantity of acetate in the perfusate exhibited a decrease rather than the slight increase noted in livers

Received for publication October 23, 1959.
[1] Scientific article No. A-808, contribution No. 3080 of the Maryland Agricultural Experiment Station.

TABLE 1

Total quantities of metabolites, and per cent liver glycogen at the beginning and end of ketotic liver perfusions

Metabolites	Acetate-1-C^{14} added		Butyrate-1-C^{14} added	
	Beginning	End	Beginning	End
	(mg.)			
Blood				
Glucose	5,982	13,074	2,022	13,200
Acetone bodies[a]	1,890	1,890	3,618	4,332
Valeric Acid	414	1,152	564	834
Butyric Acid	1,458	4,986	1,878	6,318
Propionic Acid	942	4,866	1,908	5,754
Acetic Acid	4,602	3,852	4,884	3,156
Formic Acid	540	1,068	504	1,002
Lactic Acid	4,578	6,414	3,702	8,058
Liver				
Glycogen	0.007%	0.001%	0.005%	0.001%

[a] Acetone bodies calculated as β-hydroxybutyric acid.

TABLE 2

Distribution of recovered C^{14} label following perfusion of the livers of cows with ketosis, in which carboxyl-labeled acetate and butyrate were added to the blood

Substance isolated	Per cent distribution of label from added metabolites	
	Acetate	Butyrate
Blood		
Valeric Acid	0.03	0.90
Butyric Acid	0.14	83.23
Propionic Acid	0.13	2.56
Acetic Acid	90.50	2.06
Formic Acid	3.51	1.18
Lactic Acid	0.33	9.43
Glucose	0.00	0.00
Acetone bodies -derived carboxyl CO_2	0.04	0.61
Liver		
Glycogen	0.06	0.49
Nucleotide fraction	1.51	0.06
Per cent added C^{14} recovered	91.0	69.0

of normal goats (4), thus indicating a reduction in the size of the acetate pool within the liver, possibly associated with reduced hepatic catabolism of lipids.

The data in Table 1 show that an increase occurred in the quantities of valeric, butyric, and propionic acids in the blood. This was a direct result of the addition of these acids to the perfusate during the experiment. In the studies with normal livers (4), there was a rapid removal of these acids from the blood. In the experiments reported here, an effort was made to simulate the normal picture of continuous absorption from the rumen by adding VFA (C_3 to C_5) to the perfusate at different times and in quantities based on an estimation of the rate of liver utilization of these acids. Unfortunately, an accurate measure of the quantities of C_3 to C_5 fatty acids added to the perfusate after the initial addition was not obtained. However, the amount added was less than the estimated utilization.

The distribution of label from C^{14}-labeled butyrate (Table 2) shows 83% of the recovered label still present in blood butyrate at the completion of the perfusion. This is in contrast with the normal liver (4), where 83% of the recovered label was isolated in glycogen, blood glucose, and lactic acid. Admittedly, the addition of unlabeled butyrate to the perfusate reduced the entrance of labeled butyrate into liver metabolism simply by dilution. However, there was over five complete passages of the perfusate through the liver before the addition of unlabeled butyrate. Because of this initially high specific activity a greater utilization than observed was to be expected. As noted previously with normal liver (4), a considerable portion of the C^{14} label from butyrate was recovered in blood lactic acid.

The perfusion studies reported here confirm some of the results previously obtained with normal goat livers (4). First, the liver is the source of the normal blood metabolite formic acid, and second, the direct conversion of butyrate to β-hydroxybutyrate is negligible in the liver.

A general decrease in hepatic metabolism associated with ketosis is indicated by these present experiments. A similar decrease in liver metabolism during ketosis has been noted by Chung *et al.* (2) in their studies with tissue slices, Sauer *et al.* (8) working with liver homogenates, and Peeters *et al.* (6) in studies of the sulphonamide-acetylating capacity of liver slices. Robertson *et al.* (7) also obtained evidence of a decreased liver function by the use of the bromsulphalein fractional clearance test. The studies by Chung *et al.* (2) also demonstrated a marked change from normal in the oxidative metabolic patterns of ketotic cow liver incubated with 1-C^{14}-labeled VFA, indicating considerable alteration in the Krebs cycle. Likewise, a lowered hepatic metabolism during ketosis is in agreement with the proposal of Bach and Hibbitt (1) that ketosis is associated with a deficiency of coenzyme A.

It has been demonstrated that the utilization of ketone bodies is not impaired in ruminants during ketosis (9, 11). Therefore, the elevated blood levels associated with the disease must result from an increased production. The present work suggests that instead of an elevated production of ketone bodies by the liver there is actually a marked reduction. The origin of these metabolites during ketosis provides a very intriguing question. The recent report of Kronfeld and Kleiber (3), showing the increased formation of ketone bodies from acetate by the mammary gland during ketosis, suggests that the metabolism of C_5 to C_2 fatty

acids by the extrahepatic tissues might provide the answer.

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REFERENCES

(1) Bach, S. J., and Hibbitt, K. G. Biochemical Aspects of Bovine Ketosis. Biochem. J., 72: 87. 1959.
J. C. Oxidative Metabolic Patterns of Normal and Ketotic Cow Livers Incubated with 1-C^{14} Labeled Aliphatic Acids. Proc. Soc. Exptl. Biol. Med., 100: 476. 1959.
(3) Kronfeld, D. S., and Kleiber, Max. Mammary Ketogenesis in the Cow. J. Appl. Physiol., 14: 1033. 1959.
(4) McCarthy, R. D., Shaw, J. C., and Lakshmanan, S. Metabolism of Volatile Fatty Acids by the Perfused Goat Liver. Proc.
(2) Chung, A. C., Hueter, F. G., and Shaw, Soc. Exptl. Biol. Med., 99: 560. 1958.
(5) McCarthy, R. D., Shaw, J. C., McCarthy, Jeanne L., Lakshmanan, S., and Holter, J. B. Production and Absorption of Organic Acids by the Perfused Goat Rumen. Proc. Soc. Exptl. Biol. Med., 99: 556. 1958.
(6) Peeters, G., Oyaert, W., Bouckaert, J., and Hoeck, H. Acetylation of Sulfanilamide in Ketonaemic Cows. Sonderdruck aus Zentralblatt fur Veterinarmedizin, Bd. V, Heft 1: 43. 1958.
(7) Robertson, W. G., Mixner, J. P., Bailey, W. W., and Lennon, H. D., Jr. Determination of Liver Function, Plasma and Blood Volumes in Ketotic Cows, Using Bromsulphalein. J. Dairy Sci., 40: 977. 1957.
(8) Sauer, F., Dickson, W. M., and Hoyt, H. H. Oxygen Uptake in Liver Homogenates Taken from Normal and Ketotic Cows. Am. J. Vet. Research, 19: 567. 1958.
(9) Shaw, J. C. A Comparison of the Acetone Body Metabolism of the Lactating Mammary Gland of the Normal Cow with that of the Cow with Ketosis. J. Biol. Chem., 142: 53. 1942.
(10) Shaw, J. C. Studies on Ketosis in Dairy Cattle. V. The Development of Ketosis. J. Dairy Sci., 26: 1079. 1943.
(11) Shaw, J. C., and Knodt, C. B. The Utilization of β-Hydroxybutyric Acid by the Lactating Mammary Gland. J. Biol. Chem., 138: 287. 1941.
(12) Shaw, J. C., and Leffel, E. C. Distillers Grains in Prepartum and Postpartum Dairy Rations. Proc. 45th Conf. Feeds of the Grain Distillers. Distillers Feed Research Council, Cincinnati. p. 46. 1949.

EFFECT OF LIQUID DIET ON DEVELOPMENT OF RUMEN PAPILLAE DURING THE FIRST ELEVEN MONTHS IN DAIRY STEERS[1]

Swanson and Harris (4) observed rumination in calves as young as 2 wk. of age and Marshall *et al.* (2) found that fermentation can be well established in the rumen of calves 20 to 30 days of age. Rumen papillation was observed by Flatt *et al.* (1) and Sander *et al.* (3) to occur when acetates, butyrates, and propionates were added to an exclusive milk diet for young calves. Tomate *et al.* (5) found that papillary development was abnormal and limited when butyric acid or an acetic–propionic acid combination was added to the diet of calves which otherwise received only milk from four days to 12 wk. of age. Essentially, no papillary development was found by any of these workers in milk-fed calves which received no dietary supplements.

In the present study, observations were made on steers from 9 to 11 mo. of age which had received a milk diet from birth. The present study involved one Holstein (No. 49) and six Jersey calves. Two calves served as controls and were fed hay and grain from birth, with milk also having been fed during the first 60 days. Five calves received whole milk supplemented daily with 1 mg. cobalt, 10 mg. copper, 25 mg. manganese, 25 mg. zinc, and 1 mg. iodine. Following the 8th wk. the solids content of the experimental diet was raised to approximately 18% by addition of dry milk solids-not-fat. In addition, 10,000 I.U. vitamin A, 500 I.U. vitamin D, 2 g. sodium chloride, and 0.6 g. magnesium were fed daily per 100 lb. of body weight. Three of the calves (No. 462, 439, and 49) received supplemental iron (3-20 mg. iron per 100 lb. body weight daily) during the last 120 days before slaughter. The calves were kept in individual pens bedded with wood shavings.

At the time of sacrifice, papillation was found in only one rumen from the milk-fed group (No. 471). The other rumens from this group contained a few fold-like structures in the mucosa but essentially no normal papillae (Figure 1). These rumens can be compared to the extensively papillated ones from the normally fed control calves shown in Figure 2. The ridges and folds in the rumens of the experimental group appear to be similar to those reported by Tomate *et al.*

[1] Florida Agricultural Experiment Stations Journal Series, No. 1068.

(5) when volatile fatty acids and certain salts were added to a milk diet.

The rumens of the experimental calves contained considerable quantities of wood shavings. Contents of the rumens from calves fed only milk and supplementary minerals contained an average of 15.2 ± 2.2% (standard deviation) dry matter of which 60.3 ± 7.1% was cellulose, compared to 17.9 ± 0.3% dry matter of which 27 ± 0.1% was cellulose for the control calves, respectively. Only the papillaed rumen in the milk-fed group and the two control rumens became distended upon removal, indicating the presence of active fermentation. Cellulose in the rumen of No. 471 which was somewhat papillated was 41.9%, compared to 53.4, 56.5, 57.8, and 62.9% for No. 462, 49, 439, and 447, respectively. This lower level of fiber in the rumen with limited papillation suggests also that fermentation was associated with the development of papillae.

All other stomach compartments appeared normal, suggesting that their development was largely a function of age. Figure 3 shows that the oldest calf (No. 447) had the most development of the omasum and that the youngest (No. 471) appeared normal. All omasa contained horny papillae and other normal tissues. These findings lend support to those of Flatt *et al.* (1), Tomate *et al.* (5), and Sander *et al.* (3). They indicate further that development of the other stomach compartments is almost independent of the type of feed, while dietary factors are extremely important in rumen development.

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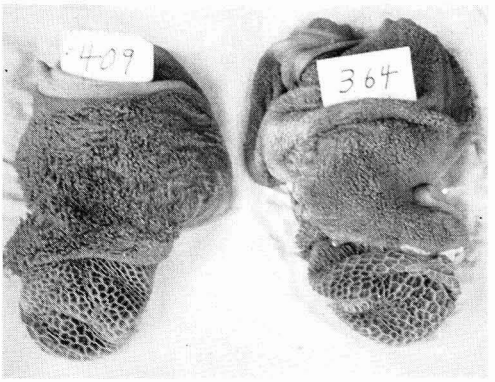

Fig. 2. Rumen and reticulum development of calves fed a normal diet.

REFERENCES

(1) Flatt, W. P., Warner, R. G., and Loosli, J. K. Influence of Purified Materials on the Development of the Ruminant Stomach. J. Dairy Sci., 41: 1593. 1958.

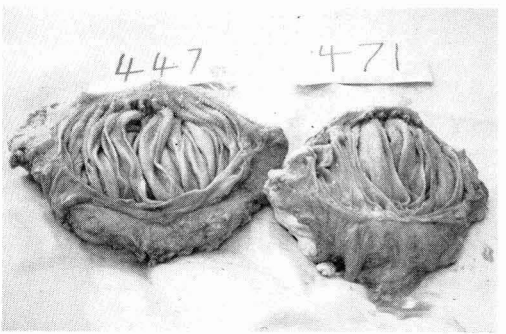

Fig. 3. Development of omasa of milk-fed calves.

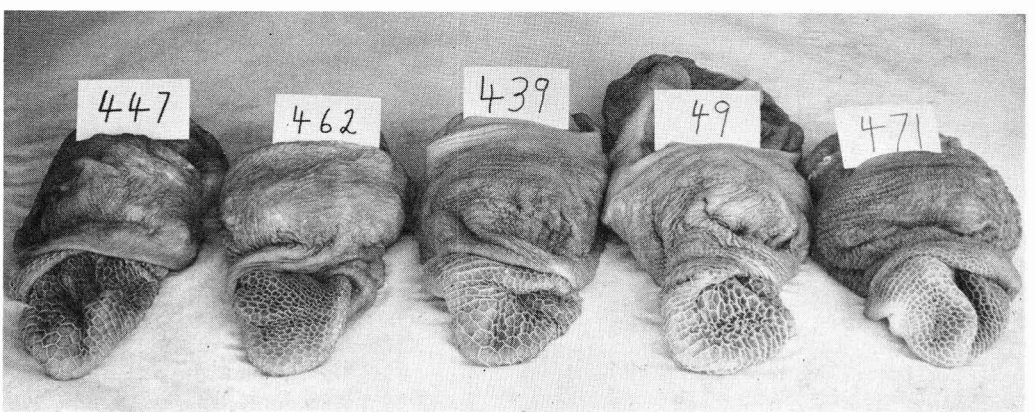

Fig. 1. Development of the rumen and reticulum of milk-fed calves.

(2) MARSHALL, S. P., ARNOLD, P. T. D., BECKER, R. B., AND WING, J. M. Post Partum Development of Bovine Stomach Compartments and Observations on Some Characteristics of Their Contents. Florida Agr. Expt. Sta., Ann. Rept. 73. 1955.
(3) SANDER, E. G., WARNER, R. G., AND LOOSLI, J. K. Effect of Administering Several Absorbable Chemical Compounds on the Development of Rumen Papillae in the Young Dairy Calf. J. Dairy Sci., 42: 1600. 1959.
(4) SWANSON, E. W., AND HARRIS, J. D., JR. Development of Rumination in the Young Calf. J. Dairy Sci., 41: 1768. 1958.
(5) TOMATE, H., MCGILLIARD, A. D., GETTY, R., AND JACOBSON, N. L. Effect of Various Substances on the Anatomical Development of the Rumen in the Calf. J. Dairy Sci., 42: 921. 1959.

CALCIUM AND MAGNESIUM DETERMINATION IN BOVINE BLOOD BY EDTA TITRATION

This study was undertaken in an attempt to establish a method for calcium and magnesium determinations which would be rapid and accurate for the routine determinations of these ions in cows' blood. A procedure which does not require the removal of serum proteins would simplify these determinations. Several methods have been reported for the determination of blood serum calcium by titration with ethylenediaminetetraacetate (EDTA) (1, 4). A method for estimating blood levels of both calcium and magnesium with this reagent was reported by Todd (5), after completion of the trials reported here. The determination of calcium and magnesium in other biological materials, together with the complexometric reactions involved, has been reported (2, 6, 7).

A total of 43 blood samples were collected for calcium analyses from 26 different dairy cows and heifers of the Holstein, Jersey, and Guernsey breeds. No samples were included from cows affected with milk fever. Magnesium determinations were made on 23 samples obtained from 16 different cows and heifers. The oxalate precipitate method of Clark and Collip, as outlined by Hawk, Oser, and Summerson (3) was selected as the standard method for calcium determination for comparison with the EDTA[1] method. Magnesium determinations were compared with the magnesium–ammonium phosphate precipitate method of Denis, as outlined by Hawk, Oser, and Summerson (3).

All the blood serum from each cow which was available after centrifugation was mixed, and served as the sample for determination by both methods. The results reported here are based on duplicate analyses for each sample (a total of 172 calcium and 92 magnesium determinations).

Calcium determinations were made by titration with EDTA, using Cal-Red[2] as the indicator. The interference of magnesium was prevented by precipitating it as the hydroxide at pH values of 12 to 12.5.

The EDTA titration method for calcium was as follows: To 2 ml. of blood serum was added 18 ml. of water and the pH adjusted to 12 with a 1% NaOH solution immediately before titration. The pH adjustment on each sample in this study was made with a Beckman pH meter, although, for routine work, a predetermined amount (about 4 ml.) of the NaOH solution will be satisfactory for all samples of this size. Sufficient Cal-Red indicator (prepared by mixing 200 mg. of indicator with 30 ml. of 95% ethanol and filtering) was added to produce a reddish tint. The solution was titrated with 0.002 *M* EDTA to a definite blue color. A standard 0.002 *M* calcium solution (prepared from A.R. $CaCO_3$) was used to standardize the EDTA solution. This solution was also added to blood serum for estimation of the percentage recovery.

Magnesium determinations were also made by titration with EDTA, using Eriochrome Black T[3] as the indicator after precipitation of the calcium as the tungstate. Magnesium was determined by adding to 2 ml. of blood serum, 18 ml. of water, 10 ml. of an NH_4Cl–NH_4OH buffer (pH 10.2), and 15 ml. of 20% sodium tungstate. The solution was boiled for 2 to 3 min., cooled, and 10 ml. of buffer solution was added and adjusted to pH 10 with 1% NaOH, if necessary, immediately prior to titration. A few drops of Eriochrome Black T indicator (prepared by mixing 50 mg. of indicator with 20 ml. of 95% ethanol and filtering) were added and the samples titrated to the first definite blue color.

The mean calcium values for the 86 samples were 11.74 and 11.72 mg.% for the EDTA

[1] Disodium ethylenediamine tetraacetate, Fisher Scientific Co., Fairlawn, New Jersey.
[2] Obtained from Scientific Service Lab. Inc., Box 175, Dallas, Texas.
[3] Obtained from J. T. Baker Chemical Co., Phillipsburg, New Jersey.

and the Clark and Collip methods, respectively. The standard deviation of the individual differences between the two methods (based on the means of two replicate determinations) was calculated to be 0.34. The average difference between the two methods was .02 mg.% with a standard error of .05, giving a nonsignificant value for "t" of .40. The correlation between these two methods was calculated to be 0.95. The repeatability calculated from the variance components (Table 1) was found to be 0.98 for each method.

Calcium recovery studies with solutions ranging from 5 to 20 mg.% showed a mean recovery of 99.6% for the EDTA method and 92.8% for the Clark and Collip method.

The mean serum magnesium values for 46 samples were 2.03 and 2.11 mg.% for EDTA and the Denis method, respectively. The standard deviation of the individual differences between the two methods (based on the means of two replicate determinations) was calculated to be 0.28. The average difference between the two methods was 0.08 mg.% with a standard error of .06 giving, again, a nonsignificant value for "t" of 1.37. The correlation between these two methods was calculated to be 0.58. The repeatability for the EDTA method calculated from the variance components was found to be 0.98, whereas the value for the Denis method was only 0.85. However, it should be pointed out that the low correlation and the low repeatability for the Denis method are in each case due to extreme variation in one set of values. The repeatability is higher (.94) if this set of values is eliminated, but still does not equal the value of .98 for the EDTA method, indicating that the latter method gives the more consistent results. Similarly, if the discrepant pair of observations is omitted, the correlation between the two methods rises from .58 to .87.

No recovery studies were made on magnesium for want of a satisfactory magnesium standard.

The results of this study show that a simple and rapid estimation of calcium and magnesium in bovine blood serum can be carried out by titration with EDTA, using the two indicators listed above. These determinations can be made in a matter of minutes as compared to several hours for the standard methods. The color changes for both titration end points are quite definite; however, these end points are not always apparent in the first few trials.

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REFERENCES

(1) BETT, ISOBEL M., AND FRASER, G. P. A Rapid Micromethod for Determining Serum Calcium. Biochem. J., 68: 13P. 1958.
(2) GEHRKE, C. W., AFFSPRUNG, H. E., AND LEE, Y. C. Direct Ethylenediaminetetraacetate Titration Methods for Calcium and Magnesium. Anal. Chem., 26: 1944. 1954.
(3) HAWK, P. B., OSER, B. L., AND SUMMERSON, W. H. Practical Physiological Chemistry. 13th ed. The Blakiston Co., Inc., New York. 1954.
(4) POLIN, D., STURKIE, P. D., AND HUNSAKER, W. The Blood Calcium Response of the Chicken to Parathyroid Extracts. Endrocrinology, 60: 1. 1957.
(5) TODD, J. R. The Determination of Calcium and Magnesium in Blood Plasma by Direct Titration. Brit. Vet. J., 115: 1. 1959.
(6) YOUNG, A., AND SWEET, T. R. Complexes of Eriochrome Black T with Calcium and Magnesium. Anal. Chem., 27: 418. 1955.
(7) YOUNG, A., SWEET, T. R., AND BAKER, B. B. Simultaneous Colorimetric Determination of Calcium and Magnesium. Anal. Chem., 27: 356. 1955.

TABLE 1
Analysis of variance of calcium and magnesium methods

	Source of variation	df	SS	M.S.	Component of variance
Calcium					
Clark and Collip Method					
	Between samples	42	78.321	1.865	0.923
	Within samples	43	0.805	0.0187	0.0187
EDTA Method					
	Between samples	42	94.010	2.238	1.110
	Within samples	43	0.742	0.0173	0.0173
Magnesium					
Denis Method					
	Between samples	22	4.471	0.203	0.0931
	Within samples	23	0.390	0.0170	0.0170
EDTA Method					
	Between samples	22	3.926	0.178	0.088
	Within samples	23	0.0426	0.0019	0.0019

FERTILITY RESULTS FROM THE USE OF BOVINE SEMEN IN A CARBON DIOXIDE EXTENDER[1]

Increasing the fertile life of spermatozoa in liquid extender has been a significant problem in the field of artificial insemination. Numerous extenders have been used in an attempt to solve this problem. Petrov and Schneerson (3) stated that carbon dioxide in an undiluted ejaculate brought the semen into a relative state of anabiosis. The use of $NAHCO_3$ at 0° C. was found by Schroder and Ramenskaja (5) to produce anabiosis. Milovanov and Hhabibulin (2) concluded anabiosis was brought about by the accumulation of CO_2 and its narcotizing effect on the spermatozoa. Trials conducted by Shettles (7) indicated that human spermatozoa could be completely immobilized by the use of CO_2. Motility was restored when the CO_2 was replaced by O_2 or air. Salisbury and VanDemark (4) concluded that the rate of glycolytic activity following inhibition with CO_2 was directly related to the completeness of the initial inhibition. A preliminary investigation on the use of the IIllinois Variable Temperature CO_2 extender was reported in 1957 by VanDemark and Sharma (9). The conception rate reported was 75.7% nonreturns on semen stored at room temperature for as long as six to seven days. Trials conducted by Swanson and McFee (8) indicated that the YCCG CO_2 extender was superior to the IVT extender. Scott and Hardenbrook (6) obtained a satisfactory conception rate with the IVT extender for as long as eight days. Dunn and Foote (1) found that the IVT extender did not compare favorably with the standard egg yolk–citrate extender.

Trials conducted by the University of Arkansas in cooperation with the Arkansas Dairy Breeders Association resulted in the successful preservation of fertile bovine spermatozoa for a period of seven days after collection.

The Arkansas extender No. 2 contained 1.0 g. glycine, 0.3 g. glucose, 0.21 g. sodium bicarbonate, 0.154 g. glutathione (reduced), 1.0 g. sodium citrate dihydrate, 0.3 g. sufanilimide, 100,000 units penicillin and streptomycin, 11 ml. of egg yolk, and 100 ml. of distilled water.

The CO_2 was incorporated into the extender with a pressure vacuum pump and measured by a spirometer at 4° C. A pH value of 6.35 was obtained by adding 74 ml. of CO_2 to 100 ml. of extender. The fresh semen was collected and allowed to cool at refrigerated temperature and added to the extender immediately after the CO_2 was incorporated. The extended semen was then stored at 4° C. in 8-cc. plastic vials.

A preliminary trial was conducted by comparing the nonreturn rate of the Arkansas No.

[1] Published with the approval of the director of the Arkansas Agricultural Experiment Station.

TABLE 1

Conception report for 60-90 day nonreturn with Arkansas CO_2 extender no. 2[a]

Breed	Age of semen in days														7-Day average	
	1		2		3		4		5		6		7			
	1st Service	% Non-return	1st Service	% Non-return	1st Service	% Non-return	1st Service	% Non-return	1st Service	% Non-return	1st Service	% Non-return	1st Service	% Non-return	1st Service	% Non-return
Angus	105	78.1	295	79.3	248	77.4	212	68.4	96	62.5	23	87.3	17	58.8	996	74.4
Guernsey	115	77.4	299	67.9	311	68.2	266	60.2	130	57.7	36	63.9	18	66.7	1,175	65.9
Holstein	127	78.7	547	77.9	581	69.2	508	65.2	294	66.7	71	67.6	38	60.5	2,166	70.5
Jersey	148	74.3	444	73.9	435	74.5	421	62.7	254	64.2	56	44.6	33	78.8	1,791	69.2
Milking S.H.[b]	45	73.3	131	80.9	138	75.4	120	67.5	59	52.5	18	66.7	3	100.0	515	71.9
Subtotal	540	76.7	1,716	75.6	1,713	72.0	1,527	64.2	833	63.0	204	61.8	110	67.3	6,643	70.0
[c]	*184	80.0	577	76.0	559	73.0	478	74.0	177	64.0	30	70.0	9	67.0	2,014	73.7
Total	724	77.5	2,293	75.7	2,272	72.2	2,005	66.5	1,010	63.2	234	62.8	119	67.3	8,657	70.9

[a] August, 1958, to June, 1959.
[b] Short Horn.
[c] First services not calculated by breeds.

TABLE 2
Conception report for 60-90 day nonreturn with egg yolk–citrate extender[a]

Age of semen in days									
1		2		3		4		4-Day average	
1st Service	% Non-return	1st Service	% Non-return	1st Service	% Non-return	1st Service	% Non-return	1st Service	% Non-return
279	73.8	646	67.5	506	62.3	50	60.0	1,481	66.6

[a] August, 1958, to November, 1958.

2 CO_2 extender with the standard egg yolk–citrate extender. The four-day 60-90 day nonreturn rate was 67.3% for 156 first services with the CO_2 extender as compared to 65.0 for 585 first services with the egg yolk–citrate extender. The data (Table 1) were obtained from field trials conducted from August, 1958, to June, 1959. All samples were stored at 4° C. and shipped to technicians of the Arkansas Dairy Breeders Association. A total of 6,643 first services was analyzed by breeds and age of the semen. An additional 2,014 first services were analyzed on the age of semen only. The nonreturn rate of 8,657 first services on semen used for seven days was 70.9%. For the corresponding months the egg yolk–citrate extender (Table 2) had a nonreturn rate of 66.6% on 1,481 first services with semen used for four days after collection.

The increase in per cent nonreturns and the length of use of the CO_2 extender over the egg yolk–citrate extender demonstrates the potential of this new extender. Trials are currently being conducted to perfect a method of adding CO_2 by a chemical process.

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REFERENCES

(1) DUNN, H. O., AND FOOTE, R. H. Fertility of Bull Semen Stored at Room Temperatures for Six Days. J. Dairy Sci., 41: 732. 1958.
(2) MILOVANOV, V., AND HHABIBULIN, K. Anabiosis of Spermatozoa and Its Utilization in Socialistic Animal Breeding. Problemy Zhivotnovodstva, 5: 83. 1933. (From Animal Breed. Abstr., 1: 225. 1933.)
(3) PETROV, E. F., AND SCHNEERSON, M. S. The Keeping of the Sperm Fluid in the Diluter GFK-4. Proc. Lening Inst. Dairy Cattle Breed., 1: 93. 1933.
(4) SALISBURY, G. W., AND VANDEMARK, N. L. Carbon Dioxide as a Reversible Inhibitor of Spermatozoan Metabolism. Nature, 180: 989. 1957.
(5) SCHRODER, V., AND RAMENSKAJA, C. T. Physico Chemical Analysis in Some Questions of the Physiology of Spermatozoa. II. Is Anabiosis of Horse Sperm Possible? Biol. Zhur., 1 (516): 13. 1932. (From Animal Breed. Abstr., 1: 233. 1933.)
(6) SCOTT, T. V., AND HARDENBROOK, H. T. Studies on the Use of Illini Variable Temperature Semen Diluent in Beef Cattle. J. Am. Vet. Med. Assoc., 133 (8): 432. 1958.
(7) SHETTLES, L. B. The Respiration of Human Spermatozoa and Their Response to Various Gases and Low Temperatures. Am. J. Physiol., 128: 408. 1940.
(8) SWANSON, E. W., AND MCFEE, A. F. Putting Bull Power to Work. A. I. Digest, 7 (10): 10. 1959.
(9) VANDEMARK, N. L., AND SHARMA, V. D. Preliminary Fertility Results from the Preservation of Bovine Semen at Room Temperatures. J. Dairy Sci., 40: 438. 1957.

INTERPRETIVE SUMMARIES OF PAPERS

CHARACTERISTICS OF PROTEIN FRACTIONS ISOLATED FROM THE FAT/PLASMA INTERFACE OF HOMOGENIZED MILK[1]

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Homogenization of milk results in a resurfacing of the fat with the proteins normally found in skimmilk. Of these, casein formed the major part of the new membrane proteins. However, in being adsorbed on the fat surface, casein was associated with a lipid fraction which was observed in the electrophoretic pattern as a trailing shoulder on the leading electrophoretic peak recognized as α-casein. Conceivably, this casein-lipid association could contribute to the characteristic properties of homogenized milk.

[1] Journal Article 2575, Michigan Agricultural Experiment Station, East Lansing.

SPECTROPHOTOMETRIC DETERMINATION OF FLURORAL AND URANINE IN MILK

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Antibiotics are introduced into a milk supply by using milk from cows which have been treated with veterinary antibiotic preparations. A simple method of detecting antibiotic-containing milk is needed. The incorporation of two fluorescent dyes, one fat-soluble (fluroral) and one water-soluble (uranine), as markers has been proposed for this purpose. These dyes tint the cream and skimmilk portions of milk from five to nine milkings after treatment, with a yellow-green color which fluoresces brilliantly under an ultraviolet lamp. To study this procedure a quantitative value for the amount of dye and antibiotic in the milk was needed. The purpose of this study was to devise simple and rapid spectrophotometric methods for determining the fluroral and uranine content of milk.

The water-soluble dye, uranine, is determined by precipitating the milk proteins, filtering the solution, and measuring the color of the filtrate in a spectrophotometer.

The fat-soluble dye, fluroral, is extracted with ether and the color of the ether solution is measured in a spectrophotometer.

Duplicate uranine determinations agree within 5% and fluroral duplicates within 8% of each other. Recovery values, obtained by adding known amounts of uranine and fluroral to milk, range from 94 to 103%.

Each of these methods was used to analyze a large number of milk samples obtained from cows that were treated with antibiotic preparations containing

125 mg. of each dye. A wide range of dye concentrations was encountered, from 2 to 500 μg. of dye per milliliter of milk, but the methods were accurate and satisfactory over the entire range.

STRONTIUM IN MILK. III. DISTRIBUTION IN CREAM, SKIMMILK, CHEDDAR CHEESE, AND WHEY

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Milk has received considerable attention as a source of Sr^{90} in the human diet. Strontium is similar to calcium in its deposition in the body and in its chemical action. One purpose of this investigation was to determine the ratios of Sr to Ca in cream and skimmilk and to compare these ratios with that in the original milk. The other purpose was to determine ratios in Cheddar Cheese and whey and to compare them with the milk from which they came.

Cream of about 40% fat content and the resulting skimmilk were found to have a Sr/Ca ratio approximately the same as the milk from which they came. The cream was rediluted and reseparated several times and the fat portion was reduced materially in strontium content.

Due to the concentration of the milk solids in cheese making, the strontium per gram of material is greater in cheese than in milk. The strontium tended to concentrate slightly more in the curd than did the calcium.

OBSERVATIONS ON THE CREAMING OF COTTAGE CHEESE

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Consumers judge the quality of Cottage Cheese by appearance and firmness as much as by flavor. Hard or mushy curd, over-creaming, lack of cream, or free whey in the carton may cause complaints. In this study, a draining test was devised to measure free liquid in cartons of creamed Cottage Cheese so that the influence of some factors affecting the retention of dressing might be observed and related to curd firmness. The test consisted essentially of a funnel in which exactly 12 oz. of creamed curd could be drained on a screen so that the free liquid, which might be cream or whey, or both, might be measured. When curd was tempered to 50° F. and then allowed to drain for 30 min. in the test funnel device, the majority of duplicate tests would usually agree within a few grams.

It was soon found in experiments with large curd that factors which tended to increase the amount of dressing retained by the curd also decreased the firmness of the curd. Such variables included increases in time of holding curd after creaming, breakage of curd, increases of salt up to 2%, increases in pH, and special homogenizing treatments or use of thickening agents like gelatin in the dressing. Increasing the amount of fat in the creamed Cottage Cheese by adding more dressing made the curd retain more dressing per 100 g. of curd and decreased the firmness of the curd, but of course more dressing drained off in the test. Increasing the fat in the dressing and keeping the fat in the creamed curd the same increased the dressing retained per 100 g. of curd and it slightly increased the firmness of the curd.

Certain general observations can be made from this series of observations. The addition of dressing to curd lowered curd firmness and increased the pH. Marked decreases in curd firmness were often accompanied by increases in the amount of dressing retained. Whenever the dressing was treated in some way to increase its viscosity, there was a marked increase in cream retained and usually some decrease in curd firmness.

The data indicate that two separate phenomena probably explain the retention of dressing by curd: one is the absorption of serum from the dressing by the curd; the other is the physical clinging of dressing to curd, an action which appears to be influenced primarily by the viscosity of the dressing. The data do not clearly show why the firmness of the Cottage Cheese is affected by creaming. It may be that firmness is related to those factors which cause the curd to swell, as it might in absorbing serum from the dressing, or by factors which cause the curd to shring, as it might if it were treated with excessive amounts of salt. Certainly, many interrelated factors are involved.

This study shows the trends of effects but has not measured them in such a way that they can be used for specific controls of the quality of the creamed Cottage Cheese. Such controls may be developed only by careful observations and extended measurements. Until that can be done, the control of retention of cream and firmness of curd must continue to be largely regulated by empirical methods and the skilled art of men responsible for producing creamed Cottage Cheese of high quality.

RELATION BETWEEN COMPOSITION AND CONSUMER ACCEPTANCE OF MILK BEVERAGES

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The addition of solids-not-fat (SNF) to milk beverages is not provided for in most legal standards. There is a general awareness, however, that the addition of from 0.5 to 1.0% SNF to either whole, low-fat, or nonfat milk beverages improves palatability, flavor, food value, and utilization.

A consumer preference study has been conducted to provide statistically reliable data on which standards and practices relating to the optimum composition of milk beverages can be based.

Taste discrimination or threshold tests using paired and triangulation techniques were conducted with adult men and women who were not trained dairy products judges. The results showed that many people can consistently differentiate between milk beverages with variations in fat and SNF contents of 0.5 and 1.0%, respectively.

Paired preference comparisons within and between each beverage class (whole, low-fat, and nonfat beverages) were then made with approximately 14,300 persons in retail food markets, public gatherings, and public schools. The addition of 1.0% SNF caused a highly significant improvement in the consumer acceptance of whole, low-fat, or nonfat milk beverages. There was a slight but significant preference for a fortified low-fat beverage compared with a nonfortified regular whole milk. No significant difference was indicated in the preference for a nonfortified low-fat beverage compared with a fortified nonfat product. A slight but significantly greater preference was shown for a regular fortified whole milk compared with a nonfortified higher fat milk.

ERRORS IN ESTIMATION OF LACTATIONAL YIELDS OF MILK, FAT, AND SOLIDS-NOT-FAT FROM INDIVIDUAL COWS

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The sampling errors in estimation of milk and fat yields of individual cows have been investigated by several workers. Likewise, the precision of the Babcock test is well known. However, no investigations have been made of the errors in estimating percentages and yields of solids-not-fat. Furthermore, no reports on the final or over-all errors of estimate, even for milk or fat, were found in the literature. For these reasons, a sampling investigation on individual cows was undertaken.

Individual milkings from 12 Holstein-Friesian cows were weighed and sampled daily through entire lactation periods. One-day composite samples were prepared. These samples were analyzed for fat by the Babcock procedure and solids-not-fat by the Watson lactometric procedure. Occasional groups of samples also were analyzed for total solids by the official A.O.A.C. gravimetric method.

The Watson lactometric procedure was found to be of comparable precision to the A. O. A. C. method, and to give practically unbiased results over whole lactations. The large and small model Watson lactometers gave results that did not differ significantly. The Watson lactometric procedure for solids-not-fat appears to equal or exceed, in precision and accuracy, the Babcock test for fat. Thus, the Watson lactometric procedure should be acceptable for use wherever the Babcock test is used at present.

The use of one-day samples, taken at monthly intervals and on median days, gave unbiased estimates of lactational yields of milk, fat, and solids-not-fat. The taking of samples as much as three days before or after the median day did not lend bias to the estimated yields. These sampling procedures, which are widely used in production recording of dairy cows, appear to be valid as well as practical.

The final or total error of estimate, for each percentage and yield, was found to consist principally of sampling error. The sampling errors for yields of fat and solids-not-fat, when expressed on a comparable basis, did not differ significantly. Therefore, it is concluded that sampling at monthly intervals for yields of solids-not-fat is quite as accurate as for yields of fat. This result suggests that production recording for yields of solids-not-fat may be legitimately included in the programs of dairy breed associations and Dairy Herd Improvement Associations.

The relative error of estimate for percentage of solids-not-fat was found to be much smaller than the error for percentage of fat. Thus, it may be permissible to sample cows less frequently for percentage of solids-not-fat than for percentage of fat. This result, however, has only limited application, since cows are normally sampled for yields, as well.

GENETIC IMPROVEMENT IN PRODUCTION ATTRIBUTABLE TO SIRES USED IN ARTIFICIAL INSEMINATION IN NORTH CAROLINA

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This study was concerned with an evaluation of the genetic improvement in production attributable to sires used in artificial insemination in North Carolina, and with an examination of the magnitude of the bias introduced when various methods of evaluation are used. A total of 6,888 North Carolina DHIA and HIR records initiated between January, 1948, and January, 1955, for herds using the state-wide artificial insemination program was utilized.

Each year was divided on the basis of high and low consecutive 6-mo. groupings, in an attempt to eliminate as much of the seasonal influence on production as possible. Subdividing the year into periods of from June through November, as compared with December through May, appeared to remove most of the seasonal differences.

The first-lactation contemporary comparison of artificially sired versus naturally sired progeny within seasons was used to evaluate the influence of artificial insemination. The animals resulting from artificial insemination appear to have been genetically superior, as was indicated by an increase in production of 366 lb. milk and 15.7 lb. of fat per lactation over their naturally sired contemporaries.

More contemporary comparisons can be made if the records of all contemporary naturally sired animals are used to compare with the first lactations of

the artificially sired ones. However, this would mean that the artificially sired animals would be compared with naturally sired animals, part of which had survived culling on the basis of their past performance. Two comparisons were made in this way to examine the effect which culling might have had on the results obtained from these data.

The first of these comparisons was between the first lactations of artificially sired animals used in the original summary and all the contemporary records on their naturally sired herd-mates. The apparent increase was reduced by 90 lb. milk and 2.4 lb. fat when compared with the results of the first-lactation contemporary comparison.

The importance of the selection bias, imposed by using second and later lactation records of naturally sired progeny, became even more evident when all possible contemporary comparisons were made using the first lactations of artificially sired animals with all available contemporary records on their naturally sired herd-mates. Included in this summary were many artificially sired animals that were omitted from the first analysis because no first-lactation naturally sired contemporaries were available. The apparent bias increased from 90 to 120 lb. for milk and from 2.4 to 3.1 lb. for fat.

The difference in the results for the two comparisons is due to the absence of first-lactation naturally sired contemporaries in some of the comparisons in the second study. In these cases, the unselected artificially sired animals are compared with naturally sired animals, all of which have been selected to remain in the herd on the basis of one or more previous records. Under these conditions, a sire whose daughters' first records even equal their contemporaries actually may be a desirable sire. Older sires, whose daughters had completed their second and later lactations, would be affected less severely by these circumstances.

The superiority of the artificially sired progeny in this study exceeded that reported in previous studies. For the most part, the results reported have not reflected the anticipated increase in production from the use of artificial insemination that was predicted initially. If the artificial insemination program is to become of real value in herd improvement, more efficient methods of proving and selecting sires must be put into practice.

AN APPROACH TO A RAPID TEST FOR ANTIBIOTICS IN MILK

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A new rapid test for antibiotics in milk makes use of filter paper assay discs as holders, whereby the cells of the bacterial test culture are packed together. Cells from broth cultures of *Streptococcus cremoris* (806) are placed on to the 0.5-in. discs. Each disc is then covered with a dry disc and the milk sample is added to give complete saturation. Following an incubation period of 10 min., 2, 3, 5-triphenyl tetrazolium chloride is added and the discs are observed for the development of a pink color.

Using this technique, reduction, as indicated by the pink color on the discs, occurred in 20-30 min. with control milk, while milk containing terramycin required a longer time for color development. This test is rapid and simple to perform. Its applicability for detecting inhibitory substances in milk, other than terramycin, is under investigation.

IMMATURE FORAGE MIXTURES WITH CITRUS PULP VERSUS MORE MATURE FORAGE WITHOUT ADDITIVE FOR SILAGE

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It has been well established that the stage of maturity at which forage is harvested has a tremendous influence on yields per acre and nutritional value. When the forage is being used for silage, the stage of maturity also may have a considerable effect on the nature of the fermentation and on the nutrient losses in the process. In an attempt to improve the type of fermentation and/or reduce the nutrient losses, various additives are frequently combined with forages which are being ensiled. It is commonly believed that these have their greatest advantage when used with immature, high-moisture forage. At more mature stages the additives are not considered to be of as much benefit.

In this study immature forages plus citrus pulp were compared with more mature forages without citrus pulp. Citrus pulp is an additive which is frequently recommended as a conditioner-preservative to absorb excess moisture in immature, high-moisture forage. The two mixtures which were compared were rye grass, crimson clover, and rye, versus rye grass, crimson clover, and oats.

The four combinations were compared for yields per acre, nutrient losses in the ensiling process, chemical changes and performance of lactating cows which were fed the silages. The more mature mixtures yielded more dry matter per acre and had lower ensiling losses. However, of the same mixture, the immature forages plus citrus pulp resulted in a higher milk production and weight gains of the cows. At the same stage of maturity the rye mixture gave a higher yield per acre than did the non-rye mixture. The more mature non-rye mixture resulted in a considerably higher yield of silage dry matter per acre than did the immature rye-mixture. The milk production of cows fed these two silages was about equal. Thus, the mature non-rye mixture was superior to the immature non-rye mixture plus citrus pulp.

When all factors are considered, it appears that the more mature mixtures without additive were a more practical combination than the immature forages plus citrus pulp. The differences in milk production favoring the less mature forages were not sufficient to offset the cost of the citrus pulp, the lower dry matter yields per acre, and the increased nutrient loss by the ensiling process. The increased milk production of 11% from cows fed the more mature non-rye mixture appeared to be sufficient to offset the 9% larger yield per acre of silage dry matter from the comparable rye mixture.

A PLASTIC REPLICA-EMBEDDING AND STAINING TECHNIQUE FOR STUDYING THE BEHAVIOR OF MICROORGANISMS ON FOOD-CONTACT SURFACES

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The techniques usually employed to determine residual bacterial populations on food-contact surfaces following a given cleaning treatment are based upon macro-colony counts by direct agar submersion of the organisms which have grown directly on the surfaces, or upon the macro-colony counts of the residual organisms removed from the surfaces and subsequently cultured in agar media. While useful as general indicators of residual bacterial populations, these techniques account only for those organisms that can be recovered from the surfaces or that grow on the surface to produce colonies which are large enough to be seen with the unaided eye or at low magnifications. It is also important to have some idea of the types of surface features in which bacteria can become lodged, and how growth proceeds from these sites; information that can be obtained only by observing conditions at the surface itself. With such information, surface finishes can be developed which provide a minimum of bacterial-lodging sites, and upon which sanitizers and other bacterial controlling agents can be given optimum conditions for contact with the residual bacteria.

The plastic-replica technique provides a simple procedure for studying bacterial populations on food-contact surfaces. It consists of coating the surface in question with an acetone solution of a celloidin-nitrocellulose mixture which makes intimate contact with the finest features of the surface. After it has dried, the plastic film is removed to obtain an exact replica of every detail of the surface. Bacteria removed with the plastic film are fixed in position on the replica of the surface feature in which they had been trapped or where they had grown. The replica, a permanent record of the surface and the bacteria on it, can be easily mounted on microscope slides, stained by conventional methods to differentiate bacteria from other surface residues, and examined under the microscope as in routine bacteriological determinations. The technique may be employed to give useful information on residual bacterial populations before or after growth of the organisms on the surface in question.

RELATIVE VALUE OF CAROTENE AND VITAMIN A FED AT MEDIUM LEVELS IN A MILK REPLACER

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Finely dividing the particles of the oil carrier of vitamin A or carotene, the latter being the parent substance from which vitamin A is formed by the animal,

has improved their utilization as evidenced by increased blood plasma and liver concentrations of vitamin A. To determine the relative values of carotene and vitamin A when fed over a range of intakes in a milk replacer diet, 24, one-day-old male Holstein calves during the first week of life were changed from whole milk to a milk replacer which contained virtually no vitamin A activity. In this study, three forms of vitamin A were compared, namely, a regular vitamin A feeding oil and synthetic carotene and vitamin A which were dispersed in a finely divided state within a gelatin beadlet. When these beadlets are dispersed in water, a stable and relatively clear suspension of the ordinarily water-insoluble carotene and vitamin A is achieved. Beginning the eighth day, each calf received for three successive seven-day periods one of three levels of water-dispersible carotene 40, 80, or 160 γ or one of three levels of water-dispersible vitamin A, 9, 12, or 15 γ, or one of two levels of vitamin A feeding oil, 9 or 15 γ, per pound of live weight per day. When the plasma vitamin A concentration of calves on the different forms of vitamin A were compared, it was found that the water-dispersible carotene at the 40-γ intake level was about one-eighth as active as the water-dispersible vitamin A; this value decreased to one-eleventh at the 80-γ carotene intake level and to one-sixteenth at the 160-γ carotene intake level. The response to vitamin A feeding oil for both the 9 or 15 γ intakes was 42% that of the water-dispersible vitamin A.

These data may serve as approximations of the relative value of carotene to vitamin A in milk replacer–fed calves, but not as exact estimates due to numerous factors which influence the utilization of carotene or vitamin A. They confirm other studies reporting widening ratios of carotene to vitamin A as the intakes are increased, as well as the enhanced utilization of water-dispersible forms of vitamin A in studies with milk-fed calves and steers.

METABOLISM OF VOLATILE FATTY ACIDS BY THE PERFUSED LIVER OF COWS WITH KETOSIS

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A knowledge of the changes in metabolism during bovine ketosis should aid in developing adequate preventative measures. Toward this goal, studies of ketotic metabolism were undertaken. The livers of two ketotic cows were surgically isolated and supplied with blood by the use of a heart-lung apparatus. Volatile fatty acids, the materials resulting from digestion in the rumen, were added to the blood perfusing the organ. In each experiment one of the acids was labeled with radioactive carbon and its fate followed with this tracer.

The results indicated that the same metabolic pathways as in the normal animal were in operation. However, there appeared to be a decrease in the rate of many of these reactions. This was demonstrated by the decreased production of normal blood formic acid, and ketone bodies. This was also supported by

the decrease in utilization of volatile fatty acids. Earlier work demonstrated that the liver was normally the major source of blood ketone bodies. Since ketosis is characterized by very high levels of these ketone bodies, it was surprising to find a decreased liver production. The utilization of ketone bodies is not impaired during ketosis; therefore, the high levels must be a result of increased production. This raises an intriguing question as to their source in this disorder.

EFFECT OF LIQUID DIET ON DEVELOPMENT OF RUMEN PAPILLAE DURING THE FIRST ELEVEN MONTHS IN DAIRY STEERS[1]

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Observations of other workers with calves no older than 12 wk. of age indicated an absence of rumen papillae in those fed milk exclusively. This work included seven calves, two of which were fed hay and grain while the five others were fed milk fortified with vitamins and minerals through 11 mo. of age. All milk-fed calves consumed some wood shavings which were used as bedding.

Four experimental rumens were essentially smooth. One of the milk-fed calves showed some papillation of the rumen, but the tissues were subnormal when compared with the controls. This organ was from the only milk-fed calf which showed active rumen fermentation at the time of slaughter. The other compartments in all calves appeared normal, suggesting that rumen development varies with type of diet, but that development of the other compartments is a function of age.

FERTILITY RESULTS FROM THE USE OF BOVINE SEMEN IN A CARBON DIOXIDE EXTENDER

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The preservation of bovine spermatozoa in a satisfactory liquid extender has been an economic problem in artificial breeding. Recently, carbon dioxide has been used for storing bovine semen under anaerobic conditions. The results of its use have been variable.

The purpose of this investigation was to determine the fertility of spermatozoa stored at 4° C. for seven days in a carbon dioxide extender. Successful preservation of semen would decrease shipping costs and provide better utilization of superior sires.

The 60-90 day nonreturn rate for 8,657 first services was 70.0% for the carbon dioxide extender used for seven days. The nonreturn rate for 1,481 first services was 66.6% for the egg yolk–citrate extender used for four days. There was an advantage in using the carbon dioxide extender.

OUR ASSOCIATION

OBSERVATIONS AND EXPERIENCES IN GERMANY

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Ever since hearing from our friends last December in regard to Dr. Hunziker's passing, I have intended writing to the American Dairy Science Association, but it seems we are always moving and there is never enough time for writing. We were very sorry that genial Dr. Hunziker was gone. We wrote to Mrs. Hunziker and were pleased to hear from her. The dairy journals in Europe paid high tribute to Dr. Hunziker, as he was probably better known here than any other American dairy scientist.

Already we have had many experiences. Mrs. Bendixen and I feel very grateful to have this opportunity to spend a year in Europe under a Fullbright research grant. Evehywhere we have been received in a most friendly manner and the weather has been excellent. The fall of 1959 was reported to have been the driest in 120 yr. of weather reporting. The entire winter has been very mild, enabling us to see much of Germany, especially since we acquired a car.

Immediately after our arrival in Bremerhaven last September, we were taken by car to Kiel and then the next day on another beautiful trip to the Harz Mountains, where a large dairy convention was in progress. Professor Schulz was on the program. He introduced me and at once I was on the stage of the convention hall speaking to over 700 German dairymen and government officials in my unpracticed German. However, they took it all graciously and later wined, dined, and entertained us royally at the evening's banquet. It happened that their main theme for the convention was a consideration of their system of dairy education and possible modifications, to better suit the needs of a highly advanced dairy manufacturing industry, a subject to which many of us in America have been giving considerable thought.

It seems that the emphasis in the dairy educational program in Germany in the past has been upon much practical training, together with vocational and technical dairy school courses of comparatively short duration. No one can direct the manufacturing and processing operation of a dairy plant unless he has a Dairy Master diploma. This is not an academic degree but is based upon extensive practical plant experience together with technical dairy school training. The general managers of large plants often have, in addition, academic degrees in agriculture, in the sciences, or in business economics.

The Dairy Master diploma may be obtained without university training. First of all a young man must go through 3 yr. of apprenticeship training in an approved plant under an approved Master. This training may begin after completing 9 yr. of common school at the age of 15 yr. or after completing 4 yr. of common school plus 6 yr. of middle school, offering a more elevated technical type of training and ending with what is called "Middle Maturity." In the case of students with Middle Maturity, the practical apprenticeship training may in some cases be reduced to 2-2-½ yr. Once annually for 3 yr. every apprentice must attend and pass a course of at least 1 mo. duration at a vocational dairy school. Attendance is at the expense of the plant to which the apprentice is assigned. The schools usually have excellent plant and laboratory equipment as well as dormitory and eating facilities for the students. The regular teaching staff is commonly supplemented by special outside lectures. At the end of the apprenticeship training period, during which the young man obtains experience in all phases of plant work under the guidance of his Master. He keeps a notebook over all operations and acquaints himself with technical dairy literature; he must pass an examination over all practical work in the plant before a committee, and oral and written tests at the dairy school. If, in addition, he can show good character references, he then receives his qualifying papers to serve as a plant assistant.

Usually he then serves 2½-3 yr. as an assistant in different plants and takes a continuation course of 3-4 mo. at the dairy school to qualify as an Advanced Assistant (Obermeier). Finally, after another period of 2½-3 yr. of plant employment he may take the 6-mo. Master course, including principally dairy plant management, economics, accounting, engineering problems, and also tours to see various types of dairies, dairy machinery factories, or other dairy establishments. Upon successfully passing the examinations in this course he receives his Dairy Master diploma (Meisterbrief). He then may obtain employment in a plant, usually as an assistant manager, and after a time may be advanced to a manager's position, if and when an opportunity develops.

College training for dairy plant personnel is comparatively rare. Middle Maturity, meaning completion of a Middle School, does not entitle a student to enter a university or col-

lege. To prepare for university training he must complete 4 yr. of common school plus 9 yr. in gymnasium, a higher level preparatory school, leading, after a stiff examination, to the "Abitur" of "Maturity." Universities, however, offer no specialized degree courses in dairying. Degrees may be obtained in agriculture, in the sciences, or in business and economics. Dairy degree courses have been proposed recently, but to date have not been approved in West Germany because of the fear of narrowing university training and of overspecialization. The philosophy prevails that specialization should come only after intensive broad education in the sciences or other disciplines. Consequently, specialized upper level dairy work usually occurs first during graduate training toward a Doctor degree. Some of the administrators of the larger dairy enterprises have received this type of training together with Dairy Master training and, of course, are very well prepared.

Most of the German dairy plants are cooperatives. Formerly they were mostly small local plants just as in the United States; however, plants in Germany have been increasing in size. City milk plants are usually very large organizations and there is today a definite trend for the smaller creameries to consolidate. Recently, for instance, 40 small butter factories in Schleswig-Holstein decided to concentrate all of their manufacturing operations at one plant and to convert the others into receiving and separating stations. This trend is likely to continue, especially in view of the developing European Economic Community (Common Market) which will place an ever-increasing premium on efficiency of production and automation.

Under these conditions the question arises in dairy circles here of whether, in the present system of dairy education, practical training might not have to be reduced somewhat, and theoretical training, especially in the fields of management, marketing, economics, law, etc. increased, even though the latter subjects have been stressed more and more recently in the vocational schools. At present a new type of dairy school is to be established at Hannover, which anticipates a 3-yr. curriculum, but which still will not have the right to grant an academic degree or prepare academic work at a college or university.

Thus, you find fewer people with college degrees in the dairy industry in Germany as compared with the United States. German universities have always strongly resisted departmentalization which, in my opinion, has advantages but also disadvantages. For instance, there seem to be no universities in Western Germany, where bacteriology is established as a major field. Usually, it is taught as a branch of botany. As a result, there seem to be comparatively few specialized teachers and research men in this important science related to dairying, even though the scientific work carried on at the German universities is, of course, on a very high level.

It is interesting to observe differences in educational organization and procedure. To date I still believe in the American plan, even though it is not perfect. In the near future we hope to visit Austria, Switzerland, Italy, and later also Belgium, Holland, and the Scandinavian countries. We have already booked a flight to Berlin and I have an invitation to visit the Humboldt University in East Berlin, where a degree course is now being offered in dairying. I was also asked to speak at the University of Madrid, Spain, but have not been able to work out a date so far.

At present Mrs. Bendixen and I are enjoying our visit in this little Bavarian town just north of Munich. The college of Agriculture and the college of Brewing of the Munich Technical College are located here. Research institutes for Dairy Production, Dairy Chemistry, Dairy Bacteriology, Dairy Engineering, and Dairy Economics are here. The Fritz continuous buttermaking machine was developed here and is now being improved. Soxhlet and Henkel worked here, and 100 yr. ago the earliest attempts at mechanical cream separation were made here. The Brewing College is probably the oldest and best known in the world. Beer is supposed to have been brewed as early as 1040 by the monks. The town of Freising is over 1200 yr. old. Its cathedral is gorgeous and we have enjoyed exploring the beautiful countryside, especially south of Munich. Munich itself, of course, is an important art center of the world.

BOOK REVIEWS

Nutrition Animale, Volume II, Données générales sur la Nutrition et l'Alimentation. Tome I. Métabolismes et Transits. By R. Jacquot, H. Le Bars, and H. Simonnet.

Nutrition Animale is the general title of a series of ten volumes scheduled to appear as part of the Nouvelle Encyclopédie Agricole which is being published by J. B. Baillière et Fils, Paris, under the direction of Jean Lefèvre and Pierre Tissot of l'Institute National Agronomique. The first three volumes bear the general title Données générales sur la Nutrition et l'Alimentation, of which Volume I, which contained sections on the principal nutrients and on digestion and digestibility, was reviewed. (Dairy Sci., 41: A19. 1958.). Volume II on metabolism and transport is to appear in two parts, of which this is the first.

In this volume are discussed at length the metabolism of carbohydrates, fats, and proteins and the transport of water and minerals. The first chapter deals with general pathways of metabolism and the concepts of high energy bonds as contained in certain acid anhydrides and elsewhere; and with enzymes, their classification, activation, and inhibition. Perhaps the most interesting facet of this volume is the attempt to tie together the classical concepts of nutrition and of physiology with the latest knowledge in biochemistry. At times, this provides rather startling contrasts in thought and methodology.

The chapter on carbohydrate metabolism covers the types and levels of carbohydrates in the blood of the monogastric animal, the ruminant, and the fetus, and in the liver and muscles, etc., and in milk and eggs; the synthesis and breakdown of glycogen; tissue respiration and muscular work and an extensive treatment of carbohydrate metabolism in spermatozoa; the regulation of carbohydrate metabolism including mechanisms, agents (vitamins, endocrines, and nervous system); and a short section on the nutritional significance of carbohydrates.

The chapter on lipid metabolism covers the types and state of lipids in the body and in milk and eggs and discusses the dynamic state of body lipids, as well as their origin, synthesis, and breakdown. In discussing the central position of acetate in fat metabolism, utilization by the ruminant is briefly reviewed. The regulation of lipid metabolism is discussed with regard to lipid balance, lipotrophic factors, vitamins, and hormones. The chapter on protein and amino acid metabolism discusses the nature and levels of the proteins of the blood, the tissues, and of milk and eggs; such concepts as reserve proteins and the dynamic equilibrium of body proteins; the biosynthesis of the amino acids, including the microbial synthesis of the essential amino acids; the biosynthesis of proteins and nucleic acids; the origin of the various body proteins (e.g., fibrinogen, etc.) and of milk and egg proteins. Included also are sections on amino acid catabolism and the regulation (by hormones and vitamins) of protein metabolism, as well as a section on the nutritional importance of protein.

The chapter on water and mineral transport is devoted principally to a discussion of the movement of liquids (water and electrolytes) in the body and to the regulation of these exchanges.

One could point out numerous minor errors, such as the direct conversion of serine to alanine, the formula of glycine written CH_3-NH_2-COOH and so on; to some glaring omissions, such as the present-day concept of protein synthesis as proposed by Zamecnic, Lippmann *et al.*, involving amino acid activation, transfer to RNA and polymerization on a template and to others, but rather we should commend the authors on attempting and carrying out so large a task as this and on the general excellence of their organization and their contribution in putting the biochemical, the classical, and the physiological sides of nutrition together into a more or less integrated whole.

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Milk Sanitation Administration—Selected Lectures. P.H.S. Publication No. 728, 1960; 208 Pages.

This volume is a collection of lectures delivered at the 1959 course on Milk Sanitation Administration, conducted at the Communicable Disease Center, U. S. Public Health Service, Atlanta, Georgia.

Included are descriptions of the results of recent research in relation to the inactivation of pathogenic microorganisms in milk and milk products exposed to ultra-high temperatures. Other subjects covered are dairy plant sanitation; vector control procedures; milk-borne diseases, including those of animal origin; and the question of additives and foreign substances in milk. The possible relationship of milk and milk products to noninfectious disease is also explored.

Administrative programs of the Public Health Service as related to the state and local procedures for the milk industry are considered in detail. Other lectures deal with the operational program of the Public Health Service and with survey and laboratory certification procedures for interstate and intra-state shipments of milk and milk products.

Although primarily developed for state and local public health administrators, these selected papers should be particularly useful to universities, the dairy industry, the armed forces, and others who deal with problems in the realm of milk hygiene.

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