Journal of

DAIRY SCIENCE

Vol. 45 December, 1962 Number 12

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JOURNAL OF DAIRY SCIENCE

OFFICIAL PUBLICATION OF THE
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INSTRUCTIONS TO CONTRIBUTORS

The Style Manual for Biological Journals[1] has been adopted as the guide for authors.[2] Publication of papers is limited to members of A.D.S.A., but only one author of a joint paper need be a member. Those of outstanding merit may be accepted from nonmembers. Papers when accepted become the copyright of the JOURNAL and can be reprinted only by the Garrard Press. Reproduction of graphs, tables, and illustrations for books and other periodicals may be authorized by the Editor-in-Chief.

ORGANIZATION OF PAPERS

1. Title should appear at the top of the first page, be as brief as possible, and be indicative of the research, followed by the author(s) name(s) and affiliation(s).
2. Summary and its preparation.
   a. There are three reasons for the summary: first, convenience to readers; second, reduce costs and expedite work of abstracting journals; and third, to disseminate scientific information.
   b. The summary should be brief, specific, and factual. It should not exceed 200 to 225 words.
   c. The opening sentence should state the research objectives, but the title *should not be repeated.*
   d. It should be intelligible without reference to the original paper and contain complete sentences and standard terminologies. It should be assumed that the reader has some knowledge of the subject.
   e. The author(s) should emphasize newly discovered facts and observations, unique apparatus and techniques, numerical data with statistics, physical-chemical constants, and new methods and their accuracy.
   f. References to earlier work should be omitted, except in most unusual cases.
3. Statement of the problem, pertinent investigations, and reasons for the study.
4. Experimental procedures.
5. Results.
6. Discussion. (5 and 6 may be combined.)
7. Conclusions.
8. Acknowledgments.
9. References. All references must have author(s) name(s), name of periodical, volume, page number, and year of publication. If a book, publisher's name and address must be added.
10. Manuscripts must be typed double-spaced[3] on 8½- by 11-inch bond paper. Lines on each page should be numbered from 1 to 26 or 28, to make it easier for the Editorial Board to review papers. The side margins should be one inch wide. Clipped-to, pasted-on, and written insertions are not acceptable. Do not staple pages together.
11. Figures (graphs) should be made with black India ink on white drawing paper, tracing paper, or blue linen and the sheets should not exceed 8½ by 11 inches. Graph papers with yellow, green, and red lines should not be used, because the lines cannot be filtered out. Curves should be identified with the symbols ○, ⊙, ●, □, ■, △, ▲, ▼, +, or ×, and they should be about 0.8 mm thick, for the axes about 0.5 mm thick, and for grid lines about 0.4 mm thick. Grid lines are necessary only if readings are to be made from the curves. Letters on the abscissae, ordinate, and the figure should be in upper case and be about 3 by 5 mm and about 0.5 mm thick, to be readable when graphs are reduced to column width. Titles for figures (graphs) must be on separate sheets. Following is a well-made figure reduced to size of the printed page:

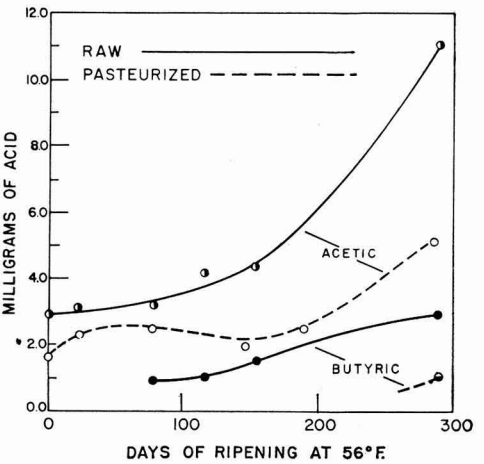

FIG. 1. Acetic and butyric acids in raw and pasteurized milk Cheddar cheese during ripening (milligrams in distillate obtained from 150 g of cheese oil).

12. Tables should be numbered on the center of the page with the title immediately below, and each table should be typed on a separate sheet of 8½- by 11-inch bond paper. They should be placed together at the end of the manuscript.

[1] American Institute for Biological Sciences, 2000 P Street, N. W., Washington, D. C. Price $3.

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Titles should indicate the content of tables and facilitate comparisons, show relationships clearly, be self-explanatory, and save space. Label heading and subheadings accurately and concisely with the data centered under them. Use correct abbreviated dimensions. Data should be referred to and discussed but not repeated in the text, and they should be presented in only significant digits within the accuracy of the methods. Use the metric system whenever possible. Do not use vertical lines and only a minimum of horizontal ones.

Tables are costly. Use graphs whenever possible.

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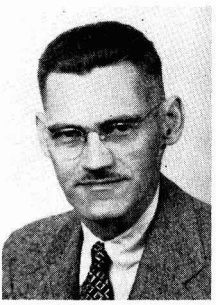
15. Terms such as Cottage cheese, Cheddar cheese, Limburger cheese, etc., should be capitalized as indicated. Butteroil, skimmilk, buttermilk, etc., should be written as one word. Milk fat has replaced butterfat.
16. Critical reading of papers, before they are submitted, by persons other than the author(s) will help to clarify statements and eliminate errors.
17. All manuscripts must be submitted to the Editor-in-Chief in duplicate.
18. Receipt of manuscripts will be acknowledged. Authors will be notified within 30 to 60 days of the action taken by the Editorial Staff.

PEOPLE AND EVENTS

F. J. Doan Retires

PROFESSOR FRANCIS J. DOAN has concluded a distinguished career of service to his university and the dairy industry. On June 30 he retired as Professor Emeritus at the Pennsylvania State University after more than 37 yr of continuous service in teaching and research.

Born in Philadelphia, Professor Doan received his education in the Philadelphia public schools and from the Pennsylvania State University, where he earned B.S. and M.S. degrees in Agriculture and Biological Chemistry.



F. J. Doan

Following service in World War I he joined the Nestlé Foods Products Company and worked as a chemist from 1919 to 1922. He then joined the teaching staff at the University of Maryland, where he taught Dairy Chemistry until joining the Penn State staff in 1925.

During his career at Penn State he carried out research work in a wide area of dairy products processing, analysis, nutritive value, keeping quality, and dairy chemistry, the results from which led to the publication of 65 research bulletins and scientific papers. He was author of numerous popular and review articles in the dairy technology and education field. He co-authored the Manual for Dairy Manufacturing Short-courses and is a contributing author in the Fundamentals of Dairy Science, now being assembled for publication.

Professor Doan has always been a highly competent teacher. He has taught courses in Market Milk, Concentrated Milks, and Judging of Dairy Products for over 30 yr and has assisted in many others when the need arose. He supervised and taught a Market Milk Short Course for over 35 yr and has assisted his associates with several other short courses each year. He coached the Penn State Dairy Products Judging Team for 23 yr and served as advisor to all junior class students for over 30 yr.

Throughout his career, he supervised the academic and thesis programs of 30 M.S. and Ph.D. candidates and was a close personal advisor of hundreds of undergraduate and graduate students. He has served Penn State in many important committees, both university and college, and his judgment has been frequently sought on academic and research matters. The design and layout of the Borland Laboratory building was largely delegated to him in 1929–32. The fact that the academic, research, and creamery sections of this building are still meeting the needs of a growing university and changing dairy industry confirms that he had good vision of the future.

Professor Doan has served the American Dairy Science Association in many capacities. Since 1926 he has been serving on A.D.S.A. Committees almost continuously. He was Chairman of the Eastern Section of A.D.S.A. in 1939; served on the Editorial Board of the Journal from 1954–61; and was named to the Journal Management Committee in 1958 and served as Chairman of that Committee in 1961. From 1959 to 1962 he served on the Executive Board.

He has been a member of many industry committees, including the Committee on Milk, Food and Nutrition Board, National Research Council from 1946–52, and has served on the Committee on Casein, American Society for Testing Materials since 1956.

He has received many honors, including the Borden Award in Dairy Manufacturing, 1949, and the Poor Richard Club Citation of Merit, 1950. The Penn State Dairy Science Club dedicated its 1962 Dairy Exposition to him as a gesture of appreciation from the students, and at their annual banquet this year the Pennsylvania Dairy Sanitarians honored Professor Doan for his many years of service as program chairman of the Annual Dairy Fieldmen's Conference. He was also honored by

his associates in the Dairy Science Department at Penn State.

Professor Doan and his wife are continuing to make their home at 711 Holmes Street, State College, Pennsylvania. He plans to carry on his active interest in the Dairy Science field and will do some traveling and writing. This, together with enduring interests in gardening, photography, bowling and other sports, will continue for him what has been a very active life.

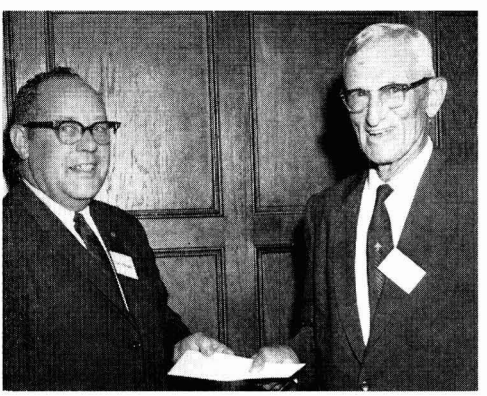
The American Dairy Science Association, we are sure, joins Professor Doan's associates in wishing him a long and happy career in retirement.

Dr. B. W. Hammer Honored

Dairy industry alumni of Iowa State University convened this fall for an enjoyable and successful reunion on the campus in Ames. The event was conceived and planned by a group of alumni in the Chicago area under the leadership of TOM G. MAYNARD and HARLIE ZIMMERMAN, both of Quality Chekd Dairy Products Association, and ORVAL H. AUSE and HAROLD BRACKET, both of H. C. Christians Company.

Apart from the fellowship which makes reunions pleasant affairs, this one became a signal event, in that the alumni chose to use the occasion to recognize a great and beloved former teacher. The reunion got under way Friday night, September 14, with a banquet honoring DR. B. W. HAMMER, who served as professor of Dairy Bacteriology at Iowa State from 1912 to 1944. More than 100 alumni and faculty members attended. Tom Maynard was toastmaster. During the program DR. RALPH W. HUSSONG, National Dairy Products Corporation, RALPH N. BAKER, Cherry-Burrell Corporation, C. Y. STEPHENS, High's Dairy, Washington, D. C., and DR. FLOYD ANDRE, Dean of the College of Agriculture, paid tribute to Dr. Hammer. DR. J. HILTON, President of Iowa State University, also attended the banquet. Highlight of the evening was the presentation by Orval Ause of a bound volume of letters of tribute from Dr. Hammer's former students. Dr. Hammer responded with an enjoyable little talk in which he not only reminisced about his work as teacher and investigator but also made some interesting observations about present and future dairy research

Orval Ause presents Dr. Hammer with volume of letters of appreciation. Dean Floyd Andre (seated) was among those who spoke during the program.



Tom Maynard presents Dr. Hammer with a gift from friends and former students.

and technology. Tom Maynard then presented Dr. Hammer with a gift from his friends and former students.

On Saturday morning the staff of the Department of Dairy and Food Industry conducted tours of the building which gave the alumni an opportunity to see the newly completed wing with facilities for food technology research. The remainder of the forenoon was given to a seminar in which DR. V. H. NIELSEN, Head of the Department of Dairy and Food Industry, discussed recruitment and training programs for the dairy industry. Members of the department faculty then entertained the alumni for lunch, whereupon hosts and guests went to Clyde Williams Field to watch the Iowa State Cyclones defeat the Drake University Bulldogs.

STUDENT NEWS SECTION

W. W. SNYDER, Editor

A Section Devoted to News of Student Members

Spotlight

The Dairy Science Club of Louisiana State University is the third club to be featured in a series of articles dealing with club histories and activities. This club has more than 30 yr of active service and is happy to share some of its outstanding accomplishments with other clubs.

The L.S.U. club won the first A.D.S.A. trophy at the annual meeting in 1959. It has been a very serious competitor since, by receiving the award of merit in both 1961 and 1962. Perhaps the activities and achievements of this club may serve to provide additional stimulation for higher goals and attainments in other affiliate chapters. —W. W. SNYDER.

The Dairy Science Club of Louisiana State University

Foundation of the Club. An idea was born in the minds of a group of foresighted dairy students at Louisiana State University a little over 30 yr ago that resulted in the formation of a dairy organization known as the Cream and Cow Club. The purpose of this organization was mainly to provide recreation and entertainment for students working on the dairy farm and in the creamery. According to Professor A. J. Gelpi, Jr., a faculty member at the time the club was formed, the boys were very enthusiastic about their big annual banquet and dance. The club also raised money to sponsor the first Dairy Products Judging Team in 1923 for the contest in Detroit, Michigan. The organization retained the original name until it received a formal charter in 1935 and became known as the L.S.U. Dairy Club. The activities and goals outlined by the new charter were much broader and more comprehensive.

The first photograph available that appeared in the 1939 edition of THE GUMBO, the L.S.U. student annual publication, reveals 63 members of the organization. An inscription underneath this photograph states that the Dairy Club was founded primarily to promote greater cooperative interest in the dairy industry. One can recognize today in this photograph a large number of very successful men including college professors, extension workers, lawyers, dairy farmers, and milk distributors.

The club did not function during World War II, but was soon reactivated after the end of the war. In 1956 the club became affiliated with the American Dairy Science Association and the same year won third place in the na-

tional chapter rating, and a certificate of merit. The first-place chapter award in 1959 was earned by the L.S.U. Dairy Science Club along with a cash award and a certificate of merit.

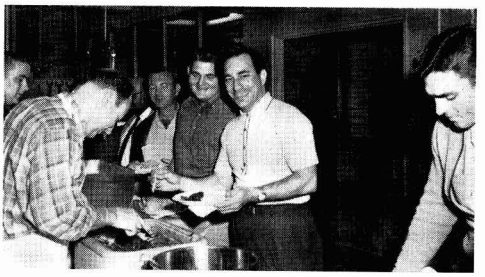
The publication of a formal annual entitled The Bayou Dairyman was a club activity for a number of years. One of the main purposes of the annual was to acquaint prospective L.S.U. students with the facilities, staff, and activities of the Dairy Science Department of the College of Agriculture, along with some information on the many types of careers available in the dairy industry to those who are qualified by aptitude and training.

Current aims of the Club. The purposes of the L.S.U. Dairy Science Club under the revised constitution of 1960–61 are:

1. To advance the dairy industry at L.S.U., throughout Louisiana and the nation.
2. To provide educational and recreational activities for students in the agricultural field interested in being members of the organization.
3. To encourage cooperative effort among the students and to maintain a feeling of mutual interest and enthusiasm in dairy work among club members and faculty members of the Dairy Science Department.
4. To develop friendship, promote scholarship, and leadership in the club members.
5. To strengthen the confidence of the student in himself and in his work.
6. To encourage and to support every bona fide organizational effort or program looking to the improvement and advancement of dairying.
7. To develop interest in the wise selection and judging of dairy animals, milk, and milk products.
8. To select an outstanding individual(s) in the field of dairying from Louisiana and to honor him (or them) at the Annual Banquet and Dance.
9. To select the outstanding Senior and Freshman each year.

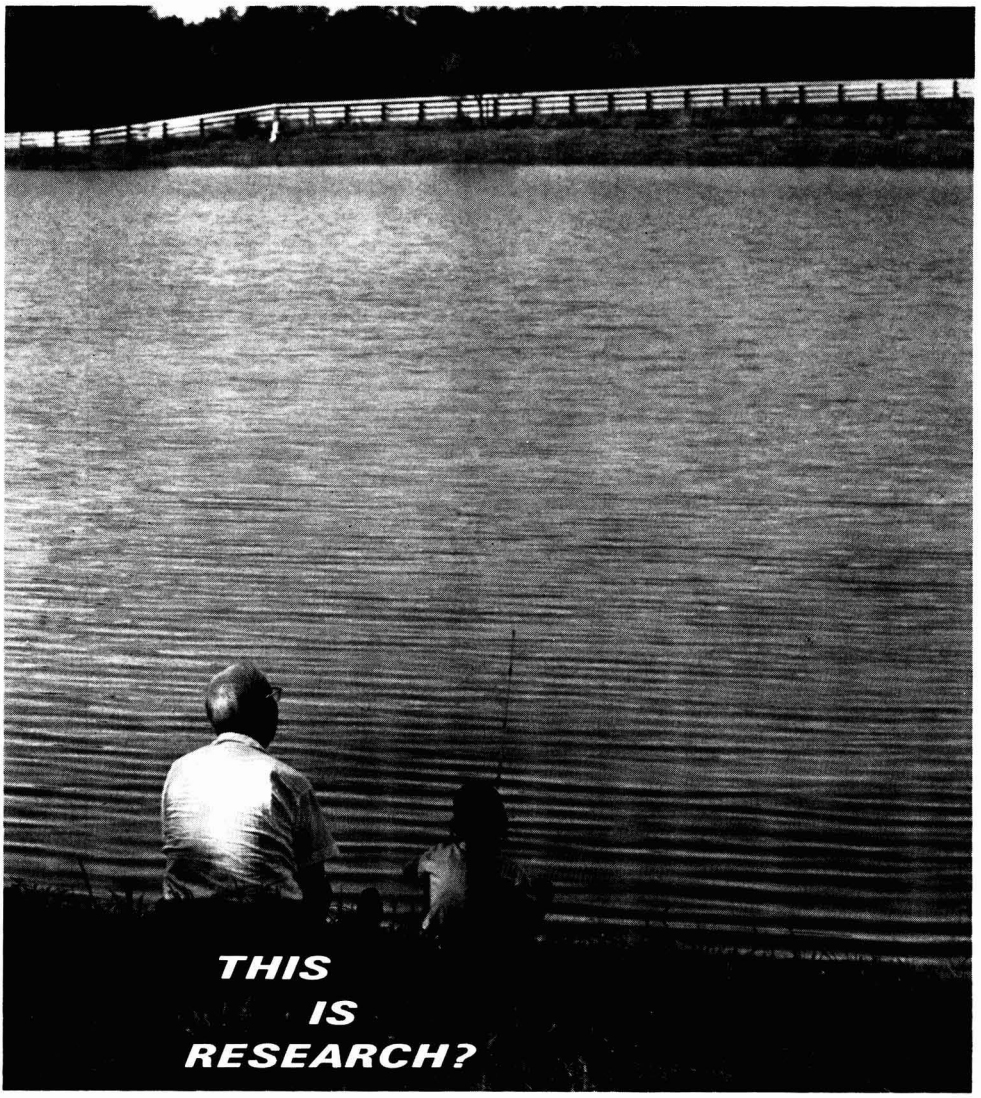
Activities of the Club. One of the first club activities in the fall is to stage a get-acquainted supper for all prospective club members. The faculty of the Dairy Science Department and the members of the Dairy Science Club all join hands in providing some type of supper and entertainment. This is usually followed by a recreational activity such as a softball game between the faculty and students. The new students and prospective members are made to feel that there is a place for them in the club.

Regular club meetings are held the first Monday night of each month and are well publicized in advance. The main purpose of these regular meetings is to transact club business, usually presented in the form of committee reports. The program committee of the club arranges to have some type of educational or recreational activity in connection with each meeting. Emphasis is placed upon well-planned meetings that properly adhere to rules of order. The club usually sponsors about three parties that are held during the year in addition to the regular meetings. These events are enjoyed by members of the faculty, staff, club members, and their guests.



Club members and guests enjoyed themselves at the get-acquainted party.

The student must go through an initiation procedure in order to become a member of the L.S.U. Dairy Science Club. University students indicating an interest in the field of dairying are eligible for membership upon receiving a majority vote from the active members of the club. The neophytes, students aspiring to membership, must wear a uniform for three days as designated by the club, which is a way of publicizing their desire to become a club



You see no test tubes, microscopes or scientists in white coats. But swing the camera 90 degrees to the right and you're looking at continuous research. For this pond is just downhill from the Purina Dairy Research Center near Gray Summit, Missouri.

Here, research is basic and practical. It may start in the Purina Research Laboratories at Checkerboard Square in St. Louis, but the next step is out here at the Research Farm. And the final test is the scrutiny of thousands of dairymen around the world who feed Purina Chows every day. When they open a Checkerboard bag or watch a Checkerboard bulk truck unload, it's something like looking at the scene above. They can't see the research behind it, but they know it's there.

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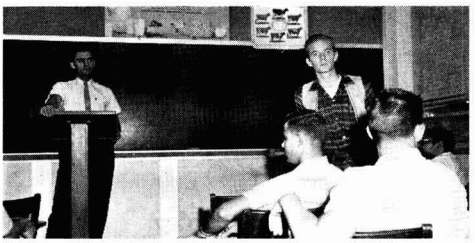
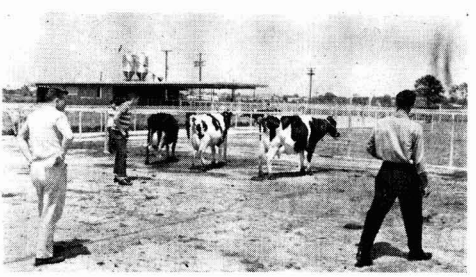


Frank Millican presents a matter of club business, with Jessie Rainey acting as club president.

member. This uniform consists of white clothing, rubber boots, and a bottle of milk with a plaque attached to it that is hung around the neck. The name of the club in large letters is placed on the plaque, and the student is required to obtain the signatures of the faculty and club members within the specified time. The initiation ends with some mild hazing and then a formal acceptance into the club.

Fund raising within the club is an important activity of the membership and is accomplished through a variety of ways. The selling of milk and cold drinks by members during the annual College of Agriculture Spring Livestock Show and Rodeo constitutes the main source of income. The club presents an award to the member who sells the largest number of drinks, and competition is always very keen for the prize. Membership dues are $3 per year, which includes a club key for each new member. The records of the club for last year indicate approximately $1,000 in total receipts.

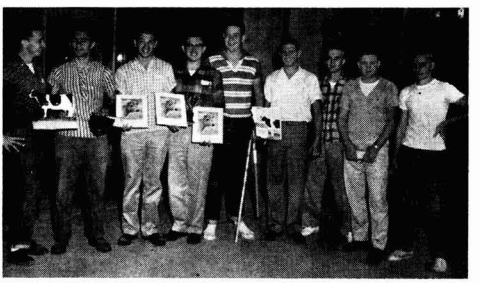
The Dairy Science Club sponsors an annual student judging contest that is usually scheduled in early spring. Any student interested in judging is eligible for participation. The contest is divided into junior and senior divisions in both dairy products and cattle. A wide variety of prizes and awards are donated by breed associations, milk plants, feed companies, dairy supply firms, and local business men. The highlight of the judging contest is usually a barbecue supper, followed by the presentation of awards and prizes. More than 40 contestants participated in the contest this year, and a gold watch was awarded to the individual with the highest score.



Students preparing for the annual judging contest.

Homecoming is an annual affair on the campus of L.S.U. and is scheduled in connection with a home football game. The Dairy Science Club participates in the decoration contest and was awarded third place last year. Club members usually find it necessary to work all night before the contest, since the rules allow them only a specified period of time. Alumni and guests are made to feel at home as they visit the department throughout the day.

Two collegiae judging teams are sponsored each year by the club, and their expenses are paid from the sale of a heifer reared as a club project. The 1962 Spring Judging Team got off to a good start by winning first place in Holsteins at the Southwestern Exposition and Fatstock Show held at Fort Worth, Texas. The Dairy Products Team competed in the Intercollegiate meets in the Southern Division and also the National Conteest. This team won first place in ice cream judging at the Southern Division.



Winners and their awards in the Dairy Science Club judging contest.

The outstanding event during May of each year is the Annual Banquet and Dance for members, faculty, and alumni. This is the largest social function of the year for the club and offers an opportunity for the club members to get together with their dates for dinner and dancing. This occasion also serves to stimulate better relations between students and alumni.

The Annual Banquet gives the club an opportunity to recognize outstanding accomplishments of individual members and dairy faculty. The program of the evening includes introduction of guests, recognition of Dairy Judging Teams, outstanding Senior of the Year, Freshman of the Year, incoming and outgoing officers, graduates and recognition of the Dairyman of the Year. The Dairyman of the Year for 1962 was Leon R. Kleinpeter, Jr., of Baton Rouge, Louisiana. The club will long remember the acceptance speech of Mr. Kleinpeter, in which he pointed out some of the very pene-

Annual Club Banquet—1962.

trating problems confronting the dairy industry today.

The Dairy Science Club of Louisiana State University is truly a student organization that has earned a high place of esteem in the College of Agriculture. The laurels of the past make the days of the future more challenging. Continued success will depend upon the spirit and cooperation so prevalent throughout the past years among the students, faculty, and all others associated with the club.

Activities of the Varsity Dairy Club University of Nebraska

The Varsity Dairy Club of the University of Nebraska held three successful events during September and October.

State Fair Booth: The operation of the State Fair Booth is an annual event. The purpose of this booth is to sell dairy products during the State Fair and to use the proceeds for the support of the Dairy Judging Teams and other activities of the Varsity Dairy Club. This year Kenneth Kast and Ron Schaffert were in charge of the project from September 1 through September 6. Dairy products from the University Creamery were featured. This year 300 gallons of ice cream were sold, gross sales amounting to $950.

Fall Picnic: The fall picnic is an annual event. The purpose of the event is to welcome freshmen and new students to the club. This year the event was held October 4. The barbecued chicken was enjoyed by the 85 faculty members and students in attendance. John Svoboda and Ron Wilton were in charge of arrangements. Miss Phyllis Riddle was an honorary guest serving in her dual role as Princess of the Varsity Dairy Club's Dairy Royal and Dairy Princess of the Nebraska American Dairy Association.

First Meeting: The first meeting, October 11, was attended by 35 persons, including 11 new members. Ike Anderson, club president, informed the club about the activities of the National Student Branch of the American Dairy Science Association and the annual meeting held at College Park, Maryland. He also showed colored slides taken during his trip to the annual meeting, especially featuring our National Capitol at Washington, D. C.

Tennessee Dairy Club Serves Free Supper

The University of Tennessee Dairy Club served a free supper for all students in the School of Agriculture who were interested in dairying but had not previously joined the Dairy Club. The free supper, held October 24, 1962, was especially planned for freshmen and new students in Agriculture.

Members of the Dairy Club at UT and members of the Dairy Department Staff were on hand to welcome the guests. Members of the Club prepared the meal headed by Foods Chairman Wayne Scharber. It was mainly a get-acquainted time for all involved.

Club president Gerald Martin served as Master of Ceremonies and also presented plaques to the winners in the annual Dairy Judging Contests. Steve Cates, Readyville, Tennessee, was high man in Products Judging; Herbert Lackey was high in Cattle Judging; John Housley, Riceville, Tennessee, was high over-all. These plaques will be placed on display in the Dairy Building, McCord Hall. Guy Walker, Friendsville, Tennessee, told the group what his experiences in the Dairy Club had meant to him as a student. The free supper and program proved to be successful.

Activities of Minnesota Dairy Science Club

The first meeting of the Minnesota Dairy Science Club was held on Wednesday, October 17. Miss Marie Jarvinen, president, presided before 36 club members and guests and six staff members.

Tom Amren reported that a milk booth was sponsored by the club at the St. Paul Campus Student Union Open House. Mr. Duane Bartons, a member of I.M.C., was the winner of a drawing and was given a pen set.

Miss Marie Jarvinen, our official delegate to the Student Branch, A.D.S.A., reported on the student affiliate activities at the convention. Congratulations were extended to Miss Jarvinen on her election by the A.D.S.A. Student Branch to the office of Student Branch Secretary-Treasurer.

Dr. Elmer Thomas spoke about the activities of the Dairy Products Judging team. The 1962 team members were Dick Angus, Tom Amren, Gerald Hasse, and David Kraft. Dr. J. B. Williams of the Dairy Husbandry Department presented the 1962 Dairy Cattle Judging team. The members were Marie Jarvinen, James Merritt, David Minar, and Russel Erickson. The coach was Mr. Roger Gerrits.

Dr. Williams introduced Mr. Harry Rajamannan of Ceylon, who entertained the audience with sleight-of-hand tricks.

Graduate Student Scientific Paper Presentation Contest

Each year at the annual meeting the American Dairy Science Association sponsors a scientific paper presentation contest for graduate students who are affiliate members of the Association. A separate contest is conducted in the Production and Manufacturing Sections, and a cash award of $100 is given to the winner in each section. Only one student per college or university may enter each contest.

The purpose of this competition is to provide graduate students with an opportunity to participate in the scientific meetings of the Association and to gain experience in the preparation and oral presentation of scientific papers. It is desired that, through such participation, each contestant will recognize the importance of adequate communication in the dissemination of research information and more fully appreciate the role of the Association and its individual members in advancing the welfare of the industry through research.

The Association has sponsored this contest for the past 4 yr. In 1959, a contest was held in the Production Section only, and there were 11 contestants. Since then, contests have been held both in the Production and in the Manufacturing Sections, with the following numbers of participants:

	1960	1961	1962
Production Section	10	14	14
Manufacturing Section	9	8	7

Several of the contestants in the previous contests are now engaged in full-time research work.

The Student Affiliate Committee feels that the contests have served to improve the general quality of the scientific paper presentations, and it is hoped that more graduate students will compete in the future. The procedure for entering the contests is outlined in the Call for Papers published in the JOURNAL OF DAIRY SCIENCE. The rules for the contests were mailed to the Department Chairman.

Each Dairy Club is urged to encourage a graduate student to enter each contest.

L. J. BOYD, Chairman
Student Affiliate Committee

UNIVERSITY NEWS

11th National Engineering Conference at Michigan State

The eleventh Annual National Dairy Engineering Conference will be held on February 26-27, 1963, at the Kellogg Center. The Conference is sponsored by the Departments of Agricultural Engineering and Food Science at Michigan State University.

This Conference will be devoted to recent developments in the processing of milk and milk products. These will be presented more or less in the order of flow through the plant. Latest engineering developments in receiving, processing, packaging, storage, and distribution of milk and milk products will be emphasized.

Rutgers University Dairy Science Graduates Accept Positions

Rutgers, The State University, New Brunswick, announces that two of their recent recipients of the Doctor's Degree have accepted positions.

PHILLIP R. WELLS, whose thesis was titled Changes Related to Casein Precipitation in Concentrated Milk During Frozen Storage, and who formerly was a graduate assistant in the Dairy Science Department at Rutgers, has accepted a research position with Colgate-Palmolive Research Center in New Brunswick.

NAGESH S. MHATRE recently accepted a research position with Miles Laboratory, Inc., Elkhart, Indiana. He was also formerly a graduate assistant and had finished his thesis entitled: Effects of Small Electric Currents upon the Heat-Induced Changes in Milk Proteins and Salt System.

Rutgers Represented at Kentucky Conference

A Rutgers University faculty member, DR. JOSEPH G. LEEDER, Professor of Dairy Industry, presented two papers at the University of Kentucky Dairy Manufacturing Conference held November 19 and 20 at Lexington. He also had charge of the Chocolate Ice Cream Clinic.

Titles of his papers were: Selection of Ingredients for Quality Ice Cream, and Observations on the Operation of a Plate-Holding HTST Pasteurizer.

Dairy Day Program at Southern Illinois University

Eighth Annual Dairy Day was featured at Southern Illinois University, Carbondale, on December 4, 1962, at the Muckelroy Auditorium, Agriculture Building.

In addition to planning the program, DR. HOWARD H. OLSON, Associate Professor, SIU School of Agriculture, Department of Animal Industries, reported on New Tests for Finding Protein and Solids-not-fat Content of Milk, discussing their accuracy and readiness for field use.

Other program participants were DR. DAVID WIECKERT, lecturer in Animal Industries, SIU, who discussed possibilities in selecting dairy cattle on the basis of protein and solids-not-fat content of milk rather than milk fat production. DR. DONALD HILLMAN, Associate Professor, Dairy Extension, Michigan State University, reported on findings in high grain feeding practices with dairy cows, as reflected in greater milk production.

Dairy Day also included discussions by GUY M. CREWS, Program Director of the American Jersey Cattle Club, and HARRY EATON, Moorman Manufacturing Company, Quincy, Illinois.

Grant to Washington State University for Mastitis Study

A $24,000 grant to study the effects of mastitis on the efficiency of milk production and profits has been made to Washington State University by the Washington Dairy Products commission. The economic penalties levied against dairy producers by the injury-induced udder infections will be determined by DRS. T. L. FORSTER, U. S. ASHWORTH, and L. O. LEUDECKE, of the Department of Dairy Science. This research team will attempt to determine costs of mastitis in terms of lowered milk production. Losses caused by varying degrees

of severity of udder damage will also be determined.

A milking machine which shunts milk from each quarter of the cow's udder into four separate containers will be used. Milk samples from 500 to 1,000 cows will be weighed and analyzed for chemical and bacteriological content. Experiment Station herds at Pullman and Puyallup, some from other state institutions, and some private herds will be studied.

New Grant Program in Cardiovascular Disease

Special Dairy Industry Board representing all segments of the Dairy Industry announces the establishment of a special grant research program for the investigation of the potential role of dairy products in the development of cardiovascular disease.

Special attention will be directed toward the metabolic behavior of different dairy products or interactions between dairy product components with regard to lipid metabolism, the development of atherosclerosis, or thrombus formation in suitable experimental animals or in man. Studies concerning the nutritional value of milk fat or the factors modifying the proportions of the major or minor components of dairy foods influencing dairy fat utilization also may be considered. Investigations which simultaneously provide fundamental information about dietary factors in cardiovascular disease and also provide guidance for the development of nutritionally superior dairy products are particularly encouraged. Scientific guidance will be provided by a committee of outstanding scientists from academic, government, and dairy industry laboratories. Interested investigators in medical or university laboratories should apply to DR. MERRILL S. READ, Secretary, Scientific Advisory Committee, Special Dairy Industry Board, 111 North Canal Street, Chicago 6, Illinois.

U.S. Public Health Service to Conduct Courses

The Public Health Service, through its Division of Environmental Engineering and Food Protection, will present two related 1-week training courses, Laboratory Examination of Foods, February 4-8, 1963, and Microbiological Examination of Dairy Products, February 11-15, 1963, in Cincinnati, Ohio. The first course presents advanced technical information of special interest to laboratory and supervisory personnel concerned with examination of foods for bacterial or chemical contaminants.

The second course is offered for professional personnel in responsible positions in state, municipal, and other laboratories engaged in milk analysis and dairy products examination.

A third related course, Recent Developments in Water Bacteriology, follows February 18-22, 1963, for the convenience of those desiring to

enroll in the three consecutive microbiological courses.

Address requests for information to the Chief, Training Program, Robert A. Taft Sanitary Engineering Center, 4676 Columbia Parkway, Cincinnati 26, Ohio, or to a PHS Regional Office.

INDUSTRY NEWS

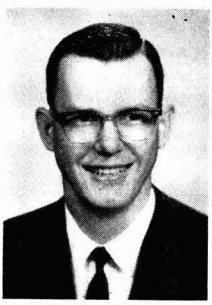
American Can Company Builds New Factory

SECRETARY OF THE INTERIOR STUART L. UDALL has announced that American Samoa has contracted to award a 20-yr lease to the American Can Company of Samoa, subsidiary of American Can Company, New York City, to build and operate a factory in the territory to supply present and future food processing plants there.

A tuna-packing plant is already operating in American Samoa. The territory governor, H. REX LEE, recently signed a contract with a second tuna-packing firm. Earlier this year he completed negotiations with a third company to build a factory for processing coconuts. The new can factory will serve both tuna canneries and the coconut factory.

Olin Mathieson Appoints Market Analyst

ALBERT A. NELSON has been appointed market analyst in the market development department, forest products operations, Packaging Division of Olin Mathieson Chemical Corporation, West Monroe, La. He has experience in packaging procurement, graphic arts, and organic chemical processing. Nelson received a B.B.A. degree in 1951 from the University of Cincinnati and an M.B.A. in 1957 from Xavier University in Cincinnati.



A. A. Nelson

Dairy Technology Societies

Central Illinois—The November 14 meeting was held in Decatur at the Elks Club, with Ralph Baker, Vice-President, Cherry-Burrell Corporation, Chicago, as speaker. His topic: Highlights of the 1962 Dairy Supplies Exposition, with Emphasis on Cherry-Burrell Developments. The December meeting featured Ladies' Night, and was at the Urbana-Lincoln Motor Inn Garden Room, Urbana.

Chicago—December meeting was held at the Furniture Club of America headquarters. Dr. John Hetrick, Director of Research, Dean Milk Company, Rockford, addressed the society, reporting on his impressions of the International Dairy Congress held last summer at Copenhagen, Denmark.

Massachusetts—John Milkne, Vice-President and General Production Manager, Dorothy Muriel Baking Company, spoke at the November 19 meeting on the uses of dairy products in a baking operation. The Question Box was conducted by Arthur Fraser.

Metropolitan—Speaker at the November 13 meeting of this group was Chris Anastos, Associate Editor of Olsen Publications, who discussed Dairy Industry of the Future.

North Carolina—Controlling Psychrophilic Bacteria in Dairy Products was the subject of a talk by Dr. M. L. Speck, Food Science Department, North Carolina State College, at the November 14 and 15 meetings.

Oklahoma—The November meeting of the society was held jointly with the Dairy Industry Conference on the campus in Stillwater. The meeting was a luncheon event, with Dr. O. S. Willham, President, Oklahoma State University, speaking on The International Aspects of Agriculture. December 14 was reserved for Ladies' Night, in Tulsa.

Philadelphia—Dr. Joseph G. Leeder, professor of Dairy Industry, Rutgers, The State University, New Brunswick, gave a speech at the November 8 meeting entitled: Sandiness in Ice Cream. A discussion period followed, in which some interesting relationships were brought out between sandiness and ingredients and flavors.

ADVERTISERS' INDEX

RESEARCH PAPERS

EFFECT OF GAMMA RADIATION ON LACTOSE. II. ISOLATION AND IDENTIFICATION OF MONOSACCHARIDES AND THEIR DERIVATIVES FORMED IN IRRADIATED LACTOSE SOLUTION[1]

SUSUMU ADACHI[2]
Department of Dairy Science
The Pennsylvania Agricultural Experiment Station, University Park

SUMMARY

The effect of gamma radiation on 0.15 M lactose solution at a dosage of 2×10^6 rep was studied, to identify the monosaccharides formed. Lactose was transformed into at least ten fragmentation products which give positive color reaction with anilinhydrogenphthalate reagent. Some of these substances were fractionated by charcoal-, celite-, and ion exchange resin-column chromatography. From this fractionation, D-galactose, D-glucose, D-lyxose, and galactonic acid were identified as crystalline derivatives. Two mechanisms for cleaving the disaccharide linkage of lactose during irradiation are proposed.

Ionizing radiation with a dosage at or above the sterilization level induces chemical changes in milk. The changes that lead to a brown discoloration and development of undesirable flavor are among those of practical importance.

Wertheim et al. (29) have reported that in radiation-induced browning of casein-lactose systems, amino groups of casein do not appear essential but that lactose plays an important role and that carbonyl compounds, the browning precursor of milk, are formed from lactose. Day et al. (8) have shown that in gamma-irradiated skimmilk a caramelized flavor originates primarily from the diffusible fraction of the skimmilk and is associated with browning; and that lactose may be a source of the acidic taste in irradiated milk. Although these observations do not necessarily lead to the conclusion that lactose is the only active factor in these changes, it seems reasonable to assume that lactose decomposition products are involved. There is no report, however, on the identification of the degradation products of lactose by ionizing radiation.

The present paper is concerned with identification of monosaccharides and their derivatives formed in irradiated lactose solution.

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[1] Authorized for publication on August 25, 1961 as Paper No. 2596 in the Journal Series of The Pennsylvania Agricultural Experiment Station.

[2] Permanent address: Department of Animal Products Technology, College of Agriculture, Tohoku University, Sendai, Japan.

EXPERIMENTAL PROCEDURE

Materials. Most of the carbohydrates used in this investigation were commercial products as follows: D-arabinose and D-galactose, Fisher Scientific Co.; D-glucose, J. T. Baker Chem. Co.; lactose, Smith, Klein & French Co.; D-lyxose and D-xylose, Nutritional Biochem. Corp. These substances gave a single spot on paper chromatograms developed with various solvent systems, and were used without further purification. Authentic lactulose was prepared from lactose by a modification (3) of the alkaline isomerization method of Montgomery and Hudson (23). Galactonic acid and gluconic acid were prepared from galactose and glucose, respectively, by bromine-oxidation in the presence of calcium carbonate. In the course of purification, calcium was removed by passing the reaction mixture through a column of Amberlite IR-120 in the hydrogen form. The calcium-free solution was concentrated and lyophilized. The products from the two sugars were syrups, both of which gave three spots on paper chromatograms that corresponded to the γ-, and δ-lactones and the free acids.

Paper chromatography. Chromatograms of carbohydrates were prepared by spotting the sample solution 2.5 cm from the end of a piece of Whatman No. 1 filter paper (43 by 28 cm), along with the solutions of authentic samples. The chromatograms were developed by unidirectional ascending technique in 45- by 14.5-cm glass jars which were kept in a constant-temperature (20 ± 1 C) room. After removal from the jars, the chromatograms were allowed

to air-dry in a hood. To increase Rf values, and to obtain better resolution of sugar mixtures on the paper sheet, the technique of multiple development (17) was sometimes carried out.

The chromatograms of free sugar were developed with the following solvent mixtures prepared each day: Solvent A, n-butanol, pyridine, and water, 3:2:1.5 by volume (17); Solvent B, ethylacetate, pyridine, and water, 40:11:6 (10); Solvent C, the top phase from the mixture ethylacetate, pyridine, and water, 2:1:2 (16). The air-dried papers were sprayed with α-naphthylamine trichloroacetate, diphenylamine trichloroacetate, resorcinol (14), or dipped into anilinhydrogenphthalate solution of Baar (5). The paper chromatograms treated with these reagents, other than resorcinol, were heated 5 min at 110 C, to produce spots indicative of the sugars. The papers sprayed with resorcinol reagent were heated 5 min at 80 C.

Paper chromatography of uronic acids, lactones, and deoxy-sugars was carried out by the methods of Gee and McCready (11), Abdel-Akher and Smith (1), and Pöhm and Weiser (28), respectively.

Measurement of melting points. All melting points were determined on a Fisher micro melting point apparatus. Melting temperatures were not corrected.

The radiation techniques used in this investigation were the same as those described previously (2).

RESULTS

General properties of the irradiated lactose solution. Before breaking the seal, the irradiated lactose solution was refrigerated four days. On opening, this solution showed no bacterial colonies by the standard agar plate method using M.P.H. medium (4).

The irradiated solution had no flavor but had an acidic taste and slightly yellow color. The pH of the solution was 2.35 and 3.1 ml of 0.1 N sodium hydroxide solution were required to neutralize 100 ml of it to the methyl red end point. Various color reactions were applied to this solution for the purpose of qualitatively detecting carbohydrates formed. According to the usual procedures, Seliwanoff's, Ihl-Pechman's and Tollen's and Bial's reagents were used for the detection of ketose and aldopentose, respectively. All of these tests were positive. However, the control lactose solution stored under the same condition as the irradiated solution yielded negative results in these tests.

The inference that ketose and aldopentose were formed by irradiation was further confirmed by the results of the following paper chromatographic analysis: Irradiated lactose solution (400 ml) was concentrated to 70 ml under reduced pressure and the sirup obtained was kept at 2 C for a day, with the addition of 150 ml of methanol. After removal of crystalline lactose by filtration, the filtrate was submitted to vacuum evaporation and the concentrate spotted on chromatography paper. The best resolution of carbohydrates and other related compounds in this sample was accomplished by multiple (four times) development, using Solvent C. When this paper chromatogram was treated with anilinhydrogenphthalate reagent, 11 spots were observed, as shown in Figure 1. The behaviors of these spots toward various coloring reagents are shown in Table 1. Thus, the conclusions from the paper chromatographic data were supported by the results of qualitative determinations by the color re-

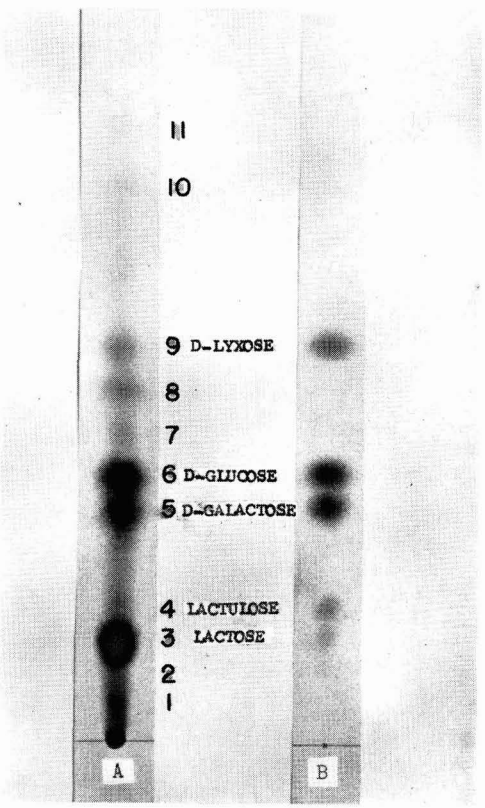

FIG. 1. Paper chromatogram of carbohydrates in irradiated lactose solution.
A = Carbohydrates in irradiated lactose solution.
B = Authentic carbohydrate.

TABLE 1

Characteristics of the spots on paper chromatograms for substances obtained from irradiated lactose solution

Spot no.	$R^*_{lactose}$	Spot** concentration	Spot color: Anilin-hydrogen-phthalate	Spot color: Diphenyl-amine trichloro-acetate	Spot color: α-Naphthyl-amine trichloro-acetate	Spot color: Resor-cinol	Correspondence
1	0.33	++	Brown	.....	.....	.....	
2	0.64	+	Brown	.....	.....	.....	
3	1.00	++++	Brown	Brown	Brown	.....	Lactose
4	1.36	++	Brown	Brown	Brown	Pink	Lactulose
5	2.18	+++	Brown	Brown	Brown	.....	D-Galactose
6	2.54	+++	Brown	Brown	Brown	.....	D-Glucose
7	2.96	+	Brown	.....	.....	.....	
8	3.40	+++	Pink	.....	Brown	.....	
9	3.80	+	Brown	Purple	Green	.....	D-Lyxose
10	4.50	±	Brown	.....	.....	.....	
11	6.05	±	Brown	.....	.....	.....	

$$^* R_{Lactose} = \frac{\text{Distance travelled by sugar}}{\text{Distance travelled by lactose}} .$$

** The spot concentration is presented as color intensity for the anilinhydrogenphthalate reagent as follows: Strong, ++++; Medium, +++; Slight, +; Faint, ±.

actions. The mobilities of these spots were found to coincide with those produced by authentic samples of D-galactose, D-glucose, lactose, lactulose, and D-lyxose. As D-lyxose is moved almost identically to D-xylose on paper developed with various solvent systems, it was generally difficult to differentiate these substances. After a number of trials, however, the possibility of D-lyxose being confused with D-xylose was completely eliminated by four successive developments of the chromatogram with Solvent B. Under these conditions, spots for the two pentoses were sharply separated.

Paper chromatography by the method of Abdel-Akher and Smith (1) showed two spots, the mobilities of which were found to correspond to those produced by galactonic acid γ- and δ-lactones. Spots for galacturonic and glucuronic acids and deoxy sugars were not observed on the paper chromatograms.

Isolation of carbohydrates formed in the irradiated lactose solution. The procedures for isolation of the secondarily formed carbohydrates from the irradiated lactose solution are outlined in Figure 2.

Step 1. After breaking the seal of the can, 6,300 ml of the irradiated solution were immediately concentrated to 600 ml by a circulating evaporator of Mitchell et al. (22). Methanol was added to the concentrate and most of the unchanged lactose that crystallized was removed by filtration, after standing in the cold. The filtrate was evaporated further and extracted with ethyl ether for 70 hr in a continuous liquid extractor. Lactose, crystallizing during the extraction, was separated by filtration. After washing the crystals with methanol, the filtrate was diluted with addition of water and concentrated to a sirup to remove the organic solvents. From the ether extract 0.2 g of a sirupy residue was obtained by evaporation of the solvent (Fraction 1).

Step 2. A modification of Machell's method

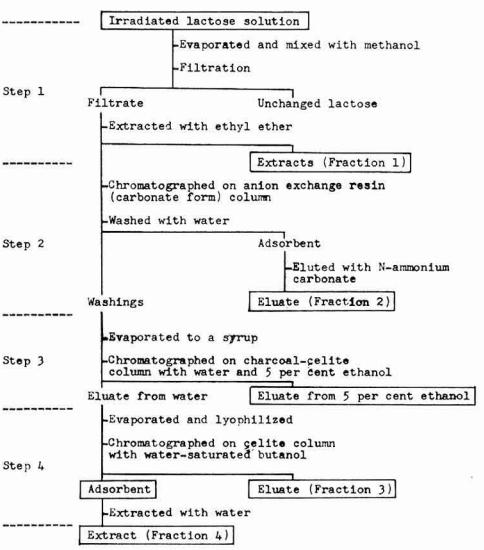

FIG. 2. Isolation scheme of carbohydrates in irradiated lactose solution.

(20) was employed for separating lactones from the aqueous concentrate obtained in Step 1.

Fifty milliliters of the concentrate was diluted to 100 ml with water and treated with 40 g of Amberlite IRA-400 carbonate for 8 hr at room temperature. The adsorbed substances were eluted from the resin with 200 ml of 1 N-ammonium carbonate. The eluate was then stirred with a large amount of Amberlite IR-120 in the hydrogen form to remove the ammonium ion. The resin was removed by filtration and washed with water, the combined filtrate and washings were evaporated to a sirup and lyophilized in a vacuum desiccator. Three-tenths grams of sirup were obtained (Fraction 2).

Step 3. The 100 ml of aqueous concentrate from Step 2 was poured into a charcoal-celite column prepared by packing with a mixture of 75 g of active carbon (Eastman Kodak Co.), 150 g of Celite (Johns-Manville), and several parts of water in a tube (58 by 4.6 cm), giving a column 37 cm in height. The carbohydrates in this solution were chromatographed at a flow speed of 60 ml/hr by successive displacement with water and 5% ethanol, according to a technique essentially similar to that of Whistler and Durso (30). Five and four-tenths and 2.1 g of amorphous powders were obtained from the water- and ethanol-eluting fractions, respectively.

Step 4. To separate further the material obtained by water elution, celite column chromatography according to the procedure of Lemieux et al. (18) was applied. The sample (0.5 g) was fractionated on a Celite column (34 by 4.6 cm) with 1,500 ml of water-saturated *n*-butanol at a flow rate of 20 ml/hr. Before use, the column was packed with 160 g of Celite and washed with 1,500 ml of water-saturated *n*-butanol, to remove completely butanol-soluble substances in the Celite. The eluate obtained (500-700 ml) was evaporated and lyophilized. Five-hundredths of a gram of a lyxose-like amorphous substance was obtained (Fraction 3). The column packing was extruded from the tube, and 0.28 g of a amorphous powder was obtained from the extracting solution of a zone of the column (Fraction 4). To obtain sufficient amounts of these fractions, the procedure was repeated twice.

Identification of carbohydrates in each fraction.

Fraction 1. The sirup contained three substances, with Rf values of 0.42, 0.37, and 0.25. The paper chromatogram was developed by the solvent mixture of *n*-butanol, formic acid, and water (10:2:5) and sprayed with 0.05% of bromeresol green alcoholic solution. The substances were not further investigated.

Fraction 2. The sirup gave two spots corresponding to galactonic acid γ- and δ-lactones on paper chromatogram and failed to reveal any spot of reducing sugars with Solvent A and spray reagents α-naphthylamine trichloroacetate and resorcinol. The sirup was heated to condense with o-phenylenediamine in a glycerin bath at 130-140 C in the manner of Moore and Link (24). After cooling, the dark-colored material was dissolved in water and then centrifuged. Concentrated ammonium hydroxide was added in to the upper supernatant solution under stirring, until a precipitate formed. The precipitate was separated by filtration, dissolved in a small amount of diluted acetic acid, and decolorized with charcoal. Concentrated ammonium hydroxide was then added to the clear filtrate until no more white crystalline substance was formed. This precipitate was filtered and washed with ethanol. After three times recrystallization, as described above, it melted at 248-250 C.

Analysis. Calculated for $C_{12}H_{16}O_5N_2$: C, 53.70; H, 602; N, 10.45.
Found: C, 53.53; H, 6.55; N, 10.39

The picrate of this substance, prepared by the method of Moore and Link (24), was identified as D-galactobenzimidazole picrate by mixture melting point determination. It melted at 217 or 219 C without decomposition.

Fraction 3. This powder contained a compound indistinguishable from D-lyxose (Rf 0.48) on the paper chromatogram developed with Solvent A. Confirmation of the suggested identity was obtained by means of the absorption spectrum in concentrated sulfuric acid by the method of Ikawa and Nieman (15) and Bath (6), with minor modification (Figure 3). From the data of Table 2, it is evident that the spectrophotometric characteristics of the compound coincide with those of D-lyxose. The preparation of D-lyxose 2,4-dinitrophenylhydrazone from this fraction was carried out by the method of Lloyd and Doherty (19). After recrystallizations from hot 95% ethanol, the 2,4-dinitrophenylhydrazone melted at 168 C.

Fraction 4. Paper chromatograms of this fraction, developed with Solvent A, gave two spots at Rf 0.39 and 0.34 by anilinhydrogenphthalate reagent. The mobilities of these unknowns were coincident with known samples of D-glucose and D-galactose, respectively.

Isolation of D-galactose phenylhydrazone and D-glucose phenylosazone from the Fraction 4.

TABLE 2

Comparison of the spectrophotometric properties of a substance obtained from irradiated lactose soluiton with those of pentoses by Bath's method (6)[a]

Substance	Max (m)	E_{max}	Min (m)
D-Arabinose	317	13,000	.....
D-Lyxose	223 288	700 9,440	233
D-Xylose	257 316	5,860 22,000	277
Sample from Fraction 3	288	8,500	.....

[a] These data were obtained from the result shown in Figure 3.

Five-tenths of a gram of Fraction 4 was dissolved in 2 ml of water, together with 1.7 g of phenylhydrazine and 2 ml of absolute ethanol. The mixture was allowed to stand at 20 C for 24 hr. Upon addition, with stirring of 3 ml of water, a white crystalline substance was obtained by filtration. The yield of dried compound was 1.2 g (Derivative A). Sodium acetate, 0.45 g and 0.9 of phenylhydrazine hydrochloride were added to the filtrate. The mixture was heated on a boiling water bath for 1 hr and then allowed to stand in a refrigerator for one day. One-half gram of a yellow crystalline substance was obtained by filtration and rinsing with water (Derivative B). After recrystallization from hot absolute ethanol, the melting point of Derivative A was found to be 161-162 C with decomposition. After recrystallization from hot 80% ethanol, the melting point of Derivative B was found to be 209 C.

Conversion of Derivative A into D-*galactose diphenylformazone.* Three-tenths gram of Derivative A was added to a solution of 0.5 g phenylhydrazine in 0.2 ml pyridine and 0.2 ml water. The solution was stirred until the crystals were dissolved. The solution was kept at 20 C for 24 hr, after which it was chilled by ice-water and shaken while fresh diazonium reagent (21) was slowly added dropwise. After standing 10 min, the solution was poured into 10 g of ice and subsequently stored in the refrigerator for 24 hr. A dark-red crystalline substance (0.4 g) was obtained by filtration and washing with a small amount of cold water. The derivative, recrystallized from hot 50% ethanol, melted at 168-169 C without decomposition.

Analysis. Calculated for $C_{18}H_{22}O_5N_4$: C, 57.74; H, 5.92; N, 14.97
Found: C, 57.41; H, 6.15; N, 16.21

Conversion of Derivative B into D-*glucose triazole.* Four-tenths of a gram of Derivative B was suspended in 100 ml of water and, with the addition of 0.3 g cuprous sulfate, was heated for 2 hr. The dark-red precipitate that formed was removed by filtration of the hot solution, and discarded. The filtrate was concentrated to 10 ml by evaporation and allowed to stand in the refrigerator for 24 hr. From this solution 90 mg of a white crystalline substance was obtained by filtration and washing with ethyl ether. The derivative was recrystallized from hot water, and melted at 168-169 C without decomposition.

Analysis. Calculated for $C_{12}H_{15}O_4N_3$: C, 54.33; H, 5.70; N, 15.84
Found: C, 54.38; H, 5.71; N, 15.82

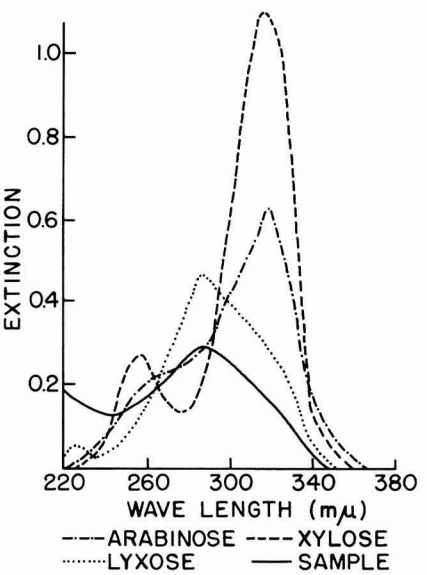

FIG. 3. Absorption spectra in concentrated sulfuric acid of pentoses and a substance obtained from irradiated lactose solution. The concentration of the authentic pentoses was 50 μg/ml.

TABLE 3

Comparison of the melting points of derivatives obtained from irradiated lactose solution with those of authentic samples

Substance	Observed	Authentic	Mixed	Reported	(Ref.)
D-Galactobenzimidazole	248–250[a]	247–248[a]	247–249[a]	245[a]	(24)
D-Galactobenzimidazole picrate	217–219	217–218	217–219	217[a]	(24)
D-Galactose phenylhydrazone	161–162	161–162	161–162	160–152[a]	(13)
D-Galactose diphenylformazone	168–169	167–168	167–168	167–168	(21)
D-Glucose phenylosazone	209	208–209	208–209	208	(9)
D-Glucose triazole	195–196	195–196	195–196	195–196	(12)
D-Lyxose 2,4-dinitrophenylhydrazone	168–169	169–170	169–170	169–170	(19)

[a] Decomposition points.

DISCUSSION

From the paper chromatographic results given herein, it is evident that lactose is degraded into at least ten fragmentation products which are color-productive with anilinhydrogenphthalate reagent. Comparisons of the melting points of the derivatives obtained from the irradiated lactose solution with those of the authentic samples and references are shown in Table 3. These data confirm that the carbohydrates isolated are galactonic acid, D-galactose, D-glucose, and D-lyxose.

The degradation of lactose in aqueous solution by ionizing radiation is so complex that a precise mechanism cannot be presented at this time. That the galactonic acid is not formed primarily from galactose liberated from lactose is evidenced by the fact that the concentration and area of spots of galactose and glucose were approximately equal on paper chromatograms. Rather, it may be reasonable to assume that galactonic acid is formed mainly from the galactose portion of lactose by oxidative degradation. It seems reasonable that D-lyxose is derived from galactonic acid by decarboxylation. In this connection, Bourne, Stacey, and Vaughan (7) have suggested that since lactic acid gives mainly acetaldehyde, with a large dose of gamma radiation in the absence of oxygen, a gluconic acid could be converted into the next-lower aldose. Phillips, Moody, and Mattok (27) have considered that the D-arabinose in gamma- and electron-irradiated glucose solution is prabably formed from gluconic acid in the same manner. Wolfrom et al. (32) recently have reported that a pentose is formed from the next higher hexitol by a selective oxidation of the terminal primary hydroxyl group of hexitol on irradiation in aqueous solution.

Based upon the above evidence, a possible mechanism for degradation of lactose in aqueous solution by gamma radiation is shown, in part, in Figure 4. If the probabilities of the cleavages of the glycosidic linkage of lactose are the same, the amounts of galactose and glucose should be equal. Although the quantitative determination of these carbohydrates was not carried out, the paper chromatographic results strongly suggest that the amounts of these sugars may be equimolar in the irradiated lactose. It appears that there should be two modes of cleavage of the glycosidic linkage in the lactose degradation by gamma radiation. Phillips and Moody (26) postulated such a mechanism for the degradation of sucrose in aqueous solution by gamma radiation. In this

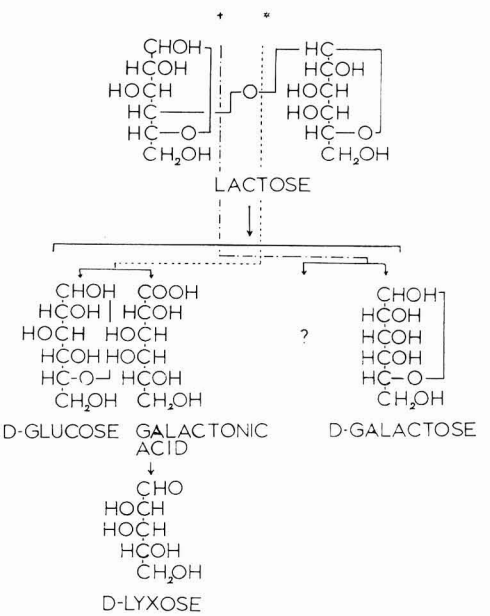

FIG. 4. A proposed mechanism for the formation of the monosaccharides in irradiated lactose solution.

way, D-glucose and galactonic acid should be produced from the cleavage at the point of *, and D-galactose and other unknown substance(s) should be formed by the cleavage of + point. In the latter case, the glucose residue of lactose may be decomposed into two three-carbon or four- and two-carbon fragments, because evidence for the presence of hexuronic acid and deoxy sugar was not found on the paper chromatograms. Some of the unknown spots on the paper chromatograms, therefore, are probably derived from this source.

In attempting to understand fully the formation of galactose and glucose, however, one cannot neglect the possibility of hydrolysis of lactose to its constituents by radiation. Phillips and Moody (25) have isolated glucose, isomaltose, isomaltotriose, gluconic acid, and glucuronic acid as major products from irradiated aqueous dextran solution; and they have suggested that one of the main processes of the dextran degradation is hydrolysis to glucose and other saccharides. Moreover, Wolfrom et al. (31, 32) have reported that sucrose, maltose, cellebiose, and raffinise are degraded to their constituent monosaccharides by ionizing radiation. They have also reported that the hydrolysis order is qualitatively that found with acid hydrolysis. Thus, these findings seem to support the possibility of lactose hydrolysis by gamma radiation.

The results of identification of a substance in the amorphous powder obtained from 5% ethanol eluting fraction on the charcoal column will be given in a paper of this series to follow.

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FACTORS RELATED TO FLAVOR STABILITY OF FOAM-DRIED MILK. II. EFFECT OF HEATING MILK PRIOR TO DRYING

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SUMMARY

The flavor of stored samples of vacuum foam-dried whole milk powders made from milk heated prior to drying was determined organoleptically. Heating milk at 165 F for 30 min, 170 F for 6 min, and 195 F for 15 sec effectively stabilized the flavor of air-packed samples during six months of storage at 80 F. These treatments had little or no effect on the flavor stability of samples packed in nitrogen containing either 1.0 or 0.1% oxygen. As oxidative deterioration during storage was checked by heat treatments in excess of pasteurization requirements, increased cooked flavor in and staling of the dried products was noted.

In our laboratory, it was found that extending the shelf life of foam-dried whole milk by gas packing required in-package oxygen levels below those obtainable with commercial gas-packaging equipment (10). Therefore, it became desirable to further study other means of stabilizing the flavor of this product.

Other authors' studies (1, 3, 5, 7) have shown that heating milk in excess of pasteurization requirements, prior to drying, conferred on spray-dried whole milk a stability against oxidative change.

This paper presents data pertaining to the flavor of stored samples of vacuum foam-dried powder made from milks subjected to a variety of heat treatments. The effect of these heat treatments on the flavor of gas-packed samples also is included.

Lack of suitable chemical and physical techniques (8) limited the evaluation of flavor quality of the samples to an organoleptic method.

METHODS AND MATERIALS

Pilot plant capacity restricted this study to four heating variables per lot of milk used. Possible variation in composition of milk during the course of the work was reduced by using mixed-herd milk from cows maintained on an invariant husbandry regime at the Agricultural Research Center located at Beltsville, Maryland.

Experimental samples of powder were produced by drying, under high vacuum, foamed concentrates made from heated milk (9). Variations in the heat treatment of the milk were carried out during the pasteurization step in all cases except one, where additional heating of the concentrate prior to drying was carried out.

Heating milk 6 min or longer was done in a steam-jacketed pan equipped with a stirrer, or in a 150-gal capacity Cherry-Burrell[1] spray pasteurizer. Plate heating and steam injection were carried out by using a custom-designed deodorizer-pasteurizer of 5,000-lb/hour capacity (4). A Mallory[1] tubular heater having a 100-gal/hour capacity was used for high temperature–short time work.

The powders containing 26% fat and approximately 2.5% moisture were packaged in hermetically sealed tin cans. Conditions were controlled in such fashion that representative samples of each type of powder were packaged in air, nitrogen containing 1.0% oxygen, and nitrogen containing 0.1% oxygen.

All powder samples were stored at 80 F and reconstituted for flavor evaluation at two-month intervals.

A ten-man taste panel evaluated the flavors of all stored samples, using the methods and score-card previously described (10). Data obtained in this fashion were analyzed statistically. Preliminary study showed such wide variance of judge response to strong off flavors

[1] 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.

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TABLE 1

Interaction of in-pack oxygen level and heat treatment observed in series No. 26. Mean flavor scores (MFS) averaged over 2, 4, and 6 months of storage at 80 F

Heat treatment	In-pack O_2 level (%)	MFS	Rank
168 F 15 sec Plate	0.1	36.14	a
163 F 15 sec Steam injection flash deodorize to 140 F	0.1	35.94	ab
163 F 15 sec Steam injection flash deodorize to 150 F	0.1	35.94	ab
165 F 30 min Holder	0.1	35.67	ab
165 F 30 min Holder	1.0	35.44	ab
163 F 15 sec Plate	1.0	35.17	ab
165 F 30 min Holder	Air	34.73	b
163 F 15 sec Steam injection flash deodorize to 140 F	1.0	33.90	c
163 F 15 sec Steam injection flash deodorize to 150 F	1.0	33.70	c
163 F 15 sec Steam injection flash deodorize to 140 F	Air	32.21	d
163 F 15 sec Plate	Air	31.95	e
163 F 15 sec Steam injection flash deodorize to 150 F	Air	31.89	e

that all scores below 30 were automatically raised to this level to normalize the data for computation.

Each series of four experiments performed, using a single lot of milk, was analyzed as a separate entity, using the analysis of variance (2). Where significance was found in the analysis, a mean comparison was made, using Duncan's Multiple Range test. Using the standard error of the mean, and the degrees of freedom for error in the analysis of variance, the values obtained from a 5% Significant Studentized Ranges table were adjusted. The resulting values were the minimum differences between means which, if exceeded, indicated that the compared means were different.

RESULTS

The reproducibility of data of the type presented in this paper was determined by triplicating a study of the flavors of stored air- and gas-packed samples of vacuum foam-dried powders made from milks receiving heat treatments equal to and in excess of those required for pasteurization. Excellent agreement in the results of these studies was noted, with the mean separations verifying the previously reported effect of decreasing oxygen level in the packages on improved flavor stability (10). The expected improvement of flavor stability in powders made from milk heated in excess of pasteurization requirements was also noted. The analysis of variance indicated interaction between heat treatment and the oxygen level in the packages. Table 1 presents this interaction and the relative rank of the mean flavor scores (MFS) of the two-, four-, and six-month-old samples. All MFS having a similar letter in common in the ranking column are statistically indistinguishable. These data pertain to one of the replicate series. All others were similar.

Similar interactions between oxygen level in packages and heat treatment are shown in Tables 2, 3, and 4. Each table represents data obtained from powders made from a single lot of milk, divided and heat-treated, using a variety of conditions designed to determine the optimum heat treatment for maximum flavor stability of the product stored in air and gas packs.

The type of flavor in the samples, as detected by the judges, is partially reported. For simplicity's sake, the flavor in each sample considered most objectionable by the judges has been tabulated. The four most commonly occurring defects are considered individually; all

TABLE 2

Interaction of in-pack oxygen levels and heat treatment observed in series No. 19. Mean flavor scores (MFS) averaged over 2, 4, and 6 months of storage at 80 F

Heat treatment	In-pack O_2 level (%)	MFS	Rank
145 F 30 min Plus 165 F 30 min on conc.	0.1	35.38	a
145 F 30min	0.1	35.32	a
165 F 30 min	1.0	35.24	a
165 F 30 min	0.1	35.23	a
185 F 30 min	1.0	35.17	a
185 F 30 min	0.1	35.09	a
145 F 30 min Plus 165 F 30 min on conc.	1.0	34.56	a
165 F 30 min	Air	34.28	ab
145 F 30 min	1.0	33.15	bc
185 F 30 min	Air	33.09	bc
145 F 30 min Plus 165 F 30 min on conc.	Air	32.20	cd
145 F 30 min	Air	31.69	d

TABLE 3

Interaction of in-pack oxygen levels and heat treatment observed in series No. 44. Mean flavor scores (MFS) averaged over 2, 4, and 6 months of storage at 80 F

Heat treatment	In-pack O_2 level (%)	MFS	Rank
150 F 6 min	0.1	36.23	a
145 F 30 min	0.1	36.00	ab
190 F 6 min	0.1	35.60	abc
190 F 6 min	1.0	35.46	abc
170 F 6 min	1.0	35.45	abc
170 F 6 min	0.1	35.33	bc
190 F 6 min	Air	35.01	cd
170 F 6 min	Air	34.97	cd
150 F 6 min	1.0	34.45	de
145 F 30 min	1.0	33.71	e
145 F 30 min	Air	30.00	f
150 F 6 min	Air	30.00	f

others have been consolidated. The effect of the oxygen level in the packages on the type of flavors found in the various groups of samples is shown in Table 5. The effect of the individual heat treatments on the frequency of flavor occurrence in all stored packs during the entire period is shown in Table 6.

DISCUSSION

Consideration of the data in its entirety demonstrates that like the flavor of spray-dried whole milk, the flavor of foam-dried whole milk samples packed in air or commercial type gas packs can be stabilized against oxidative changes during storage by applying a variety of heat treatments in excess of conventional pasteurization. The flavor of the more stable products, a combination of definite cooked and slight stale, cannot be considered like that of fresh market milk. However, the optimum heat treatments did produce readily soluble products which can be considered palatable.

TABLE 4

Interaction of in-pack oxygen levels and heat treatment observed in series No. 50. Mean flavor scores (MFS) averaged over 2, 4, and 6 months of storage at 80 F

Heat treatment	In-pack O_2 level (%)	MFS	Rank
275 F 15 sec	1.0	36.01	a
165 F 30 min	0.1	35.79	ab
275 F 15 sec	0.1	35.48	ab
165 F 30 min	1.0	35.45	b
250 F 15 sec	1.0	35.44	b
225 F 15 sec	0.1	35.43	b
225 F 15 sec	1.0	35.40	b
165 F 30 min	Air	35.35	b
250 F 15 sec	0.1	35.25	b
250 F 15 sec	Air	34.62	c
225 F 15 sec	Air	34.54	c
275 F 15 sec	Air	33.92	d

While a relatively simple picture of change in flavor is presented by mean flavor score ranking, it must be realized that it is highly deceptive without a consideration of the types of flavor which, in a somewhat arbitrary fashion, dictate the magnitude of the given score. For instance, in the scoring system used in this study, a definite cooked flavor in milk produces a relatively higher score than a slight oxidized flavor. If both defects were equally weighted, the results of the investigation, as presented by mean flavor scores alone, would have been somewhat different. Therefore, the type of flavor detected in the samples by the judges must be considered in evaluating the data.

The consumers' acceptance of the flavors typical of foam-dried powder made from heated milk is undergoing study at present, along with the relationships between consumer acceptance and the weighting factors used in the development of the score-card used in this study.

CONCLUSIONS

The flavor stability of vacuum foam-dried whole milk packed in air can be improved by heating milk to 165 F for 30 min, 170 F for 6 min, or 195-225 F for 15 sec. Heating above these levels results in no real further improvement of product, since the high levels of cooked flavor become objectionable.

The stabilizing effect of heat treatment decreases with decreasing oxygen level in gas packs. Heating milk in excess of pasteurization requirements, prior to drying, actually had little or no effect on the stability of products packaged in nitrogen containing 0.1% oxygen.

As oxidative changes in the samples were checked, stale flavor became the predominant defect in stored samples.

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TABLE 5

Effect of in-pack O_2 level on frequency of dominant off-flavor in products of all series. All storage times and heat treatments averaged together for computation

Series no.	In-pack O_2 level (%)	Per cent total judgments per specific flavor				
		Astr.	Cooked	Oxidized	Stale	Others
19	0.1	8.3	19.2	43.3	11.7	17.5
19	1.0	5.0	12.5	56.7	12.5	13.3
19	Air	2.6	13.8	62.9	13.8	6.9
26	0.1	11.7	20.0	24.2	20.0	24.1
26	1.0	5.8	15.8	44.2	15.8	18.4
26	Air	1.7	10.8	70.0	11.7	5.8
44	0.1	7.5	35.0	5.0	34.2	18.3
44	1.0	5.8	28.3	26.7	31.7	7.5
44	Air	3.0	19.0	40.0	22.0	16.0
46	0.1	5.9	37.0	17.6	24.4	15.1
46	1.0	6.7	34.2	24.2	25.0	9.9
46	Air	1.8	23.6	28.2	30.9	15.5
50	0.1	4.2	33.3	5.0	43.3	14.2
50	1.0	10.0	30.0	7.5	40.8	11.7
50	Air	6.7	18.3	16.7	41.7	16.6

TABLE 6

Effect of heat treatment of milk on frequency of dominant off-flavor in products of all series. All storage times and oxygen levels averaged together for computation

Series no.	Heat treatment	Per cent total judgments per specific flavor				
		Astr.	Cooked	Oxid.	Stale	Others
19	145 F 30 min	4.5	4.5	68.5	12.4	10.1
19	145 F 30 min + 165 F 30 min on conc.	4.5	12.4	61.8	9.0	12.3
19	165 F 30 min	4.5	24.7	51.7	10.1	9.0
19	185 F 30 min	7.9	19.1	34.8	19.1	19.1
26	163 F 15 sec	5.6	11.1	50.0	12.2	21.1
26	163 F 15 sec SIFD[a] to 140 F	7.8	8.9	60.0	16.7	6.6
26	163 F 15 sec SIFD[a] to 150 F	6.7	4.4	51.1	16.7	21.1
26	165 F 30 min	5.6	37.8	23.3	17.8	15.5
44	145 F 30 min	2.5	8.8	48.8	30.0	9.9
44	150 F 6 min	7.5	12.5	40.0	31.2	8.8
44	170 F 6 min	4.4	38.9	4.4	34.4	17.9
44	190 F 6 min	7.8	47.8	3.3	23.3	17.8
46	165 F 15 sec	6.3	5.1	59.5	20.2	8.9
46	165 F 30 min	5.6	46.7	11.1	25.6	11.0
46	195 F 15 sec	5.6	34.4	16.7	30.0	13.3
46	225 F 15 sec	2.2	37.8	10.0	30.0	20.0
50	165 F 30 min	7.8	35.6	6.7	35.6	14.3
50	225 F 15 sec	7.8	30.0	5.6	42.2	14.4
50	250 F 15 sec	4.4	20.0	15.6	45.6	14.4
50	275 F 15 sec	7.8	23.3	11.1	44.4	13.4

[a] SIFD: Steam injection flash deodorized.

of the manuscript by S. R. Hoover also are acknowledged.

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FACTORS AFFECTING THE APPARENT ACTIVITY AND HEAT SENSITIVITY OF XANTHINE OXIDASE IN MILK [1]

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SUMMARY

The xanthine oxidase activity, expressed as μl O_2/ml/hr, was measured by the Warburg manometric technique. The activity in fresh milk increased as a result of storing at 4 C, heating to 70 C for 5 min, homogenization, and incubation with commercial enzymes of proteolytic and lipolytic nature. These increases were accompanied by increases of activity in the skimmilk phase, with a corresponding decrease of activity in the fat phase. The changes in apparent activity were attributed to the release of aggregates of enzyme microsomes from the fat globule membrane.

The heat sensitivity of xanthine oxidase in milk increased on storage and on homogenization. The increase in heat sensitivity is believed to be due to a solubilization of the enzyme microsomes. In this case, a disintegration of the microsome seems to be involved, whereas increases in apparent activity seem to involve a simple dispersal of microsomal aggregates.

It has been known for about 60 yr that an oxidizing enzyme, now generally called xanthine oxidase, exists in rather large amounts in milk and various milk products. The characteristics of the concentrated or purified enzyme have been studied extensively. However, the behavior of xanthine oxidase as it occurs in milk is not clearly elucidated. Relatively large variations in the activity, heat sensitivity, and distribution of the enzyme between the fat and skimmilk phases have been reported. This study was undertaken to determine the factors responsible for these variations.

HISTORICAL

Wieland and Macrae (19) observed that storage at low temperature resulted in an increase in xanthine oxidase activity. They reported that the xanthine oxidase activity of fresh milk increased slowly, reaching a maximum after two to four days, when the activity measured three to four times the original value. They also found that shaking the milk in air or nitrogen atmosphere increased the activity two to three times. Bengen and Bohm (3) also reported that storage of fresh, raw milk at 0-5 C resulted in an apparent increase in xanthine oxidase activity. They suggested that the oxygen in milk was used up by bacteria during storage, so that there was less oxygen available to reoxidize the methylene blue, thus decreasing time needed for discoloration. Such an effect would be interpreted as indicating an increase in enzyme activity.

Booth (6) was not able to confirm Wieland's observation that the activity increased on standing. In fact, he observed a slight decline. In 1939, Ball (1) confirmed that the enzyme activity increased on standing. Worden (21) observed a four- to sevenfold increase in xanthine oxidase activity after 36-hr storage at low temperature.

Ball (1) reported that a large part of the xanthine oxidase was associated with the fat fraction. Since the portion in the skim fraction increased as the milk aged, he suggested that the enzyme was adsorbed on the fat droplets, and could be forced into solution by causing the fat droplets to coalesce. This was in agreement with the explanation of Wieland and Macrae (19). They believed that in fresh milk the finely divided fat globules adsorb the enzyme so that it is largely passive, but when the fat surface decreases, such as by coalescing, the enzyme is released and becomes active. Wieland and Rosenfeld (20) reported that on separating milk most of the xanthine oxidase went with the cream and there was a four- to

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[1] These data were taken in part from a thesis presented by the senior author in partial fulfillment of the requirements for the degree of Doctor of Philosophy, Cornell University.

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fivefold increase in the over-all activity. They attributed this increase to an activation by the fat. However, Kiermeier and Vogt (10) in 1956 stated that there was no increase in the total activity as a result of separation.

In 1947, Polonovski et al. (16) found that on cooling milk there was a marked increase in enzyme activity when the milk reached 20 C. This increase leveled out at 15 C, which is approximately the solidification point of the milk fat. Polonovski and coworkers (15) also found that treating milk with alkalis, organic solvents, and surface-active compounds released the enzyme from the fat globule.

The nature of the relationship of xanthine oxidase with the fat was explained by Morton (11) in 1953. He reported the isolation from milk of lipoprotein particles with which xanthine oxidase, alkaline phosphatase, and several other enzymes were associated. He suggested that these particles should be called milk microsomes, since they are quite similar to animal microsome particles, and especially the ones found in the cow's mammary gland. He found that the microsomes, which range in sizes from 30-200 mμ, have a tendency to be adsorbed on the proteinaceous milk fat globule membrane. In another paper he (11) concluded that milk xanthine oxidase is wholly bound to the milk microsomes, and considered it quite possible that alkaline phosphate was bound to the same particle. Zittle et al. (22) confirmed Morton's results, but added that xanthine oxidase activity was probably connected to a different microsome, and that the xanthine oxidase microsomes were smaller and less easily sedimented. The milk microsomes, of course, are colloidal and, therefore, not in true solution in the milk.

Various investigators have reported on the temperature necessary to inactivate xanthine oxidase in milk, either partially or completely. The reports do not agree very well and are difficult to compare. Bergel and Bray (4) stated that the pure enzyme is inactivated at 56 C for 3 hr. Booth (5) reported that the enzyme in his preparation was partially inactivated at 67 C for 1 hr, but on more prolonged holding the enzyme was completely destroyed at that temperature. According to Schwarz (18), the enzyme in buttermilk is completely inactivated after momentary heating to 80 C. Dixon and Thurlow (8) found that by heating at 70 C for 5 min 70% of the enzyme was destroyed. Barthel (2) reported that the enzyme was destroyed by heating to 75 C for 15 min or 80 C for 5 min. Brand (7) stated that the enzyme was completely inactivated at 80 C. Pien (14) determined various time and temperature relationships necessary for inactivation. He found that 10 sec at 80 C, 30 sec at 79.5 C, 2 min at 77 C, etc., inactivated the enzyme. Kiermeier and Vogt (9) studied the inactivation of xanthine oxidase in a large number of samples of milk. They found that significant irregularities in the inactivation occurred in the region of 60-70 C, both in milk and cream. In some samples they even observed increases in the activity of the enzyme, when heated to temperatures in this region. Temperatures of 70 C or above always decreased the activity, but in order to completely inactivate the enzyme, temperatures of over 85 C (for 35 sec) were necessary. These investigators did not explain the anomalies which they observed. Polonovski, Robert and Robert (17) also studied the effect of elevated temperatures on xanthine oxidase of milk. They found that by subjecting fresh, uncooled milk to short exposures of heat, activation occurred at first, but when exposed for longer periods of time, inactivation occurred, if the temperatures were over 60 C. For example, a sample heated to 69 C reached a maximum after 2 min of heating. After 3 more min of exposure, only 80% of the maximum activity remained. Recently, Pereira, Kristoffersen, and Harper (13) reported that heating to 170 F for 18 sec resulted in a 27% decrease in activity. They did not reveal the previous temperature history of their samples.

EXPERIMENTAL PROCEDURE

Milk samples. The samples of milk were collected from individual cows in the University herd. The samples were immediately brought to the laboratory and were either analyzed at once without cooling or after definite periods of storage in the refrigerator.

Separation. The milk was separated at 32 C in a small DeLaval (Model 100) separator. The fat content of the creams obtained in this way varied from 25–28%. The fat content of the skimmilks ranged from 0.01-0.06%. All fat percentages were determined by the Babcock method.

Homogenization. Samples were homogenized at 35 C, using a small, two-piston laboratory homogenizer (Logeman).

Heating of samples. A pyrex jar 18 in. high and 12 in. in diameter was used in making a water bath. An aluminum cover with a minimum of openings for instruments was placed over the jar to retard evaporation. The bath temperature was controlled by means of a thermostat and circulation pump. The samples were heated in the following way: A glass

U-tube (4 mm internal diameter) with a horizontal sidearm was placed in the bath so that the bulk of the U-tube was immersed. The sidearm was then connected to a 250-ml Erlenmeyer flask, partially immersed in water at room temperature. Suction was applied to the flask by means of a water aspirator. After allowing the U-tube to equilibrate in the water bath for at least 5 min, approximately 8 ml of sample was poured through a funnel into the U-tube in such a way that the sample was in the immersed portion of the tube. The internal diameter of the funnel was necessarily smaller than the diameter of the U-tube to minimize entrapment of air in the milk. Both ends of the heating tube were left open to the air during the filling operation for the same reason. The milk was held in the tube for exactly 5 min. By applying suction the sample was rapidly drawn into the Erlenmeyer flask, and thus cooled to room temperature immediately.

Determination of xanthine oxidase activity. The activity of xanthine oxidase was determined by the method of Ball (1). This method consists of measuring rate of oxygen uptake during a period of the enzyme-catalyzed reaction with a suitable substrate, using the Warburg manometric apparatus. The Warburg flasks contained the following:

1. Main chamber:
   a. For milk and skimmilk: 1.0 ml 0.1 M phosphate buffer (pH 7.2), 1.6 ml milk or skimmilk
   b. For cream: 1.6 ml 0.1 M phosphate buffer (pH 7.2), 1.0 ml cream
2. Sidearm: 0.4 ml of a 0.05 M solution of hypoxanthine[3] in 0.05 M NaOH
3. Center well: 0.2 ml 5% KOH (aqueous solution).
4. Total volume in the flasks: 3.2 ml.

The Warburg bath was maintained at 37 C. Atmospheric oxygen was used as the source of oxygen in the reaction. Only 1-ml samples were used in the cream, to reduce any possible error due to the higher viscosity of cream as compared with milk or skimmilk.

The flasks were equilibrated in the Warburg bath for at least 15 min before tipping the contents of the sidearm into the main compartment. After tipping, readings were taken at 5-min intervals for a period of 30 min. It was found that the oxygen uptake during the first 5-min period was erratic; the first reading was, therefore, omitted. The reaction rate had generally started to decline markedly after 25 min. It was, therefore, decided to use only the four highest readings for the calculations of activity. Generally, these fell between the first and the fifth readings.

The changes in pressure read on the manometers were then converted to microliters of oxygen, and the results expressed as μl O_2/ml/hr. The formula for the conversion is as follows:

$$\mu\text{l } O_2/\text{ml/hr} = \frac{R \times 3 \times K}{S}$$

Where:

R = Sum of four highest readings (i.e., μl O_2/ml/5 min)
K = Flask constant
S = Size of sample in milliliters

Since the fat percentages of the creams varied so much that their enzyme activities could not easily be compared, it was necessary to calculate the activities per gram of fat. Activities could then readily be compared. In these calculations it was assumed that milliliters and grams of fat were equal. This may distort the true activities somewhat, but since the error is similar in all the calculations, and the purpose was only to compare the values, it was felt that this assumption was justified. The following formulas were derived for the calculations of activity in the fat:

1. $$\text{Activity per g of fat} = \frac{(a \times b) - (c \times d)}{e \times 10}$$

Where:

a = Activity per ml of cream
b = Ml of cream per 1,000 ml of milk (see Formula 2)
c = Ml of skimmilk in cream (see Formula 3)
d = Activity per ml of skimmilk
e = Fat per cent of milk

2. $$b = \frac{1{,}000 \times \text{fat \% in milk}}{\text{Fat \% in cream}}$$

3. c = ml cream − ml fat

These formulas are only valid if there is 100% recovery of the fat in the cream. For all practical purposes, this was the case.

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EXPERIMENTAL RESULTS

Variations in enzyme activity. The first experiment was conducted to obtain more information concerning the increases in xanthine oxidase activity which have been reported to occur after cooling and storing milk. For this purpose ten samples of individual cows' milks were collected and the activities were determined before cooling (within 2 hr of milking) and after storage at 4 C for 24 hr. They were then stored for an additional 24-hr period and activities determined once again. Table 1 shows

TABLE 1

Increase in xanthine oxidase activity of milk after storage at 4 C

Sample no.	Apparent enzyme activity: Fresh uncooled milk	After 24 hr	After 48 hr	Average increase after storage
	——(μl O_2/ml/hr)——			(%)
1	53	70	80	49
2	68	114	121	74
3	72	142	133	92
4	73	138	142	92
5	73	166	147	116
6	83	200	202	142
7	84	166	160	94
8	90	200	204	126
9	103	228	224	120
10	105	184	179	74

the results from this experiment. Generally, the activity had reached a maximum level after 24 hr at 4 C. At that time the activity usually had about doubled, although the increase was by no means uniform. Although not shown in the table, several of the samples were tested once more after five days of storage at 4 C. The activities of these samples remained constant within the experimental error.

The increases in xanthine oxidase activity on storing and cooling have been attributed by Ball (1) to a release of xanthine oxidase from the fat globule membrane. In view of this theory, additional experiments were conducted to determine if other treatments which might be expected to bring about a release from the fat globule would produce an increase in activity. The treatments chosen for this purpose were heating, homogenization, and enzymatic action.

Seven samples were tested in the experiments involving heat treatment. The xanthine oxidase activities were determined in the fresh, uncooled samples and in the samples after they had been heated to 70 C for 5 min. Preliminary experiments had indicated that heating to 70 C produced a greater increase in activity than either higher or lower temperatures. Table 2 shows the increases produced by the 70 C treatment. The increases varied from 53 to 102%. The effect of homogenization is also shown in Table 2. Homogenization of fresh, uncooled milk resulted in increases in activity from 59 to 89%. Pereira, Kristoffersen, and Harper (13) claimed that homogenization reduces activity. However, they did not indicate whether the raw control had been cooled before testing.

Lipolytic and proteolytic enzymes also increased the xanthine oxidase of uncooled milk. The effect of four enzymes is shown in Table 3.

Variations in the distribution of enzyme. The effect of cooling, heating, homogenization, and enzymatic action on the activity of xanthine oxidase might be explained on the basis of the release of the enzyme from the fat globule membrane. To test this hypothesis, experiments

TABLE 2

Increase in xanthine oxidase activity of fresh uncooled milk on heating to 70 C and on homogenization

Sample [a] no.	Effect of heating: Fresh raw	Heated to 70 C for 5 min	Increase	Effect of homogenization: Fresh raw	Homogenized uncooled	Increase
	——(μl O_2/ml/hr)——		(%)	——(μl O_2/ml/hr)——		(%)
1	67	127	90	61	108	77
2	72	140	94	73	116	59
3	73	140	95	73	138	89
4	78	134	72	76	126	66
5	84	146	74	82	140	71
6	103	211	105	87	162	87
7	105	168	60	105	178	70

[a] With the exception of Samples 3 and 7, the samples used for the homogenization trials were different from those used in the heating trials.

TABLE 3

Increase of xanthine oxidase activity of fresh, uncooled milk on incubation with some commercial lipolytic and proteolytic enzymes

Enzyme added	Activity expressed as μl O_2/ml/hr: Fresh uncooled	After incubation [a] with enzyme	Per cent increase
Steapsin [b]	67	132	97
Lactivase [c]	73	153	110
Pancreatin [d]	67	128	91
Pancreatin [d]	73	131	80

[a] Samples were incubated at room temperature for 90 min.

[b] Eimer & Amend.

[c] Paul-Lewis Laboratories.

[d] Bausch & Lomb (two separate preparations).

were conducted to determine the effect of various treatments on the distribution of xanthine oxidase between the fat and skimmilk phases of milk.

To determine the normal amounts of activity in the fat phase and the skimmilk phase of cooled milks, nine samples which had been stored at 4 C for 24 hr were separated mechanically. The activities of the milks, creams, and the skimmilks were then determined. Fat percentages of the milks, creams, and skimmilks were also determined. The activities per liter of the original milk were compared with the amount recovered in the corresponding skimmilk and fat phases. Table 4 shows that there was remarkable consistency in the percentage of xanthine oxidase activity found in the skimmilk phase. It varied only from 66-80%. The sum of the recovered activities generally was quite close to the theoretical. The variations in recovered activities and percentage of activity in the skimmilk were of about the same order of magnitude.

To study possible redistribution of the enzymatic activity between the fat and the skimmilk phases on cooling of fresh milk, three samples of individual cow's milk were collected. Each sample was divided into three portions. One portion was separated and tested immediately without cooling. The second portion was separated immediately and stored for 24 hr at 4 C before testing. The third portion was cooled to 4 C and stored for 24 hr before separating. As is shown in Table 5, the activity of both the milk and cream increased on storage, whereas there was no activity increase in the skimmilk. However, skimmilk which was obtained by separating after 24 hr storage showed an increase in activity, whereas the corresponding cream showed a decrease. To determine if further redistribution between the skim and fat phase would occur on prolonged storage, a portion of the third sample was held 120 hr before separating and testing. Two additional samples were also stored 24 and 120 hr before separating. The results, as shown in Table 5, indicate that no significant redistribution took place after 24 hr.

To show whether heating also produced redistribution, three samples of fresh, raw milk were collected and divided into two portions. One portion of each sample was separated im-

TABLE 4

Relative amounts of xanthine oxidase activity [a] in whole milk, fat phase, and skimmilk phase from milks separated after at least 24 hr storage at 4 C

Sample no.	Amount in milk [b]	Amount in fat phase [c]	Amount in skim phase [d]	Recovered total [e]	Per cent in skimmilk [f]
1	91,000	26,000	60,000	86,000	70
2	161,000	44,000	97,000	141,000	69
3	182,000	34,000	140,000	174,000	80
4	168,000	52,000	99,000	151,000	66
5	168,000	49,000	107,000	156,000	69
6	224,000	54,000	163,000	217,000	75
7	232,000	50,000	173,000	223,000	78
8	133,000	38,000	96,000	134,000	72
9	127,000	49,000	91,000	140,000	65

[a] Activity computed from μl O_2/ml/hr.

[b] Activity per milliliter milk $\times$ 1,000.

[c] Activity per gram of fat $\times$ fat per cent of milk $\times$ 10.

[d] Activity per milliliter of skimmilk $\times$ milliliter skimmilk per 1,000 ml of milk.

[e] C plus [d].

[f] Activity in skimmilk phase as per cent of total recovered activity.

TABLE 5

Comparison between the xanthine oxidase activity in skimmilk and fat phase from milks separated uncooled and milks separated after storage at 4 C

Sample no.	Hr stored at 4 C		Activity in μl O_2/ml/hr		
	Before separation	After separation	Milk	Fat [a]	Skimmilk
1	0	0	72	1,060	43
	0	24	127	1,930	36
	24	0	127	980	100
2	0	0	86	1,000	33
	0	24	162	2,800	33
	24	0	162	1,080	123
3	0	0	103	1,140	42
	0	24	224	1,900	42
	24	0	224	1,070	179
	120	0	232	1,010	182
4	24	0	174	800	82
	120	0	163	800	92
5	24	0	168	935	107
	120	0	168	910	107

[a] Activities of the creams were converted to activities per milliliter of fat to compensate for differences in the fat content of the creams. See Experimental Section for method of conversion.

mediately and the other portion was heated momentarily to 70 C, then separated. The activities of the skimmilks obtained from the unheated portions were 43, 32, and 43 μl O_2/ml/hr and 87, 61, and 69 for the corresponding heated portions. Thus, it appears that heating produces a redistribution of the enzyme.

To test the effect of homogenization on the distribution of enzyme, two samples were collected and divided into two portions. One portion was separated immediately and the other was homogenized first and then separated. The activity in the skimmilks from the unhomogenized sample was 32 and 43, whereas the values for the corresponding homogenized portions were 74 and 71. (Incidentally, it was necessary to run the homogenized milk through the separator 20 times to reduce the fat test in the skimmilk to a reasonable level.)

Variations in heat sensitivity. The results obtained in the studies of the effect of heat on xanthine oxidase activity of uncooled milk (see Table 2) suggested that there were variations in the heat sensitivity of the enzyme. Additional experiments were conducted to obtain more information on this effect.

In the first experiment a sample of milk was stored for 24 hr at 4 C, then subjected to temperature treatments at 2.5 C intervals from 60-80 C, with a 5-min holding time at each temperature. The activities of the heat treated and raw samples were then determined. An aliquot of the sample was stored for four more days and the temperature treatments and measurements repeated. The results were expressed as percentage of the raw milk activity which remained after heating and storage. Figure 1 shows that the heat sensitivity of the enzyme is increased by aging of the milk, and that this increase is greatest in the region of 70 C.

To determine whether aging generally produces an increase in heat sensitivity, seven more samples were stored for 24 hr at 4 C. They were then heated to 70 C for 5 min and the activities of both the raw and the heat-treated samples determined. Aliquots of the raw samples were stored at 4 C for four more days,

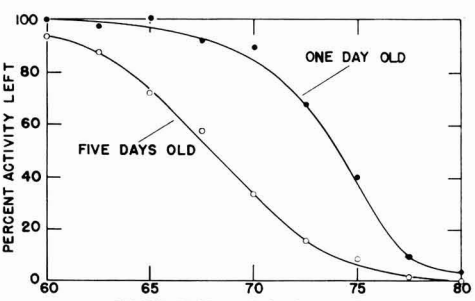

FIG. 1. Comparison of the heat inactivation of xanthine oxidase in a sample of milk after one and five days of storage at 4 C.

TABLE 6

Effect of storage at 4 C on the heat sensitivity of xanthine oxidase in milk

Sample	Per cent activity left [a] after heating [b]	
	Days of storage before heating	
	1	5
1	88	47
2	73	42
3	73	40
4	86	50
5	91	49
6	82	55
7	90	51

[a] The activity left is expressed as percentage of the activity in the comparable unheated sample.

[b] Samples were heated to 70 C for 5 min.

then heated, and the activity determinations repeated. Again, the raw samples showed no change on storage, and all results were expressed as percentage of the raw. As may be seen from Table 6, the sensitivity of the samples after one day's storage varied somewhat from sample to sample. But the heat sensitivity of the enzyme in each sample was significantly greater after aging for five days.

To ascertain whether the change in sensitivity was a gradual one, two samples were heat treated (70 C for 5 min) fresh and after storage at 4 C for one, two, and five days. Approximately the same results were obtained for both samples. The average percentages of activity left after the heat treatment were 95, 80, 60, and 45 for the fresh, one-, two-, and five-day-old samples, respectively. These results indicate that the heat sensitivity of the enzyme gradually increases with age.

To see if the same changes occurred in cream and skimmilk, a portion of a milk sample was separated after storage at 4 C for two days. The activities of the raw samples were determined and also the activities remaining after holding each of these at 70 C for 5 min. These samples were then stored for three additional days and the treatments and measurements repeated. The increase in sensitivity proceeded in a similar manner in the milk, cream, and skimmilk.

The effect of homogenization on the heat sensitivity of xanthine oxidase in milk is shown in Table 7. It may be seen that initially, homogenization had very little effect on the heat sensitivity of the enzyme. However, after standing for 24 hr at 4 C, the homogenized samples were considerably more sensitive to the heat treatment than the unhomogenized samples.

An experiment was conducted to determine if the sensitization of the enzyme in both homogenized and unhomogenized samples could be increased by increasing the temperature of storage. The activities of fresh, uncooled samples of homogenized and unhomogenized milk were determined both before and after heating to 70 C for 5 min. The samples were then divided into two portions each. One portion was incubated at 37 C and the other portion was cooled immediately to 4 C. After 4-hr storage aliquots from each portion were tested before and after heating. The enzyme in the portion held at 37 C had become more sensitive to heat than in the portion held at 4 C, as shown in Table 8.

DISCUSSION

The increases in apparent xanthine oxidase activity which occurred when fresh milk was subjected to cooling, heating, homogenization, and enzymatic activity (Tables 1, 2, and 3) indicate that some of the enzyme is not active in fresh uncooled milk. On the average, these treatments produced about a twofold increase in activity as determined by the manometric technique. Some workers (19, 21) using the methylene blue reduction test have observed three- to sevenfold increases. Results obtained in this study as well as those of others (1, 10, 15, 16, 20) indicate that initially the enzyme is bound in some form to the fat phase. The increase in enzyme activity is always accompanied by a great increase in activity in the skimmilk phase. This observation has given rise to the hypothesis that the activity increase is related to the redistribution of the enzyme. Inasmuch as Morton (11) has shown that the

TABLE 7

Effect of homogenization on the heat sensitivity of xanthine oxidase in milk

Age of samples	Per cent activity left [a] after heating [b]	
	Unhomogenized	Homogenized
Fresh	100	100
24 hr	96	70
Fresh	95	86
24 hr	80	29
Fresh	84	77
24 hr	80	62

[a] Expressed as percentage of the same sample raw and cooled.

[b] Samples were heated to 70 C for 5 min.

TABLE 8

Comparison of the heat sensitivity of xanthine oxidase in samples of milk incubated 4 hr at 4 and 37 C

Sample	Per cent activity left [a] after heating to 70 C for 5 min			
	Unhomogenized		Homogenized	
	4 C	37 C	4 C	37 C
1	....	....	85	59
2	80	66	73	64
3	82	75	76	58

[a] Expressed as percentage of the same sample raw and cooled.

enzyme occurs in microsomes, it is assumed that a desorption of the microsomes from the fat is involved.

This study indicated that only a part of the microsomes in question are held tenaciously by the fat portion of the milk. In none of the samples studied was there less than 20% and usually about 30% of the enzyme associated with the fat in cooled milks (Table 4). It was also demonstrated that there was essentially no change in the amount of activity per gram of fat between 24 and 120 hr (Table 5). This indicated that a rather definite portion of the microsomes was held much more tenaciously than the rest. Perhaps the reason that some are not held as tightly as others is that of position, i.e., they are not in contact with those substances on the fat globule membrane which best absorb them. Perhaps they are aggregated around the ones that are more tightly bound, and form layers or clumps of microsomes. If this is the case, penetration of the substrate or the hydrogen acceptor to the site of reaction might become a limiting factor in the determination of activity. Once the outermost layers of microsomes have been shed, such as by cooling, the total activity of the enzyme is easily measured. In the manometric method the samples are shaken continuously during the reaction, perhaps improving the penetration somewhat. In methods other than the manometric one, no agitation is employed. As a result, one might expect to be able to measure more of the enzyme in uncooled milk with the Warburg method than with the other methods. Calculations of data reported by Worden (21) lend support to this theory. His data show that after 36-hr storage at low temperatures a 1.5-2.8-fold increase in activity was observed when he used the manometric method; whereas, a 4.0-7.2-fold increase occurred when he used the methylene blue reduction test. Obviously, one should be careful in interpreting results obtained by different methods of analysis of xanthine oxidase, especially in the case of uncooled milk.

As was pointed out by Kiermeier and Vogt (9) the literature on the heat sensitivity of xanthine oxidase shows gross inconsistencies, especially in the region of 60-70 C. The discrepancies have not been explained at all, or have been attributed to differences in the general composition of the milks.

Data obtained in this study indicate that the heat sensitivity of the enzyme gradually increases with age. Since even a few hours' difference in age affects the heat sensitivity, it seems reasonable to assume that some of the discrepancies in the literature may be due to the effect of aging. Earlier investigators were not aware of the aging effect and consequently they did not report the age of their samples. Another possible misleading factor is the increase in activity of uncooled samples upon heating (Table 2). It should be pointed out that the heat treatment of cooled samples never resulted in an increase of activity.

One is led to speculate on the cause for the increase in heat sensitivity on aging. It could be that protective substances are gradually destroyed, or that substances which accelerate the destruction by heat are produced. Another and more likely possibility is that the enzyme becomes gradually solubilized. This is particularly true in view of the microsomal nature of milk xanthine oxidase, since microsomal or particulate enzymes are more heat-stable than those in aqueous solution. Xanthine oxidase in milk, for example, generally appears to be completely inactivated only if subjected to temperatures of over 80 C. Holding milk 4 hr at 60 C does not affect the enzyme activity at all (17), whereas purified solutions have been reported (4) to be destroyed at 56 C for 3 hr. In the present study the milk enzyme was destroyed after 5 min holding at 66-68 C after butanol, a well known solubilizing agent for many enzymes, had been added to the milk.

To solubilize xanthine oxidase from the microsomal particles, the materials holding the particles together must be disrupted. It was suspected, because of the gradual nature of the process, that it was enzymatic, i.e., that an enzyme or enzymes in the milk slowly attacked the cementing materials, thus releasing the enzyme from the microsomes.

The increase in sensitivity produced by homogenization (Table 7) supports the solubilization theory. Furthermore, homogenization, as is well known, speeds up milk lipase activity,

and could possibly speed up the activity of other enzymes. The fact that samples incubated at 37 C for 4 hr exhibited greater heat sensitivity than their counterparts stored at 4 C also seems to support the enzymatic solubilization theory.

The authors believe that there is a distinct difference between the dispersal of microsomes and the solubilization or disruption of individual microsomes. The dispersal of microsomes is believed to be associated with increases in activity, whereas solubilization is associated with increases in sensitization to heat. The evidence indicates that dispersal can be brought about by relatively mild treatment in a short period of time. On the other hand, the studies pertaining to heat sensitization suggest that solubilization requires more time or more vigorous treatment.

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FACTORS AFFECTING THE SUSCEPTIBILITY OF MULTIVITAMIN MINERAL MILK TO OXIDATION

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SUMMARY

The flavor of multivitamin mineral (MVM) milk was not affected when it was processed in a conventional HTST pasteurizer-homogenizer system. However, when it was subjected to a steam-vacuum treatment, in addition to pasteurization and homogenization, an oxidized flavor developed. The off-flavor developed regardless of whether the MVM preparation was added before or after processing. This indicates that alteration of the milk, rather than the MVM preparation, was involved in the development of the oxidized flavor.

It was observed that homogenized-treated milk from another milk plant did not develop an oxidized flavor when fortified with MVM mix. Results indicated that the difference in susceptibility between the homogenized-treated milk from the two plants was due to processing differences.

The iron compound, ferric pyrophosphate soluble, was identified as the ingredient in the MVM preparation causing the oxidized flavor. Fortification of homogenized and homogenized-treated milks with ferric and ferrous compounds showed that both milks developed oxidized flavors when fortified with ferrous iron. However, the homogenized-treated milk was more susceptible than the homogenized milk when ferric iron was added. It was suggested that the difference in susceptibility between homogenized and homogenized-treated milk is a difference in the capacity of these milks to reduce ferric iron to the stronger prooxidant ferrous form. Measurements of the relative amounts of iron reduced by these milks substantiate this explanation.

In recent years, multivitamin mineral (MVM) milk has become an important commercial product. As used in this paper, MVM milk refers to milk containing at least 4,000 USP units of vitamin A, 400 USP units of vitamin D, 1 mg thiamine, 2 mg riboflavin, 10 mg niacin, 10 mg iron, and 0.1 mg iodine per quart.

Some dairy plants reported that off-flavors develop in MVM milk. The first time this product was prepared at the University dairy plant it developed an oxidized flavor. In this first run the milk had been subjected to a steam-vacuum treatment in addition to conventional HTST pasteurization and homogenization. Subsequent processing trials revealed that the off-flavor did not develop when the product was merely subjected to conventional pasteurization and homogenization. Milk processed in this latter manner will be referred to as homogenized milk, whereas milk subjected to the additional steam-vacuum treatment will be referred to as homogenized-treated milk.

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This study was undertaken to determine the factors responsible for the oxidized flavor development in the homogenized-treated milk. The initial objective was the identification of the component or components in the MVM concentrates which were involved in the off-flavor development. The second objective was to elucidate the effect of the steam-vacuum treatment on the susceptibility to oxidation.

EXPERIMENTAL PROCEDURE

Multivitamin-mineral concentrates. The multivitamin-mineral (MVM) concentrates used in this study were commercial preparations supplied by General Mills, Inc., Minneapolis, and the Nopco Chemical Company, Harrison, New Jersey. The term MVM mix, as used subsequently, refers to the water-soluble components of a multivitamin-mineral preparation. The iron compound in the preparations was present as ferric pyrophosphate soluble.

Preparation of samples. Unless otherwise stated, the preparation of samples was as follows: Additives were weighed out and trans-

ferred into sanitized amber bottles. The bottles were half-filled with milk, capped, and shaken to dissolve the additive. The bottles were then filled with milk and poured back and forth from one bottle to another to insure mixing. After capping, bottles were stored at 40 F until judged.

Processing equipment. Commercial processing equipment was used in this study. The milk was heated at 169 ± 1 F for 16 sec in a HTST pasteurizer, then homogenized at 1,700 psi pressure. After homogenization, the milk was cooled to 40 F in the regeneration and cooling sections of the HTST unit. Milk processed in this manner is referred to as homogenized milk. The term homogenized-treated milk applies to milk which had been exposed to a steam-vacuum treatment in addition to the regular homogenizer treatment. The vacuum treatment was accomplished with a double-chamber Cherry-Burrell Aro-Vac Model AV-A unit. An Ultra-High Temperature (UHT) direct steam injector was used to heat the milk to 200 F. The milk was first pasteurized at 169 ± 1 F in the HTST unit, then heated to 200 F and held for about 1 sec in the UHT heater. The milk passed into the first chamber of the Aro-Vac unit, where the temperature dropped to approximately 190 F. The milk was drawn from the first to the second chamber by the lower pressure in the second chamber. The temperature of the milk in the second chamber was approximately 165 F. A centrifugal pump was used to pump the milk to the homogenizer, where it was homogenized at 1,700 psi pressure. The milk was then cooled to 40 F in the regeneration and cooling sections of the HTST unit.

Homogenized-treated milk processed through different equipment at a dairy plant in Albany, New York, was used for comparative purposes in this study. The sequence of processing homogenized-treated milk was the same in the Albany plant as in the Cornell dairy plant. However, there were some differences in equipment and processing rates. The vacuum treatment was accomplished with a single-chamber Cherry-Burrell Aro-Vac unit at the Albany plant, whereas a double chamber unit was used at Cornell. The milk was held at 190 F or above for 3-4 sec in the double-chamber unit (4). A holding time of .5-.6 sec at 200 F was calculated for the single-chamber unit. This calculation was based on the pipe diameter and average flow rate. Milk was processed at a rate of 20,000 lb/hr at Albany as compared to 4,000 lb/hr at Cornell.

Oxygenation-deoxygenation experiment. Approximately 20 qt of milk were put into a 40-qt stainless-steel milk can. Deoxygenation was accomplished by introducing nitrogen gas into the bottom of the milk can through tygon tubes and dispersed into the milk through fritted glass aerators on the ends of the tubes. It took approximately 30 min to deoxygenate to the desired oxygen content (i.e., 3-4 mg per liter). Deoxygenation was carried out in a 40 F cooler, so that the temperature of the milk would not be affected. Milk was oxygenated by shaking a quart bottle one-half full of milk and then pouring the milk from one bottle to another several times.

To minimize changes in the oxygen levels in the samples, special handling was used. In this case, the iron was added in a concentrated solution rather than as a dry preparation. Furthermore, samples were siphoned into the sanitized bottles through tygon tubes, so that the bottles could be filled from below the surface of the milk in the bottle.

Flavor evaluations. An experienced panel was used to evaluate the samples of milk. A code was used so that the judges did not know the identity of the samples. The samples were tempered to 60 F before they were judged.

Iron determinations. A method devised by Ruegamer, Michaud, and Elvehjem (6) was used to determine the iron content of milk. Glass-distilled water was used in making up the reagents. Glassware was soaked in a dilute versene solution for 24 hr prior to use. The versene solution was rinsed from the glassware with distilled water and the glassware was allowed to dry. The amount of iron present in the samples was calculated by reference to a standard curve (2).

Reduced ascorbic acid determinations. Titrations with 2,6-dichloroindophenol of metaphosphoric–trichloroacetic acid filtrates prepared according to Doan and Josephson (1) were used to determine reduced ascorbic acid. The dye was prepared and standardized as recommended by Stewart and Sharp (9).

Oxygen. Dissolved oxygen was determined by the ascorbic acid oxidase method of Sharp, Hand, and Guthrie (7). The crude ascorbic acid oxidase was concentrated by pervaporation.

2,2′-Bipyridine test to determine ferrous iron. The following procedure was devised to measure the relative reducing capacities of different milks. Different aliquots of milk to be tested were fortified with ferric and ferrous iron. For this purpose, 10 mg of ferrous sulfate and the equivalent amount of ferric pyrophosphate soluble in the form of the MVM mix were added per quart of milk.

The fortified milks were stored at 40 F for 6 hr. Then 25 ml of each aliquot were pipetted into 125-ml Erlenmeyer flasks containing 1 ml of a .21% 2,2′-bipyridine solution. 2,2′-Bipyridine reacts specifically with ferrous iron to produce a pink color (2). Two drops of chloroform were added to each flask to prevent bacterial action. Stoppers were put on the flasks; the flasks were left in a dark place at room temperature for 6 hr for color development. Twenty-five milliliters of fully saturated ammonium sulfate were pipetted into each flask to precipitate the casein. The flasks were gently swirled to mix the contents. The contents of each flask were then filtered through Whatman No. 40 filter paper into a second 125-ml Erlenmeyer flask. The amount of color in each filtrate was determined in a Bausch and Lomb Spectronic 20 colorimeter at 520 mμ. The filtrate of a milk that had no iron added was used for the blank. The optical densities of the filtrates showed the relative amounts of ferrous iron in the milks which indicate their relative reducing capacities for the iron system. This method will subsequently be referred to as the 2,2′-bipyridine test.

Preliminary experiments indicated that the method of handling the milk could affect the 2,2′-bipyridine test. Bottled homogenized-treated milk was found to give higher values than homogenized-treated milk collected in a milk can. Therefore, the method of handling milk for the 2,2′-bipyridine test was standardized as follows: Samples were collected in stainless-steel milk cans from a valve located on the output side of the cooling section of the HTST unit. The ingredients to be added to the milk were weighed out and placed in sanitized amber bottles. The milk was siphoned into the bottles through a piece of tygon tubing and the ingredients were mixed by placing plastic-covered magnets into the bottles and mixing for several minutes on magnetic mixers. The magnets were removed from the bottles and the bottles were capped and stored at 40 F.

RESULTS AND DISCUSSION

Preliminary experiments confirmed the report that homogenized-treated MVM milk developed an oxidized flavor. Furthermore, when MVM concentrates were added to homogenized-treated milk an oxidized flavor also developed. The fact that the off-flavor developed without the MVM concentrates having been exposed to the steam-vacuum treatment indicates that the steam-vacuum treatment causes a change in the milk rather than in the MVM concentrates, which makes homogenized-treated MVM milk susceptible to oxidized flavor. This made it possible to continue the investigation by adding concentrates to homogenized-treated milk rather than by processing the fortified milks after the concentrates had been added.

Selective addition of various components of MVM mixtures. There are two parts to the complete MVM concentrates. One part contains the fat-soluble vitamins A and D and the other part contains the water-soluble vitamins and minerals. These two parts were added separately to homogenized-treated milk. An oxidized flavor developed in the milk to which the water-soluble ingredients (MVM mix) had been added. The milk containing only the fat-soluble ingredients did not develop an off-flavor.

Special MVM mixes were prepared so that each mix was minus one of the water-soluble components. The milk fortified with the mix lacking iron did not develop an oxidized flavor. All of the other special mixes did cause oxidized flavor. This indicates that the iron compound is the component responsible for causing the off-flavor. In his review of the literature on oxidized flavor, Riel (5) points out that the pro-oxidant effects of iron have been demonstrated by many investigators.

The possibility that the UHT direct steam injector was adding iron to the milk was considered. Iron determinations were run on milk before and after it passed through the direct steam injector. No significant difference was observed in the iron content of the milk taken before and after the injector.

Comparison of susceptibility of vacuum-treated milk from two different plants. Reports had been received that homogenized-treated MVM milk from a plant in Albany, New York, did not develop an oxidized flavor. To verify this report homogenized-treated milk from both the Albany and Cornell dairy plants were fortified with MVM mix. The treated milk from Cornell developed an oxidized flavor, whereas the milk from Albany did not develop an oxidized flavor.

There are two major differences between the homogenized-treated milk at Cornell and the homogenized-treated milk from Albany. The two milks come from different sources and are processed through different equipment. In an attempt to determine which variable was responsible for the difference in the susceptibilities between the two milks, milk from the Albany source was processed through the Cornell milk plant. Raw milk from Albany was transported to Cornell in stainless-steel milk

TABLE 1

Flavor evaluations[a] of five-day-old homogenized-treated milk from two different sources processed in two different plants

Milk source	Processing plant	Additive	Trial 1	Trial 2
Cornell Univ.	Cornell Univ.	None	0	0
Cornell Univ.	Cornell Univ.	MVM mix	3	4
Albany	Cornell Univ.	None	0	0
Albany	Cornell Univ.	MVM mix	3	2
Albany	Albany	None	0	0
Albany	Albany	MVM mix	0	0
No. of judges			3	4

[a] The values indicate the number of judges who criticized the sample for being oxidized.

cans. The temperature of the milk raised from 34 to 40 F in transit in Trial 1 and from 36 to 60 F in Trial 2. The milk was steam-vacuum-treated at the Cornell plant and fortified with MVM mix. For comparison, milk from the same storage tank was treated at Albany and transported to Cornell under refrigeration. This milk was also fortified with MVM mix. Table 1 shows that the milks processed at Cornell developed an oxidized flavor, whereas the milk processed at Albany did not. This indicates that a difference in the process between the two plants is involved in the difference in susceptibilities between the two milks.

Both steam-vacuum units were operated at 200 F but, as indicated earlier, the milks are held at the elevated temperatures for a longer period of time in the Cornell steam-vacuum unit than in the Albany unit. Whether this time-interval at the elevated temperature is critical in the development of oxidized flavor is a subject that needs further study. Of course, it is possible that a difference in susceptibility between the two raw milks also may be involved.

Differences between homogenized and homogenized-treated milk. Kleyn and Shipe (3) studied the differences between homogenized-treated and homogenized milks. They found that homogenized-treated milk had a lower oxygen level, higher sulfhydryl content, and a longer retention of reduced ascorbic acid than homogenized milk. These differences were studied to see if they account for the difference in susceptibility between homogenized-treated and homogenized milks fortified with MVM mix.

Homogenized milk with an initial oxygen content of 10.7 mg per liter was deoxygenated to 3.35 mg per liter. Homogenized-treated milk with an initial oxygen content of 3.05 mg per liter was oxygenated to 8.20 mg per liter.

The difference in sulfhydryl values between homogenized milk and homogenized-treated milk was reported (3) to be approximately 3.3 mg per liter, expressed as cysteine hydrochloride. Therefore, 3.3 mg per quart of cysteine hydrochloride were added to homogenized and deoxygenated homogenized milk.

Flavor evaluations of these samples are shown in Table 2. The fortified deoxygenated homogenized milk did not develop an oxidized flavor and the fortified oxygenated homogenized-treated milk did develop an oxidized flavor. The fortified homogenized milks with added cysteine hydrochloride also failed to develop an oxidized flavor. This indicates that the difference in oxygen content, by itself or with added cysteine, does not account for the difference in susceptibility between the fortified homogenized-treated and the fortified homogenized milk.

Reduced ascorbic acid determinations were performed on fortified homogenized milk, fortified homogenized-treated milk, and fortified homogenized-treated milk containing hydrogen peroxide (.025 ml of 30% hydrogen peroxide per quart of milk). Table 3 shows that the MVM mix caused an increase in the rate of disappearance of reduced ascorbic acid in homogenized and homogenized-treated milk. The addition of hydrogen peroxide prevented the development of oxidized flavor, but it increased the rate of disappearance of reduced ascorbic acid. The fortified homogenized-treated milk had a more stable ascorbic acid system than the other fortified milks, yet it was the only fortified milk that developed an oxidized flavor. One interpretation of this is that the milk with the stronger reducing system is more prone to develop an oxidized flavor when fortified with MVM mix.

Addition of ferric and ferrous iron compounds to homogenized and homogenized-treated milk. In view of the reports (10, 11) that ferrous iron is a much stronger pro-oxidant than ferric iron, different ferric and ferrous compounds were added to homogenized and homogenized-treated milk. The flavor evaluations in Table 4

TABLE 2

Effect of oxygen content and added cysteine hydrochloride [a] on the development of oxidized flavor

Sample	Additive [b, c]	Oxygen content	Flavor evaluation		
			1 day	2 days	5 days
		(mg per liter)			
Homogenized-treated	None	3.05	0	0	0
Homogenized-treated	Iron		3	4	3
Homogenized-treated (oxygenated)	None	8.20	0	0	0
Homogenized-treated (oxygenated)	Iron		2	3	4
Homogenized	None	10.70	0	0	0
Homogenized	Iron		0	0	0
Homogenized	Iron + cysteine HCl		0	0	0
Homogenized (deoxygenated)	None	3.35	0	0	0
Homogenized (deoxygenated)	Iron		0	0	0
Homogenized (deoxygenated)	Iron + cysteine HCl		0	0	0
No. of judges			4	4	4

[a] The values indicate the number of judges who criticized the sample for being oxidized.
[b] Iron additive—10 mg of iron in the form of an aqueous solution of ferric pyrophosphate soluble.
[c] 3.3 mg of cysteine HCl per liter.

show that homogenized-treated milk is susceptible to oxidized flavor development when fortified with either ferrous or ferric iron. However, homogenized milk is much more susceptible when fortified with ferrous iron than when fortified with ferric iron. One way of explaining the difference in susceptibility between homogenized-treated and homogenized milk when fortified with ferric iron is that the ferric iron in the homogenized-treated milk was reduced to ferrous iron. This is in line with the observation of Smith and Dunkley (8), that reduction of copper was involved in the development of copper-induced oxidized flavor.

The addition of ferric orthophosphate and sodium ferric pyrophosphate to homogenized-treated milk failed to produce an oxidized flavor. However, further investigation revealed that the solubility of these compounds was less than 10 mg iron per quart. A previous experiment indicated that levels of ferric iron below 5.0 mg per quart did not produce an oxidized flavor in homogenized-treated milk. Therefore, the low solubility of these compounds could explain why they did not produce an oxidized flavor in homogenized-treated milk.

Determination of reducing capacities of different milks. The 2,2′-bipyridine test was used

TABLE 3

Reduced ascorbic acid contents and the flavor evaluations [a] of fortified homogenized milk, fortified homogenized-treated milk, and fortified homogenized-treated milk plus hydrogen peroxide

Milk	Additive [b]	Reduced ascorbic acid *(mg per liter)*			Flavor evaluation		
		1 day	2 days	5 days	1 day	2 days	5 days
Homogenized	None	13.1	13.3	11.0	0	0	0
Homogenized	MVM mix	11.3	11.0	5.7	0	0	0
Homogenized-treated	None	13.5	13.5	13.3	0	0	0
Homogenized-treated	MVM mix	12.5	12.2	11.4	3	3	3
Homogenized-treated	MVM mix plus H_2O_2	9.1	8.5	7.2	0	0	1
No. of judges					4	4	3

[a] The values indicate the number of judges who criticized the sample for being oxidized.
[b] .025 ml of 30% H_2O_2 per quart of milk.

TABLE 4

Flavor evaluations [a] of homogenized and homogenized-treated milks fortified with ferrous and ferric iron compounds

Milk	Additive [b]	Flavor evaluation		
		1 day	2 days	5 days
Homogenized-treated	None	0	0	0
Homogenized-treated	Ferrous sulfate	4	4	4
Homogenized-treated	Ferrous ammonium sulfate	4	4	4
Homogenized-treated	Ferric sulfate	3	3	2
Homogenized-treated	Ferric ammonium sulfate	4	4	4
Homogenized	None	0	0	0
Homogenized	Ferrous sulfate	4	4	4
Homogenized	Ferrous ammonium sulfate	2	2	3
Homogenized	Ferric sulfate	0	0	1
Homogenized	Ferric ammonium sulfate	0	0	1
No. of judges		4	4	4

[a] The values indicate the number of judges who criticized the sample for being oxidized.
[b] The ferrous and ferric compounds were added in such quantities that approximately 10 mg of iron was added per quart of milk.

to determine the effect of treatment on the ability of milk to reduce ferric to ferrous iron. For this purpose, the reducing capacities of homogenized milk, homogenized-treated milk, and homogenized-treated milk with added hydrogen peroxide were compared. The optical densities of the filtrates from these milks are shown in Table 5. These results show that the filtrates from the homogenized-treated milk contained more ferrous iron than the filtrates from the other milks, regardless of whether the iron was added in the ferric or ferrous form. This indicates that the homogenized-treated milk had a stronger reducing system for iron than did the two other milks.

The fact that homogenized-treated milk reduces more ferric iron to the stronger pro-oxidant ferrous form could explain why homogenized-treated milk is more susceptible to oxidized flavor when fortified with certain ferric compounds.

TABLE 5

Relative amounts of ferrous iron in different milks fortified with MVM mix and ferrous sulfate

Milk	Optical densities when fortified with:	
	MVM mix [a]	Ferrous sulfate [b]
Homogenized-treated	.0757	.1973
Homogenized-treated + H_2O_2 [c]	.0269	.0969
Homogenized	.0410	.0783

[a] Iron compound in MVM mix: Ferric pyrophosphate–soluble.
[b] 10 mg of iron in the form of ferrous sulfate.
[c] .025 ml of 30% H_2O_2 per quart of milk.

The addition of hydrogen peroxide retarded the reduction of iron in fortified homogenized-treated milk (Table 5). This could explain the observation that adding hydrogen peroxide to fortified homogenized-treated milk prevented the development of oxidized flavor (Table 4).

The increased susceptibility of homogenized-treated milk appears to involve a three-step process. First, the steam-vacuum treatment increases the reducing properties of the milk. Second, added ferric iron is reduced to the stronger pro-oxidant ferrous form. Third, the ferrous iron, being a strong pro-oxidant, catalyzes the oxidation of the milk.

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FORCED-CONVECTION HEAT-TRANSFER CHARACTERISTICS OF FLUID MILK PRODUCTS DURING COOLING

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SUMMARY

The cooling characteristics of four milk products were investigated. A tubular heat exchanger (cooled with refrigerated water) equipped with wall and stream thermocouples was used. The mass flow rates were regulated to effect a Reynolds Number range of 5,000 to 250,000.

The Nusselt-type equations which described the cooling characteristics of each of the products investigated follow: skimmilk (8.8% SNF), $Nu = 0.204\ Re^{0.586}Pr^{0.4}$; whole milk (3.8% fat, 8.7% SNF), $Nu = 0.118\ Re^{0.609}Pr^{0.4}$; 10% milk (10% fat, 7.5% SNF), $Nu = 0.108\ Re^{0.613}Pr^{0.4}$; 25% cream (25% fat, 6.7% SNF), $Nu = 0.026\ Re^{0.571}Pr^{0.4}$.

A general equation which described the cooling characteristics of all four products was $Nu = 0.11\ (Re^a)^{0.686}Pr^{0.4}$ where a was a factor for correcting the water equation to reflect the cooling characteristics of the dairy products. The a values for the products were: skimmilk—0.8454; whole milk—0.8032; 10% milk—0.8234; 25% cream—0.8594.

The objective of the study was to develop thermodynamic expressions which describe the forced-convection cooling characteristics of four fluid milk products.

EXPERIMENTAL PROCEDURE

Equipment. An apparatus similar to the one used in earlier work (5) was utilized in this study. The cooling portion of the apparatus was the testing section and consisted of a 10-ft length of stainless steel tubing (1-inch diameter) with six equally spaced wall and stream thermocouples. The cooling medium was refrigerated water at 34 F. The dairy product under study was recirculated through the testing section by means of a centrifugal pump and flow rate was controlled with a throttling valve ahead of the pump. The heating medium was saturated steam at 40 psi. The temperature range over which the experiment was conducted varied from 100 to 200 F and the approximate flow range was from 1,000 to 15,000 lb per hour. A sample size of approximately 120 lb of product was used in each trial and a water-dilutable dispersion of a silicone defoaming agent (5 ml per 10 gal) was added, to prevent the product from foaming during the experiment.

Viscosity determination. A MacMichael viscosimeter was used for making viscosity measurements. The instrument was calibrated against sucrose and viscosity determinations were made on several samples of each product to be investigated. The various viscosities compared favorably with those reported in earlier work (5).

Dairy products. Duplicate trials of 20 runs each were made on each of the following products: 25% cream (25% fat and 31.7% TS), 10% milk (10% fat and 17.5% TS), 3.8% milk (3.8% fat and 12.5% TS), and reconstituted skimmilk (8.8% MSNF).

The thermal conductivity and specific heat values were obtained from published information (3).

Standardization of instrument readings. Fifty trials were made with water and the results obtained were utilized in standardizing the instrument readings to correspond with the Nusselt type expression for water obtained in earlier work (5). The expression was $Nu/Pr^{.4} = .11Re^{0.686}$. This was in accordance with the recommendations of Brown and Marco (1), in that the recommended formula for describing the cooling characteristics of water is the same as that for describing the heating characteristics of that product.

RESULTS AND DISCUSSION

After establishing the accuracy of the apparatus, the heat-transfer characteristics of four fluid milk products were investigated. The summary graphs which represent the relation-

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ships of Reynolds (Re), Nusselt (Nu), and Prantl (Pr) numbers for whole milk and skimmilk are shown in Figure 1, and similar rela-

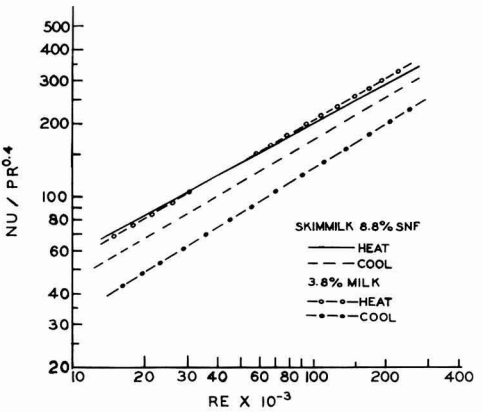

FIG. 1. Comparison of the forced-convection cooling characteristics of skimmilk and whole milk.

tionships for the two high-fat products are given in Figure 2. The heating curves in these two figures were taken from earlier work (5), for the purpose of comparing the heating characteristics to the cooling characteristics of the various products. These figures show that the rates at which heat can be transferred from the dairy products during cooling are less at particular Reynolds numbers than the rates at which heat can be transferred to the products during heating.

For example, from Figure 1 it can be seen that at Reynolds number of 10,000, the $Nu/Pr^{.4}$ value for heating reconstituted skimmilk containing 8.8% SNF was approximately 57; whereas, the $Nu/Pr^{.4}$ for that product during cooling at the same Reynolds number was 45. Also, from Figure 2, during heating the $Nu/Pr^{.4}$ value for milk containing 10% fat was approximately 240 at a Reynolds Number of 100,000; whereas, the corresponding $Nu/Pr^{.4}$ value during cooling was about 160. Similar results are shown for the other products.

Analysis of these data reveal that, in general, the heat-transfer coefficients for the respective products during cooling are from 65 to 75% as great as are the heat-transfer coefficients during heating at any particular Reynolds number within the experimental range of this study. This is in agreement with the findings of workers who have done similar work with other products (2).

The data represented by Figures 1 and 2 were tested by regression analysis techniques and the evaluations pertaining thereto are shown in Table 1. The values for b and a in this table were utilized in formulating Nusselt-type expressions for describing the cooling characteristics of the dairy products. The resulting expressions are given below. It can be seen that the expressions differ remarkably between products of varying compositions.

Skimmilk (8.8% MSNF)
$Nu = 0.204\ Re^{0.586}Pr^{0.4}$

Whole milk (3.8% fat; 8.7% MSNF)
$Nu = 0.118\ Re^{0.609}Pr^{0.4}$

10% milk (10% fat; 7.5% MSNF)
$Nu = 0.108\ Re^{0.631}Pr^{0.4}$

25% cream (25% fat; 6.7% MSNF)
$Nu = 0.260\ Re^{0.571}Pr^{0.4}$

A single expression which described the cooling characteristics of the several products was developed according to the method used by Peeples and Eastham (4). Essentially, the water equation which is $Nu/Pr^{.4} = 0.11(Re^{0.686})$ was modified as follows: $Nu/Pr^{.4} = 0.11\ (Re^{a})^{0.686}$ where a is a parameter for modifying the water equation in such a manner that it will describe the cooling characteristics of each of the individual products.

To obtain values for a, the values of $Nu/Pr^{0.4}$ and Re were taken from the original data; then, using the values for $Nu/Pr^{0.4}$, corresponding

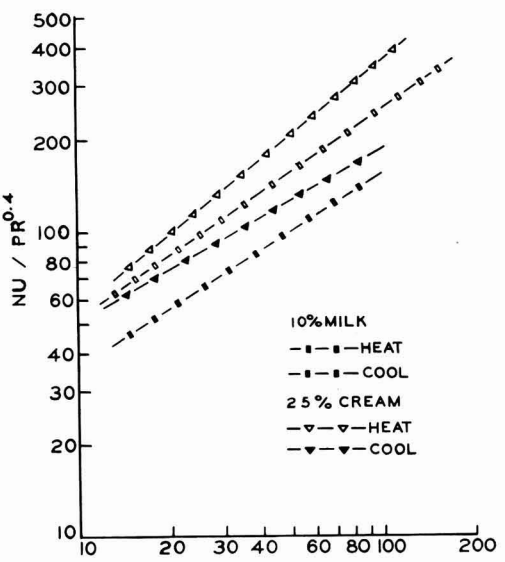

FIG. 2. Comparison of the forced-convection cooling characteristics of milk products containing 10 and 25% fat.

TABLE 1

Statistical evaluation pertaining to regression analysis of the logarithmic relationship of $Nu/Pr^{0.4}$ to Re for the dairy products

Product	$y = bx + a$		
	b	a	Sy
Reconstituted skim (8.8%)	.5863	− .991	.0714
Whole milk (3.8% F; 8.7% SNF)	.6090	−1.230	.05798
Cream (25% F; 6.7% SNF)	.5710	− .877	.06884
Milk (10% F; 7.5% SNF)	.6321	−1.264	.0462

$y = \log Nu/Pr^{0.4}$; $x = \log Re$.

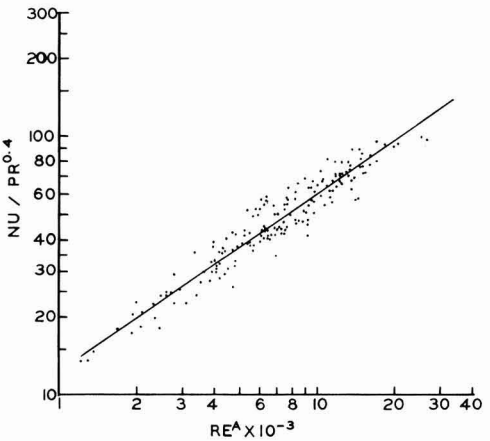

FIG. 3. Results of treating experimental data according to the equation $Nu = 0.11(Re^a)^{0.686}$ compared to the plot of $Nu/Pr^{0.4}$ vs. Re for water.

Reynolds numbers were calculated from the water equation. After obtaining these values, a was calculated by the following formula and the average a value determined for each product.

$$a = \log Re_1 / \log Re_2$$

where Re_1 = Reynolds number as calculated from the water equation using experimental values for $Nu/Pr^{0.4}$

and Re_2 = Experimental Reynolds number corresponding to each value of $Nu/Pr^{0.4}$

The values of a are shown below for the various products:

Product	a value
Reconstituted skimmilk (8.8% MSNF)	0.8454
Whole milk (3.8% fat; 8.7% MSNF)	0.8032
10% milk (10% fat; 7.5% MSNF)	0.8234
25% cream (25% fat; 6.7% MSNF)	0.8594

To show the accuracy with which the modified equation describes the cooling characteristics of the dairy products, a graphical representation was made (see Figure 3). The solid line in this graph represents the water equation, and superimposed over this line are the various points obtained by treating the experimental data according to the modified equation. A study of this graph reveals that the modified equation has practical value, in that the heat-transfer coefficient at a particular Reynolds number for any of the dairy products studied can be estimated.

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AMPEROMETRIC TITRATION OF SULFHYDRYL AND DISULFIDE GROUPS IN MILK PROTEINS [1]

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SUMMARY

Sulfhydryl (–SH) and disulfide (–SS–) groups in milk proteins were estimated amperometrically with silver nitrate. Cysteine, β-lactoglobulin, milk, and whey were titrated at different pH levels in sodium acetate, ammonium nitrate, and tris buffer solutions. This was done to study the fate of –SH and –SS– groups in some fluid milk products heated at high temperatures and their possible relationship to flavor and physical defects that appear during storage. Cysteine yielded results 5 to 50% higher than the theoretical values, depending on the titrating conditions. The cysteine content of β-lactoglobulin in sodium acetate buffer at pH 10.25 was 95.7% of the theoretical amount. Adding 1% sodium lauryl sulfate to the titrating medium made more –SH groups available, whereas 8 M urea decreased them. Decreased titers of –SH groups in heated milk coincided with the denaturation of serum proteins and they were oxidized to –SS– groups. Disulfide was stable at 95 C for 30 min. During sterilization of concentrated milk, titratable –SH decreased and –SS– increased.

Sulfhydryl (–SH) groups are the most reactive ones in proteins. Investigators have studied them in milk in relation to the heated flavor resulting from high-temperature processing (8, 11, 18). The –SH groups are also oxidized to disulfide (–SS–) or interchanged with another –SS–, resulting in deformation of native protein molecules (10, 12).

Sulfhydryls have been estimated by methods other than amperometric (9, 14, 15). Hutton and Patton (11), Zweig and Block (18), and Burton (4) investigated heat activation and distribution of –SH groups and their relationship to the heated flavor in milk. These investigators used variable titrating procedures, resulting in somewhat different conclusions.

Experimental conditions for titrating –SH and –SS– groups in casein, β-lactoglobulin, and milk, and the effect of two denaturing agents were investigated. This was done to study the fate of –SH and –SS– groups in fluid milk products subjected to sterilization temperatures and their possible relationship to flavor and physical defects that appear in these products during storage.

EXPERIMENTAL PROCEDURES

The electrolyte solutions used were sodium acetate, ammonium nitrate, and tris (tri-hydroxy-methyl aminomethane) adjusted to pH levels of 7 to 11, by the addition of concentrated ammonium hydroxide. All of them were 0.1 M and contained 10^{-4} M ethylenediamine tetraacetic acid disodium salt (EDTA). It was not necessary to purify the urea when EDTA was added (6, 17). Oxygen-free nitrogen gas was passed into the buffer solution through a sintered glass bubbler and only before the sample was added. The pH of the titrating medium was determined with a Beckman Model G instrument. Silver nitrate, 0.001 M, was added at half-minute intervals until the end point was attained. The method for making the amperometric titrations has been described (1).

β-Lactoglobulin was dissolved by adjusting the pH to 6.6 with 0.1 N NaOH. No salt was used. Its concentration was 0.7753%, estimated by the Kjeldahl method, N × 6.38. The milk used in these experiments was obtained from the University Farm.

Sodium caseinate was prepared by acidifying raw skimmilk with 1.0 N HCl, collecting the casein by filtering, redissolving with 1.0 N NaOH, and precipitating again at pH 4.6. It was washed three times with demineralized water and dispersed with 1.0 N NaOH at pH 6.6. The casein concentration was 2.72% (N × 6.38).

RESULTS AND DISCUSSION

Titration with cysteine. Some mercaptans like cysteine do not yield theoretical –SH values by the amperometric titration (1, 5, 16). Figure 1 shows the results of titrating cysteine in three buffers at different pH levels. The results are expressed in percentages of the theoretical. The end points of titration differed greatly

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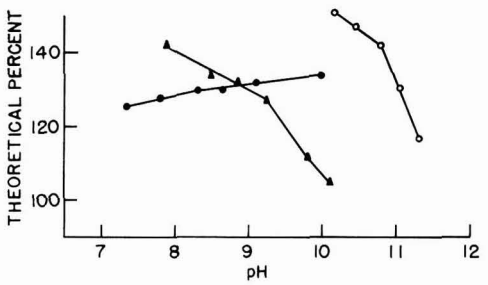

FIG. 1. Amperometric titration of cysteine HCl H_2O in sodium acetate -○-, ammonium nitrate -▲-, and tris -●- buffer solutions.

and the theoretical value was not obtained in the buffers used. Five to fifty per cent error resulted from the excess binding of silver ions as RS Ag (mercaptide), forming complexes like $(RS\ Ag)_2\ Ag^+$, $(RS\ Ag)_4\ Ag^+$, or $(RS\ Ag)_{10}$-Ag^+ (1, 13). Because titrating with Ag^+ is not specific for -SH groups, Burton (4) expressed his results in an arbitrary way, without giving a specific meaning to the end point unless an independent verification of the -SH content was available. Our results are expressed as cysteine or cystine, to make them comparable.

Titrating milk and β-lactoglobulin. Figures 2 and 3 show that sodium acetate at pH 10.25 yielded the highest values for both milk and β-lactoglobulin. The titratable -SH groups were 0.235 mM per liter of milk as compared to 0.264 mM reported by Zweig and Block (18). The highest value obtained was 0.235 and the lowest was 0.185 mM -SH per liter of milk at pH 10.25. Such variations have been reported (18). The highest titers in ammonium nitrate and tris buffers were 0.18 and 0.22 mM -SH per liter of milk, respectively. The -SH groups in raw milk titrated in ammonium nitrate buffer were higher than the data reported by Hutton and Patton (11) for titrations made in 50% alcohol.

Brand et al. (3) reported 1.11% for the -SH content of β-lactoglobulin (cysteine); whereas, our titrations in sodium acetate at pH 10.25 yielded 1.0626%. This figure was obtained by titrating 5 ml of a 0.7753% solution of β-lactoglobulin, which required 3.40 ml or an equivalent of 680 ml of silver nitrate for 7.753 g of this protein per liter. Therefore, 8.7708 ml of M silver nitrate will combine with 100 g of β-lactoglobulin ($680 \times 100/7.753 =$ 8,770.8 of 0.001 M or 8.7708 ml of M silver nitrate) and this is equivalent to 1.0626 g of cysteine ($121.15 \times 8.7708/1,000 = 1.0626$). This figure is 95.7% of the one reported by Brand et al. (3) ($1.0626/1.1100 \times 100 = 95.7\%$). Titrations with tris and ammonium nitrate buffers yielded 70.3 and 86.5%, respectively, of the cysteine in β-lactoglobulin.

Tris buffer at pH 7.4 was used in the amperometric titration of -SH groups in proteins (2). However, at a higher pH more -SH groups were titrated. According to Burton (5), more -SH groups in β-lactoglobulin were titrated in tris buffer as the pH was decreased, but we obtained fewer -SH groups in cysteine, β-lactoglobulin, and milk with this buffer when the pH was decreased.

Effect of denaturing agents. When protein is denatured, inactive -SH groups in the native state are expected to become titratable. These groups were titrated in the presence of 1% sodium lauryl sulfate (SLS) and in 8 M urea. Results are shown in Figures 4 and 5.

Adding 1% SLS to three solutions increased the titratable -SH in both skimmilk and β-lactoglobulin, and especially in ammonium nitrate and tris buffers. The highest titers for milk and β-lactoglobulin in each buffer with SLS agreed closely.

Lower -SH titrations were obtained in sodium acetate and tris buffers which contained 8 M urea. The effect of urea was dependent on the

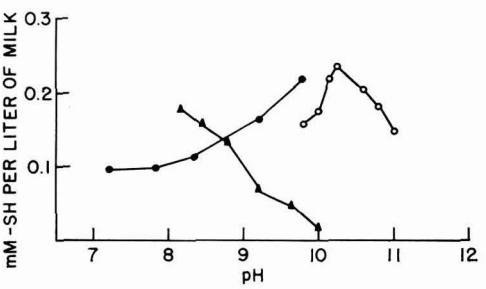

FIG. 2. Amperometric titration of raw skimmilk in sodium acetate -○-, ammonium nitrate -▲-, and tris -●- buffer solutions.

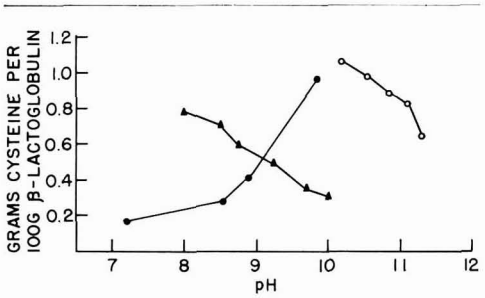

FIG. 3. Amperometric titration of β-lactoglobulin in sodium acetate -○-, ammonium nitrate -▲-, and tris -●- buffer solutions.

TABLE 1

Effect of urea and SLS in sodium acetate buffer on titration of –SH groups in raw milk [a]

Urea moles	mM –SH per liter	Per cent	SLS %	mM –SH per liter	Per cent
0	0.185	100	0	0.185	100
2	0.165	89	0.2	0.195	105
4	0.147	80	0.6	0.215	116
6	0.137	74	1.0	0.210	113
8	0.133	72	1.5	0.215	116
			2.0	0.210	113

[a] Amperometric titrations were made in sodium acetate buffer at pH 10.25.

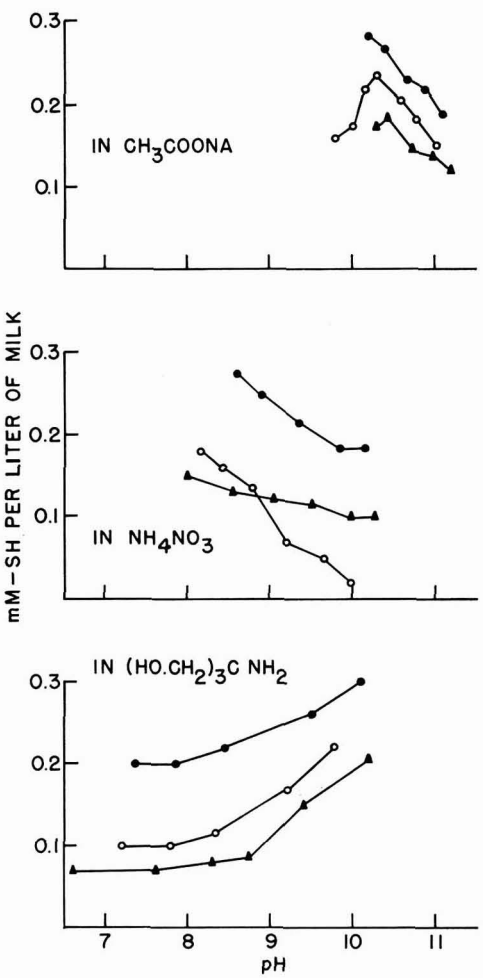

FIG. 4. Amperometric titration of raw skim-milk in the presence of denaturing agents. (–○– buffer only, –▲– 8 M urea in buffer, and –●– 1% SLS in buffer.)

pH of the ammonium nitrate buffer as lower –SH titrations were obtained in the more alkaline solution.

Sodium lauryl sulfate was more effective in lower concentrations for activating –SH groups in raw milk proteins; whereas, urea decreased the –SH titer (Table 1). Benesch et al. (2) used tris buffer containing 8 M urea at pH 7.4 and found that –SH groups in some proteins were not activated.

Effect of heat. Individual samples of the same milk were heated at 65, 75, 85, and 95 C for 30 min in a tightly closed container. Whey was prepared from each one by precipitating the casein with HCl at pH 4.6. Titrations for –SH groups in the milks and wheys are shown in Table 2. There was no activation of –SH groups in the heated milks. Their oxidation may have exceeded their activation. It is evident that the heat-denaturable serum protein precipitated with casein at pH 4.6 did not contain –SH groups. When milk was heated at 85 and 95 C, the serum protein which still had free –SH groups was precipitated with the casein.

Disulfide determinations. The decrease of free –SH groups in heated milk is due to oxidation to –SS– or production of other sulfur compounds which cause the cooked flavor. The –SS– groups in milk proteins were estimated

TABLE 2

Effect of heating for thirty minutes at different temperatures on the –SH groups in milk and its whey [a]

Temp	Milk	Whey	Whey N
(C)	*(mM –SH per liter)*		*(g per liter)*
Raw	0.195	0.217	1.147
65	0.162	0.185	1.072
75	0.092	0.110	0.770
85	0.072	0	0.544
95	0.062	0	0.518

[a] Amperometric titrations were made in sodium acetate buffer at pH 10.25.

by reducing them to –SH with sodium sulfite and urea, 1 M –SS– yields 1 M –SH as follows:

$$RSSR + SO_3^{--} \rightarrow RS^- + RSSO^-_3$$

thiol S-alkyl-thiosulfate

The disulfide is the difference between the –SH content before and after reduction of –SS–.

Varying concentrations of urea and sodium sulfite for estimating disulfide in milk proteins were investigated. The effect of SLS with the sulfite to reduce –SS– was also determined, because it was more effective than urea for activating –SH groups. Results are shown in Table 3. A concentration of 8 M urea was the most effective with 0.05 M sulfite to reduce disulfide, as shown in Table 4.

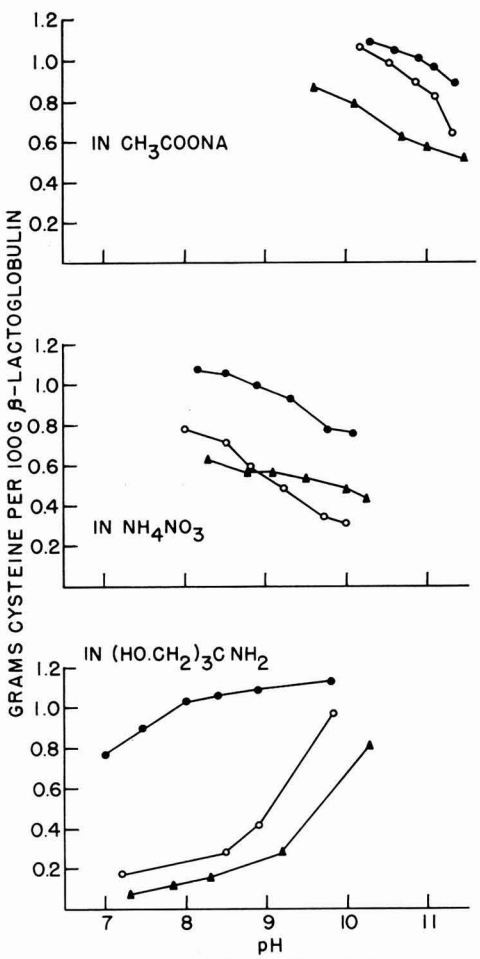

FIG. 5. Amperometric titration of β-lactoglobulin in the presence of denaturing agents. (–○– buffer only, –▲– 8 M urea in buffer, and –●– 1% SLS in buffer.)

–SH and –SS– in casein, β-lactoglobulin, and milk. Sodium caseinate and β-lactoglobulin solutions and milk were heated at 65, 75, 85, and 95 C for 30 min in a tightly closed container and –SH and –SS– groups were estimated. Results are shown in Tables 5, 6, and 7.

There were no –SH groups in casein, which has been reported (7, 11, 18). The cystine content of casein has been reported to be 0.34% (7), but we obtained 0.265% by titrating a solution containing 27.2 g of casein per liter, which yielded 0.300 mM of –SS–. In 100 g of

TABLE 3

Effect of concentration of sodium sulfite on the reduction of disulfide in milk and whey [a]

Na_2SO_3 mole	Milk		Whey	
	8 M Urea	1% SLS	8 M Urea	1% SLS
	(mM –SH + –SS– per liter)			
0	0.20	0.26	0.19	0.26
0.01	1.00	0.51	0.71	0.35
0.03	1.11	0.54	0.81	0.41
0.05	1.11	0.57	0.81	0.43
0.10	1.06	0.66	0.78	0.43

[a] Amperometric titrations were made in sodium acetate buffer containing urea or SLS at pH 10.25.

TABLE 4

Effect of concentration of urea on reduction of disulfide in milk and whey by 0.05 M sodium sulfite [a]

Urea mole	Milk	Whey
	(mM –SH + –SS– per liter)	
Control [b]	0.20	0.22
0	0.37	0.26
2	0.56	0.33
4	0.95	0.64
6	1.13	0.82
8	1.20	0.88

[a] Amperometric titrations were made in sodium acetate buffer containing urea at pH 10.25.
[b] Contained neither urea nor Na_2SO_3.

TABLE 5

Determination of –SH and –SS– in casein solutions heated at different temperatures for thirty minutes [a]

Temp	Cysteine	Cystine
(C)	*(%)*	
Raw	0	0.265
65	0	0.265
75	0	0.265
85	0	0.265
95	0	0.265

[a] Amperometric titrations were made in sodium acetate buffer containing 8 M urea and 0.05 M Na_2SO_3 at pH 10.25.

TABLE 6

Determination of –SH and –SS– in β-lactoglobulin solution (0.548%) heated at different temperatures for thirty minutes [a]

Temp	Cysteine	–SH	△ –SH	–SH + SS	Cystine	–SS–	△ –SS–
(C)	(%)	(mM per liter)			(%)	(mM per liter)	
Raw	0.807	0.365	0.0	0.820	2.00	0.455	0.0
65	0.774	0.350	−0.015	0.820	2.06	0.470	+0.015
75	0.707	0.320	−0.045	0.800	2.11	0.480	+0.025
85	0.597	0.270	−0.095	0.770	2.19	0.500	+0.045
95	0.531	0.240	−0.125	0.770	2.32	0.530	+0.075

[a] Titration conditions were the same as for Table 5.

casein there would be 1.103 mM of –SS– (0.300 mM × 100/27.2 = 1.103). The molecular weight of cystine is 240.30 g; therefore, 240.30 × 0.001103 = 0.265 g cystine, as compared to 0.340 g per 100 g of casein (7). This difference was due to urea which activated fewer –SH groups. The –SS– groups in casein were stable at 95 C (Table 5).

The –SH groups decreased and –SS– increased in the β-lactoglobulin solutions as the temperature was raised. At 75 C the decrease in –SH was 0.045 mM, which agrees with the increase of 0.0225 mM of –SS–, theoretically, and the observed value was 0.025 mM of –SH. At 85 and 95 C, increases in disulfide were close to calculated values. However, increases in disulfide in heated milk cannot be explained entirely by the oxidation of –SH groups (Table 6).

The –SH contents of raw milk and β-lactoglobulin in raw milk are lower than those in Figures 2 and 3, because the titrations were made in the presence of urea.

–SH and –SS– groups in concentrated milk. Skimmilk was preheated at 88.9 ± 0.5 C for 4 sec and concentrated 3:1. It was sterilized at 145.5 ± 0.5 C with no holding time. Titrations for –SH and –SS– groups were made on the raw, preheated, and reconstituted concentrated milks before and after sterilization and also on their caseins and wheys. The titrations were done in sodium acetate, pH 10.25, with 1% SLS and 8 M urea. Results are shown in Tables 8 and 9.

One per cent SLS was effective only for activating –SH groups in raw milk and its whey. The sum of the –SH and –SS– groups in milk remain almost constant during processing, with disulfide increasing in the heated milk to a higher value than expected. Decreased –SH groups in the acid whey of sterilized concentrated milk resulted from the precipitation of serum protein and, as expected, casein had a high cystine content. Since the casein was washed only once with water, some whey may have been occluded, which accounted for the higher –SS– content in the raw milk than that shown in Table 5.

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TABLE 7

Determination of –SH and –SS– in milk heated at different temperatures for thirty minutes [a]

Temp	–SH	△ –SH	–SH + SS	–SS–	△ –SS–
(C)	(mM per liter)				
Raw	0.175	0.0	1.150	0.975	0.0
65	0.110	−0.065	1.150	1.040	+0.065
75	0.075	−0.100	1.160	1.085	+0.110
85	0.020	−0.155	1.130	1.110	+0.135
95	0.015	−0.160	1.130	1.115	+0.140

[a] Titration conditions were the same as for Table 5.

TABLE 8

Changes in –SH and –SS– groups in milk caused by preheating, concentrating, and sterilizing [a]

Treatment of milk	–SH			–SH + –SS–	Cystine
	Control	1% SLS	8 M Urea		
	(mM per liter)				
Raw	0.200	0.260	0.192	1.150	0.958
Preheated	0.195	0.187	0.132	1.140	1.008
Whey from:					
Raw	0.205	0.285	0.185	0.860	0.675
Preheated	0.190	0.210	0.132	0.704	0.572
Reconstituted concentrated milk					
Unsterile	0.165	0.175	0.115	1.130	1.015
Sterile	0.110	0.117	0.062	1.100	1.038
Whey from: Reconstituted concentrated milk					
Unsterile	0.150	0.175	0.112	0.660	0.548
Sterile	0.040	0.050	0.042	0.300	0.258

[a] Titration conditions were the same as for Table 5.

TABLE 9

Determination of –SS– in casein from sterilized concentrated milk [a]

Casein from	Protein	–SS–	Cystine	Cystine
	(%)	(mM per liter)	(g per liter)	(%)
Raw milk	2.40	0.330	0.0793	0.330
Preheated milk	2.32	0.390	0.0937	0.405
Reconstituted concentrated milk				
Unsterile milk	2.27	0.370	0.0890	0.391
Sterile milk	2.45	0.634	0.1523	0.622

[a] Titration conditions were the same as for Table 5.

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WHEY PROTEIN PATTERNS OF MILK FROM COWS WITH EXPERIMENTALLY PRODUCED MASTITIS[1]

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SUMMARY

Whey protein patterns were determined on milk samples from cows in which experimentally induced mastitis was being studied. In two trials in which the cows showed increased temperatures, decreased production, and grossly abnormal milk, there were marked changes in the whey protein patterns. In the third trial, the attempt to produce mastitis resulted in increased cell counts and CMT scores, but no other definite evidence of mastitis. The changes in the whey protein patterns of samples collected during this trial were slight. In the third trial, it appeared that increases in cell numbers and CMT scores were more sensitive than changes in whey proteins as indicators of inflammation of the mammary gland. Whey protein analysis may be useful in studying the physiological responses in attempts to experimentally induce mastitis under controlled conditions.

Blood serum albumin, immune globulins, α-lactalbumin and β-lactoglobulin are proteins found in milk whey. The first two are blood proteins which pass into the milk; the latter two are synthesized in the mammary gland (1). Increased capillary permeability during inflammation may allow the passage of blood proteins from capillaries to milk at a rate faster than normal. Under these conditions the relative proportion of blood proteins in the whey could increase. Increases or decreases in the rate of protein synthesis in the mammary gland would likewise influence the relative proportion of each of the fractions present in the whey. It has been suggested that increases in the blood protein fractions in whey, especially the blood serum albumin fraction, may be a sensitive indicator of increased capillary permeability which occurs in inflammation (2).

Leece and Legates (2) determined whey protein patterns in milk from 43 cows with suspected cases of mastitis, using paper electrophoresis. The most consistent and obvious change in mastitis milk was the appearance of a protein fraction which migrated at the rate of blood serum albumin. An increase in the relative proportion of immune globulins also was noted. Weigt (5), using similar techniques, found that blood serum albumin was present in the whey in chronic mastitis. Her statement that this fraction is absent in milk from acute cases of mastitis is difficult to understand. Weigt noted increases in immune globulins and concluded that chronic mastitis was indicated when the relative portion of immune globulin reached 25% or higher. She also reported an absolute increase in total whey protein, proportional to the severity of mastitis. Nilsson (3) examined milk samples from normal cows and from cows with chronic mastitis in which *Streptococcus agalactiae* or *Staphylococcus aureus* infections were involved. In comparing mastitis milk with normal milk he did not detect any changes in the amount of total protein in the milk serum of mastitic cows. Electrophoretically separated milk serum proteins stained with Amido Black 10 B always revealed four fractions in normal milk and in milk from quarters with chronic infections. However, when ninhydrin staining was used six fractions were usually seen in normal milk and seven fractions were present in milk from animals with chronic mastitis. The additional fraction represented 11 to 12% of the total whey protein, and it was postulated that the extra fraction may represent antibodies formed locally in mammary tissue.

The objective of this study was to investigate the possibility that changes in whey protein patterns may be sensitive and reliable indi-

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cators of mild inflammation or inflammation in its early stages in the mammary gland. Frequent sampling of milk from normal quarters and from quarters in which mastitis was induced made it possible to study the early changes which resulted from induced mastitis.

Sizable numbers of cows were involved in the studies by Leece and Legates (2) and Nilsson (3). Milk from quarters with chronic bacterial infections or clinical mastitis was compared with milk from normal quarters. In this study a comparable number of samples was examined, but only three cows were involved. To determine the pattern of changes in whey proteins due to attempted experimental production of mastitis, quarter samples were examined prior to inoculation and at frequent intervals thereafter. Samples from uninoculated quarters as well as preinoculation samples served as controls. The whey protein studies were conducted in conjunction with other experiments on induced mastitis and, to a certain extent, the choice of cows and inoculums was determined by the objectives of these experiments.

MATERIALS AND METHODS

Experimental cows. The cows used in these trials were high-producing Holsteins in the first to third month of their third lactation. Complete records of bacteriological examinations, leukocyte counts, California Mastitis Tests (CMT), and milk production were available. The quarters were identified as follows: A—right front, B—right rear, C—left front, and D—left rear.

At the beginning of Trial I, Cow 2481 was shedding *Aerobacter aerogenes* from the A quarter. CMT reactions for the quarter prior to this trial varied from one to three and the highest cell counts were over one million. At the same time no organisms of significance were cultured from B, C, and D quarters. Only occasional trace CMT reactions were recorded for these quarters. Quarter samples from Cow 2414 used for Trial II were low in cell counts, negative to the CMT test, and negative bacteriologically except for occasional nonhemolytic, coagulase-negative micrococci. Milk from the C quarter of Cow 2507 used in Trial III was negative to CMT tests and low in cell counts. The A, B, and D quarters showed low to moderate cell counts. The CMT reactions for these quarters were frequently trace and occasionally one. Bacteriological examinations of milk from all four quarters were negative for pathogenic organisms.

TABLE 1

Relative per cent of whey proteins, CMT scores, and cell counts in Trial I

Inoculum	Micrococcus		Saline		Micrococcus		Saline	
Quarter	A	C	B	D	A	C	B	D
Time after inoculation	Serum albumin				Immune globulin			
−15 hr	3.5	1.3	1.4	2.6	29.8	30.3	27.4	33.3
36 hr	13.7	12.8	6.4	4.2	58.2	62.2	36.4	37.9
60 hr	3.2	4.0	1.1	1.3	61.5	61.8	27.7	28.8
6 days	3.6	4.9	2.1	2.1	49.4	52.9	29.5	33.0
9 days	4.3	3.7	5.1	1.2	47.9	49.6	34.3	38.1
14 days	2.9	2.6	3.5	3.0	41.3	36.2	34.9	33.6
30 days	2.4	2.2	3.1	4.0	38.6	36.0	36.1	38.0
	β-Lactoglobulin				α-Lactalbumin			
−15 hr	50.9	50.0	50.7	47.4	15.8	18.4	20.5	16.7
36 hr	19.6	17.4	43.6	45.3	8.5	7.6	13.6	12.6
60 hr	24.4	23.0	52.1	50.0	10.9	11.2	19.1	20.0
6 days	34.9	32.4	52.6	50.0	12.1	9.8	15.8	14.9
9 days	36.8	34.8	46.5	47.6	11.1	11.9	14.1	13.1
14 days	42.3	47.4	47.7	49.5	13.5	13.8	14.0	13.9
30 days	43.4	47.2	45.4	44.0	15.7	14.6	15.5	14.0
	CMT score				Cell count × 10^6			
−15 hr	1	0	0	0	2	0	0	0
36 hr	4++	4++	1	1	56	59	.2	0
60 hr	4	4+	0	0	34	34	0	.2
9 days	2+	2+	1	1	3.5	3	1.5	1
9 days	2+	2+	T	0	3.5	3	1.5	1
14 days	0	0	0	0	.3	.2	.2	.2
30 days	0	0	T	T	0	0	0	0

TABLE 2

Relative per cent of whey proteins in milk from quarters inoculated with *Aerobacter aerogenes* in Trial II

Quarter.....	Serum albumin				Immune globulin				β-Lactoglobulin				α-Lactalbumin			
	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
Time after inoculation																
(hr)																
−2	0.9	0.9	0.8	1.3	26.8	25.9	32.3	33.3	50.9	50.9	46.6	42.7	21.4	22.2	20.3	22.7
0	0.9	1.8	1.5	2.0	25.2	25.5	26.2	31.1	51.4	47.3	49.2	46.6	22.4	25.5	23.1	20.3
2	1.0				32.0				43.0				24.0			
4	6.9	5.6			29.9	28.9			44.8	44.4			18.4	21.1		
6	5.5	5.6	19.0		35.6	29.6	33.1		41.1	45.1	27.3		17.8	19.7	20.7	
8	3.6	5.7	16.2	5.7	32.7	31.8	33.3	37.2	43.6	37.5	30.5	35.7	20.0	25.0	20.0	21.4
10	5.7	8.9	17.3	7.0	37.1	41.8	36.0	41.9	35.7	25.3	27.3	27.9	21.4	24.1	19.4	23.3
22	5.1	6.5	9.1	8.7	46.8	41.5	43.9	44.3	32.9	34.1	30.3	33.9	15.2	17.9	16.7	13.0
24	5.8	5.7	18.3	9.1	54.2	58.6	52.0	59.1	25.8	21.8	16.6	18.2	14.2	13.8	13.1	13.6
46	1.0	1.7	4.4	2.5	44.4	47.0	48.2	55.5	35.4	34.2	29.9	26.9	19.2	17.1	17.5	15.1
70	1.1	2.1	4.1	1.9	46.1	49.3	43.9	47.2	36.0	33.6	34.1	35.2	16.9	15.1	17.9	15.7
94	1.2	1.7	1.8	1.1	40.2	43.7	44.5	51.1	39.0	35.3	36.4	31.8	19.5	19.3	17.3	15.9
(days)																
8	0	2.2	1.1	2.6	29.8	32.2	36.3	33.6	49.3	47.3	42.9	45.7	20.9	18.3	19.8	18.1
10	0	1.2	1.3	2.9	31.7	42.2	40.8	54.7	45.0	39.8	42.1	32.4	23.3	16.9	15.8	10.1
30	0	1.1	0	0	27.9	33.0	29.0	32.4	50.0	47.3	50.0	46.5	22.1	18.7	21.0	21.1

Cultures. The culture used for Trial I was a nonhemolytic, coagulase-negative micrococcus isolated from the D quarter of the cow used in this trial. The inoculum was prepared from a 24-hr growth on tryptose agar washed twice with saline. The saline suspension contained approximately six billion organisms per milliliter. *A. aerogenes* which originally was isolated from a milk sample and later subcultured from an experimental case of mastitis was used in Trials II and III. The inoculums were saline dilutions of an 18-hr serum broth culture and contained one and two million organisms per milliliter in Trials II and III, respectively.

Inoculations. In Trial I (Cow 2481) Quarters A and C were inoculated with 1 ml of a saline suspension of micrococci. B and D quarters were infused with 1 ml of saline. In Trial II (Cow 2414) each quarter was inoculated with a 1-ml suspension of *A. aerogenes*. In Trial III (Cow 2507) Quarters A and D were inoculated with 1-ml suspensions of *A. aerogenes*. The B quarter was infused with 1 ml of undiluted serum broth and the C quarter was maintained as a control.

Sampling procedures. In Trial I, 20 ml of foremilk was collected twice daily immediately before regular milking and the next 20 ml from each quarter was collected for whey protein determinations. In Trial II the first 20 ml of milk from each quarter was collected for bacteriological examination. From 2 hr preinoculation to 24 hr post-inoculation, samples for whey protein analysis were taken from the 150-ml quantities of milk drawn from each quarter following collection of samples for bacteriological examinations. Thereafter, 20 ml of milk for whey protein determinations was collected immediately after sampling for bacteriological examination. On the day of inoculation the preinoculation samples were taken at the regular milking time and about two-thirds of the milk was removed by machine from the udder at that time. The cow was completely milked following the 10- and 22-hr post-inoculation samplings. The intervals of sampling are shown in Table 2.

In Trial III each quarter was milked separately after collecting samples for bacteriological examination. Milk used for whey protein determinations was an aliquot of the complete milking from each quarter.

CMT scores and cell counts. CMT reactions were scored from 0 to 4++, as recommended by Schalm (4). A 4 was the strongest CMT reaction and the + symbol indicated alkalinity of the sample. Cell counts of less than 20,000 were recorded as 0.

TABLE 3

CMT scores and total cell counts in milk from quarters inoculated with *Aerobacter aerogenes* in Trial II

		CMT				Cell count × 10^6			
Quarter		A	B	C	D	A	B	C	D
Time after inoculation									
Hours	−2	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0
	2	0				0			
	4	3	3			9.0	5.0		
	6	3	3	3		34.5	31.5	26.0	
	8	3	3	3	3	6.5	15.0	15.5	10.5
	10	3	3	3	3	5.5	10.0	7.0	6.0
	22	3	3	3	3	14.0	25.0	22.0	22.0
	24	3	3	3	3	84.0	86.0	56.0	90.0
	46	3	3	3	3	5.5	8.0	9.0	8.0
	70	2	2	2	2	4.5	3.5	5.0	4.5
	94	1	2	2	2	1.6	3.0	4.0	2.5
Days	8	0	0	0	1	0	0	0	.8
	10	0	0	0	3	0	0	0	18.0
	30	0	0	0	1	0	0	0	.5

Preparation of samples. The milk samples were centrifuged and the cream layer removed. Whey was prepared from a 20-ml milk sample by adding 0.3 ml of a 1:50 dilution of rennin and 0.2 ml of saturated calcium chloride, holding at 37 C for 20 to 30 min, and centrifuging. The whey was filtered and refrigerated or frozen until used for determining whey protein patterns by paper electrophoresis.

Paper electrophoresis. A Spinco Model R Paper Electrophoresis System was used for protein fraction determinations. The procedures followed were those outlined [3] for blood serum, except that it was usually necessary to increase the volume of sample applied to the strips to .05 to .07 ml. When whey from quarters with clinical mastitis was used a smaller application was adequate. Veronal buffer, pH 8.6, was used and the separation was conducted at 2.5 ma. for 16 hr. Strips were stained with bromphenol blue and a Spinco Model RB Analytrol was used to evaluate the stained strips.

RESULTS

A clinical case of mastitis was produced in Experiment I. Quarters A and C, inoculated with a micrococcus, were swollen and the milk was grossly abnormal. The B and D quarters, infused with saline, produced normal-appearing milk. Total milk production dropped to about 60% of the preinoculation level on the day following inoculation, but quickly returned to near the preinoculation level.

[3] Spinco Division, Beckman Instruments, Inc., Palo Alto, California.

In examining data in Table 1, it can be seen that following inoculation the relative per cent of blood proteins in the whey from all quarters increased, whereas those fractions synthesized in the mammary gland decreased. The changes which occurred in the quarters inoculated with micrococci were more pronounced and persisted for a longer time than those observed in the quarters infused with saline. The blood proteins present prior to inoculation constituted about one-third of the total whey proteins; whereas, after inoculations with micrococci they made up more than two-thirds of the total whey proteins.

In comparing whey protein patterns, CMT scores, and cell counts, it can be seen that most values for the A and C quarters gradually returned toward the preinoculation levels during the 14 days after inoculation, and that there was a marked drop in the per cent blood serum albumin between 36 and 60 hr after inoculation. Results of a later trial suggest that the peak of the change in blood serum albumin may have been missed in this trial.

In Trial II there were no apparent differences in the quarters of the experimental animal prior to inoculation and all quarters received similar inoculations. The time of the first sampling of individual quarters following inoculation varied from 2 hr post-inoculation for the A quarter to 8 hr post-inoculation for the D quarter. The blood protein fractions increased at the expense of the fractions synthesized by the mammary gland. As in Trial I the most evident early change was in the blood

TABLE 4

Relative per cent of whey proteins, CMT scores, and cell counts in milk from quarters inoculated with *Aerobacter aerogenes* (A and D) or infused with broth (B) in Trial III

	A	D	B	C	A	D	B	C	A	D	B	C	A	D	B	C
	Serum albumin				Immune globulin				β-Lactoglobulin				α-Lactalbumin			
Before inoculation																
6 days	2.5	2.1	2.5	1.9	25.7	18.3	21.6	16.9	53.3	61.8	56.9	61.7	18.6	17.9	19.1	19.6
8 hr	1.0	1.1	0.8	0	18.4	18.1	18.9	16.3	65.0	59.6	63.1	67.3	15.5	21.3	17.2	16.3
2 hr	1.8	1.0	0.9	1.7	21.2	16.7	21.3	20.0	58.4	62.5	60.2	60.8	18.6	19.8	17.6	17.5
After inoculation																
4 hr	1.1	1.8	2.0	0.5	22.2	20.5	19.4	23.0	57.8	60.7	61.2	59.5	18.9	17.0	17.3	17.0
10 hr	2.1	3.9	2.3	1.2	28.8	27.4	22.7	23.3	50.5	52.0	60.2	59.3	18.6	16.7	14.8	16.3
16 hr	1.7	2.5	2.3	1.0	22.3	20.7	17.1	19.6	62.8	63.6	67.0	66.7	13.2	13.2	13.6	12.7
22 hr	1.8	3.6	1.8	1.9	19.3	24.3	23.6	21.2	64.0	58.6	60.9	60.6	14.9	13.5	13.6	16.3
28 hr	1.1	2.9	2.5	1.2	27.3	24.8	25.0	22.9	50.0	55.2	55.0	56.6	21.6	17.1	17.5	19.3
34 hr	0.6	2.2	1.3	0.6	19.5	24.4	20.0	18.1	63.0	57.8	63.7	64.4	16.9	15.6	15.0	16.9
46 hr	0.4	0.8	0.8	0.9	28.3	22.0	26.2	13.8	58.3	60.2	62.3	68.8	13.0	17.1	10.7	16.5
58 hr	0.8	1.9	1.6	0.8	18.3	18.7	18.9	17.8	64.2	63.6	63.9	64.4	16.7	15.9	15.6	16.9
7 days	0.8	2.1	1.6	1.0	17.9	23.2	14.7	13.1	60.2	59.9	65.9	68.7	21.1	14.8	17.8	17.2

serum albumin fraction. Increases in blood serum albumin values for the C quarter were considerably greater than those for the three other quarters. Serum albumin and immune globulin constituted about 27 to 35% of the total whey proteins in all four quarters prior to inoculation. In samples taken between 10 and 70 hr after inoculation, these two fractions increased to about 50% of the total, except in the 24-hr sampling, where the values were 60 to 70%. However, the 24-hr samples were not considered to be comparable to samples taken at other times, because the cow had been completely milked 2 hr before this sampling.

Indications of a clinical case of mastitis in the D quarter at ten days after inoculation are shown by the data in Tables 2 and 3. Increased cell counts and CMT scores were found in the D quarter only. The whey protein values at this time (Table 2) indicate that the trend toward the preinoculation values was somewhat affected in all four quarters at ten days after inoculation. The most pronounced changes were the increase in per cent immune globulin and the decrease in lactoglobulin and lactalbumin in the whey from the D quarter.

Although the organism used in the attempt to produce mastitis in Trial III was from the same culture used in Trial II, and the number of organisms per milliliter inoculum was twice that used in Trial II, the only definite effects produced were increases in cell counts and CMT scores. The quarters which were inoculated and the one infused with broth showed slight swellings and a few flakes were present in the milk. At 6 to 10 hr after inoculation there was a slight elevation in temperature. Milk production was depressed slightly in the individually milked quarters at 4 hr post-inoculation, but at the next milking, 10 hr post-inoculation, production returned to normal and remained normal (Table 5). In examining the whey protein data in Table 4, there are some suggestions of changes in the percentages of blood serum albumin and immune globulin at 10 hr post-inoculation. However, these changes are not much greater than those which appear to be due to normal variations. In this trial it is of interest to note that the attempt to experimentally produce mastitis depressed milk production only slightly, caused little or no change in whey protein patterns and, in general, produced little evidence of mastitis other than the increases in milk cell counts and CMT scores. The CMT scores and cell counts for the control quarter did not change throughout the sampling period (Tables 4 and 5).

TABLE 5

CMT scores, total cell counts, and milk production in Trial III

Quarter.....	A	D	B	C	A	D	B	C	A	D	B	C
Before inoculation	CMT				Cell count $\times 10^6$				Pounds milk			
6 days	1	T	T	0								
8 hr	T	T	T	0	.3	0	.2	0	3.5	3.2	3.2	3.1
2 hr	T	0	0	0	.1	0	0	0	3.7	3.3	3.3	3.1
After inoculation												
4 hr	3	3	2	0	38	35	4.8	0	2.4	1.9	2.0	2.0
10 hr	3	3	2	0	22	12	4.5	0	3.7	3.1	3.3	2.9
16 hr	3	3	2	0	8	18	2.5	0	3.0	3.0	3.2	2.8
22 hr	3	3	2	0	5	10	2.5	0	3.6	3.1	3.1	3.1
28 hr	1	3	1	0	2	7	1.0	0	3.1	2.8	2.9	2.6
34 hr	1	2	1	0	1.5	2	.8	0	3.2	3.0	3.1	2.8
46 hr [a]	1	1	1	0	1.0	.5	.3	0	6.4	5.3	5.4	5.6
58 hr	T	0	0	0	.3	.3	0	0	6.8	6.0	6.4	5.8
7 days	0	1	T	0	0	3	.3	0	6.8	5.3	5.8	6.0

[a] Milking interval changed from 6 to 12 hr.

DISCUSSION

Whey protein patterns were determined for milk samples obtained before and after attempts to experimentally produce mastitis. In two trials where the experimentally produced mastitis resulted in a rise in temperature, reduced milk production, and grossly abnormal milk, there were definite changes in whey protein patterns. The most marked early change was an increase in the relative per cent of blood serum albumin in the whey. This increase was of short duration. The other blood protein present in whey, immune globulin, increased more slowly, continued at its peak value after the relative per cent of blood serum albumin had declined, and remained at considerably above preinoculation levels for several days. The difference in the rates of change for these two fractions suggests that the mechanisms responsible for these changes are not identical. One possible explanation for this difference is that the increase in blood serum albumin may be due entirely to changes in capillary permeability, whereas increases in immune globulin may be due to active transfer from blood to milk or to local production, as suggested by Nilsson (3). The short duration of the increase in blood serum albumin may offer an explanation of a failure to find it in certain suspected cases of mastitis.

If it is assumed that the increase in cell counts and CMT scores in Trial III accurately reflect inflammation resulting from the attempt to induce mastitis, it would appear that changes in whey protein patterns are less sensitive indicators of inflammation than are the CMT scores and cell counts. It is of particular interest to note that in this trial, in which only slight changes in whey protein patterns occurred, the attempt to produce mastitis had very little effect on milk production.

Attention should be called to the fact that in this study as well as those of others relative changes in whey protein fractions were considered. Depression in mammary tissue synthesis of protein fractions, due to mastitis, would result in an apparent increase in the blood protein fractions. Attempts to determine absolute changes in the four whey protein fractions in Trial III failed when the whey protein patterns remained essentially unchanged.

While the small number of cows involved in this study does not allow definite conclusions, the results do point out differences in the rate of change in the different whey protein fractions following inoculation of the quarters. In addition, it appears that irritation sufficient to increase cell numbers in milk may not alter whey protein patterns.

Whey protein patterns may be of value in determining some of the physiological responses of mammary tissue to a specific cause of inflammation. Attempts to use these procedures to detect mastitis milk due to a variety of causes or to diagnose mastitis during various stages of the course of the disease are likely to yield variable results.

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EFFECT OF AD LIBITUM OR FORCE-FEEDING OF TWO RATIONS ON LACTATING DAIRY COWS SUBJECT TO TEMPERATURE STRESS [1]

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SUMMARY

Comparison of constant intake with ad libitum feeding of dairy cows demonstrated that: (1) The major decrease in milk production at high ambient temperatures is due to reduced feed intake. (2) High ambient temperature per se causes a decrease in production. Part of this effect may be due to decreased rate of feed passage through the rumen. (3) Differences in fiber or molasses content of rations at low or high temperatures showed no significant influence on amount or composition of milk produced. (4) Increase in temperature caused a significant decrease in production. (5) High temperatures caused a significant decrease ($P < .05$) in efficiency of energy utilization for milk production. (6) Significant interaction of ration and temperature on efficiency of energy utilization for milk production indicates that ambient temperature should be considered in evaluating ration effect.

The influence of environmental temperature on the production of milk by the dairy cow has been studied extensively. Interest in this factor which affects a dairy cow's response is increasing, and new facilities are being developed to study the problem more intensively.

According to reviews by Hancock (7), Larson (13), and Legates (14), most of the studies on the influence of controlled temperature and relative humidity upon milk production and composition were conducted at the Missouri Station (3-6, 16-20). Larson concluded from this work that the decrease in feed intake at higher temperatures is apparently the major reason for decreased milk production. None of the reported studies were designed to determine whether elevated temperatures had a deleterious effect upon lactation other than that which appeared to be directly associated with decreased feed intake.

The work of Stott and Moody (22) showed that studies of response to environmental temperature must consider the type of ration. They reported that during the summer in Arizona there was a highly significant change in cow response as hay was decreased and concentrates were increased to provide the same level of energy and protein. Body temperature, respirations per minute, and milk fat test were decreased and 4% fat-corrected milk was increased. These data suggest that when environmental temperatures are high, the lactating dairy cow can maintain nearly normal response, except for fat test, if the energy level of the ration is kept constant and the fiber content is decreased.

Information on the effect of temperature per se on lactation response under different feeding conditions would increase our ability to understand the component factors of the physiology of milk secretion and their interrelation. This study was designed to explore these problems.

EXPERIMENTAL PROCEDURE

Six Holstein cows just past the peak of lactation were allotted at random to two groups and assigned to a switchback design of three 6-wk periods (1). Each 6-wk period consisted of 3 wk at low temperature followed by 3 wk at high temperature. Relative humidity was held constant at 50%.

The base temperature used was 65 F and the high temperature was predetermined as that which would cause a 2 F rise in rectal temperature. Previous studies (10) showed that a constant temperature of 85 F was required to obtain a 2 F rise in rectal temperature for most lactating cows.

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[2] Present address: Department of Animal Science, University of Hawaii, Honolulu. (Paper No. 588, approved by Hawaii Agricultural Experiment Station.)

The cows were housed in one chamber of the Missouri psychroenergetic or climatic laboratory, which provides for maintenance of six cows per chamber under conditions of controlled temperature and relative humidity. They were kept in individual stalls arranged to permit collection of separate feed and water consumption data on each cow, as described by Brody (3).

TABLE 1

Rations fed

Ingredient	Ration A	Ration B
Cane molasses	10.0	30.0
Alfalfa hay (1-2-in. cut)	45.0	35.0
Soybean meal (44% protein)	5.5	5.5
Shelled corn, No. 2 (¾-in. grind)	37.5	27.5
Salt	1.0	1.0
Bonemeal	1.0	1.0
Total	100.0	100.0

Two complete mixed rations of chopped hay, concentrates, and molasses, as shown in Table 1, were fed during the trial to provide a test of the influence of reduced fiber at high and low temperatures. Both rations, when eaten in normal amounts, provided more protein and energy than the recommended allowances for the cows on test. Proximate analysis and energy content of the rations are shown in Table 2.

Two cows in each group were provided with rumen fistulas, closed with a lucite cannula having an inside diameter of 3¾ in., to permit force-feeding. The third cow in each group was left intact as an ad libitum control.

During a preliminary 2-wk period at 65 F and 50% relative humidity, the cows were all fed ad lib. on the 10% cane molasses ration shown in Table 1. The amount of feed eaten during this period determined the level of feed intake of the fistulated cows during the 18 wk of the trial. Any feed refused by these cows during the experiment was weighed back and inserted through the cannula. In an attempt to provide uniformity in the amounts fed through the cannula throughout the trial, 20% of the daily allowance of each fistulated cow was allotted for cannula feeding.

Feed was mixed at the beginning of each 3-wk interval of the trial, and allowances for each fistulated cow were weighed into heavy paper bags for each feeding. Amounts to be fed through the cannula were bagged separately from the oral offering. Feed was weighed out at each feeding for the ad lib-fed controls and weighbacks from the previous feeding were recorded. No allowance was made for the slight difference in estimated TDN of the two rations in switching from one to the other.

As feed was bagged for the fistulated cows, grab samples were taken from each bag prior to weighing and were composited for proximate and energy analyses. Energy values determined by bomb calorimetry were used to compute the gross energy intake of each cow.

The cows were milked at 11- and 13-hr intervals. Milk was recorded to the nearest tenth of a pound for each milking. Individual samples of milk were collected from each cow on Sunday, Tuesday, and Thursday evenings, and Monday, Wednesday, and Friday mornings, to provide six samples per cow per week. Samples were refrigerated immediately.

Fat percentage was determined on individual samples by Babcock test and total solids by Watson Pattern Lactometer. Herrington (9) concluded that the lactometer is not a desirable technique for determining total solids on samples of milk from individual cows. To obtain greater accuracy with the lactometer, concurrent analyses were made by the Mojonnier method and a correction factor determined. This factor, −0.268 was then applied to the lactometer formula:

TABLE 2

Proximate analysis and gross energy content of rations fed

Ration	Water	Protein	Ether extract	Crude fiber	Ca	P	Energy
	(%)						(kcal/g)
A	12.95	13.1	2.26	16.92	0.908	0.278	3.5109
B	15.69	10.9	1.77	14.98	0.893	0.295	3.3314

Note: Proximate analyses were performed by Dr. C. W. Gehrke, Experiment Station Chemical Laboratories, and energy content determined by Dr. W. H. Pfander, Department of Animal Husbandry, University of Missouri, Columbia.

Per cent total solids =

$$1.33\ F + \frac{273\ L}{L + 1{,}000} - 0.40$$

Where F = Fat percentage by Babcock test

L = Lactometer degrees at 102 F

Yields of fat, total solids, and solids-not-fat were computed for each cow for each milking, using morning fat and total solids values for all morning milk, and evening values for all evening milk. The energy value of the total solids was computed from these data using the following formula:

kcal/gm of solids = 4.516 + 0.321 × fat test, as developed by Lofgreen and Otagaki (15)

These yields were then summed into weekly totals for analysis.

Body weights were recorded after milking and before feeding each Thursday and Friday morning. Rectal temperatures, pulse, and respiration rates were recorded twice daily on a regular schedule Monday through Friday. Rectal temperatures were observed over the weekend during the high-temperature intervals to guard against overheating the cows.

Concurrent studies which will be reported by other investigators studied the effects of the applied stress upon metabolism as measured by open circuit calorimetry and thyroxine I^{131} disappearance rates in the third week of each temperature period. Adrenal function and metabolic end products were also investigated, using one 24-hr collection of urine obtained with internal catheters during the third week of each temperature period.

RESULTS

At the end of 48 hr of the first high-temperature period, it was apparent that 85 F was not warm enough to provide the desired temperature stress. The temperature was, therefore, adjusted upward at the rate of 1 F per day. This was as rapid as the chamber could be precisely adjusted and the influence of the increase checked. It was found that 88 F was the temperature necessary at 50% relative humidity to cause a 2 F rise in rectal temperature for the cows used. When the ambient temperature exceeded this level, rectal temperatures rose rapidly and the cows suffered excessively from the heat. After this initial adjustment, the chamber was maintained as near 88 F as possible during high-temperature intervals.

Feed intake decreased when the chamber temperature was raised. This is shown in Figure 1. The decrease was greater and more abrupt on the 10% molasses ration. This was most apparent in the voluntary intake of the force-fed cows. During the first high-temperature period, the force-fed cows receiving the 30% molasses ration consumed all feed offered them through the seventh day. The force-fed cows on the 10% molasses ration stopped cleaning up their feed by the third day and dropped to a voluntary intake of less than 1 lb per day in the 2nd wk. The decrease was less in the 2nd and 3rd high-temperature periods. The average decrease in voluntary feed intake by force-fed cows at 88 F from the preceding 65 F period was 47.6 and 18.6% on the 10 and 30% molasses rations, respectively. In comparison, the ad lib.–fed cows decreased their total feed intake by 31.8 and 18.8% on the same rations.

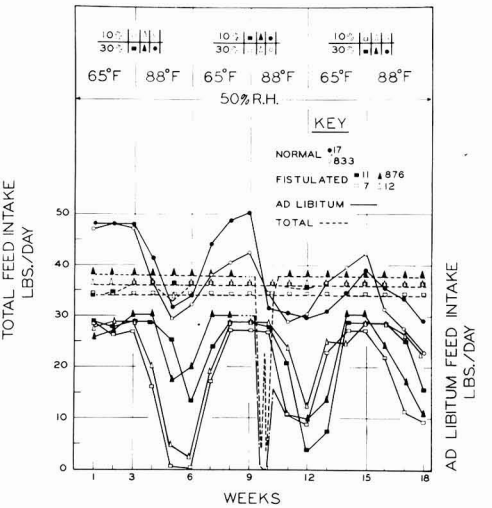

FIG. 1. Average daily feed intake by weeks. The voluntary and total intake are shown separately for the force-fed cows.

As shown in Figure 2, the milk production of both force-fed and ad lib.–fed animals decreased at 88 F. The ad lib.–fed cows showed a greater decrease in production at 88 F and a stronger recovery in the succeeding 65 F period. Regression analysis showed a decline in average daily production of 0.869 and 1.919% of actual milk each week by the force-fed and ad lib.–fed cows, respectively. Average daily 4% FCM declined at the rate of 1.036 and 2.425% each week for the two groups of cows, respectively. The decrease due to temperature was statistically significant.

Production by the ad lib.–fed cows followed

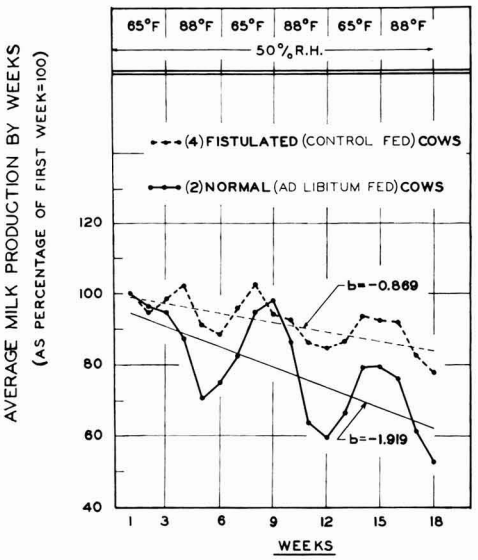

FIG. 2. Average actual milk production by weeks for the fistulated and control cows. Values are shown as per cent of production in the first week.

their pattern of feed consumption very closely. However, the force-fed cows also followed a pattern of decline in production at high temperature and recovery in the succeeding low-temperature period but of decreased amplitude. Inasmuch as their feed intake was held constant, the fluctuation was due to factors other than feed intake. One of these factors was the apparent reduced rate of feed passage through the animal at high temperature. This was manifested by the increase in weight of the force-fed cows at 88 F and the decrease at 65 F, as compared with the reverse pattern for the ad lib.–fed cows (11). It was also apparent in the increasing difficulty associated with force-feeding through the cannula within high-temperature periods.

The production of total solids, solids-not-fat, and fat varied with temperature, as shown in Figure 3. Inasmuch as each of these is a function of the amount of milk produced, the variation was smaller for the force-fed cows. Per cent total solids, solids-not-fat, and fat were not significantly affected by temperature or ration, or by their interaction.

There was a wide variation in the partial efficiency for milk production, as shown in Table 3. Differences due to rations were not statistically significant. Increase in temperature caused a statistically significant decrease in efficiency of feed utilization for milk production ($P < .05$). Interaction of ration and temperature was significant ($P < .02$).

DISCUSSION

The response to force-feeding in this study demonstrates that cows will reduce their feed intake, whenever possible, under high-temperature stress. Davis and Merilan (6) reported a decrease of approximately 20% at an ambient temperature of 90 F with 50% relative humidity. The ad lib.–fed cows of this study had a comparable decrease in feed consumption on the 30% molasses ration. However, on the higher fiber, 10% molasses ration, their feed intake decreased an average of 31.8%. The greater decrease on the 10% molasses ration, as compared with previous studies, is probably due to method of ration formulation. Each ration was a complete, mixed ration which was eaten as a unit. In previous studies, hay was fed ad lib. and concentrate according to milk production. With this method of feeding, the consumption of hay decreased rapidly under high-temperature stress and concentrate intake remained fairly constant. When the cow was unable to be selective, total intake decreased more on comparable rations. The smaller decrease in consumption of the 30% molasses ration shows a greater tolerance at high ambient temperatures for a ration with lower fiber and higher readily available energy (22).

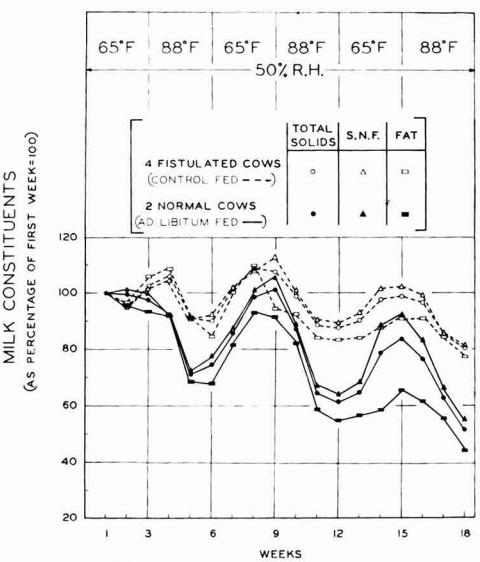

FIG. 3. Weekly production of total solids, fat, and solids-not-fat for fistulated and control cows shows as per cent of production in the first week of the trial.

TABLE 3

Per cent efficiency for milk production during the second and third week of each temperature period [a]

Temperature	Period no.	Group I [b] Cow no. 7	12	833	Group II [c] Cow no. 11	876	17
(F)							
65	1	17.9	22.3	16.3	22.0	18.4	24.0
88	1	16.6	18.8	19.3	19.9	17.8	24.3
65	2	19.1	24.0	16.9	23.4	20.6	23.9
88	2	15.2	21.3	18.1	17.8	16.1	21.6
65	3	15.4	21.4	18.1	21.7	19.1	20.6
88	3	11.6	19.3	15.2	18.0	17.4	21.6

[a] $\frac{\text{Milk energy produced during period}}{\text{Feed energy consumed during period}} \times 100.$

[b] 10% molasses ration in Periods 1 and 3, 30% molasses ration in Period 2.

[c] 30% molasses ration in Periods 1 and 3, 10% molasses ration in Period 2.

Summary of mean values

	10% molasses ration	30% molasses ration	Mean
65 F	20.0	20.7	20.3
88 F	17.3	19.2	18.3
Mean	18.7	20.0	19.4

The marked decrease in voluntary feed intake at high ambient temperatures when total intake is maintained constant should be considered in the design of future studies which involve force-feeding. In the first 88 F period, there were two days during which it was impossible to force all the feed into the rumen of Cow 12. This caused a decrease in her total intake for the week from 36 lb per day to an average of 33 lb. At the end of the 9th wk, Cow 876 stopped eating and her milk production dropped off precipitously. The cause was not determined other than that her cannula was pulled out on the stall divider. Force-feeding of this cow was stopped temporarily. After three days, it was resumed and gradually increased to full feed on the fifth day. Her milk production increased with the increase in feeding.

The influence of ration upon production followed the same trend reported by Breidenstein et al. (2) and Stott and Moody (22), although differences in fiber content of the rations were not as great as reported by these investigators. The average decreases in 4% FCM between 65 and 88 F in the 2nd and 3rd wk of each period were 8.72 and 5.32 lb on the 10 and 30% molasses, respectively. This difference due to rations was greater than reported by the above authors but was not significant. However, the differences due to temperature were significant on both rations.

The effect of temperature alone on milk production can be seen in a comparison of the response of ad lib.- and force-fed cows. Because of the difference in levels of production and numbers of fistulated and control cows, the effect is shown in Figure 2 as per cent of production in the first week. The force-fed cows decreased average 4% FCM from 33.5 to 28.1 on the 10% molasses ration and from 33.3 to 29.5 on 30% molasses at 65 and 88 F, respectively. The ad lib.–fed cows decreased from 41.2 to 25.9 and from 39.0 to 30.6 in the same order. The decrease in production by the ad lib.–fed animals was 15.3 and 8.4 lb on the 10 and 30% molasses rations, respectively. In comparison, the force-fed cows declined 5.4 and 3.8 lb in the same order. The large difference confirms that the major factor involved in decreased production at elevated temperature is lowered feed intake (12). It also shows that high temperature per se adversely influences milk production. Part of this is due to decrease in the rate of feed passage through the rumen at high temperatures. In addition, the force-fed cows expended less energy per lb of 4% FCM produced (11).

Cobble and Herman (5) maintained dairy cattle under controlled temperature and humid-

ity with alfalfa hay fed ad lib. and grain fed according to production. They reported a highly significant decrease in solids-not-fat at temperatures above 90 F. Fat percentage increased abruptly at the same time, but not significantly. Merilan and Bower (16) reported variable results under similar conditions. Other investigators (8, 12) reported that solids-not-fat are lowest in summer and highest in winter under natural climatic conditions. In this study there were no differences in milk composition due to ration or temperature. The method of feeding probably accounts for lack of agreement with previous work. Force-fed cows showed less variability in milk composition than those fed ad lib.

The significant interaction of temperature and ration in partial efficiency for milk production confirms that ambient temperature should be considered in the formulation of dairy cattle rations to obtain maximum efficiency (22).

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ELECTROPHORETIC CHARACTERIZATION OF PREGASTRIC ESTERASE FROM THE CALF [1, 2]

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SUMMARY

This investigation was conducted primarily to determine whether pregastric esterase from the calf is a single enzyme or a group of enzymes. Accordingly, sham-fed water and extracts of various alimentary and related tissues were subjected to electrophoresis in starch gel, which was stained histochemically to locate individual esterases. The results indicate that pregastric esterase is composed of at least six different enzymes. Resolution of these esterases was obtained in gel containing 8 g of starch per 100 ml of buffer, but not in that containing 15 g per 100 ml. Thus, the components of pregastric esterase are believed to be of high molecular weight. Moreover, these esterases precipitate at a slightly acid pH, thereby providing a means of separating pregastric esterase from nonsecretory esterases that are present also in tissue extracts.

Pregastric esterase is an enzyme found in the oral secretions of the young calf. The secretion of pregastric esterase and its importance in fat digestion have been considered previously (3, 7, 9, 11, 12), though it has not been ascertained whether this esterase is a single enzyme or a group of enzymes. Recent studies with starch gel electrophoresis (2, 4), however, indicate that many animal tissues contain more than one esterase. Thus, the primary objective of the present investigation was to study the electrophoretic identity of pregastric esterase, in order to establish its singular or multiple nature.

The initial phase of this study was a survey of the electrophoretic distribution of esterases in extracts of various tissues believed to secrete pregastric esterase. Other alimentary and related tissues were included also for comparative purposes. In the latter phase of this study, efforts were continued toward the identification of pregastric esterase and the isolation of this enzyme from nonsecretory esterases. The experimental procedures and the results and discussion for each area of investigation are presented separately.

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Part I—Survey of Esterases in Various Tissues

EXPERIMENTAL PROCEDURES

Preparation of tissue extracts. For the initial survey, various tissues were obtained from four calves ranging in age from 1 to 8 wk. This selection included tissue from three areas, previously defined (6), that are believed to secrete pregastric esterase: tongue in the region of the vallate papillae, glosso-epiglottic space, and pharyngeal end of the esophagus. The other tissues were tongue in the region of the fungiform papillae, parotid salivary gland, submaxillary salivary gland, cardial end of the esophagus, reticulum, rumen, omasum, fundic region of the abomasum, pyloric region of the abomasum, duodenum, pancreas, and liver. Extracts were prepared by adding water to the fresh tissue, 2 ml/g, and homogenizing this mixture. The homogenate was centrifuged for 1 hr at 7,500 × g, and a portion of the supernatant extract was frozen for subsequent analysis.

Electrophoretic analysis for esterases. The tissue extracts were subjected to vertical electrophoresis in starch gel for 16 hr at room temperature according to the procedure of Smithies (10). The voltage gradient was 5 v/cm of gel length. The gel was prepared with borate buffer, pH 8.55 (0.025 M boric acid, 0.005 M NaOH), and the ratio of starch to buffer in the gel was 15 g/100 ml. An extract of rat liver was included in each analysis as a reference material. The use of a reference material seemed advisable because the rate of protein migration is dependent on the temperature

of the gel during electrophoresis, over which there was no precise control in this study. After electrophoresis, the gel was sliced with stainless steel wire, 40-gauge, and its cut surface was stained for esterases in a manner similar to that described by Markert and Hunter (2). The gel slice was kept in the staining solution until the bands of esterase were well-defined, for several hours if necessary. The staining solution was prepared immediately before use by dissolving either 100 mg of Blue B (tetrazotized o-dianisidine) or 200 mg of Blue RR (diazotized 4-benzoylamino-2,5-dimethoxyaniline) in a mixture of 200 ml of distilled water, 2 ml of a stock solution of substrate (1-naphthyl butyrate), and 20 ml of 0.2 M phosphate buffer, pH 6.0. This solution was filtered prior to use. The stock solution of substrate contained 1 ml of 1-naphthyl butyrate, 12.5 g of melted Brij 35,[3] and 87.5 ml of 1,4-dioxane. When refrigerated, the stock solution of substrate is stable for months. After the gel was stained, the electrophoretic mobility of each esterase was determined by measuring the distance between the point at which the tissue extract had been inserted (sample slot) and the leading edge of each band. These values were expressed subsequently as percentages of the distance recorded for the most rapidly migrating esterase in rat liver, the reference material. The foregoing substrate, 1-naphthyl butyrate, identifies esterases that hydrolyze simple esters but not necessarily triglycerides. Previous results, however, have indicated that pregastric esterase hydrolyzes both simple esters (8, 9) and triglycerides (3, 7-9, 11, 12).

RESULTS AND DISCUSSION

Each tissue extract was subjected at least twice to electrophoretic analysis. The distribution of esterases in corresponding tissues was similar for all calves, irrespective of age. Thus, a mean was computed for the relative mobility of each esterase, and such values for each tissue are presented in Figure 1. Only the position of the leading edge of each band is shown in this figure; no attempt has been made to illustrate either the width of the bands or the intensity of their staining.

Two esterases, one migrating slowly, ca. 14, and the other migrating rapidly, ca. 93, were common to nearly all of the calf tissues. In fact, these esterases appeared to be the only ones present in five of the tissues: tongue in the region of the fungiform papillae, cardial end of the esophagus, reticulum, rumen, and omasum. Abomasal tissue, however, contained

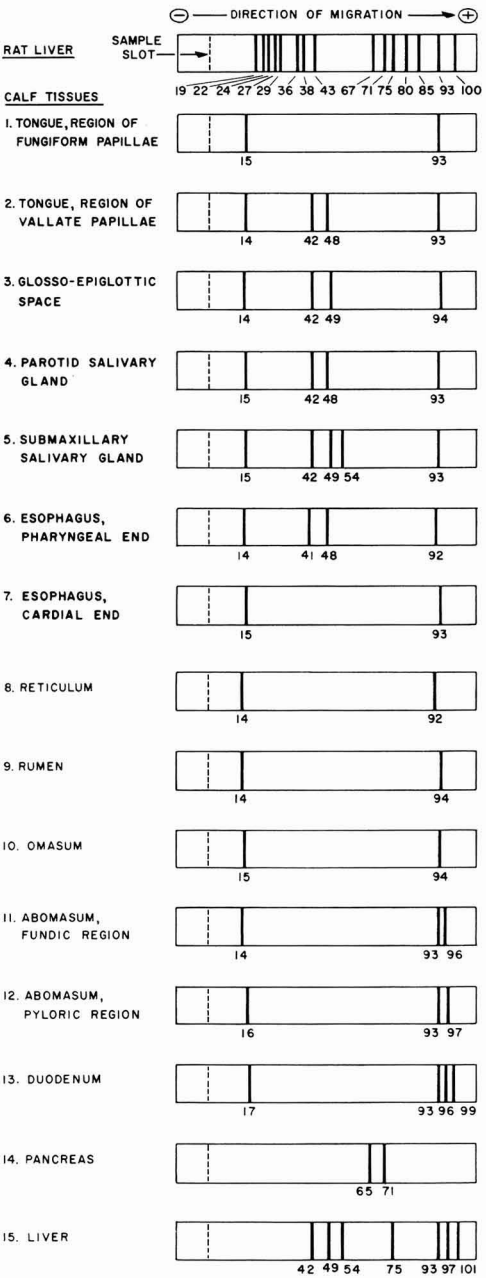

FIG. 1. Electrophoretic distribution of esterases in extracts of various tissues from the calf, with rat liver as the reference material. (Composition of gel, 15 g starch/100 ml buffer.)

[3] A nonionic surfactant manufactured by Atlas Chemical Industries, Inc., Wilmington 99, Delaware.

a third esterase, ca. 96, whereas duodenal tissue exhibited two additional esterases, ca. 96 and 99. Two esterases having an intermediate rate of migration, ca. 42 and 48, were found in tissue from the tongue in the region of the vallate papillae, glosso-epiglottic space, pharyngeal end of the esophagus, parotid salivary gland, and submaxillary salivary gland. The last gland also contained a third esterase of intermediate mobility, ca. 54. Seven esterases were detected in the liver, and six of these, ca. 42, 49, 54, 93, 97, and 101, appeared to have the same electrophoretic mobility as some of the esterases in other tissues from the calf. The resolution of pancreatic esterases was indistinct, and only two bands, ca. 65 and 71, were visible on the gel. The difficulty in resolution may have resulted from the hydrolysis of starch in the gel by pancreatic amylase and the concomitant alteration in gel structure. Rat liver, though not of primary interest in this study, contained at least 15 different esterases, approximately twice the number found in calf liver.

Previous observations (6) suggested that at least three of the foregoing tissues are involved in the secretion of pregastric esterase: tongue in the region of the vallate papillae, glosso-epiglottic space, and pharyngeal end of the esophagus. As shown in Figure 1, all of these tissues exhibited the same pattern of esterases: ca. 14, 42, 48, and 93. Since 14 and 93 were found in many tissues, it seemed improbable that these two enzymes are part of pregastric esterase. On the other hand, the esterases of intermediate mobility, ca. 42 and 48, were found only in pregastric tissues, with the exception of the liver. Thus, these two enzymes appeared to be constituents of pregastric esterase, though this identification was regarded only as tentative, for the following reason. Histochemical studies of animal tissues have revealed that esterases are present both in secretory cells and in nonsecretory cells. Tissue extracts, such as used in the previous survey, may contain esterases from either type of cell or from both. Since the cellular origin of esterases cannot be ascertained by electrophoresis per se, further investigation was required to establish the identity of pregastric esterase.

Part II—Identification and Isolation of Pregastric Esterase

EXPERIMENTAL PROCEDURES

Sources of pregastric esterase. The identification of pregastric esterase required a source of this enzyme free from other esterases. Such was obtained by nipple-feeding 5 lb of water to a rumen-fistulated calf and collecting the mixture of water and saliva from the cardial end of the esophagus. This procedure is known as sham-feeding (11). To increase the concentration of pregastric esterase, the sham-fed water was dialyzed against an aqueous 30% solution of polyethylene glycol[4] for 18 hr at 2 C.

The isolation of pregastric esterase from a tissue extract was attempted also. For this purpose, two of the tissue areas were combined: glosso-epiglottic space and tongue in the region of the vallate papillae. Such tissue from ten calves was pooled for the preparation of an extract as outlined in Part I. The approximate range in age of the calves was two to three months. This tissue extract will be referred to hereafter as that from the base of the tongue.

Electrophoretic analysis for esterases. Initially, the electrophoretic distribution of esterases in both the concentrated sham-fed water and the tissue extract was studied with the vertical starch gel procedure (10) mentioned previously. For reasons that will be presented later, a modified technique of starch gel electrophoresis (5) was employed subsequently. The change of primary importance in the latter procedure was a lesser concentration of starch in the gel, 8 g/100 ml buffer. Such gel is quite fragile and must be handled with special care. Electrophoresis is conducted in a horizontal plane, in contrast to the vertical procedure, so that the gel is not dependent on its own mechanical strength for support. This technique also utilizes a gel of smaller dimensions, 25 by 150 by 1.5 mm. Furthermore, the gel is not sliced prior to staining. Other changes involved a higher voltage gradient, 12 v/cm, and a shorter time for electrophoresis, 2 hr. The method of staining per se remained unchanged.

Determination of lipolytic activity. On various occasions, some of the esterase preparations were incubated with milk fat to determine their lipolytic activity. Afterwards, the free fatty acids of the incubation mixture were recovered on a silicic acid column, either as total acids (12) or as two fractions: butyric acid and higher fatty acids (8).

RESULTS AND DISCUSSION

The electrophoretic pattern of esterases for the concentrated sham-fed water is illustrated in the upper part of Figure 2. Though the staining was pronounced, particularly in the

[4] Carbowax 20000, Union Carbide Chemicals Company, 270 Park Avenue, New York 17, New York.

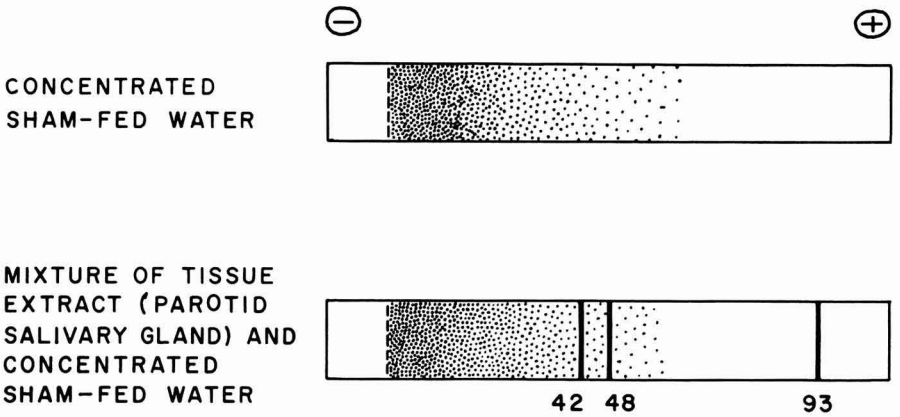

FIG. 2. Electrophoretic distribution of esterases in sham-fed water alone and in a mixture of sham-fed water and an extract of the parotid salivary gland. (Composition of gel, 15 g starch/100 ml buffer.)

region near the sample slot, there was no resolution into discrete bands. Moreover, the anodic face of the sample slot stained markedly, as if part of the esterase never migrated into the gel. Strangely enough, this pattern seemed to bear no resemblence to those of tissues believed to secrete pregastric esterase. Thus, an explanation was sought for the apparently anomalous behavior of pregastric esterase as found in sham-fed water.

One possible explanation was that sham-fed water contains some unkonwn material that interferes with the migration of pregastric esterase. To test this hypothesis, extracts of several pregastric tissues were mixed with sham-fed water, and these mixtures were subjected to electrophoresis and subsequent staining for esterases. A typical result is illustrated in the lower part of Figure 2. No interference in migration was indicated, as evidenced by the fact that the bands of the tissue extract were clearly superimposed on the general staining of the sham-fed water. The slowest esterase, ca. 14, usually stained too lightly to be seen against the dark background near the sample slot. These results established two facts: that the general staining of the sham-fed water is indeed the pattern of pregastric esterase, and that the two esterases, ca. 42 and 48, are not components of pregastric esterase.

Now that the identity of pregastric esterase on starch gel was known, though admittedly not well defined, the question arose as to whether this pattern was present in any of the tissue extracts. Light background staining had been noted during the survey described in Part I, though at the time it was believed to be an artifact and was accorded little significance. Thus, electrophoretic analyses were conducted on the extract of tissue from the base of the tongue, which contains two areas believed to secrete pregastric esterase. To darken the pattern of pregastric esterase, if present, the gel was incubated in the staining solution for a longer period of time than normal. Results of this analysis are illustrated in the upper part of Figure 3. The background staining of pregastric esterase was plainly visible, in addition to the four bands seen previously.

Efforts were then directed toward the separation of pregastric esterase in the tissue extract from the other esterases present. This was achieved by acid precipitation. First, the tissue extract was chilled in an ice bath, and its pH was reduced to 5.0 by adding citric acid. The acidified extract was centrifuged at 3,000 × g for 1 hr at 2 C, after which the supernatant was decanted from the precipitate. The pH of the supernatant, which had dropped to approximately 4.0 during centrifugation, was adjusted to 7.0 by adding tris(hydroxymethyl)aminomethane. The precipitate, on the other hand, was crushed and suspended in a volume of water equal to that of the original tissue extract. After adjusting the pH to 7.5 with tris(hydroxymethyl)aminomethane, the suspension was stirred at 0 C until solution of the precipitate was complete, which usually required several hours. The electrophoretic patterns of both the supernatant and the precipitate fractions are illustrated in the lower part of Figure 3. The pattern of the precipitate was identical to that of pregastric esterase in sham-fed water (Figure 2), whereas the other esterases were present in the supernatant.

The diffuse pattern of pregastric esterase,

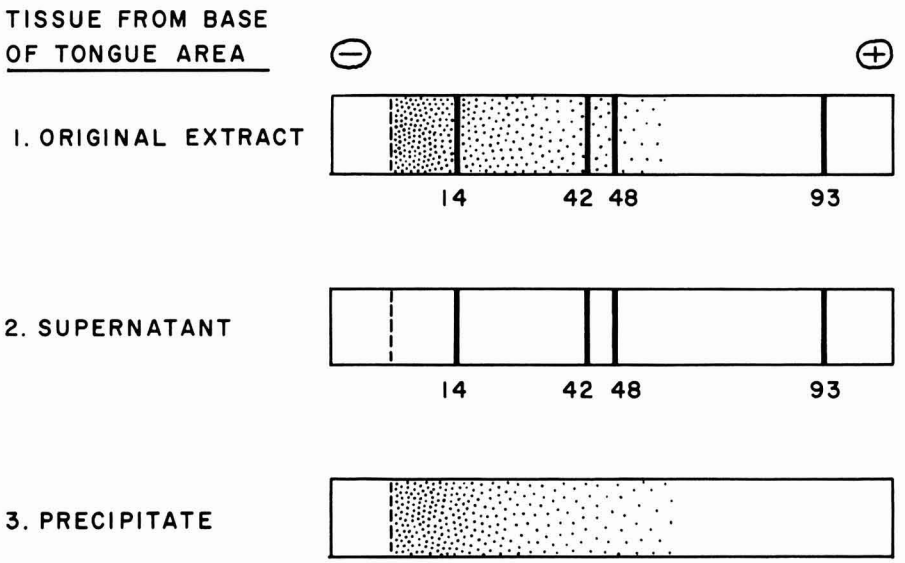

FIG. 3. Electrophoretic distribution of esterases in an extract of tissue from the base of the tongue and of the supernatant and precipitate fractions thereof. (Composition of gel, 15 g starch/100 ml buffer.)

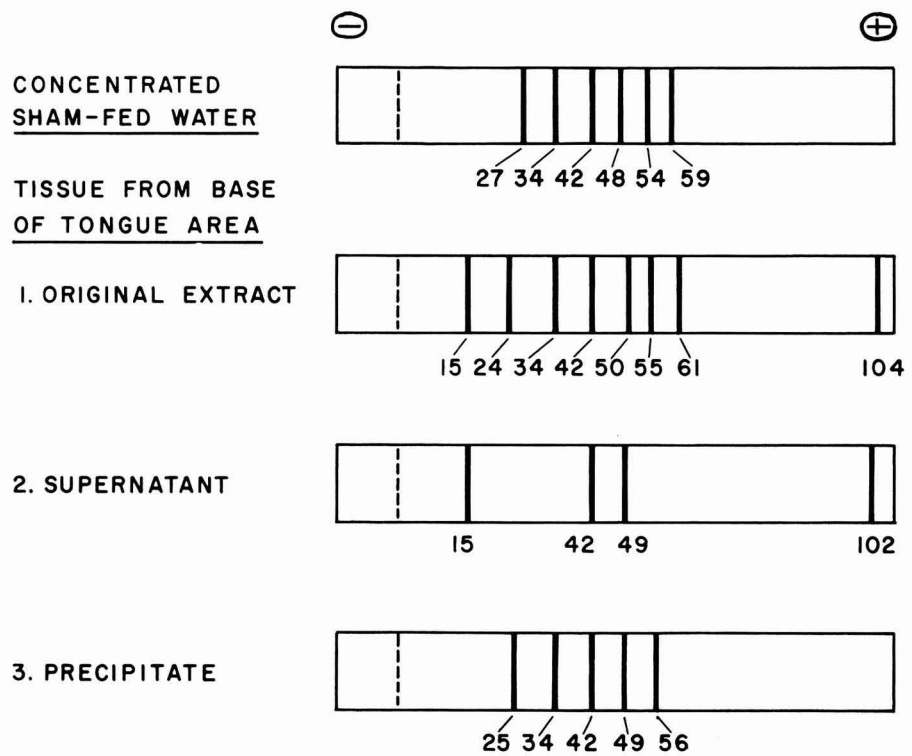

FIG. 4. Electrophoretic distribution of esterases in sham-fed water and in an extract of tissue from the base of the tongue, including the supernatant and precipitate fractions thereof. (Composition of gel, 8 g starch/100 ml buffer.)

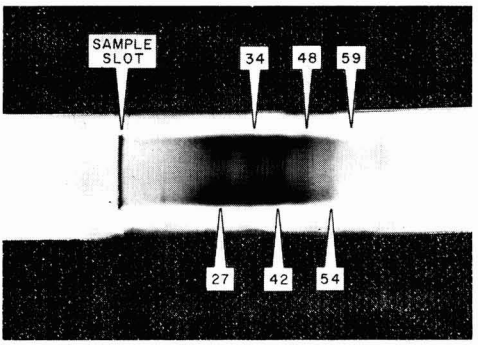

FIG. 5. Electrophoretic pattern of pregastric esterase in sham-fed water. (Composition of gel, 8 g starch/100 ml buffer.)

though recognizable, did not indicate the number of enzymes involved. In seeking an explanation for the lack of resolution, the following hypothesis was offered. The molecular dimensions of pregastric esterase are greater than those of the other esterases and are of such magnitude that this enzyme can pass through only the larger pores of a gel containing 15 g of starch per 100 ml of buffer. Hence, the smaller pores block the migration of some of the pregastric esterase molecules. This hypothesis is in accord with the staining noted on the anodic face of the sample slot, as if part of the pregastric esterase never migrated into the gel. It also explains why the staining on the gel surface becomes progressively lighter as one moves away from the sample slot toward the anode.

If the foregoing hypothesis is correct, the resolution of pregastric esterase should be enhanced by enlarging the pores of the gel. Such a change in gel structure was attempted by using less starch to prepare the gel, as indicated in the description of the modified technique of electrophoresis. The gel for this procedure contained only 8 g of starch per 100 ml of buffer, in contrast to 15 g used previously. The results from using such gels to analyze the sham-fed water and the extract of tissue from the base of the tongue, including the supernatant and precipitate fractions thereof, are illustrated in Figure 4. Six esterases were visible in the pattern of sham-fed water, thus establishing the multiple nature of pregastric esterase. A photograph of this pattern is presented in Figure 5. The most rapidly migrating esterase, ca. 59, stained very lightly, however. A similar pattern, except for the absence of 59, was noted also for the precipitate, thus confirming the identity of this fraction as pregastric esterase. For the supernatant, the four esterases observed previously were seen again, though the relative position of one, ca. 104, seems to have shifted slightly. Two enzymes of the pregastric esterase system, ca. 42 and 48, have the same mobility as two esterases that are present in the supernatant, ca. 42 and 49. They are not the same enzymes, however. The esterases of the supernatant resolve in gel containing either 8 or 15 g of starch per 100 ml of buffer, and they remain in solution at a slightly acid pH. In contrast, the components of pregastric esterase resolve only in gel containing 8 g of starch per 100 ml of buffer, and they precipitate at a slightly acid pH. Thus, the original tissue extract, which appears to have only eight esterases in Figure 4, actually has ten.

Though resolution of pregastric esterase was enhanced greatly by using less starch in the gel, it still was not so sharp as desired. Further reduction in the quantity of starch could not be effected, however, because 8 g per 100 ml of buffer is approximately the lowest concentration of starch from which a gel can be obtained. In addition, the resolution of pregastric esterase in the precipitate was never so sharp as that from either the sham-fed water or the original tissue extract. The factors involved are un-

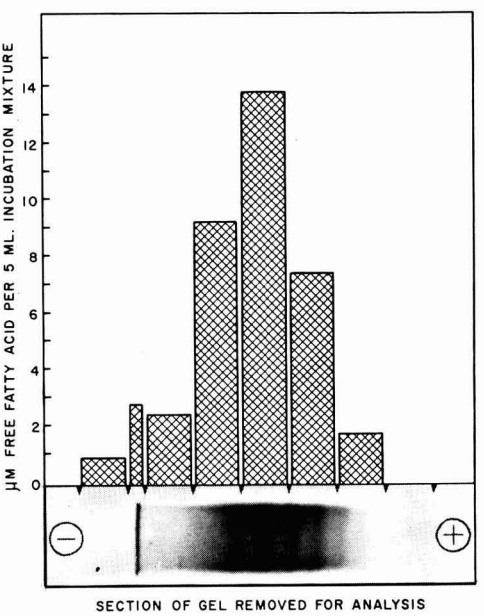

FIG. 6. Electrophoretic distribution of the lipolytic activity of pregastric esterase from sham-fed water. (Composition of gel, 8 g starch/100 ml buffer.)

known, though it is probable that this change was induced by the acid precipitation.

At this point in the investigation, an attempt was made to locate pregastric esterase on starch gel by using milk fat as the substrate. Since no technique was known for the histochemical detection of triglyceride hydrolysis, enzyme location was approached in the following manner: Concentrated sham-fed water was subjected to electrophoresis in gel containing 8 g of starch per 100 ml of buffer. After electrophoresis, the gel was cut into eight serial sections: one from the cathodic side of the sample slot, one at the slot, and six from the anodic side of the slot. To increase the concentration of enzyme, corresponding sections from five gels were combined and homogenized in 5 ml of 1.0 M phosphate-citrate buffer, pH 6.0. The homogenate for each of the eight sections was mixed with 10 ml of evaporated milk and incubated at 35 C for 2 hr. After incubation, 5 ml of each mixture was analyzed for total free fatty acids, according to the procedure described by Young et al. (12). The results were then compared, as shown in Figure 6, to gels that had been stained in the usual manner with 1-naphthyl butyrate. In general, the concentration of free fatty acids paralleled the intensity of staining in each section. The trace of lipolysis on the cathodic side of the sample slot probably resulted from cross-contamination during removal of the gel sections. The detection of hydrolysis by titrating free fatty acids is markedly less sensitive than the staining procedure involving 1-naphthyl butyrate. Thus, with enzyme concentration as a limiting factor, the serial sections of gel could not be made narrow enough to resolve the individual components of pregastric esterase. Nevertheless, these results indicate that the same esterases are responsible for the hydrolysis of both simple esters and triglycerides.

Milk fat was used also to investigate the substrate specificity of both the supernatant and the precipitate fractions of tissue from the base of the tongue. A mixture of 1 ml of enzyme, 10 ml of evaporated milk, and 5 ml of 1.0 M phosphate-citrate buffer, pH 6.0, was incubated 2 hr at 35 C. Afterwards, the free fatty acids in 5 ml of each mixture were separated on a silicic acid column (8) into two fractions: butyric acid and higher fatty acids. As shown in Figure 7, most of the total lipolytic activity was found in the precipitate (pre-

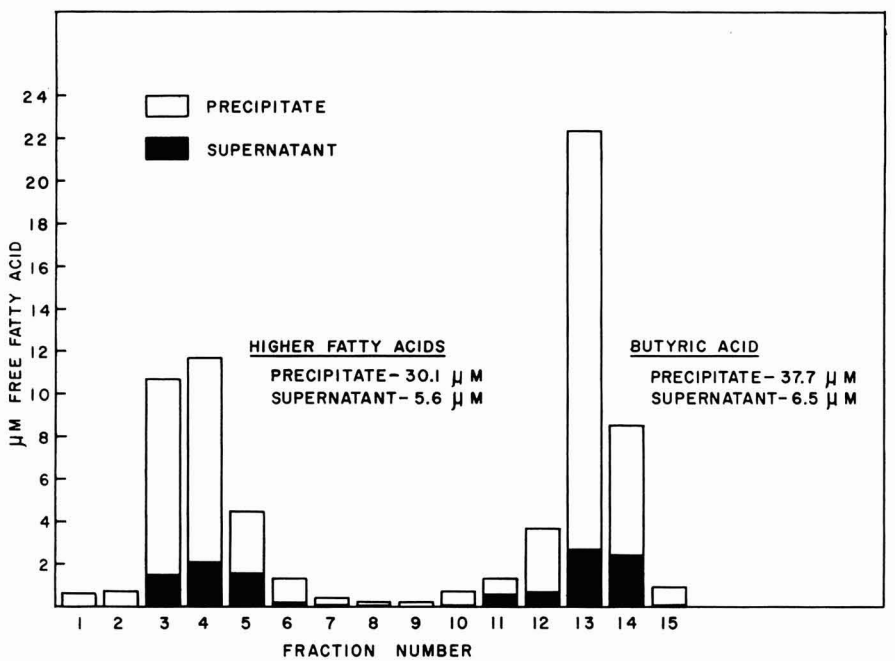

FIG. 7. Substrate specificity of the supernatant and the precipitate fractions of tissue from the base of the tongue. (Size of the eluate fractions collected from the silicic acid column, 15 ml.)

gastric esterase). In addition, this fraction exhibited a marked preference for esters of butyric acid. Over 50% of the total acid liberated by the precipitate was butyric, even though milk fat averages only 10.4% of this acid (1). This pattern of substrate specificity is in accord with previous observations on pregastric esterase as found in sham-fed milk (8). The specificity of the supernatant was similar to that of the precipitate.

CONCLUSIONS

Results of this investigation have established that pregastric esterase consists of at least six different esterases. Four of these enzymes, ca. 27, 34, 42, and 48, appear to be secreted in greater quantities than the other two, as judged from the intensity of their staining shown in Figure 5. Moreover, all of these esterases probably are of relatively high molecular weight, as suggested by their improved resolution in gel that contains a low concentration of starch. The presence of pregastric esterase in tissue from the base of the tongue identifies this area as one that is involved in secretion. The presence or absence of this enzyme system in other tissues will need to be established accordingly in future studies.

Some esterases in animal tissues are known to differ in their biochemical properties. Thus, the multiple nature of esterases in the calf, including pregastric esterase, emphasizes the need for separating such enzymes if their biological functions are to be studied and accurately understood.

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METABOLISM OF AMINO ACIDS IN THE BOVINE RUMEN[1]

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SUMMARY

Catabolic intermediates of L-arginine, L-lysine, and DL-tryptophan in vivo were compared with previously determined dissimilation products of these amino acids in vitro. The relative concentrations of amino acids and amines of jugular vein–blood plasma were examined at 0, 1, 2, and 4 hr after administration of each amino acid, and rumen ingesta was assayed for amino acids, ammonia, indole, and skatole for a 24-hr period. DL-tryptophan (265.5 g), L-arginine · HCl (274 g), or L-lysine · HCl (237 g) dissolved in one liter of water was mixed with the rumen ingesta of one of two rumen-fistula cows 2 hr after feeding 12 lb hay and 8 lb grain.

Rumen ammonia concentrations were the largest 6 hr following arginine administration (32 mg NH_3-N/100 ml), whereas tryptophan and lysine yielded smaller, but detectable, amounts of ammonia. Arginine yielded ornithine; the ruminal concentration of these two amino acids became equal within 6 hr after arginine administration. The ruminal lysine concentration decreased 50% within 6 hr of administration and this decrease was accompanied by an increase in delta-amino valeric acid concentration. Ruminal tryptophan concentrations were not markedly reduced until 8 to 10 hr after administration, at which time indole and skatole concentrations were 3 to 5 μg/ml rumen fluid. Plasma indole and skatole did not exceed 0.25 μg/ml through 4 hr. Arginine and lysine supplementation increased the concentration of several plasma amino acids within 1 hr after addition to the rumen, whereas tryptophan had little or no effect. Plasma lysine was markedly increased 4 hr after lysine administration.

Limited information exists as to the fate of amino acids in the rumen per se. Hueter et al. (10), comparing in vitro and in vivo techniques, reported deamination of DL-aspartic acid, DL-lysine, and a mixture of DL-alanine and glycine. Five amino acids, β-alanine, DL-aspartic acid, L-glutamic acid, L-lysine and glycine, were fed individually to a fistulated steer by Looper et al. (14). The first three were readily deaminated, whereas L-lysine and glycine failed to raise ammonia levels over those of the control ration. Neither study reported intermediates arising from the catabolism of amino acids in vivo.

Results of initial studies indicated that amino acids were poorly absorbed from the rumen (2, 19). Recent investigations, however, revealed that the rumen may be a major site of amino acid absorption (6, 8).

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This investigation had two purposes: The first was to compare the catabolic pathways of arginine, tryptophan, and lysine in vivo with results previously obtained in vitro (11, 12). The second was to examine qualitatively the relative concentrations of the amino acids and amines of jugular vein–blood plasma after administering individually these three amino acids into the rumen via a fistula.

MATERIALS AND METHODS

Animal management and analytical methods were reported earlier (11), except for the following modifications. The two 1,200-lb rumen-fistula cows used in this study were fed once daily 12 lb alfalfa hay and 8 lb of concentrate mixture.

For purposes of calculating the amount of each amino acid to be added via the fistula, the following procedure was employed: The assumption was made that the rumen contents constituted 14% of total body weight (3) and were 85% water, by weight. Using these figures, the rumen would contain 65 liters of aqueous phase. Equivalent amounts of DL-tryptophan,

L-arginine, HCl or L-lysine HCl (265.5, 274, and 237 g, respectively) were dissolved in a liter of water and added to the rumen, giving approximately a 0.02 M solution of the amino acid. DL-tryptophan was solubilized in 800 ml of 1 N NaOH and made up to one liter with water, prior to fistular administration.

Each amino acid solution was added 2 hr after feeding and thoroughly mixed with the rumen ingesta by stirring with the arm and fist for 5 min. The endogenous (no amino acid administered) L-lysine and DL-tryptophan investigations were performed using one cow, whereas the other cow was used for investigation of L-arginine. A portion of rumen ingesta strained through a double layer of cheese cloth served as the zero (0) hr sample. Portions of rumen liquor were sampled at 1, 2, 3, 4, 6, 10, 13 or 14, and 23 or 24 hr after the addition of each amino acid. Samples were obtained at 8 hr in the endogenous and tryptophan studies but not in the lysine and arginine trials.

Blood samples were obtained by jugular venipuncture 0, 1, 2, and 4 hr after fistular administration of the amino acid solution. Fifty milliliters of ethanol were added to 5 ml of plasma. After standing 15 min, the tubes were centrifuged 5 min at 250 × G to remove the plasma proteins. The supernatant was decanted and evaporated at less than 50 C under vacuum to dryness. The residue was dissolved in 5 ml of distilled water.

Ninhydrin-reactive components of the plasma were purified and separated into three fractions, employing the technique of Thompson et al. (18). Each fraction was evaporated under vacuum to dryness (< 50 C) and dissolved in 5 ml of 50% ethanol–50% water (v/v). A 1.5-ml portion of this solution was spotted on Whatman No. 3 MM paper (16½ x 22½ in.) for one- or two-dimensional descending chromatography.

Basic amino acids and amines were chromatographed unidimensionally, employing pyridine/acetic acid/water (50/35/15), phenol/water (80/20) in a 1% ammonia atmosphere or ethanol/dimethylamine (77/1) as solvents. Neutral and acidic amino acids were chromatographed, using the phenol/water mixture described above as the first solvent and n-butanol/acetic acid/water (62/16/26), containing 0.1% ninhydrin, as the second. The chromatograms were air-dried and allowed to develop overnight in the dark.

Individual amino acids were identified by comparison with Rf values of standard amino acids; color reactions of each amino acid when sprayed with the cupric nitrate-ninhydrin reagent of Moffat and Lytle (16); individual tests for proline (isatin), arginine (Sakaguichi) and citrulline (Ehrlich's); and by comparison with the chromatograms of bovine plasma amino acids by Coulson et al. (7).

The relative color intensities of each resultant ninhydrin-reactive component from plasma 1, 2, or 4 hr were compared to those drawn before amino acid addition. Preliminary studies, involving visual comparison of paper chromatograms, indicated little effect on endogenous levels of amino acids in jugular plasma employing identical sampling-time sequences.

RESULTS

Effects of the addition of DL-tryptophan, L-lysine, and L-arginine to the rumen on rumen ammonia concentration are summarized in Figure 1. Arginine had the most pronounced effect upon rumen ammonia concentrations which rose for at least 6 hr after the addition of arginine but had decreased by 10 hr. L-lysine had little effect upon rumen ammonia levels for 3 hr following its administration, but thereafter tended to keep rumen ammonia levels at much higher levels than the endogenous ration. Ammonia levels following lysine addition, however, were still considerably below those following arginine supplementation. The addition of DL-tryptophan had little effect upon rumen ammonia concentration until about 6 hr after its administration; however, these levels were much lower than those noted for arginine and lysine. After 13 hr, rumen ammonia levels were identical between the endogenous and tryptophan trials and appeared to remain so thereafter. The rumen ammonia levels in the endogenous, tryptophan, and arginine studies returned

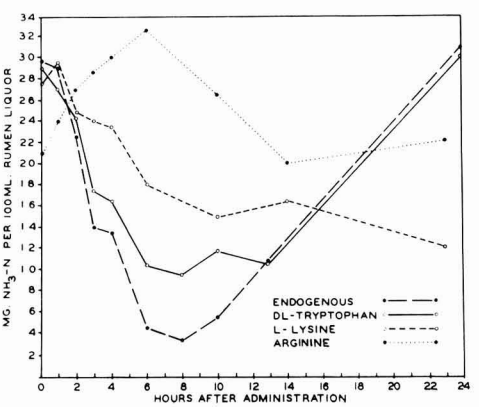

FIG. 1. Levels of ammonia nitrogen in rumen liquor after the administration of three amino acids.

TABLE 1

Relative effects on jugular plasma amino acid concentrations of L-arginine addition to the rumen

Amino Acid	Relative intensity	Intensity relative to the 0-hr sample at 1 hr	2 hr	4 hr
Aspartic Acid	Very weak	S[b]	S	S
Aspartic Acid	Strong	+[c]	S	S
Serine	Strong	+	+	S
Glycine	Strong	+	+	+
Threonine	Weak	+	S	S
Citrulline	Moderate	+	+	+
Glutamine	Moderate	+	+	+
Alanine	Very strong	+	S	S
Tyrosine	Weak	S	S	S
Valine	Strong	+	+	+
Methionine	Moderate	+	S	S
Leucine	Moderate	+	S	S
Phenylalanine	Moderate	S	−[a]	S
Proline	Weak	S	S	S
Unknown	Weak	S	S	S
Histidine	Weak	S	S	S
Lysine	Moderate	+	+	+
Arginine	Moderate	+	+	+
AABA[d]	Weak	S	S	S

[a] − = Less than other sample.
[b] S = Equal to other sample.
[c] + = More than other sample.
[d] Alpha amino butyric acid.

within 23 or 24 hr to those levels prior to the initiation of the experiments; whereas, in the lysine experiment, they failed to return to the pre-experimental concentration.

Whenever samples were prepared for ammonia analysis, rumen liquor samples were likewise prepared for chromatographic analysis. Arginine (arg.), glutamic acid (g.a.), alanine (al.), and δ-amino-valeric acid (d.a.v.a.) were present in rumen liquor immediately after the addition of L-arginine to the rumen. Ruminal concentrations of these amino acids changed as a function of time in the following manner:

1-hr: arg. decreased; ornithine (orn.) was formed; g.a., al., d.a.v.a. increased

2-hr: arg. decreased; g.a., al., orn., d.a.v.a. increased

3, 4, 6-hr: arg., al., g.a. decreased; orn., d.a.v.a. increased

10-hr: arg., al., g.a., orn. decreased; d.a.v.a. increased

14, 23-hr: only trace amounts of all five amino acids present

Equal amounts of arginine and ornithine appeared to be present on the chromatogram of the 6-hr sample.

The qualitative effects of ruminal arginine administration on plasma amino acids are tabulated in Table 1. Lysine (ly.), glutamic acid, alanine, and δ-aminovaleric acid were present in rumen liquor immediately after addition of L-lysine to the rumen. Ruminal concentrations of these amino acids changed as a function of time in the following manner:

1-hr: ly. decreased; al., g.a., d.a.v.a. increased

2-hr: ly. decreased; d.a.v.a. increased; al., g.a. remained the same

3-hr: ly., al. decreased; d.a.v.a. increased; g.a. remained the same

4, 6, 10-hr: ly., al. decreased; d.a.v.a., g.a. remained the same

14, 23-hr: ly., al. trace; d.a.v.a., g.a. remained the same

The color intensity of the lysine spot decreased to approximately 50% of the original level on the chromatogram of the 6-hr rumen sample. Lysine altered plasma amino acid concentrations as shown in Table 2.

Alanine and glutamic acid were present in low concentrations in both studies; the largest increases per time interval were noted 1 hr after

TABLE 2

Relative effects of jugular plasma amino acid concentrations of L-lysine addition to the rumen

Amino Acid	Relative intensity	Intensity relative to the 0-hr sample at 1 hr	2 hr	4 hr
Aspartic Acid	Very weak	−[a]	−	−
Glutamic Acid	Strong	+[c]	S[b]	S
Serine	Strong	+	S	S
Glycine	Strong	+	S	S
Threonine	Weak	++[d]	+	+
Citrulline	Moderate	+	S	S
Glutamine	Moderate	+	S	S
Alanine	Very strong	+	+	+
Tyrosine	Weak	+	+	+
Valine	Strong	++	+	+
Methionine	Moderate	++	+	+
Leucine	Moderate	++	+	+
Phenylalanine	Moderate	++	+	+
Proline	Weak	−	−	−
Unknown	Weak	S	+	+
Histidine	Weak	−	−	−
Lysine	Moderate	S	−	++
Arginine	Moderate	S	−	−
AABA[e]	Weak	S	S	−

[a] − = Less than 0-hr sample.
[b] S = Equal to 0-hr sample.
[c] + = More than 0-hr sample.
[d] ++ = More than +.
[e] Alpha amino butyric acid.

TABLE 3

A comparison between concentrations of indole and skatole in the rumen in the absence and presence of DL-tryptophan

Time	Endogenous		DL-Tryptophan	
	Indole	Skatole	Indole	Skatole
	($\mu g/ml$)			
0 hr	0.4	0	0.3	0.0
1 hr	1.0	0	0.9	0.0
2 hr	0.8	0	1.6	0.0
3 hr	1.2	0	1.8	0.0
4 hr	1.2	0	2.2	0.0
6 hr	0.9	0	3.6	0.0
8 hr	0.9	0	4.8	3.6
10 hr	0.4	0	4.2	2.6
13 hr	0.2	0	1.5	0.0
24 hr	0.3	0	0.4	0.0

lysine administration and 2 hr after arginine addition. Cadaverine and putrescine were not present in detectable concentrations in either study.

Chromatograms of the catabolism of DL-tryptophan in vivo failed to reveal the formation of other ninhydrin-reactive products. Glutamic acid and alanine were present but decreased in concentration more rapidly than in the arginine and lysine studies. Tryptophan concentrations decreased only slightly the first 3 hr after administration and then a gradual decrease ensued until by the 10th hr the tryptophan concentration was markedly reduced. A comparison of indole and skatole rumen fluid concentrations between the endogenous and DL-tryptophan studies are present in Table 3. Indole concentrations did not exceed the endogenous levels until 2 hr after tryptophan administration and thereafter steadily increased to 4.8 μg/ml in the 8-hr sample. This maximum was followed by a decline to endogenous levels by the 24-hr sample. Skatole was detected only in the 8- and 10-hr samples which corresponded to the time of highest indole concentrations.

Jugular-venous serum levels of indole and skatole were less than 0.25 μg/ml at 0, 1, 2, and 4 hr following addition of DL-tryptophan to the rumen. These samples, however, were taken prior to maximal concentrations of indole and skatole in the rumen. Ethanolamine was detected in the plasma from each experiment, but its concentration appeared to be unaffected by treatment.

DISCUSSION

Paper chromatograms of blood plasma from the jugular vein were similar to those of Walker (20) and Coulson et al. (7), and likewise failed to reveal tryptophan in the venous plasma. The two most significant observations were the lack of effect of tryptophan supplementation on other plasma amino acids and the marked increase in lysine concentration of jugular-venous plasma 4 hr after adding L-lysine to the rumen. Arginine and lysine supplementation increased the concentration of several plasma amino acids within 1 hr following their addition to the rumen. Plasma aspartic acid, proline, histidine, and α-amino butyric acid, however, failed to show a detectable increase. Addition of L-arginine to the rumen resulted in an increase in venous arginine concentrations of jugular plasma.

The catabolisms of L-arginine, L-lysine, and DL-tryptophan in vivo were in excellent agreement with studies in vitro utilizing these same three amino acids (12). Arginine produced δ-aminovaleric acid and ornithine, whereas lysine yielded δ-aminovalerie acid. Indole and skatole were formed from tryptophan. The qualitative identification of these catabolic products from the aforementioned amino acids in vivo, coupled with quantitative measurements of certain of these metabolites, necessitates the recognition of these compounds as normal intermediates in the catabolism of tryptophan, lysine, and arginine.

Results of this investigation definitely show that individual amino acids differ in their rates of deamination in the rumen. Rumen ammonia levels in vivo paralleled expectations from studies in vitro. Arginine, which had previously been shown to have the most pronounced effect on ammonia levels in vitro (11), increased most markedly rumen ammonia levels in vivo. Similar analogies were demonstrated for the effects of lysine and tryptophan on rumen ammonia levels. Lysine was shown in vitro to be an amino acid which was intermediate in its dissimilation rate, whereas tryptophan was catabolized slowly in vitro (11). These same rate effects were noted in vivo. The presence of alanine and glutamic acid in rumen fluid 2 hr after feeding, a period of maximal ammonia concentrations in the rumen, and a subsequent increase in ruminal levels of these two amino acids following the addition of the readily catabolizable amino acids, L-arginine and L-lysine, suggest that the former two amino acids play a significant role in protein anabolism by rumen microorganisms.

The only amino acid employed in this investigation that had been previously studied in vivo was lysine. Hueter et al (10) and Looper et al. (14) reported little dissimilation of lysine in vivo, which is contrary to the results reported here. Looper's study and the present one both

showed that the peak of NH_3-N occurred within 1 hr after lysine addition to the rumen. The major difference between the two studies was that endogenous ammonia levels changed markedly with time in the present study and were more stable in Looper's experiment. Hueter et al. (10) used the production of volatile fatty acids as the criterion of DL-lysine dissimilation. DL-lysine has been shown to be more slowly catabolized in vitro than the L-enantiomorph (11).

Limited information exists on the production, absorption, and excretion of indole in the ruminant. The present study, coupled with a previous investigation in vitro (12), suggests tryptophan as a major precursor of indole in the rumen. When a goat was changed from an alfalfa to a *Lepidium* diet, blood indole levels rose from 0 to 1.0 ppm and indole appeared in the milk fat (5). Indole appears to be detoxified by the liver and excreated as indoxyl toxified by the liver and excreted as indoxyl a positive relationship between the proportions of legumes in the ration and indoxyl substances in the urine. One might conclude that the higher indole levels on leguminous rations were due to the greater tryptophan content of these plants or the greater availability of the tryptophan to the rumen microorganisms, or both.

The blood amino acid results are difficult to interpret, due to the limited number of analyses and the poor sensitivity of the analytical technique. Convincing evidence now exists which demonstrates that the pattern of amino acids in the diet markedly influences the level of some amino acids in the blood. In poultry, Charkey et al. (4) and Almquist (1) obtained good correlations between amino acid levels in chick blood and composition of dietary protein. Denton et al. (9) and Longenecker and Hause (13) likewise demonstrated that plasma amino acid changes in the dog were directly dependent upon the amino acid composition of the protein and supplemental amino acids ingested. Cook (6) and Demaux et al. (8) recently demonstrated the absorption of glycine and mixtures of amino acids from the rumen by means of the rumen perfusion technique and venous samples from the ruminal area of lightly anesthesized goats and sheep. The present study suggests the absorption of the added amino acids from the rumen, but also demonstrates that catabolizable amino acids, such as L-arginine and L-lysine, had a positive effect on levels of several plasma amino acids rather than just the one administered. Since the plasma samples were obtained from the jugular vein, the amino acid pattern encompassed the entire metabolism of the body rather than the rumen itself. Tryptophan was absent on all chromatograms of plasma amino acids as occurred in samples prepared by Walker (20) and Coulson et al. (7). It would appear that tryptophan was lost during the extraction process.

McMenany et al. (15) reported that only 25% of the total tryptophan is present in plasma in an unbound form.

Further studies on amino acid absorption via the rumen must be performed in a natural physico-chemical environment and with animals in normal physiological status. In addition, results need to be quantitative and extensive rather than qualitative and fragmentary. Blood amino acid patterns must be related to all gastric reservoirs, rather than just the one being studied.

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CONGENITAL ABNORMALITIES IN CATTLE: THEIR ASSOCIATION WITH HEREDITARY AND ENVIRONMENTAL FACTORS[1]

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SUMMARY

The purpose of this paper is to indicate the frequency of congenital anomalies in cattle and to record data dealing with the influence of environmental and hereditary factors on their occurrence.

Approximately 5,000 calves of the Holstein, Guernsey, and Jersey breeds were divided into normal and abnormal animals. Abnormality was defined as any anatomical defect observed by the cooperating dairymen. The abnormalities reported were divided into five major groups: general abnormalities, abnormalities of ectodermal, mesodermal, and neurectodermal derivatives and calves born dead with no other observable defect. Environmental and genetic factors were tested for an association with the number of abnormal animals reported and the type of abnormality reported, by the chi-square test of independence; 6.26% of the calves born were abnormal. A significant association of sire, with the number of abnormal animals reported, was demonstrated. A highly significant association of twins and abnormal gestation length with the number of abnormal animals was shown. Twins were highly significantly associated with type of abnormality reported, as was breed. No significant association was found between sex, number of services required for conception, breed, level of herd production or inbreeding, and the number of abnormal animals reported. No significant association could be shown between number of services required for conception or sex and type of abnormality reported.

Congenital abnormalities are an economic burden to the dairyman. Every abnormal calf is one less replacement for cows that leave the herd. In addition, the birth of an abnormal calf may be accompanied by severe dystocia.

The purpose of this paper is to indicate the frequency of congenital abnormalities in cattle and to record data dealing with the influence of environmental and hereditary factors on their occurrence.

The problem of congenital abnormalities can be approached from two points of view. The diagnosis and study of the genetic history of individual abnormalities may be undertaken. This method has been used in the early reports of abnormalities, reported in cattle for over 100 yr (4). In 1934, a list of eleven abnormalities occurring in cattle was published (9). This list has been increased by numerous authors so that the most recent review (18) included 34 lethals and 14 sublethals. Other reviewers (5, 7, 8, 15) have considered the general problem of abnormalities and have grouped reports by type of tissue affected, while trying to find some connection among the anomalies cited.

Mead, Gregory, and Regan (12) tested six sires at random in the California Experiment Station herd and reported eight deleterious recessive genes carried among these bulls. The Production Division of the Milk Marketing Board of Great Britain (13), on the other hand, has reported few recessively inherited abnormalities carried per bull for the A.I. centers of Great Britain.

All congenital abnormalities are not genetically initiated. They have been attributed to disease (10), poisons (15), vitamin deficiency (6), and other environmental factors, as well as interactions of genes and the environment. Brum (2, 3), with information on 601 abnormalities affecting 337 cattle, concluded that general environment and inherited influences affected either the frequency of abnormal ani-

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mals born or was associated with particular types of abnormalities. The Milk Marketing Board of Great Britain reported the following breakdown on general type of abnormality, noting, however, that environmentally caused anomalies and inherited traits of but little economic importance are included, e.g., flexed pasterns. Of all cases reported, skeletal abnormalities accounted for 55.7%; head, thorax, and abdominal soft tissue, 27.5%; skin, hair, and muscle anomalies, 8.9%; reproductive abnormalities, 5.9%, and udder and teat abnormalities, 1.5%.

MATERIALS AND METHODS

Source of data. Animals involved in this study were the purebred dairy cattle in 87 Ohio herds during the 3.5-yr period from January, 1957, to July, 1960. During this time, 4,980 calves were born, 2,004 Guernsey, 1,706 Holstein, and 1,270 Jersey. For each calf born, the dairyman completed a birth report card on which was given a number of attributes regarding the calf, its gestation and parentage. If the calf were described as abnormal, a multiple check-list type of abnormality form supplied for this purpose was later completed. Some calves had more than one abnormality reported, so that more abnormalities were reported than the total number of abnormal calves. Only those abnormalities apparent at birth were taken into consideration. This excluded such traits as porphyria or lumpy jaw. The normal animals on which birth reports were received were used as controls in this study, except for two of the analyses in which the next normal calf born in the same herd following the birth of an abnormal animal was used as a paired control for the abnormal calf.

Abnormality, described as any anatomical deviation from the norm, grossly apparent at birth, or shortly thereafter, was determined by the individual dairymen. Abnormalities reported were divided into five major categories, based primarily on derivation of the tissue affected. They included (a) general abnormalities, i.e., those that affected the animal as a whole; (b) bone, muscle, cartilage, and joint defects; (c) nerve and eye abnormalities; (d) epithelial anomalies such as skin, hair, and genitalia defects; (e) those calves born dead, but with no other grossly observable defects, i.e., stillborn calves.

Analyses of data. The statistical analyses were based on the chi-square test of independence (16). If differences between expected and observed values were significant at the 5% level of probability, the hypothesis of independence was rejected and an association was assumed between the factor being tested and the number of abnormal animals born or the frequency of the type of abnormality. Where applicable, a heterogeneity test of the data in the different breed groups was carried out. If the data were homogeneous, the groups were combined for subsequent analysis.

The factors tested for an association with either the number of abnormal animals reported or frequency of type of abnormality can be divided into those that were genetic or environmental. Among the genetic factors tested were breed (Guernsey, Jersey, or Holstein), sire (any sire with at least 100 offspring reported), sex, and inbreeding. In the inbreeding analysis, the next normal calf born in the herd was matched with the abnormal calf and used as a control. The coefficient of inbreeding (21) was calculated for each animal, using a three-generation pedigree. The two groups were then divided into inbred and noninbred calves to make the chi-square test of independence.

The environmental factors tested for an association with either number of abnormal animals reported or frequency of type of abnormality included services required per conception, in which normal and abnormal calves were divided into groups based on whether one, two, or three or more services were required for their conception. Another environmental factor tested was abnormal gestation length. In this analysis, the group of controls was again the next normal calf born in the same herd following the birth of an abnormal calf. This control group was used to calculate a mean gestation length for each breed group. The standard deviation was calculated for each breed group and an abnormal gestation length was taken to be any gestation exceeding two standard deviations from the breed mean. The abnormal animals were then scored for normal or abnormal gestation length by breed and the two groups tested against the expected values for normal animals of each breed. Twinning was also tested for an association with abnormal animals and frequency of type of abnormality. Finally, the abnormal animals were tested for an association with the level of production of the herds from which they were reported. Forty-eight herds for which production records were available were divided by breed. Herds from each breed were then separated into those on DHIA test and those on HIR test. Within each of the six categories so formed (three breeds each with two types of records), the 1957-1959 4% fat-corrected average production per herd was calculated. The standard devia-

TABLE 1

Proportion of abnormal animals by breed

	Guernsey I		Guernsey II		Holstein		Jersey		Total	
	(no.)	*(%)*	*(no.)*	*(%)*	*(no.)*	*(%)*	*(no.)*	*(%)*	*(no.)*	*(%)*
Abnormal	58	5.16	63	6.56	113	6.63	78	6.14	312	6.26
Normal	986	94.44	897	93.44	1,593	93.37	1,192	93.86	4,668	93.74

$X^2 = 1.4459$; Df = 3; P = .70-.60.

tion for each of the six groups was calculated and those herds more than one standard deviation below the mean of their group were considered low-producing herds, and those more than one standard deviation above the mean of their group were considered high-producing herds. All others constituted a third group (average-producing herds). All herds in each of the low-producing groups, i.e., low DHIA Holstein, low HIR Holstein, low DHIA Jersey, low HIR Jersey, etc., were combined, as were all high group herds and all average herds. These three classes were then tested for an association with the number of abnormal animals reported.

RESULTS AND DISCUSSION

Of the 4,980 calves reported, 312 (6.26%) were abnormal. Of these, 44.2% were born dead with no other observable defects, i.e., stillborn.

No significant association of breed with the number of abnormal animals reported could be demonstrated (Table 1), i.e., all three breeds had approximately the same proportion of anomalous calves. However, a highly significant association of breed with the frequency of the class of abnormality reported was found (Table 2), i.e., a definite association of certain types of abnormalities with specific breeds was demonstrated. The large contributions to the high chi-square value were attributable in part to the Holsteins and Guernseys which had many less and more than expected, respectively, of muscle, bone, cartilage, and joint abnormalities. In addition, the Holsteins and Jerseys had respectively more and less than expected of stillborn calves. The Jersey dairymen have reported only 31.9% of the total abnormalities as stillborn calves, whereas the figure is 50.4% for Holsteins. On the other hand, the Jersey dairymen reported 35.2% of the total abnormalities as general anomalies which includes weak calves and small calves, whereas the Holstein dairymen reported only 22.3% in that class. Thus, the difference between the breeds may be due in part to a tendency of Jersey breeders to report stillborn calves as small and of Holstein breeders to report them only as stillborn.

Especially interesting in the association of frequency of class of abnormality with breed is the wide range of frequency of muscle, bone, cartilage, and joint defects among the breeds. These results generally agree with literature reports in which specific abnormalities have been described as predominating in certain breeds (7).

A significant association was found between sire and the number of abnormal animals born (Table 3). This is a verification of the theory that there are differences among bulls not due to chance as to the number of abnormal calves sired if, as in this study, the bulls are bred to cows at random with respect to the genes they carry for abnormalities. This difference may be caused by the frequency in the cow population of recessive genes for which the bull is a carrier. Any inherited abnormality conditioned by recessive genes would occur quite often in the progeny of a heterozygous sire if he were

TABLE 2

Proportion of abnormalities in each class by breed

Class of abnormality	Guernsey		Holstein		Jersey		Totals	
	(no.)	*(%)*	*(no.)*	*(%)*	*(no.)*	*(%)*	*(no.)*	*(%)*
General	40	28.8	27	22.3	32	35.2	99	28.2
Muscle, bone, joint, cartilage	47	33.8	19	15.7	25	27.5	91	25.9
Nerve (eye)	1	0.7	4	3.3	3	3.2	8	2.3
Epithelium	2	1.4	10	8.3	2	2.2	14	4.0
Stillborn	49	35.3	61	50.4	29	31.9	139	39.6

$X^2 = 25.2816$; Df = 8; P = .01-.001.

TABLE 3

Proportion of normal and abnormal calves by sire

	Sires																		Totals	
	A		B		C		D		E		F		G		H		I			
	(no.)	(%)	(no.)	(%)	(no.)	(%)	(no.)	(%)	(no.)	(%)	(no.)	(%)	(no.)	(%)	(no.)	(%)	(no.)	(%)	(no.)	(%)
Abnormal	7	4.4	17	15.7	5	3.8	7	6.3	21	8.4	11	8.4	10	8.2	13	9.8	16	6.6	107	7.7
Normal	151	95.6	91	84.3	125	96.2	104	93.7	228	91.6	120	91.6	112	91.8	120	90.2	225	93.4	1,276	92.3

$X^2 = 16.6416$; Df = 8; P = .05-.01.

mated to a random selection of the cow population in which the frequency of the recessive genes was fairly high. Therefore, he would have more abnormal progeny than another sire, also a carrier of genes for a recessively conditioned abnormality, if the frequency of these recessive genes in the cow population were low.

The third genetic factor tested was sex. No significant association could be demonstrated between sex and either the number of abnormal animals reported (Table 4) or frequency of types of abnormality (Table 5). The results reported here are in agreement with the expectation of the hypothesis that if any positive association could be demonstrated between number of abnormal animals and sex, the male sex should have the more than expected number of abnormal animals. This is due to the fact that sex-linked recessives causing abnormality would necessarily appear in half the male progeny of a female carrier bred to a hemizygous normal male, whereas none of the female offspring would exhibit the abnormality. Although the results agree with this hypothesis, the values are not significant. Considering the fact that cattle have 30 pairs of chromosomes and the difference between male and female is one in 60 chromosomes, an extremely large sample would be needed to attain significant differences attributable to sex-linked genes.

The final genetic factor analyzed was inbreeding. No association of inbreeding with the number of abnormal calves reported could be demonstrated (Table 6). These results are contrary to expectation based on the theory of recessive inheritance. However, an explanation of these results may be the small amount of inbreeding in the sample. There were only 46 inbred calves compared with 510 noninbred individuals in the analysis, i.e., only 8.3% of the population was inbred.

Among the environmental factors tested for an association with the number of abnormal animals and the frequency of type of abnormality was the number of services required per conception. It was thought that a high number of services might indicate early abortion and, therefore, a tendency toward a full-term calf being abnormal. The chi-square values for an association of number of services required per conception with both the number of abnormal animals and the frequency of type of abnormality were not significant ($p = .50\text{-}.40$; $p = .20\text{-}.10$, respectively). Thus, an association is not indicated.

The second environmental factor tested was abnormal gestation length. The data for the

TABLE 4

Proportion of normal and abnormal calves by sex

	Male		Female		Total	
	(no.)	*(%)*	*(no.)*	*(%)*	*(no.)*	*(%)*
Abnormal	161	6.4	141	5.7	302	6.1
Normal	2,336	93.6	2,330	94.3	4,666	93.9

$X^2 = 1.1988$; Df = 1; P = .30-.20.

TABLE 5

Proportion of abnormalities in each class by sex

Class of abnormality	Male		Female		Total	
	(no.)	*(%)*	*(no.)*	*(%)*	*(no.)*	*(%)*
General	42	24.6	48	28.2	90	26.4
Muscle, bone, joint, cartilage	44	25.7	47	27.6	91	26.7
Nerve (eye)	2	1.2	6	3.5	8	2.3
Epithelium	6	3.5	7	4.1	13	3.8
Stillborn	77	45.0	62	36.6	139	40.8

$X^2 = 3.3048$; Df = 4; P = .40-.30.
Yates correction applied (Snedecor, 1955).

TABLE 6

Proportion of normal and abnormal calves in inbred and outbred groups

	Inbred		Outbred		Total	
	(no.)	*(%)*	*(no.)*	*(%)*	*(no.)*	*(%)*
Abnormal	22	47.8	256	50.2	278	50.0
Normal	24	52.2	254	49.8	278	50.0

$X^2 = .0948$; Df = 1; P = .99-.975.

TABLE 7

Proportion of calves with abnormal gestation lengths by breed

	Holstein		Jersey		Guernsey		Total	
	(prop.)	*(%)*	*(prop.)*	*(%)*	*(prop.)*	*(%)*	*(prop.)*	*(%)*
Abnormal animals	27/96	28.1	25/69	36.2	36/98	36.7	88/263	33.5
Normal animals	6/96	6.3	2/69	2.9	3/98	3.1	11/263	4.2

$X^2 = 78.4000, 261.0205, 363.5465$; Df = 1; $P < .001$ for each breed.

TABLE 8

Distribution of twins and singles between normal and abnormal calves

	Twins		Singles		Totals	
	(no.)	*(%)*	*(no.)*	*(%)*	*(no.)*	*(%)*
Abnormal	66	23.7	247	5.3	313	6.3
Normal	212	76.3	4,462	94.7	4,674	93.7

$X^2 = 152.6335$; Df = 1; $P < .001$.

TABLE 9

Distribution of abnormalities between twins and singles

Class of abnormality	Twins		Singles		Totals	
	(no.)	*(%)*	*(no.)*	*(%)*	*(no.)*	*(%)*
General	14	24.6	84	29.1	98	28.3
Muscle, bone, joint, cartilage	2	3.5	86	29.8	88	25.4
Nerve (eye)	0	0	7	2.4	7	2.1
Epithelium	0	0	14	4.8	14	4.0
Stillborn	41	71.9	98	33.9	139	40.2

$X^2 = 32.8737$; Df = 4; $P < .001$.

various breeds were not homogeneous and, therefore, are shown separately (Table 7). The chi-square value for the distribution of the abnormal and normal calves between normal and abnormal gestation lengths was highly significant for each breed. Thus, an association of gestation length with the number of abnormal animals reported is indicated. The fact that the data for the different breeds were not homogeneous indicates differences between breeds in the association of abnormal animals and abnormal gestation lengths. A cause of the heterogeneity may be the variation in mean gestation length between breeds. It should be noted that in contrast to the literature in which the reports are almost exclusively on cases of prolonged gestation (1, 11, 19), the abnormal gestation lengths in this survey were predominantly short rather than prolonged.

Another factor tested for an association with both the number of abnormal animals reported and the frequency of type of abnormality was twinning. The chi-square value for an association of twinning with the number of abnormal animals reported was highly significant (Table 8). When the frequency of type of abnormality was tested for an association with twinning, the results were also highly significant (Table 9). The categories that contributed to the high chi-square value were the more-than-expected numbers of stillborn calves among twins and the less-than-expected number of twins with muscle, bone, cartilage, and joint defects. These results corroborate results reported in the literature. Woodward and Clark (20) noted that 8.1% of all stillbirths were twins among range Herefords. Stalleup et al. (17) found that only 2.54% of all calves were twins. Thus, more than a proportional share of stillbirths are twins and an association between twinning and stillbirths is indicated.

The fourth environmental factor tested for an association with the number of abnormal calves reported was the level of production of the herd in which the abnormal calf was reported. The chi-square value of a test of independence of level of production with the number of abnormal animals reported did not reach significance (Table 10). No association is indicated from these data. The negative result may be explained by the fact that environmentally caused abnormalities usually have a threshold value for nutritional levels and other management factors. The herds in this survey probably are all above the threshold value for basic environmental considerations.

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TABLE 10

Proportion of abnormal and normal animals in high-, average-, and low-producing herds

	High		Average		Low		Totals	
	(no.)	*(%)*	*(no.)*	*(%)*	*(no.)*	*(%)*	*(no.)*	*(%)*
Abnormal	30	5.4	148	7.1	42	6.3	220	6.6
Normal	528	94.6	1,950	92.9	622	93.7	3,100	93.4

$X^2 = 2.1297$; Df = 2; P = .40-.30.

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EFFECT OF BODY WEIGHT AND AGE AT CALVING ON MILK PRODUCTION IN HOLSTEIN CATTLE [1]

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SUMMARY

The 180-day production records of 1,344 lactations of Holstein cows including records of lactations one through eight, collected from six herds, were used to determine the independent influences of age and weight on production. A multiple regression analysis of first lactation data indicated that for a constant age milk production increased 134 lb and fat 7.8 lb for each 100-lb increase in body weight, and for a constant weight each increase of one month of age was accompanied by an increase of 46 lb of milk and 1.2 lb of fat. When all lactations were combined and the number of the lactation ignored, milk production increased by 400 lb and fat production by 14.4 lb for each 100-lb increase in weight. For a constant weight each month increase in age was accompanied by an increase of 28.4 lb of milk and 0.9 lb of fat.

Heritability estimates based on 385 daughter-dam pairs for body weight, milk production, and fat production were .29, .44, and . 0, respectively, when obtained from first lactation data, and were .19, .43, and .41 for all lactations 1 through 4 combined.

Genetic correlations between total milk and fat production were of the order of .90. Those between body weight and production ranged from near zero in the first lactation to −.53 in the second lactation. The genetic correlation between weight and milk was −.12 and that between weight and fat −.23, when the first four lactations were combined. Because of the negative correlations, less progress would be expected in selecting for both increased body weight and production than if they were independent or positively correlated.

Analysis of the data indicated that as much and probably more of the variation in production was associated with body weight as with age.

Breeders of dairy cattle have talked much about size, and size has received considerable attention in most type-score cards; however, no cattle breeders and few researchers have investigated the genetic relationships between size and production. Some have considered adjusting production records for weight instead of age, but not both jointly.

Body weight has always been considered a good measure of size, as it is an easy measurement to obtain and has received considerable attention in the dairy field (2, 6, 7, 10, 29, 30, 36, 37). The body weight of an animal is influenced by its age, genotype, and the environmental conditions under which it is raised. Environmental conditions which are conducive to large size also contribute to high levels of production; thus, large cows may give more milk, not only because they are large but because they are maintained under better management conditions than smaller cows.

There is evidence in the litreature (3, 14, 28, 35, 37) that a number of characteristics other than actual milk and fat production have been studied to find a measure that will be a reliable indication of eventual or present production. To be an effective measure in bringing about genetic gain in production, there must be a

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[1] A portion of the data for this study was taken from the Dairy Cattle Crossbreeding Project being conducted at the Illinois Agricultural Experiment Station. This project is a cooperative project between the Dairy Cattle Research Branch of the ARS, USDA, and the Department of Dairy Science, University of Illinois, and is a contributing project to Regional Project NC-2, The Improvement of Dairy Cattle Through Breeding.

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high genetic correlation between the character used and production.

The objectives of the present study were to determine the relationships between weight, age, and production and to explore some of the consequences and uses of these relationships.

SOURCE AND DESCRIPTION OF DATA

Data for the present study were obtained from six different Holstein herds. Two of the herds are located at the University of Illinois, Urbana; one of these is the main Holstein dairy herd, the other consists of the Holstein cattle maintained as a part of the crossbreeding project. The four other herds are owned by the Canada Department of Agriculture, Research Branch, and are located at Fredericton, New Brunswick; Ottawa, Ontario; Lethbridge, Alberta; and Agassiz, British Columbia.

The six herds have been maintained at their various locations for breeding, feeding, management, and demonstrational projects. In all cases a high level of feeding and management has been maintained.

Data used for the present study were collected as part of the routine procedure maintained in the different herds. The following data were collected or calculated and placed on I.B.M. cards for each lactation of each animal: the number of the herd, identification of the animal, its sire and dam, year of birth, year of calving, age at calving in months, lactation number, calving weight, 180-day total milk yield, average milk fat test, 180-day total fat yield, and 180-day fat-corrected milk.

The body weight was obtained by actual weighing with a scale. Body weight for the Research Branch cows was a single weight taken within 24 hr after calving, and that for animals at the University of Illinois was the average of three weighings taken on the three consecutive days following parturition.

All of the herds were on twice-a-day milking. The 180-day milk production was obtained from the daily recorded milk weights, whereas the fat tests were obtained from an official testing program and conducted at approximately monthly intervals. Total fat production was calculated from the fat tests and the milk produced. Only cows that completed records of at least 180 days were included in this study. Production records were not adjusted for environmental factors such as age, weight, or season of calving.

There were 1,344 lactation records with accompanying weights available for inclusion in this study. A summary of the records used and their source is given in Table 1.

A summary of all locations combined for averages and standard deviations for age at calving, weight at calving, 180-day milk and fat yield, and 180-day fat-corrected milk is given in Table 2.

To study genetic correlations and heritabilities, 385 daughter-dam pairs distributed throughout the first four lactations were obtained from the original data. The number of pairs in later lactations was so small that these pairs were not included in estimating the genetic correlations and heritabilities. The source and number of daughter-dam pairs are listed in Table 3.

Partial lactation records, as a basis for selection, have been used by several workers (9, 17, 25). Lerner and Cruden (21) found that in chickens the genetic correlations between part and complete production records were high. They found that there was a greater gain in the average production rate when partial records were used as the basis for selection than when complete records were used. The genetic correlation between the 180-day and the 305-day production has been found to be near unity (17, 25). Correlations between the 180-day and 305-day production are of the order of 0.70 to 0.90 (5, 16, 17, 25, 26, 32, 39); thus, the shorter period of the record can be used as a basis for selecting with little loss in efficiency. The

TABLE 1

Source of data by lactation, location, and years collected

Location	Lactation no.								Total	Years collected
	1	2	3	4	5	6	7	8		
Lethbridge	90	75	42	34	20	10	11	6	288	1937–1955
Ottawa	150	112	64	39	26	8	6	2	411	1935–1956
Agassiz	32	22	11	8	5	2	....	5	85	1954–1956
Fredericton	44	37	27	11	13	9	7	2	150	1936–1955
Illinois	100	78	42	29	20	8	6	3	295	1946–1956
NC-2	44	27	21	15	7	1	....	..	115	1949–1956
Total	473	351	207	136	91	38	30	18	1,344	

TABLE 2

Averages and standard deviations for age at calving, weight at calving, 180-day milk, fat, and fat-corrected milk production

Lactation no.	No. obs.	Age			Weight			180-day yield							
			S.D.			S.D.			S.D.			S.D.			S.D.
		Mean	(a)	(b)	Mean	(a)	(b)	Milk	(a)	(b)	Fat	(a)	(b)	FCM	(a)
		(months)			*(lb)*			*(lb)*			*(lb)*				
1	473	30.1	3.6	3.3	1,187	102	93	6,553	1,202	1,135	236	42	41	6,163	1,077
2	351	44.0	4.9	4.7	1,306	110	106	7,675	1,587	1,607	273	55	35	7,164	1,424
3	207	57.0	5.4	4.6	1,391	118	100	8,466	1,576	1,568	301	64	59	7,922	1,523
4	136	71.3	6.7	6.5	1,430	135	129	8,784	1,536	1,536	314	59	53	8,221	1,439
5	91	84.0	7.5	7.1	1,434	123	108	9,003	1,382	1,243	316	58	46	8,338	1,354
6	38	99.0	7.9	6.7	1,431	128	133	8,966	1,380	1,435	315	53	54	8,309	1,322
7	30	114.1	9.8	8.3	1,411	108	90	8,832	1,406	1,281	305	56	44	8,105	1,382
8	18	126.7	9.6	2.7	1,432	126	77	9,752	1,850	957	330	56	30	8,846	1,488
(c)	1,344	60.9	23.4	4.6	1,306	150	102	7,694	1,723	1,394	274	62	49	7,188	1,547

(a). Standard deviations on a within lactation basis.

(b). Standard deviations on a within lactation, within herd, within year of calving basis.

(c). The standard deviations in this line under the (a) columns were calculated on an over-all basis.

TABLE 3

Source and number of daughter-dam pairs

Location	Lactation no. 1	2	3	4	Total	Years collected
Lethbridge	39	38	14	10	101	1942–1953
Ottawa	63	43	17	10	133	1939–1956
Fredericton	23	14	9	4	50	1942–1953
Illinois	41	21	6	2	70	1951–1956
NC-2	19	7	4	1	31	1952–1956
Total	185	123	50	27	385	

shorter record has several advantages over the longer record, as it allows time for evaluating records before the next calving and the records can be grouped more easily. In addition, more records are available at an earlier date for sire evaluation.

DATA ANALYSES AND RESULTS

An analysis of variance, according to Snedecor (34), was conducted to determine if there were significant differences in body weight and production associated with herds and lactation number. The analyses revealed that there were significant differences ($P < .01$ or $P < .05$) between herds and lactations for the three criteria tested. The accumulation of the data covered a considerable period of time (Table 1). Therefore, any trend in environment, common to a herd, would tend to increase the within herd variability of the collected observations.

To eliminate the above sources of variation, the regressions were derived on a within herd, within lactation, within year of calving basis. The smallest subgroup then became the group of cows in a given herd that calved for the same lactation number in a given year. To obtain the genetic correlations and heritabilities, the year of calving of the daughter was used as the year of calving class.

Multiple regression analysis. To determine the relationships between weight and age on the one hand and milk and fat production on the other, a multiple regression analysis according to Snedecor (34) and Johnson (19) was used. The various regression coefficients and their standard errors calculated from the data are tabulated in Tables 4 and 5. Table 4 contains the factors for milk and Table 5 those relating to fat. The partial regression coefficients indicate the unit change in production per unit change in weight or age, independently of the other. From Table 4 the regression coefficient bMW.A is read as the regression of milk on weight independently of age. The other partial regression coefficients are read in a similar way.

The standard partial regression coefficients indicate that in most cases for cows in the

TABLE 4

Partial and standard partial regression coefficients of milk production on weight and age

Lactation	D.F.	Partial regression coefficients bMW.A	S.E.	bMA.W	S.E.	Standard partial regression coefficients b′MW.A	b′MA.W
1	400	1.34*	.65	46.0*	18.2	.11	.13
2	283	1.31	.94	9.0	21.0	.09	.03
3	141	2.52	1.57	−50.4	33.8	.16	−.15
4	85	4.04**	1.22	5.8	24.3	.34	.02
5	43	3.92*	1.75	7.5	26.8	.34	.04
6	11	3.09	3.44	−27.1	62.4	.33	−.13
7	10	4.32	2.34	6.2	46.4	.30	.04
8	4	6.30	6.11	205.7	170.5	.51	.59
1 to 8	991	2.50**	.43	3.0	9.5	.18	.01
1 to 8 (a)	1,268	4.00**	.27	28.4**	1.7	.35	.39

M = milk production; W = weight; A = age.

(a) Calculations in this line are on a within herd, within year of calving basis; all other calculations are on a within herd, within lactation, within year of calving basis.

* Significant at 0.05 level of probability.

** Significant at 0.10 level of probability.

TABLE 5

Partial and standard partial regression coefficients of fat production on weight and age

Lactation	D.F.	Partial regression coefficients				Standard partial regression coefficients	
		bFW.A	S.E.	bFA.W.	S.E.	b'FW.A	b'FA.W
1	400	.078**	.023	1.18***	.60	.18	.10
2	283	.007	.031	1.93**	.71	.01	.17
3	141	.109*	.048	−.74	1.29	.18	−.06
4	85	.146**	.042	.41	.84	.35	.05
5	43	.157*	.064	−.13	.98	.37	−.02
6	11	.101	.133	.92	2.42	.23	.12
7	10	.084	.047	.66	1.65	.17	.12
8	4	.060	.191	6.32	5.33	.16	.58
1 to 8	991	.085**	.015	.70*	.34	.18	.07
1 to 8 (a)	1,268	.144**	.009	.90*	.07	.36	.35

F = fat production; W = weight; A = age.

(a) Calculations in this line are on a within herd, within year of calving basis; all other calculations are on a within herd, within lactation, within year of calving basis.

* Significant at 0.05 level of probability.

** Significant at 0.01 level of probability.

*** Significant at 0.10 level of probability.

same lactation, weight is more closely associated with production than is age. The exceptions to this are milk production for Lactation 1 and both milk and fat production for Lactation 8. When the lactations are combined (Lines 9 and 10 of Tables 4 and 5), weight is generally more closely associated with production than is age.

The statistics for the last lines of Tables 4 and 5 were derived when the effect of lactation number was not removed, and the age of the animals in a within herd, within year of calving class would vary over wide extremes. When this occurred, age and weight were of about equal importance in influencing production. Curvilinear regressions of production on age would generally more adequately measure the influence of age on production when all lactations are combined; however, in these data, the linear regression should be sufficient as 1,167 of the 1,344 lactation records are 1st, 2nd, 3rd, or 4th lactation records, during which period the relationship between production and age appears to be almost linear (24).

To determine whether more of the variation of production was associated with weight or age, analyses of variance according to Snedecor (34) were conducted for each lactation or group of lactations combined. This method provides a test of the influence of each variable, weight, or age, after the influence of the other has been removed. Listed in Tables 6 and 7 are the mean square values obtained from the separate analyses for those due to the regression of milk and fat on age and weight, those due to weight alone, and those due to age alone. The degrees of freedom and levels of significance are listed in the tables.

From a study of the mean square values of the last lines of Tables 6 and 7, where all lactations are combined on a within herd, within year of calving basis, the values are all significantly different from zero at $P < .01$ level of probability, and age and weight appear to account for approximately equal shares of the variation in production.

From the significant mean square values obtained in Tables 6 and 7, and from a study of the standard partial regression coefficients in Tables 4 and 5, it appears that in this study body weight and age at calving account for approximately equal parts of the variation in production in Lactation 1. This is somewhat similar to the report of Miller and McGilliard (30), who found that weight accounted for slightly more of the variation of production than age for individual production records over a group of many herds but that, on a within herd, within lactation basis, age was a slightly more important factor than weight. These authors were dealing only with first-lactation records, whereas the present study deals with Lactations 1 through 8, so comparisons for later records cannot be made. In Lactation 2, age accounts for a significant amount of the variation in fat production. During the later lactations, and when lactations are combined and the analyses done on a within lactation basis, weight accounts for more of the variation in production than age.

Gowen (13) reported that the regression of milk production on age was not linear but a

TABLE 6

Mean square values to test the effect of each independent variable age and weight on milk production

(Mean squares are rounded to the nearest ten thousand)

			Source of variation		
Lactation	DF (1)	Error	Due to regression $R^2_{M \cdot WA}$ (S^2_M) on weight and age (2)	$(R^2_{M \cdot WA} - r^2_{MA})$ S^2_M Weight alone (3)	$(R^2_{M \cdot WA} - r^2_{MW})$ S^2_M Age alone (3)
1	400	124	1,060**	531*	792*
2	283	258	352	498	47
3	141	230	1,352**	591	513
4	85	212	1,215**	2,269**	11
5	43	141	449	700*	11
6	11	219	138	175	57
7	10	180	90	180	3
8	4	100	81	103	140
1 to 8	991	188	3,318**	6,348**	19
1 to 8 (a)	1,268	182	65,251**	39,663**	48,496**

(a) Mean squares in this row were calculated on a within herd, within year of calving basis; all other mean squares are on a within herd, within lactation, within year of calving basis.
(1) Degrees of freedom for error mean square.
(2) Degrees of freedom for numerator -2- in all cases.
(3) Degrees of freedom for numerator -1- in all cases.
* Significant at 0.05 level of probability.
** Significant at 0.01 level of probability.

TABLE 7

Mean square values to test the effect of each independent variable age and weight on fat production

(Mean squares are rounded to the nearest hundred)

			Source of variation		
Lactation	DF (1)	Error	Due to regression $R^2_{F \cdot WA}$ (S^2_F) on weight and age (2)	$(R^2_{F \cdot WA} - r_{FA})$ S^2_F Weight alone (3)	$(R^2_{F \cdot WA} - r^2_{FW})$ S^2_F Age alone (3)
1	400	16	180**	181**	51*
2	283	29	127*	1	217*
3	141	34	125*	111*	11
4	85	25	166**	298**	25
5	43	19	63	112**	0.2
6	11	33	10	19	5
7	10	23	5	7	4
8	4	9	8	2	14
1 to 8	991	24	474**	736**	96*
1 to 8 (a)	1,268	24	7,474**	5,156**	4,913**

(a) Mean squares in this row were calculated on a within herd, within year of calving basis; all other mean squares are on a within herd, within lactation, within year of calving basis.
(1) Degrees of freedom for error mean squares.
(2) Degrees of freedom for numerator -2- in all cases.
(3) Degrees of freedom for numerator -1- in all cases.
* Significant at 0.05 level of probability.
** Significant at 0.01 level of probability.

logarithmic function. He considered that the increase in milk production with age depends chiefly on an increase in size of the mammary gland caused by an increase in growth. A somewhat similar point of view is taken by Gaines (11), who states, "As a cow grows older from first calving to maturity, she gives more milk, not because she grows older but because she grows larger." Gaines et al. (12) found the influence of weight on yield, independently of age, to be important, whereas the influence of age on yield independently of weight was negligible. On the other hand, Farthing (6) and Farthing and Legates (7) found that the age of the animal was more effective than weight in accounting for the variance in actual production.

Heritabilities. Heritabilities for body weight and milk and fat production were determined from 385 daughter-dam pairs. A covariance analysis was used and calculations were determined on a within herd, within lactation, within year of calving basis. Presented in Table 8 are the estimated heritabilities for body weight, milk and fat production for Lactations 1 and 2 and for all Lactations 1 to 4 combined.

TABLE 8

Heritability estimates for body weight, milk production, and fat production

Lactation	D.F.	Heritabilities		
		Weight	Milk	Fat
1	137	.29	.44	.40
2	72	.05	.37	.38
1 to 4	233	.19	.42	.41

The estimates of the heritability of body weight of 0.29 for the first lactation and 0.19 for all lactations combined are lower than those reported by other authors (3, 28, 30, 37), which range from 0.37 to 0.67. Miller and McGilliard (30) found a heritability of 0.67 for body weight of Holstein cattle taken during the first lactation when body weight was estimated by taped chest-girth measurements.

The heritability estimates of approximately 0.40 for milk and fat production in the first and second lactations are higher than most of the estimates reported in the literature (18, 22, 27-31, 33, 35-37, 40), which are of the order of 0.20 to 0.30. Miller and McGilliard (30) reported a heritability of 0.50 for milk production in first-lactation Holstein heifers. In the present study only the production of the first 180 days of the lactation was used in the calculations and the data were analyzed on a within herd, within lactation, within year of calving basis. These conditions would be expected to reduce the environmental variance; consequently, the heritability estimates for milk and fat should be larger than in some of the other reports. Heritability of first-lactation production appears to be higher than that of later lactations. Freeman (8) reported heritabilities of first, second, and third lactations of 0.36, 0.24, and 0.26 for milk, and 0.43, 0.35, and 0.26 for fat.

Genetic correlations. To measure the genetic correlation between two characteristics, it was necessary to correlate one trait in one animal with the other trait in a relative. Data from the group of daughter-dam pairs used in the previous heritability studies were employed to obtain the genetic correlations.

The method of analysis was according to the procedure outlined by Hazel (15) and by Touchberry (37). The formula to obtain the correlations in its simple form is:

$$r_{G_x G_y} = \sqrt{\frac{(Sxy')\ (Sx'y)}{(Sxx')\ (Syy')}}$$

The x and y refer to two traits of the dam and the primes to the corresponding two traits of the daughter. When one figure in the numerator was negative and the other positive, the arithmetic mean of the two figures was used. If both figures in the numerator were negative, the geometric mean was used but the genetic correlation was given a negative sign. The various genetic correlations between weight, milk, and fat are listed in Table 9. A genetic correlation outside of the range -1 to $+1$ was ob-

TABLE 9

Genetic correlations between body weight, milk, and fat

Lactation	D.F.	Between		
		Weight and milk	Weight and fat	Milk and fat
1	137	.02 ± .34	.05 ± .33	.86 ± .08
2	72	−.53 ± .79	−1.20 ± .54	.91 ± .08
1 to 4	233	−.12 ± .33	− .23 ± .32	.89 ± .05

tained from the calculations. Since the sampling error of the genetic correlation coefficient is large, such variations are to be expected with a relatively small number of degrees of freedom.

The genetic correlations between milk and fat are of the order of 0.90, which are similar to those reported in the literature (35, 37, 38). The genetic correlations between weight and milk or fat production are zero or negative. The correlations in this study are based on unadjusted records, whereas most others used adjusted records.

The genetic correlations between body weight and fat production reported in the literature (30, 37) are of the order of 0.30. However, Blackmore et al. (3) found a genetic correlation of −0.02 between body weight at 2 yr and FCM production. Touchberry (37) and Blackmore et al. (3) reported negative genetic correlations between various body size measurements and fat production.

The over-all genetic correlation between milk and weight of −0.12 falls midway between the correlations reported in the literature (3, 28-30, 37). Mason et al. (28) found a −0.43 genetic correlation between rate of gain during the lactation period and milk production. A genetic correlation of 0.50 indicates that about half of the genes that influence one trait also influence the other in a positive direction (assuming equal gene frequency and equal effects of genes). A negative genetic correlation indicates that the genes for the two traits are antagonistic. Some of the problems of adjusting production for differences in weight will be elaborated in the discussion of this paper.

DISCUSSION AND CONCLUSIONS

From the findings of this study and those of Miller and McGilliard (30), it can be concluded that sizeable amounts of variation in production are associated with both weight and age. Although several sets of age-correction factors (16,20) are in common use, and most production records and sire comparisons are expressed on a mature equivalent basis, the authors are unaware of any adjustments being made in records for differences in the weight of animals at calving. Gaines et al. (12) stated, "as rapidly as feasible initial live weight at each lactation should be made a part of all dairy records and FCM/W (or similar principle) should supersede the biologically unsound principle of age correction." It should be pointed out that selection on such a measure of efficiency as FCM/W may be economically unsound when labor and capital investment are considered.

Lush and Shrode (24) point out that the purpose of correction factors is to remove phenotypic differences that occur because the environmental conditions are not uniform. They also state that the presently used correction factors tend to select the early-maturing individuals and thus give an advantage to a sire that has early-maturing daughters.

The present authors are not presenting an argument against the present age-correction factors nor against early-maturing dairy cattle, but rather an argument that body weight as soon after calving as possible be recorded and used to help evaluate records. To obtain sufficient data to derive weight adjustment factors or to determine how production should be adjusted for weight differences would require weight data to be collected as a part of a recognized testing program, so that the data would cover a wide range of conditions and include many cows.

The problem of adjusting production records for body weight is one that should be considered with special care. One might first ask why records are adjusted for variations in weight. If they are adjusted so as to more accurately estimate the breeding value of the cows involved, then it would seem that the adjustments should be concerned only with the environmental and nonadditive genetic relationships between milk production and body weight. In this case, one would want the partial regression of milk production on weight independently of the breeding value for milk. This regression is:

$$(1)\quad b_{MW\cdot GM} = \boxed{\frac{r_{MW} - h_M\, r_{G_MG_W}\, h_W}{1 - r^2_{G_MG_W}\, h^2_W}}\ \frac{\sigma_M}{\sigma_W}$$

where h_M and h_W are the square roots of the heritabilities of milk and weight, respectively, and r_{MW} and $r_{G_MG_W}$ are the phenotypic and genetic correlations between milk and weight, respectively.

If one is adjusting milk production for variations in both weight and age for the purpose of more accurately estimating the breeding value of cows for milk production, then it would seem that the appropriate partial regression coefficients to use would be:

$$(2)\quad b_{MW\cdot G_MA} = \frac{r_{MW} - r_{MW}\, h^2_A\, r^2_{G_MG_A} - r_{MA}\, r_{WA} + r_{MA}\, h_A\, r_{G_MG_A}\, h_W\, r_{G_MG_W} + h_M\, h_A\, r_{G_MG_A}\, r_{WA} - h_M\, h_W\, r_{G_MG_W}}{1 + 2\, r_{WA}\, h_A\, r_{G_MG_A}\, h_W\, r_{G_MG_W} - h^2_A\, r^2_{G_MG_A} - r^2_{WA} - h^2_W\, r^2_{G_MG_W}} \cdot \frac{(\sigma_M)}{(\sigma_W)}$$

$$(3)\quad b_{MA\cdot G_MW} = \frac{r_{MA} - r_{MA}\, h^2_W\, r_{G_MG_W} - r_{WA}\, r_{WM} + r_{MW}\, h_W\, r_{G_MG_W}\, h_A\, r_{G_MG_A} + r_{WA}\, h_M\, h_W\, r_{G_MG_W} - h_M\, h_A\, r_{G_MG_A}}{1 + 2\, r_{WA}\, h_A\, r_{G_MG_A}\, h_W\, r_{G_MG_W} - h^2_A\, r^2_{G_MG_A} - r^2_{WA} - h^2_W\, r^2_{G_MG_A}} \cdot \frac{(\sigma_M)}{(\sigma_A)}$$

The symbols in coefficients (2) and (3) are analogous to those described for (1).

If one is willing to assume that the variation in age is entirely environmental and nonadditive genetic variance, which is probably a valid assumption, then Regressions 2 and 3 reduce to:

$$(4) \qquad b_{MW \cdot G_{MA}} = \frac{r_{MW} - r_{MA}\, r_{WA} - h_M\, h_W\, r_{G_{MG_W}}}{1 - r^2_{WA} - h^2_W\, r^2_{G_{MG_W}}} \cdot \frac{\sigma_M}{\sigma_W}$$

$$(5) \qquad b_{MA \cdot G_{MW}} = \frac{r_{MA} - r_{MA}\, h^2_W\, r_{G_{MG_W}} - r_{WA}\, r_{WM} + r_{WA}\, h_M\, h_W\, r_{G_{MG_W}}}{1 - r^2_{WA} - h^2_W\, r^2_{G_{MG_W}}} \cdot \frac{\sigma_M}{\sigma_A}$$

By observing Formula 1, it can be seen that if the genetic correlation between weight and milk is zero, the partial regression becomes the linear regression of milk on weight. Similarly, if we assume that all the variation in age is environmental and nonadditive genetic variation or that $h^2_A = 0$ and that $r_{G_{MG_W}} = 0$, the partial regressions shown in Formulas 2 and 3 reduce to the partial regressions shown in Table 4.

In considering the data in the present study for Lactation 1, it can be shown from Table 6 that $r_{MW} = .160$; $r_{MA} = .175$, and that $r_{WA} = .383$. In Tables 8 and 9, it is shown that $h^2_W = .29$, $h^2_M = .44$, and $r_{G_{MG_W}} = .02$. Substituting these values in Formulas 4 and 5, it is found that $b_{MW \cdot A G_M} = 1.23$ and $b_{MA \cdot W G_M} = 46.8$ as compared to the values $b_{MW \cdot A} = 1.34$ and $b_{MA \cdot W} = 46.0$ as shown in Table 4. Since we have assumed $h^2_A = 0$ and $r_{G_{MG_W}} = .02$, which differs little from zero, the values obtained by substituting in Formulas 4 and 5 should differ little from those shown in Table 4. The standard partial regressions of milk on weight and milk on age were $b'_{MW \cdot A G_M} = .101$ and $b'_{MA \cdot W G_M} = .136$, whereas those in Table 4 were .109 and .133, respectively, before rounding.

Assume now that the genetic correlation between milk and weight is $-.50$ and that the other correlations and heritabilities have the same values as shown above. Substituting these values in Formulas 4 and 5, it is found that $b_{MW \cdot A G_M} = 4.24$ and $b_{MA \cdot W G_M} = 9.22$. Changing the genetic correlation between milk and weight from .02 to $-.50$ has caused approximately a fourfold change in the numerical values of Formulas 4 and 5. Thus, before one adjusts production records for differences in weight, the genetic and phenotypic correlations between production and weight and the heritabilities of each should be precisely estimated.

There is evidence in Table 4 that the partial regressions of milk on weight for Lactations 1 through 8 differ. There is also evidence (8) that the heritabilities differ and probably the genetic correlations differ from one lactation to another. It has been shown (12, 13, 20, 24) that the relationship between milk production and age is curvilinear when this relationship is derived on an over-all basis ignoring differences between lactation number. Because of the above evidence, it would seem desirable to derive partial regressions of milk on weight and milk on age for each lactation, 1 through 7 or 8. With the relatively narrow ranges of age and weight for cows milking in the same lactation, the linear regressions of milk on age and weight are probably sufficient for adjusting milk production to a constant age and weight for each lactation. For example, all first lactations in this study could be adjusted to an age of 30 months and a weight of 1,190 lb. Second and later lactations could likewise be adjusted to a constant weight and age. The adjusted production records for different lactations could then be compared by further adjusting all lactations to a common basis. For example, suppose it was desirable to convert everything to a first-lactation basis. With the present data, first lactations are approximately 85, 77, 75, 73, and 74%, respectively, of the second through the sixth lactations. Thus, if each adjusted production record for Lactations 2 through 6 was multiplied by the appropriate factor, it could be converted to a first-lactation basis.

It seems important to have a large volume of data involving many herds to determine accurately the partial regressions of production on weight and age independently of the breeding value for production. This should be done for each lactation. In addition, unbiased factors for converting different lactations (1 through N) to a common basis should be derived.

One might do essentially the same type of adjustment by deriving curvilinear regressions of milk and fat on age and weight, ignoring lactation number, but this method is likely to be less precise. It is possible that there are differences between first and second lactations, etc., that are not caused by age and weight differences but become only partially confounded with age and weight differences.

The question of body size in dairy cattle selection programs demands careful consideration. The dairyman is mainly interested in producing milk at a profit. From a study of various reports (4, 12, 31), it may be concluded that milk production and the return over feed and labor costs are higher for the large cows in a breed than for the small ones. On the other hand, it has been frequently observed that the small cows in a herd or breed, which remain after culling, are more efficient than large ones [when efficiency is measured by the formula FCM/W described by Gaines et al. (12)]. This is probably because culling is usually carried out on the basis of production, and the small cow must produce almost as much milk as the large one, regardless of her size, or she will be culled for low production. By culling on a mature equivalent basis alone, the smaller cows in a breed may have been dealt a more severe injustice, genetically speaking, than is commonly thought. This is especially true if the genetic correlation between weight and production is negative and of considerable magnitude.

In some cases, it is difficult to place a true value on weight. It has frequently been observed that when cattle are sold for breeding purposes, size is emphasized and a pound of body weight in these cases might be worth many times the value of a pound of milk.

The question is frequently asked whether it would be advantageous to delay first calving beyond the usual practice, to obtain extra production in the first lactation from the accompanying increase of age and weight. By using the regression coefficients from Table 4, and feed requirements from Morrison (31), the amount of extra milk obtained from delaying calving for a definite time and the feed requirements necessary to obtain this milk can be estimated. In general, the extra costs for feed and labor required for maintenance and gain caused by the delaying of calving exceed the value of the added production attributable to the delay of the first lactation. Miller and McGilliard (30) indicated that larger heifers had little or no economic advantage in their first lactation if calving was delayed to obtain additional weight.

The delaying of calving also tends to increase the generation interval. The age to breed for first calving follows a fairly well defined pattern depending on the breed, but whether this is based on fact or tradition is hard to ascertain. Lush (23) is of the opinion that breeding efficiency can be lowered by postponing first calving to a late age. He further states that heifers bred early may appear stunted in their first lactation, but their size when mature is affected very little. There is need for further experimentation in this field.

By using the regression values from Table 4 for later lactations, it can be shown that the increase in production associated with increased weight will more than offset the cost of the increase in feed required.

It is apparent from the present study and others recently reported that the relationships between body size per se and production in dairy cattle need to be more precisely evaluated. This is especially true if production records are to be adjusted for the body weight of the cow.

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EFFECT OF INCOMPLETE RECORDS ON SIRE EVALUATION

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SUMMARY

First-lactation records of artificially sired Holstein daughters were examined to determine the effect of excluding incomplete records from sire evaluation. Results indicate that the average difference of incomplete records from stable-mate averages is different from herd level to herd level. The average difference is greater in high-producing herds than in lower-level herds. Incomplete records of daughters of high-level sires are closer to their stable-mate averages than are incomplete records of daughters of low-level sires. The effects of herd level and sire level are apparently additive in determining the average difference of incomplete records from their stable-mate records. Although the evidence indicates that a small differential bias would result in favor of low-level sires at the expense of high-level sires, if incomplete records are excluded from sire evaluation this bias is so small that it is unimportant, because of the approximately constant fraction of incomplete records of all first records over all sire levels and because of the small fraction of incomplete records (5-7%).

The inclusion of all available information for the evaluation of sires is usually necessary or at least desirable for accurate evaluation. The effect of selection, especially on later daughter records, as a possible bias in estimating the breeding value of sires, is well understood. Similarly, culling of daughters before completion of their first records can bias sire evaluation if these records are not included in the data used for the evaluation. To eliminate the bias, these incomplete records should be extended by factors free of bias. The purpose of this paper is not, however, to examine the appropriateness of the extension factors but rather to examine the effect of the inclusion or exclusion of incomplete extended first-lactation records on sire evaluation. Since only first records are studied, inappropriate extension factors are not likely to influence the results much, if at all.

DATA

The records of artificially sired Holstein daughters included in the sire file of the New York Dairy Records Processing Center as of January 1, 1961 (including cows freshening up to November, 1959) were used in this study. Incomplete records are included in this file, but are identified by special coding. Incomplete records are defined as those terminated short of 305 days for any reason other than drying off. All records were extended 2×, M.E., 305-day records. Only the records of cows freshening prior to 36 months of age were included. This grouping should essentially limit the study to first-lactation records, with the possibility of only a few second-lactation records being included. The level of milk production of the stable-mates of cows which had incomplete records relative to the Holstein DHIA season average was also recorded. Herd Level One exceeded the season average by more than 1,000 lb of milk, Level Two varied between the season average and plus 1,000 lb, Level Three varied between the season average and minus 1,000 lb of milk, and Level Four was more than 1,000 lb of milk below the season average. The seasons and season averages used are listed in Table 1.

Sires were grouped into six classes according to how much their daughter milk averages exceeded the corresponding stable-mate averages. Sire Level One included those whose daughters exceeded their stable-mates by more than 750 lb of milk; Level Two, between 750 and 350 lb of milk; Level Three, between 0 and 350 lb of milk; Level Four, between 0 and −350 lb of milk; Level Five, between −350 and −750 lb of milk; and Level Six, below −750 lb of milk.

Daughter levels of sires were estimated from first-lactation milk records, both excluding and including incomplete records. The index procedure is that used by the New York Dairy Records Center and has been described in detail

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TABLE 1

Seasons and Holstein DHIA season milk averages used in determining herd levels

Season	Average milk	Season	Average milk
	(lb)		*(lb)*
September 1950—February 1951	12,140	March 1956—August 1956	11,650
March 1951—August 1951	11,690	September 1956—February 1957	12,120
September 1951—February 1952	12,190	March 1957—July 1957	11,950
March 1952—August 1952	11,840	August 1957—November 1957	12,470
September 1952—February 1953	12,280	December 1957—March 1958	12,610
March 1953—August 1953	11,800	April 1958—July 1958	12,200
September 1953—February 1954	12,180	August 1958—November 1958	12,530
March 1954—August 1954	11,540	December 1958—March 1959	12,530
September 1954—February 1955	11,990	April 1959—July 1959	12,030
March 1955—August 1955	11,790	August 1959—November 1959	12,440
September 1955—February 1956	12,320		

by Henderson (2), and Heidhues, VanVleck, and Henderson (1). For completeness, the computing formula is given as:

$$EDL = AI + \frac{n}{n + 15}[D - .9(SM - DHIA) - AI]$$

where AI = the AI breed average for the current evaluation = 12,668 lb of milk for the January 1, 1961 report,

D = the sire daughter average of 2×, 305-day M.E. records,

n = the number of daughters,

SM = the adjusted stable-mate average [Henderson, Carter, and Godfrey (4)]

.9 = the intra-sire regression of daughter records on their adjusted stable-mate averages [Henderson (3)], and

DHIA = the DHIA 5-yr breed average for the current evaluation = 12,277 lb of milk for the January 1, 1961 report.

RESULTS AND DISCUSSION

Herd level. The incomplete record differences from stable-mate averages are shown in Table 2 by herd level. A pattern is evident, in that the higher the herd level the greater the average superiority of stable-mate records over the incomplete records. The difference is more than twice as great in the high-level herds as in the low-level herds.

This result suggests that if culling rate were the same in all herds the variation in high-level herds is greater than in low-level herds. If, as would seem likely, the culling rate were greater in high-level herds, then this would suggest with more emphasis that within-herd variation is greater in high-level than low-level herds, since the average difference of the incomplete records from herd average would be expected to be less with heavier culling. (The homogeneity of within-herd variance is presently being studied.) An alternate explanation is that selection intensity is less in high-level herds because of superior management. This would tend to produce a larger difference from the stable-mate average.

TABLE 2

Average differences of incomplete first-lactation records from their stable-mate averages according to the herd-season level of milk production

Herd level	Average difference from stable-mate averages		Average M.E. record		No. of records
	Milk	Fat	Milk	Fat	
	(lb)				
1 (High)	−2,898	−100.5	11,307	413.0	748
2	−2,270	− 78.0	10,412	378.4	683
3	−1,793	− 66.7	9,990	356.2	639
4 (Low)	−1,404	− 50.4	8,962	321.3	469
Over-all	−2,176	− 76.7	10,306	372.6	2,538

TABLE 3

Average difference of incomplete first-lactation milk records from their stable-mate averages categorized according to herd-season milk level and sire daughter level above stable-mate averages—sires having at least 20 daughters with first records

Sire level	Herd level								Over-all	
	1 (High)		2		3		4 (Low)			
	(lb)	*(no.)*	*(lb)*	*(no.)*	*(lb)*	*(no.)*	*(lb)*	*(no.)*	*(lb)*	*(no.)*
1 (High)	−1,773	49	−1,503	26	− 543	36	− 266	21	−1,144	132
2	−2,307	100	−1,411	105	− 675	100	− 677	94	−1,308	379
3	−2,501	102	−1,579	78	−1,521	73	−1,325	62	−1,814	315
4	−2,807	206	−2,226	201	−1,777	183	−1,455	144	−2,126	734
5	−3,504	149	−2,638	161	−2,240	139	−1,800	85	−2,643	534
6 (Low)	−3,416	131	−3,337	102	−3,042	96	−1,909	76	−3,032	404

Another possibility is that the factors used to extend incomplete records are not suitable for all herd levels. If it is assumed that the factors apply correctly to an average herd, then it would seem the factors may underestimate incomplete records in higher-level herds and may overestimate them in lower-level herds.

Sire level. The differences of incomplete records from stable-mate averages are less for daughters of high-level sires than for low-level sires. This result is shown in the right-hand columns of Table 3. This pattern is not unexpected, since the records of daughters of low-level sires will average lower than those of high-level sires, which produces a situation in which the incomplete records also average lower. A counterforce is that selection pressure on daughters of high-level sires would be less than on low-level daughters. Such an event might lessen the deviation for low-level sire progeny and increase it for high-level progeny groups, giving rise to a bias in estimating the genetic value of the sires if incomplete records are excluded from the evaluation. The evaluation of high-level sires would be biased downward and that of low-level sires biased upward relative to average sires.

Sire level by herd level. By superimposing the herd-level pattern and the sire-level pattern, the sire-level by herd-level situation appears in Table 3. The expected occurs in that daughters of high-level sires in the low-level herds have incomplete records, averaging only slightly below their stable-mate averages, and also in that daughters of low-level sires in high-level herds have incomplete records averaging much less than their stable-mates. The effects of sire and herd level appear to be additive in determining the difference of incomplete daughter records from their stable-mates. An unweighted squares of means analysis of the means in Table 3 resulted in a small F value (.46) for the sire-herd level interaction and highly significant F values for the sire and herd level effects, 26.15 and 23.06, respectively.

What bias would result if incomplete records were not included in the evaluation of sires? Results in Tables 4 and 5 partly answer this question. The right-hand columns of both tables indicate that the fraction of incomplete records is about the same for all levels of sires. Comparison of Tables 4 and 5 also points out that the average fraction of incomplete daughter records is greater for young sires or those sires which have not been used heavily. Sires with more than 200 daughter records are likely to be those selected for heavy use and, therefore, could be considered more popular than those not retained for use or those which are young and untested.

The effect of deleting incomplete records from evaluations of sires with more than 200 daughter records would be to raise the estimates for high-level sires by an average of 117 lb of milk and the low-level sires by 113 lb. Those in other levels would be raised by similar amounts. The average daughter difference from stable-mate averages would be raised by 122 lb of milk for the high-level bulls and 114 lb for the low-level.

The effect of excluding incomplete records from evaluation of sires having fewer daughters—20 to 50, the usual number when decisions are first made to use bulls in artificial insemination studs or to cull—is shown in Table 5. In this group of sires the high-level evaluations were raised by an average of 99 lb of milk and the low-level estimates by 183 lb of milk by excluding incomplete records. The intermediate levels were raised from high to low by 143, 48, 116, and 69 lb, respectively. The corresponding increases in average difference from stable-mate averages were 189, 219, 72, 164, 90, and 222 lb of milk, respectively, from high- to low-sire level. It is thus apparent that the bias due to

TABLE 4

Summary of daughter deviations from stable-mate averages and of estimated daughter levels for sires categorized into six levels according to average daughter milk yield differences from stable-mates—sires with number of daughter records ≥ 200

Sire level	No. of sires	Average daughter difference from stable-mate averages			Average estimated daughter levels		Average fraction incomplete
		A	B	C	D	E	
		(lb milk)					
1 (High)	3	1,050	−1,538	1,172	13,354	13,471	.050
2	9	501	−1,489	587	12,792	12,875	.040
3	8	176	−1,873	276	12,470	12,566	.047
4	4	−189	−2,205	− 67	12,121	12,239	.056
5	11	−541	−2,643	−408	11,771	11,900	.059
6 (Low)	7	−958	−2,775	−844	11,365	11,478	.056

A = Average of sire progeny averages of differences of all first-lactation daughter records from their stable-mates.

B = Average of sire progeny averages of differences of incomplete first-lactation daughter records from their stable-mates.

C = Average of sire progeny averages of differences of all first-lactation daughter records except incompletes from their stable-mates.

D = Average of estimated daughter levels calculated from daughter records in A.

E = Average of estimated daughter levels calculated from daughter records in C.

excluding incomplete records is largely constant for all sire levels.

The estimated correlations between sire evaluations based on all first records and on first records excluding incomplete records are shown in Table 6 for sires having more than 200, more than 20, and between 20 and 50 daughter first-lactation records. The correlations between all first and incomplete records are all positive, but do not approach one, as is also the pattern for the correlations between all first except incompletes and incompletes. The correlations, however, between all first and first except incompletes are very near unity. Slightly lower correlations, .97 and .98, are estimated for the sire groups having between 20 and 50 daughters.

Since the critical group of sires is the one having between 20 and 50 daughter records for

TABLE 5

Summary of daughter deviations from stable-mate averages and of estimated daughter levels for sires categorized into six levels according to average daughter milk yield differences from stable-mates—sires with number of daughter records between 20 and 50 inclusive

Sire level	No. of sires	Average daughter difference from stable-mate averages			Average estimated daughter levels		Average fraction incomplete
		A	B	C	D	E	
		(lb milk)					
1 (High)	6	1,477	− 238	1,666	13,465	13,564	.073
2	6	568	−2,459	787	12,829	12,972	.073
3	8 (5)[a]	191	−1,438	263	12,541	12,589	.047
4	12 (10)[a]	− 131	−2,152	33	12,358	12,474	.077
5	16 (13)[a]	− 535	−1,674	−445	12,045	12,114	.064
6 (Low)	16	−1,151	−3,293	−929	11,623	11,806	.099

A = Average of sire progeny averages of differences of all first-lactation daughter records from their stable-mates.

B = Average of sire progeny averages of differences of incomplete first-lactation daughter records from their stable-mates.

C = Average of sire progeny averages of differences of all first-lactation daughter records except incompletes from their stable-mates.

D = Average of estimated daughter levels calculated from daughter records in A.

E = Average of estimated daughter levels calculated from daughter records in C.

[a] = Number of sires having some daughters with incomplete records.

TABLE 6

Estimated correlations of (A) sire daughter averages of deviations from stable-mate averages and (B) of sire estimated daughter levels between (1) all first-lactation daughter records, (2) incomplete first-lactation daughter records, and (3) all first-lactation daughter records except for incomplete records

No. of daughters per sire (N)	No. of sires	Correlations r_{12}		r_{13}		r_{23}	
		A	B	A	B	A	B
$N \geqslant 200$	53	.70	.59	.99	1.00	.58	.53
$N \geqslant 20$	177	.64	.37	.98	.99	.49	.30
$50 \geqslant N \geqslant 20$	56	.49	.48	.97	.98	.28	.31

evaluation, it appears that incomplete records should be included, although little loss of accuracy is likely to result if they are excluded. On the other hand, if it is suspected that the extension factors are not suitable then it might be more appropriate to exclude incomplete records from sire evaluation.

The small changes in evaluation are somewhat surprising because of the wide spread in average differences between incomplete records and their stable-mate records for the high-sire levels and the low-sire levels, as shown in Tables 4 and 5. The only explanation apparent is that the fraction of first records which is incomplete is small and that the fraction is quite constant over all sire levels. This explanation would lead to the conclusion that not including incomplete records would lead to a differential bias in evaluating high- and low-level sires, but that the differential bias is so small it cannot be readily detected.

CONCLUSIONS

Incomplete records from high-level herds are much more below their stable-mate averages than are incomplete records from low-level herds. The reason for this is not clear. This situation suggests that more research is needed to determine whether herd management or culling practice is the reason, or whether extension factors presently used may be inappropriate for high- or low-level herds, or both.

Incomplete records of daughters of high-level sires are less different from their stable-mate averages than incomplete records of daughters of low-level sires. The pattern of herd-level and sire-level effects appears additive. The fraction of incomplete records of all first records seems to be nearly constant for all sire levels, although daughters of sires with more than 200 daughters have a smaller fraction incomplete than sires having between 20 and 50 daughters. This result suggests that a factor of popularity due to having an evaluation in artificial insemination may be involved.

No noticeable differential bias results in excluding incomplete records from sire evaluations in spite of the large difference of average differences of incomplete records from their stable-mate averages between high-level and low-level sire groups. Apparently, a small differential bias is in action but is largely obscured by the relatively small fraction of incomplete records and the apparent independence of sire level and fraction of incomplete records. Evidently, most dairymen allow first-lactation cows to complete their lactation, regardless of performance.

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REPRODUCTIVE CAPACITY OF DAIRY BULLS. VII. MORPHOLOGY OF EPIDIDYMAL SPERM[1]

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SUMMARY

Fourteen normal, mature bulls were sexually rested (SR), five ejaculated six or seven times weekly, and 15 depleted (SR-D) before slaughter. Significant, but small, treatment effects were noted in the percentages of abnormal heads, abnormal midpieces, and tailless sperm in the epididymis. The most pronounced effects were lower ($P < 0.01$) percentages of unstained or live sperm (eosin-aniline blue) and sperm with distal protoplasmic droplets in the cauda epididymis for SR-D as compared with SR bulls. Unilateral vasectomy of nine other bulls did not significantly affect any morphological characteristic. When values for 85 epididymides were averaged, ignoring preslaughter treatment, percentages of abnormal heads, abnormal midpieces, tailless sperm, bent or broken tails, coiled tails, proximal droplets, and distal droplets for the caput, corpus, and cauda epididymis were, respectively, 3.4, 2.5, 2.4; 1.1, 1.4, 0.8; 5.1, 10.1, 2.1; 4.4, 6.5, 8.4; 0.0, 0.8, 0.4; 44.4, 1.9, 2.1; and 7.5, 29.6, and 67.9%. Percentages of unstained sperm for the caput, corpus, and cauda for 40 epididymides were 77.0, 51.3, and 72.5. When diluted in 0.9% NaCl, 5.3% of the sperm from the corpus and 32.1% from the cauda were motile. Eosinophilia of sperm from the corpus epididymis apparently is not associated directly with cell death but, rather, with a change in membrane permeability. The best morphological indication of impending cell death appeared to be a high incidence of bent or broken tails, followed by complete loss of the tail and increased eosinophilia.

Most morphological studies of sperm removed from the bovine epididymis have been limited to protoplasmic droplets. These were reviewed by Bialy and Smith (7). Concerning abnormalities of epididymal sperm, Branton and Salisbury (10) found that deformities of the head were the most prevalent. The total incidence of morphologically abnormal sperm, excluding tailless sperm, for 30 epididymides averaged 13.9, 14.2, and 15.0% for the caput, corpus, and cauda, respectively. The differences were not statistically significant.

Most bulls used for the few published studies on the morphology of sperm from the epididymis were of unknown age and reproductive history. This limitation might preclude detection of changes in sperm morphology associated with their passage through the epididymis or with impending death and destruction of sperm within the epididymis. In conjunction with other detailed studies on the reproductive capacity of bulls (1, 2, 4, 5), the morphology of epididymal sperm from normal bulls was reinvestigated. Collection of about 20 consecutive ejaculates in 2 hr has been shown to remove virtually all sperm previously present in the cauda epididymis and, at the same time, to induce very rapid passage of sperm from the caput and corpus into the cauda epididymis (1). Thus, to gain further information on epididymal physiology, the influence of preslaughter ejaculation frequency and surgical intervention on the morphology of sperm from the epididymis also was investigated.

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EXPERIMENTAL PROCEDURE

Based on age and preslaughter treatment, the 47 bulls were grouped in five major classes. Of the 34 normal mature bulls, 14 were sexually rested for a minimum of 30 days (SR), five were collected at a frequency of six or seven ejaculates per week for at least 6 wk (6-7×), and 15 were depleted by collecting 11 to 26 consecutive ejaculates just prior to slaughter (SR-D). The fourth group consisted of nine

TABLE 1

Morphology of sperm in samples removed from epididymides of normal bulls (%)[1]

Portion of epididymis	Pre-slaughter treatment	No. epid.	Abn. head	Abn. mid-piece	Tail-less head	Bent or broken tail	Coiled tail	Total abn.	Protoplasmic droplets		Un-stained[2]	Total motile[2]
									Prox.	Distal		
Caput	SR	28	3.3^{ac}	0.6^{a}	4.2	5.1	0.1	13.3	48.4	8.3	86.4	0.3
	6-7×	10	1.4^{a}	1.2	5.7	6.4	0.1	14.8	36.1	10.0	.....	....
	SR-D	30	3.5^{ac}	1.3^{a}	4.7	2.8	0.0	12.2	48.4	6.6	75.0	0.1
Corpus	SR	28	2.8	0.6^{a}	8.4	5.2	0.3	17.3	2.0	33.6	64.1^{b}	2.6
	6-7×	10	1.2	1.4^{a}	10.0	7.2	0.2	20.0	4.7	37.7	.....	....
	SR-D	30	2.2	1.5	9.2	6.7	1.7	21.4	1.5	24.1	47.7^{b}	5.3
Cauda	SR	28	1.9	0.6	1.8	9.5	0.2	14.0	2.7	77.2^{b}	83.6^{b}	20.1
	6-7×	10	1.8	1.1	0.7^{b}	4.7	0.1	8.4	2.3	75.4^{a}	.....	....
	SR-D	30	2.3	0.8	3.2^{b}	3.4	0.3	9.9	1.8	55.6^{ab}	61.4^{b}	31.8
Caput	Mean	68	3.1^{a}	1.0	4.6^{b}	4.3	0.0^{ab}	13.0^{bc}	46.6^{b}	7.8^{b}	80.7^{ac}	0.2^{b}
Corpus	Mean	68	2.3	1.2	9.0^{b}	6.2	0.9^{a}	19.5^{b}	2.2^{bc}	30.0^{b}	55.9^{a}	4.0^{b}
Cauda	Mean	68	2.0^{a}	0.7	2.2^{b}	6.1	0.2^{b}	11.4^{bc}	2.3^{bc}	67.4^{b}	72.5^{ac}	26.0^{b}

[1] Statistical significance among the three treatment groups within a portion of the epididymis in the upper part of the table and among the three portions of the epididymis in the lower part of the table for each characteristic is indicated as follows: significant at the 5% level, ^{a}; significant at the 1% level, ^{b}; and not significant, ^{c} or no superscript.

[2] Mean for ten epididymides per treatment.

36-months-old unilaterally vasectomized Holstein bulls. For 20 wk prior to slaughter, trios had been sexually rested (SR) or collected at a frequency of two ejaculates per week (2×) or eight ejaculates per week (8×). Detailed information on the breed, age, body weight, gonadal, and extra-gonadal sperm reserves, and preslaughter treatment of the 34 mature bulls has been presented (1). Similar data and those for semen characteristics and quantitative testicular histology are available for the unilaterally vasectomized bulls (2, 4, 5).

The last group consisted of four sexually rested 25-months-old Holsteins (1,330 lb at slaughter) which had unilateral lesions placed with an electrocautery in the area of the caput-corpus and corpus-cauda epididymis junctions. The ipsilateral ductus deferens was isolated by resection and ligation 2 to 3 cm from its junction with the cauda epididymis and its junction with the ampulla. Assuming that the ductus epididymis is a single continuous duct, electrocautery was intended to isolate sperm in the corpus and cauda epididymis with less trauma and vascular disturbance than that resulting from isolation by ligature. Pairs of bulls were sexually rested for 24 to 70 days between surgery and slaughter. The total number of sperm in each portion of the epididymis was determined (3).

The bulls were trucked to a local abattoir and the reproductive system was removed in toto (3), chilled with crushed ice, and returned to the laboratory. About 4 hr after death, sperm samples were obtained from the caput, corpus, and cauda of the 94 epididymides (Figure 1). After making a small incision, epididymal fluid either flowed out, or was expressed. In most cases, about 2 cm of the corpus epididymis had to be opened to obtain sufficient spermatozoa. For unilaterally vasectomized bulls, a sample also was taken from the spermatocele which formed just proximal to the site of ligation (5). The undiluted sperm suspensions were transferred with a wire loop of appropriate size and mixed with 0.3 ml of eosin-aniline blue staining mixture (24) on a glass slide. The stained sperm suspension was immediately smeared with a second slide and dried within 30 sec, with a hot plate and fan. Separate counts of 100 cells were made to determine the percentages of sperm which were morphologically abnormal, which had protoplasmic droplets, and which were unstained or live. Tailless sperm heads were excluded when counting sperm with protoplasmic droplets and headless tails were never counted. For the SR-D bulls, similar data were available for the ejaculates collected before slaughter. In many cases, after making the stained smears, epididymal fluid was diluted with 0.9% NaCl and the percentage of total motile sperm was estimated at 37 C. Except for data in Table 2, when analyses of variance were used, statistically significant differences were determined by "t"-tests.

RESULTS

Spermatozoa in samples removed from epididymides of normal mature bulls (Table 1) showed certain significantly different morphological characteristics in each of the three segments of the epididymis. In only a few categories were differences among preslaughter treatment combinations significant. The caput epididymis of 6-7× bulls contained a lower percentage of sperm with abnormal heads than that of SR or SR-D bulls. Although this suggests that regular ejaculation was beneficial, for the cauda epididymis the differences in primary abnormalities were not significant among the three treatment groups. Since preslaughter treatment affected only a few morphological classes, the data were pooled and analyzed to detect differences among epididymal locations. Primarily because of an increase in tailless sperm, the incidence of total abnormal sperm in the corpus epididymis was greater ($P < 0.01$) than in either the caput or cauda. Although the difference between the percentage of total abnormal sperm in the caput and cauda

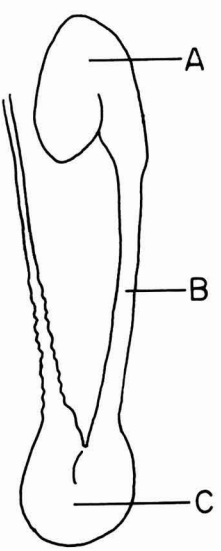

FIG. 1. Typical right epididymis showing where samples were removed from the caput epididymis, a; corpus epididymis, b; and cauda epididymis, c.

TABLE 2

Comparison of sperm morphology in samples from the cauda epididymis and in the ejaculated semen from 15 depleted bulls (%)[1]

	Cauda epididymis	First ejaculate	Tenth ejaculate	Last ejaculate[2]
Abnormal head	2.3	3.0	3.3	2.5
Abnormal midpiece	0.8	0.9	1.0	1.3
Tailless head	3.2	3.9	3.8	4.8
Bent or broken tail	3.4	3.1	2.5	3.5
Coiled tail	0.3	0.7	0.5	0.2
Total abnormal	9.9	11.6	11.1	12.4
Proximal droplet	1.9[a]	1.1	0.7[ac]	0.5[ac]
Distal droplet	55.6[b]	0.8[bc]	1.2[bc]	0.9[bc]

[1] Differences among the indicated values in a row are: significant at the 5% level, [a]; significant at the 1% level, [b]; and not significant, [c] or no superscript.

[2] The 15 depletion trials averaged 19 ± 1 ejaculates which contained a total of 38 ± 3.5 billion sperm.

was not significant, the caput contained a significantly higher percentage of sperm with abnormal heads.

The incidence of sperm with protoplasmic droplets[2] was influenced by preslaughter treatment and especially location in the epididymis (Table 1). In the caput, proximal droplets were more prevalent; in the corpus and cauda, distal droplets predominated. However, the total percentage of sperm with protoplasmic droplets was lower ($P < 0.01$) in the corpus than in the caput or cauda. The marked reduction in the percentage of distal droplets in the cauda epididymis of SR-D bulls may have resulted from rapid movement of sperm into the cauda from the corpus and caput epididymis. Excluding protoplasmic droplets, the incidence of abnormal sperm in the cauda epididymis of SR-D bulls was not significantly different from that in semen collected during the preslaughter depletions (Table 2). Ejaculated semen consistently had less than 3% protoplasmic droplets.

For five SR and five SR-D bulls, data were available for unstained sperm and total motile sperm (Table 1). Percentages of unstained sperm for the caput and cauda were not significantly different within either the SR or SR-D groups, but in both groups the corpus epididymis contained fewer unstained sperm than either the caput or cauda ($P < 0.01$). However, the corpus and cauda epididymis contained a highly significantly lower percentage of unstained sperm in SR-D than in SR bulls. Preslaughter treatment did not significantly affect the percentages of motile sperm, but the differences among the three portions of the epididymis all were highly significant. The percentage of motile sperm consistently was much lower than the percentage of unstained sperm.

Since there were only three epididymides per ejaculation frequency, data for the nine unilaterally vasectomized bulls were pooled by intact and vasectomized sides (Table 3). Data for sperm from the spermatocele of the ductus deferens at the site of vasectomy were not recorded for one bull and a second bull (no. 169) had no spermatocele. None of the differences for a specific morphological sperm characteristic between the intact and vasectomized sides within a portion of the epididymis was statistically significant when data for the cauda epididymis on the vasectomized side of Bull 169 were excluded. Possibly as a consequence of sperm stasis in the cauda, as evidenced by the absence of a spermatocele in the ductus deferens, this bull had 84% tailless sperm heads in the cauda epididymis.

In all portions of the epididymis abnormalities of the sperm tail accounted for more than 65% of the total abnormal sperm. The corpus epididymis had a higher ($P < 0.01$) percentage of tailless sperm heads than either the caput or cauda. For the 18 epididymides, 12 had more than 13% tailless heads in the corpus. In the cauda, the incidence of tailless heads was low, but sperm with bent or broken tails were more numerous than in either the caput or corpus epididymis. Seven of the 17 caudae had more than 20% and 11 caudae more than 10% bent or broken sperm tails. Apparently resulting from bull differences and not vasectomy or ejaculation frequency, the five cauda epididymides from 8× bulls averaged 29% bent or broken sperm tails, as compared to only 13% for the 12 intact and vasectomized caudae from SR and 2× bulls. This difference was signifi-

[2] Protoplasmic droplets are considered a normal morphological feature of epididymal sperm.

TABLE 3

Morphology of sperm in samples removed from the reproductive system of bulls 23 wk after unilateral vasectomy (%)[1]

Portion of epididymis	Side	No. epid.	Abn. head	Abn. mid-piece	Tail-less head	Bent or broken tail	Coiled tail	Total abn.	Protoplasmic droplets		Un-stained	Total motile[2]
									Prox.	Distal		
Caput	Intact	9	5.3	0.9	5.1	2.8	0.1	14.2	45.1	7.9	77.6	0.0
	Vasect.	9	3.7	2.4	8.9	7.2	0.0	22.2	27.6	4.4	69.0	0.0
Corpus	Intact	9	2.4	1.9	15.8	8.0	0.2	28.3	0.4	25.9	51.1	5.1
	Vasect.	9	4.1	3.1	13.0	7.2	0.4	27.9	1.0	29.8	46.8	9.0
Cauda	Intact	9	3.7	1.6	1.6	16.2	1.6	24.6	1.8	72.2	77.3	48.8
	Vasect.[3]	8	3.8	0.8	2.0	18.8	0.5	25.6	1.1	67.0	74.8	37.9
Caput	Mean	18	4.5	1.7	7.0^{b}	5.0^{bc}	0.1	18.2^{ab}	36.3^{b}	6.2^{ab}	73.3^{bc}	0.0^{abc}
Corpus	Mean	18	3.3	2.5	14.4^{b}	7.6^{bc}	0.3	28.1^{b}	0.7^{bc}	27.8^{b}	48.9^{b}	7.1^{abc}
Cauda	Mean	17	3.7	1.2^{a}	1.8^{ab}	17.4^{b}	1.0	25.1	1.5^{bc}	69.8^{b}	76.1^{bc}	43.7^{b}
Spermatocele[4]		7	2.4	3.7^{a}	23.6^{a}	9.7	1.1	40.6^{a}	0.6^{bc}	2.9^{ab}	55.1	7.4^{bc}

[1] Differences among the indicated values in a column in the lower portion of the table are: significant at the 5% level, [a]; significant at the 1% level, [b]; and not significant, [c] or no superscript. All differences between intact and vasectomized sides were not significant.

[2] Data were not recorded for Bull 159 (SR) and, therefore, values represent means for one less than the indicated number of epididymides.

[3] Excluding the data for Bull 169 (8×) which had no spermatocele but a very large accumulation of sperm in the cauda epididymis. Cauda epididymis values for this bull were 1, 1, 84, 2, 1, 89, 0, 0, 6, and 5, respectively.

[4] Excluding Bull 159 (SR) for which data were not recorded and Bull 169 (8×) which had no spermatocele. If the value of 79% tailless heads in the spermatocele for Bull 160 (SR) is excluded, the remaining six spermatoceles averaged 14.3% tailless heads and 33.2% total abnormal sperm. Similar exclusion of the value of 2% unstained sperm for Bull 160 resulted in an average of 64.0% unstained sperm in the spermatocele.

cant ($P < 0.05$). Nevertheless, sperm ejaculated by 2× and 8× bulls during the 4 wk immediately before slaughter averaged less than 3% tail abnormalities and 6% total abnormalities (excluding the 2.6% protoplasmic droplets). In the spermatocele the incidence of tailless heads was greater ($P < 0.05$) than in the cauda epididymis. However, if the value of 79% for Bull 160 was excluded, the remaining six spermatoceles averaged 14.3%, rather than 23.6% tailless sperm heads. Five of the seven spermatoceles had more than 60% morphologically normal sperm. These sperm may have been released from the testes as much as 24 wk previously.

Percentages of sperm with proximal and distal protoplasmic droplets on the midpiece were not greatly influenced by preslaughter treatment. Although not significant, the incidence of proximal droplets was lower in the caput epididymis on the vasectomized side than on the intact side for eight of nine bulls. Exclusion of the value of 94% for Bull 169 resulted in an average of 19% proximal droplets in the remaining eight capita from vasectomized sides, as compared to 45% for nine capita from intact sides. The consistent values of less than 8% total protoplasmic droplets in the spermatocele show that protoplasmic droplets can be eliminated within the reproductive system as well as following ejaculation.

Not shown in Table 3 are the percentages of unstained sperm recorded for 16 of the 18 testes. These averaged (± SE) 58.4 ± 1.9%. In general, the caput and cauda epididymis contained a similar percentage of unstained sperm; 23 of the 36 capita and caudae had more than 70% unstained sperm. However, only three of the 18 corpus epididymides contained more than 60% unstained sperm. Percentages of unstained sperm were not markedly affected by vasectomy. Motility after dilution of sperm in samples from the cauda epididymis on the vasectomized side usually was lower than for the intact side, but the difference was not significant. In contrast to the high percentage of unstained sperm in the spermatocele, only one sample from this area had more than 10% motile sperm.

When preslaughter treatment was ignored and values for all 85 epididymides from mature and vasectomized bulls averaged, the percentages of abnormal heads, abnormal midpieces, tailless sperm heads, bent or broken tails, coiled tails, proximal droplets, and distal droplets for the caput, corpus, and cauda epididymis were, respectively, 3.4, 2.5, 2.4; 1.1, 1.4, 0.8; 5.1, 10.1, 2.1; 4.4, 6.5, 8.4; 0.0, 0.8, 0.4; 44.4, 1.9, 2.1; and 7.5, 29.6, and 67.9%. Likewise, percentages of unstained sperm for the caput, corpus, and cauda for the 40 epididymides evaluated were 77.0 ± 1.8, 51.3 ± 2.7, and 72.5 ± 2.6, respectively. The percentages of motile sperm were 5.3 ± 1.2 for the corpus and 32.1 ± 2.8 for the cauda. Occasional motile sperm were seen in samples from only two caput epididymides.

Data for bulls with unilateral isolation of the corpus and cauda epididymis were quite variable and, therefore, are presented individually. Not shown in Table 4 are the changes in number of sperm per gram of testis (1, 3) which were −66, −13, −9, and −26% for Bulls 191, 192, 193, and 194, respectively. Thus, the decrease in sperm content of the isolated caput epididymis from Bulls 191 and 194 probably was associated with a marked disturbance of spermatogenesis. Necropsy, sperm counts, and morphological data suggested that the lesion at the caput-corpus junction did not effectively block sperm passage in Bull 194. However, in the remaining cases, there was a marked decrease in sperm content of the corpus and cauda epididymis. Although morphological studies were not made, there was no evidence from sperm counts suggesting a disappearance of sperm from the isolated ductus deferens (exclusive of the ampulla).

In most cases, percentages of abnormal heads and midpieces were increased in the caput and corpus epididymis on the experimental side; such abnormalities were rare in the cauda. The high incidence of bent or broken tails in the control side of bulls killed 3 wk after surgery may have resulted from postsurgical inflammation. The incidence of such cells in control epididymides was much lower after 10 wk. Nevertheless, the combined percentage of tailless sperm and sperm with bent or broken tails was greater for experimental than for control sides. In general, it was greater after 10 wk than after 3 wk and was higher in areas where the change in the sperm content of the isolated epididymal segments was most pronounced. Excluding the corpus from Bull 194, the combined incidence of tailless and bent or broken tailed sperm averaged 43% in the corpus and 61% in the cauda. The differences between control and experimental sides for total abnormal sperm were 33 and 44 percentage units for the corpus and cauda.

Surgery did not affect markedly the percentages of sperm with protoplasmic droplets and unstained sperm in the caput epididymis. In the isolated corpus, however, there appeared to be a decrease in distal and an increase in

TABLE 4

Morphology of sperm in samples removed from and changes in sperm content of multiple occluded epididymides compared with that in the contralateral normal epididymides (%)

Portion of epididymis	Bull	Days [a]	Treatment	Change in sperm content [b]	Abn. head	Abn. mid-piece	Tail-less head	Bent or broken tail	Coiled tail	Total abn.	Protoplasmic droplets		Unstained
											Prox.	Distal	
Caput	191	22	Control		2	1	4	17	0	24	82	0	87
			Isolated [c]	−10	23	2	6	2	1	34	52	1	64
	192	26	Control		2	5	8	12	0	27	3	44	70
			Isolated [c]	+51	8	3	29	5	0	45	38	0	62
	193	70	Control		0	0	2	3	0	5	81	5	88
			Isolated [c]	+134	9	1	5	1	0	16	84	1	77
	194	70	Control		1	1	3	9	0	14	47	7	81
			Isolated [c]	−60	6	2	16	1	0	25	93	0	84
Corpus	191	22	Control		2	1	6	20	0	29	0	19	63
			Isolated	−46	29	2	17	7	0	55	19	2	52
	192	26	Control		2	1	4	32	0	39	0	27	56
			Isolated	−40	13	5	14	11	1	44	16	1	58
	193	70	Control		3	1	4	5	0	13	0	16	69
			Isolated	−74	0	0	48	32	0	80	0	0	0
	194	70	Control		1	4	7	7	0	19	2	8	61
			Isolated?	+266	8	5	11	0	0	24	23	0	49
Cauda	191	22	Control		0	0	0	46	0	46	4	63	84
			Isolated	−32	2	0	1	20	0	23	0	2	59
	192	26	Control		0	2	2	23	0	27	0	44	77
			Isolated	−67	0	0	17	62	9	88	0	1	57
	193	70	Control		0	1	2	2	0	5	2	89	74
			Isolated	−66	0	3	76	10	1	90	0	0	0
	194	70	Control		1	1	0	14	0	16	2	89	76
			Isolated	−45	3	1	49	10	5	68	21	0	11

[a] Interval in days between surgery and slaughter.
[b] Based on comparison of the total sperm count for the isolated segment with that for the similar segment from the contralateral epididymis.
[c] Caput epididymis isolated from corpus epididymis but not from testis.

proximal protoplasmic droplets. Protoplasmic droplets were virtually absent in the isolated cauda epididymis. In both the isolated corpus and cauda epididymis there was a marked decrease in the percentage of unstained sperm after 70 days, but only a slight decrease in samples taken earlier. Percentages of unstained sperm in control epididymides were similar to those in Tables 1 and 3.

DISCUSSION

The 3- to 4-hr delay between slaughter and sample preparation may have influenced the results. However, the similarity, except for protoplasmic droplets and total motile sperm, between data for the cauda epididymis of mature bulls (Table 1) and typical ejaculated semen suggests this was not a serious problem. Conditions were similar for all 47 bulls studied and should permit valid comparisons. Branton (9) found that bovine reproductive tracts could be held several hours without noticeable changes in the fluids contained therein. Nevertheless, the extent of postmortem change should be investigated.

Branton and Salisbury (9, 10) found no significant differences in the percentages of total abnormal sperm, excluding tailless sperm, among the caput, corpus, and cauda of the epididymides from 15 slaughter house bulls. In the present study, the incidence of total abnormal sperm in the corpus epididymis was highly significantly greater than in either the caput or cauda. In contrast to the work of Branton and Salisbury (10), tailless sperm were included in the analysis and the significant differences found were due primarily to an increase in tailless sperm in the corpus epididymis.

When values for the three portions of the epididymis were pooled, Branton (9) found a mean of 9.0% abnormal sperm heads as compared to only 2.5% reported herein. Also in contrast to Branton's study, the present data revealed a significant decrease in the incidence of abnormal sperm heads from the caput to the cauda epididymis. In addition to this change, the cauda epididymis contained significantly lower percentages of sperm with coiled tails and tailless sperm heads than either the corpus or caput. However, for epididymides from mature SR bulls, only the percentages of tailless sperm were different ($P < 0.05$) between the caput and corpus, the caput and cauda, and the corpus and cauda ($P < 0.01$).

It can be concluded that abnormalities of the head are less prevalent than abnormalities of the tail. Although certain tail abnormalities may be preparative artifacts, it seems unlikely that significant differences among the three portions of the epididymis would result from artifacts.

Mori (18-20), in extensive studies with rats, found that strain of rat and season, as well as artificial cryptorchidism, had an effect on morphology of sperm from the cauda epididymis. He concluded (19) that the number of abnormal sperm decreased during passage through the epididymis but increased again in the ductus deferens, perhaps as a result of degeneration.

Among others, Glover (13, 14, 16) has suggested that an increase in certain abnormalities was indicative of incipient sperm degeneration. He found (13) that the pattern of degeneration in ejaculated rabbit semen was characterized by an increase in coiled tails, followed by an increase in decapitated and stained sperm. Data in Tables 3 and 4, however, do not suggest that sperm with coiled tails are associated with cell degeneration. Although this could constitute a difference between ejaculated and epididymal sperm, it might rather be a technique difference or merely a problem of semantics. In the present work, coiled tail refers to a distinct coiling (with at least one complete loop) of the distal portion of the tail. Bent or broken tail refers to a condition varying from a distinct, fairly sharp bending of the principal piece without a complete loop to a complete break. Similar conditions occurring in the midpiece were classified under abnormal midpieces. Using these criteria, the present data suggest that if sperm degeneration can be detected morphologically, it would be characterized by bent or broken tails and then tailless heads and eosinophilic sperm. The order of the last two changes appears to vary. Glover (15) reported that sperm eosinophilia and the incidence of tailless sperm heads for six normal bovine epididymides was higher in the corpus than in the cauda. This agrees with the present data. Thus, most recently, Glover (16) used a combination of the percentages of tailless sperm heads and stained sperm to evaluate sperm degeneration in the epididymis.

Values for protoplasmic droplets in sperm from the epididymides of SR bulls are similar to those reported by Bialy and Smith (7), although somewhat at variance with certain values discussed in their extensive review. That much of this variability may be associated with preslaughter treatment is suggested by the lower ($P < 0.01$) incidence of droplets in the cauda epididymis of SR-D bulls (Table 1). With slaughter house bulls, the possibility of repeated mounting and ejaculation shortly before slaugh-

ter cannot be excluded. Also, different stains give different results for protoplasmic droplets (9, 12). There is, however, little doubt that as sperm pass through the epididymis there is a shift from a predominance of proximal droplets in the caput to distal droplets in the cauda. It appears that the shift starts in the distal portion of the caput epididymis (17).

The low incidence of droplets in the corpus epididymis has been attributed to a stretching of the protoplasm along the sperm midpiece (23). Recent electron microscope studies neither support nor refute this suggestion. However, it has been demonstrated (6, 8) that the proximal, and apparently also the distal, protoplasmic droplets are enclosed within the cell membrane. This leads to the question of how the droplets eventually become detached. Physiologically, final loss of droplets apparently occurs in two areas; there is a marked decrease in droplets in the ampulla (7, 9) and virtually complete elimination after mixture with the seminal plasma at ejaculation (7). Data in Tables 3 and 4 show that in cases of sperm stasis droplets are eliminated within the reproductive system.

Many workers have reported on changes in eosinophilia of sperm associated with their passage through the epididymis. Based on three bulls, Brochart and Debatène (11) reported a fourfold increase in unstained sperm from the caput to the cauda epididymis. Branton and Salisbury (9, 10) found virtually no difference between the caput and cauda for 12 epididymides. These conflicting reports may result from widely different staining techniques, sampling differences, and ejaculation frequency. However, the present data for SR bulls are in general agreement with the detailed report by Ortavant (22), who prepared smears within 5 min after slaughter. Thus, it must be concluded that the percentage of unstained sperm in the corpus is markedly less than in either the caput or cauda epididymis.

Several interpretations have been given for the high incidence of stained sperm in the corpus epididymis. First, these may represent dead sperm. This contention has been advanced by Glover (13, 14, 16), along with the corollary that the distal corpus epididymis is the site of active sperm destruction and resorption. Others (11, 22) have contended that the increased eosinophilia is a result of an increase, presumably transient, in membrane permeability. The data reported herein do not resolve this controversy but favor the membrane permeability theory.

In the vasectomized bulls, the testis on the vasectomized side was producing sperm normally but all of these sperm were eliminated within the epididymis and proximal 25 cm of the ductus deferens (2, 5). Sperm counts of isolated and contralateral ductuli deferentia established that sperm resorption probably does not occur in the ductus deferens (5). Therefore, if stained sperm are indicative of cell death, they should be more prevalent in the vasectomized than in the intact epididymides. This was not the case. There were no significant differences for any morphological characteristic between intact and vasectomized sides within a portion of the epididymis. Even in the 8× bulls there were no apparent gross differences between sides. This suggests that death and destruction of an individual sperm cell within the epididymis is extremely rapid; so rapid that a single sperm either appears essentially normal morphologically, occasionally tailless, or has disappeared. If this is true, increased eosinophilia of sperm in the corpus epididymis can not be associated with cell death but rather with sperm membrane permeability. That a change in membrane permeability is involved is also suggested, but not proven conclusively, by the data on normal bulls (Table 1) for unstained sperm and sperm with protoplasmic droplets. Furthermore, these observations emphasize the limitations of studying sperm resorption based only on the morphology of epididymal sperm.

Although the destruction of an individual sperm may be very rapid, the data in Table 4 indicate that isolated portions of the epididymis will not void themselves of sperm even over an extended period of time. Isolation apparently was complete in seven of the eight corpora and caudae. In these seven epididymal segments about 50% of the sperm present at the time of surgery had disappeared. However, in all four isolated segments from bulls killed 3 wk post-operational more than 50% of the sperm remained unstained; only for the two caudae were the values lower than on the control side. For the three segments isolated 70 days, unstained sperm (11%) were found in only one cauda. The virtual elimination of protoplasmic droplets from sperm in the four caudae and the decrease in sperm content indicate that these segments were actually isolated. Other than the disappearance of protoplasmic droplets and the variable decrease in unstained sperm, the most predominant feature of sperm from the isolated corpus and cauda epididymis was abnormalities of the sperm tail. However,

the loss or breaking of the sperm tail was not necessarily associated with eosinophilia. Some unstained, tailless sperm heads were observed.[3] Glover (14) previously suggested that by blocking the entrance of sperm into the epididymis, normally masked degenerative processes might be observed.

The composite data suggest that the best indication of impending sperm death and destruction within the epididymis is not an increase in stainability, but rather a high incidence of tailless sperm or sperm with bent or broken tails. The latter characteristic could be an intermediate step between normal morphology and taillessness or an artifact. The data in Table 4 suggest it is not an artifact.

The entire physiological significance of the changes in sperm morphology in various portions of the epididymis and with various treatments is not clear. However, combined with the complex morphology of the ductus epididymis (21), it suggests the great diversity of physiological phenomena which occur in the epididymis.

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TECHNICAL NOTES

LIPOLYSIS OF SYNTHETIC TRIGLYCERIDES AND MILK FAT BY A LIPASE CONCENTRATE FROM MILK [1]

Investigations of unconcentrated milk lipases have revealed that these enzymes preferentially attack the primary ester positions of triglycerides (3-5). A more concentrated milk lipase, B-esterase (21 times on protein N), resembled unconcentrated milk lipase in lipolytic behavior (6) and, in addition, did not show specificity for short-chain acids when synthetic triglycerides containing both long- and short-chain acids were used as substrates (8). Although these studies have yielded information on the action of milk lipase, it is preferable to use highly purified preparations when studying enzyme specificity and reaction rates. Chandan and Shahani (1) have made available a more highly concentrated milk lipase (88 times on protein N) which showed essentially a single homogeneous protein moiety in ultracentrifuge studies and in moving boundary and gel electrophoresis. The availability of this highly purified milk lipase encouraged study of its lipolytic habits, using as substrates milk fat, olive oil, and synthetic triglycerides, so that comparisons of the three forms could be made. Results are reported herein.

Milk fat, olive oil, and simple triglycerides were used as substrates in studies at the University of Nebraska laboratories. Emulsions of the simple triglycerides were prepared by adding 5 g of each to 50 ml of a 10% aqueous gum arabic solution and passing this mixture through a hand homogenizer five times at room temperature. Tributyrin, tricaproin, tricaprylin, trilaurin, and triolein emulsified readily, whereas the solid triglycerides gave satisfactory emulsions only when they were dispersed at approximately 40 C. Milk fat and neutral olive oil emulsions were similarly prepared. The comparative rates of hydrolysis of milk fat, olive oil, and simple triglycerides were determined by the potentiometric method of Marchis-Mouren et al. (10). Twenty-five milliliters of the emulsion was tempered to 37 C and brought to pH 9.0 by the addition of a few drops of 1 N NaOH, and 1 ml of the enzyme preparation was added. Hydrolysis was followed for 20 min by continuously titrating the system every 1 to 2 min and maintaining a constant pH of 9.0. The titer value per minute in the different substrates was compared with that obtained for milk fat.

Lipolysis of glyceryl 1-oleate 2,3-dicaproate (OCC) and glyceryl 1-palmitate 2,3-dioleate (POO) was carried out at the University of Connecticut laboratories. The lipase concentrate was sent air mail–special delivery from the University of Nebraska in a Styrofoam shipper containing a block of dry ice. The material arrived in a frozen state and was stored at −20 C. The triglycerides, OCC and POO, were synthesized by acylation of pure 1-monoolein or 1-monopalmitin with caproyl or oleoyl chloride. The digestion mixtures were prepared by adding 600 mg of OCC or POO to 30 ml of tris buffer (pH 8.0) containing 10% gum arabic. After 5 min of stirring in a Waring Blendor, 10 ml of the mixture was transferred to a digestion flask and 1 ml of lipase concentrate added. The flasks were incubated with shaking in a water bath at 38 C for 5, 15, and 30 min. Two trials were run on POO and four on OCC. The free fatty acids (FFA), monoglycerides (MG), and diglycerides (DG) resulting from lipolysis were separated by column and thin-layer chromatography. The component fatty acids of the fractions were determined by gas-liquid chromatography. These procedures have been described (8).

The relative concentrations of free fatty acids liberated from milk fat, olive oil, and different simple triglycerides by the lipase concentrate are presented in Table 1. Acid production from each substrate is recorded on a relative basis with the acid production from the milk fat as 100%. The enzyme hydrolyzed tributyrin faster than milk fat or any other triglycerides. Triolein was also readily hydrolyzed. Tripalmitin and tristearin were attacked more slowly than the other triglycerides. Therefore, the enzyme was relatively more active on the short-chain and unsaturated fatty acid triglycerides than on the long-chain fatty acid triglycerides. This apparent selectivity of the enzyme might be

TABLE 1

Activity of milk lipase on milk fat, olive oil, and various simple triglycerides

Substrate	Titer value (*ml N/10 NaOH/20 min*)	Acids liberated (*μM/min*)	Relative hydrolysis [a] (%)
Milk fat	4.04	20.2	100
Olive oil	2.42	12.1	60
Tributyrin	5.16	25.8	128
Tricaproin	3.80	19.0	94
Tricaprylin	2.70	13.5	67
Trilaurin	2.22	11.1	55
Trimyristin	2.02	10.1	50
Tripalmitin	0.88	4.4	22
Tristearin	0.88	4.4	22
Triolein	2.78	13.9	69

[a] Hydrolysis of olive oil and various simple triglycerides is recorded on relative basis considering 100% for the control (milk fat).

[1] Supported in part by NIH research grant A-2605.

attributed to the existence of the more easily lipolyzed triglycerides in liquid state at 37 C. Possibly, the physical state of the triglyceride contributes to the development of enzyme-substrate complex at the oil-water interface. Kelly (9), working with a crude milk lipase system, observed that the rate of hydrolysis of various simple triglycerides appeared to decrease as the chain length increased. Also, Frankel and Tarassuk (2), using lyophilized skimmilk as the lipase source, observed that the enzyme lipolyzed tributyrin at a much faster rate than milk fat. The lipase concentrate behaved similarly to the crude milk lipase, in that the rate of hydrolysis of simple saturated triglycerides depended on the chain length of the fatty acids in the triglycerides.

The results of the liberation of FFA, MG, and DG from POO and OCC by the lipase concentrate are given in Table 2. The close relationship between the theoretical fatty acid compositions and the results obtained in these experiments (Table 2) indicated absence of specificity for any of the fatty acids involved when POO and OCC were lipolyzed by the lipase concentrate. These results may be contrasted to those in Table 1, which show apparent specificity for short-chain and unsaturated acids and graphically illustrate the pitfalls which may be encountered if fatty acid specificity studies are made using simple triglycerides of widely differing molecular weight. Preferential hydrolysis of the primary ester positions was shown by the large preponderance of 18:1 (POO) and 6:0 (OCC) in the monoglycerides. Further evidence for preferential hydrolysis of the primary ester positions was provided when the monoglycerides in lipolyzed milk fat were analyzed (5) and found to be almost 100% the 2-isomer. The lipase concentrate formed 2-monoglyceride from milk fat at the rate of 9.45 mM per 100 g fat in one-half hour. When this figure is compared to about 2 mM released in 2 hr in mixtures of raw and homogenized milks (7), the relatively high activity of the concentrate is apparent. The specific activity, mM of monoglyceride liberated per 100 ml milk per minute per milligram lipase protein, was 22.2.

The milk lipase concentrate possessed several of the attributes of unconcentrated milk lipase and B-esterase, in that it preferentially attacked the primary ester positions of milk fat and synthetic triglycerides, released both short- and long-chain fatty acids from both simple and diacid triglycerides, and did not distinguish between short- and long-chain acids esterified to the primary alcohol positions of glycerol when short periods of digestion were used.

TABLE 2

Fatty acid composition of the FFA and mono- and diglycerides formed during the lipolysis of glyceryl 1-palmitate 2,3-dioleate (POO) and glyceryl 1-oleate 2,3-dicaproate (OCC) by a lipase concentrate (Shahani) from milk [a]

Length of incubation	FFA		Monoglyceride		Diglyceride	
	Substrate: POO					
	16:0	18:1	16:0	18:1	16:0	18:1
(min)	(M%)					
5	52.2	47.8	0	100	30	70
15	47.4	52.6	4.6	95.4	25	75
30	46.3	53.7	1.7	98.3	23.7	76.3
	Substrate: OCC					
	18:1	6:0	18:1	6:0	18:1	6:0
5	53.5	46.5	5.3	94.7	24.4	75.6
15	49.7	50.3	4.4	95.6	21.0	79.0
30	43.7	56.3	2.0	98.0	28.3	71.7
Theoretical [b]	50	50	0	100	25	75

[a] Lipolysis conditions. 200 mg substrate, 1 ml lipase concentrate, and 10 ml tris buffer (pH 8.0) with 10% gum arabic; incubated with shaking at 38 C. Two trials with POO, four with OCC.

[b] Assuming specificity for primary ester linkages, but not fatty acid specificity. Applies to both substrates.

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PRODUCTION OF PSYCHROPHILIC MUTANTS FROM MESOPHILIC BACTERIA BY ULTRAVIOLET IRRADIATION

In studies to elucidate why some microorganisms grow at low temperature and others do not, the most usual approach has been to compare the growth and activity of a psychrophile with that of a mesophile (1-3). The compared psychrophile and mesophile, however, have been, at best, different species of the same genus and differed substantially in characteristics probably unrelated to their responses to growth temperature. Ideally, the psychrophile and mesophile to be compared in such studies should be identical except for their ability to grow at low temperature and except for those characteristics which bear directly upon this ability. This study was initiated to determine if it might be possible to produce such a pair of microorganisms by ultraviolet irradiation.

Heavy cell suspension of ten different mesophiles were irradiated 1 min at 15 cm from a 15-watt General Electric ultraviolet lamp. The irradiated suspensions were diluted with phosphate buffer at pH 7.0, surface streaked on trypticase soy agar, and incubated at 6 C. Any colonies appearing on these plates within 48 hr were considered to be potential psychrophilic mutants. The mutants were then isolated and grown at 0 C on trypticase soy agar containing glycerol, to prevent freezing of the plates. A potential mutant was not regarded as an actual mutant until it had been extensively compared to the parent culture to eliminate the remote possibility of contamination.

By this procedure a psychrophilic mutant was obtained from each of the three mesophilic microorganisms; *Pseudomonas aeruginosa* ATCC 9027, *P. aeruginosa* B100 obtained from the University of British Columbia, and *P. aeruginosa* F2 obtained from the National Research Council, Canada. The mutation rate for each of the mutants was about 10^{-8}.

None of the three parent mesophilic cultures produced visible growth on trypticase soy agar in ten days at 10 C, whereas all three of the psychrophilic mutants produced visible colonies on trypticase soy agar in two days at 6 C and in eight days at 0 C. Apart from the ability to grow at low temperature, each of the psychrophilic mutants was found to be indistinguishable from its respective mesophilic parental culture. However, each of the three parent cultures, and hence their respective psychrophilic mutants, differed slightly from one another. *P. aeruginosa* F2 grew more rapidly at 42 C and more slowly at 20 C than the two other strains. Also, it was nonmotile and nonflagellated, whereas the two other strains were motile, with two to three polar flagella, as demonstrated by Leifson flagella stains. *P. aeruginosa* B100 differed from *P. aeruginosa* ATCC 9027 by forming raised rather than convex colonies and by having smaller cell dimensions. All six cultures were further characterized by being gram-negative rods which formed irregular, translucent colonies with entire edges. They produced an alkaline reaction in litmus milk, liquefied gelatin, did not produce gas from glucose, oxidized gluconic acid to 2-keto gluconic acid, produced pyocyanine, and a fluorescent, diffusible, greenish pigment.

This is the first report of the production of a substantial change in the ability of a microorganism to grow at low temperature. Attempts to adapt bacteria to grow at low temperature by serial transfers to progressively lower temperatures have been unsuccessful (4), and the use of mutagenic agents for this purpose has not been previously reported. Further, the resulting three pairs of microorganisms, each pair consisting of a mesophile and a psychrophilic mutant of the mesophile, are ideal test microorganisms for growth studies at low temperatures.

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ANOTHER MEDIUM FOR ENUMERATING CITRATE-FERMENTING BACTERIA IN LACTIC CULTURES

Galesloot et al. (1) developed agar media for isolating and enumerating aroma bacteria in starters. We found their whey agar with casaminoacids and 0.5% calcium lactate (WACCA-½%) to be adequate but troublesome to prepare. Therefore, we altered the WACCA½% by substituting commercial Tomato Juice Agar[1] (TJA) for the whey agar containing casaminoacids. The streptococci inhibitor, calcium lactate, and the aroma bacteria indicator, calcium citrate, were retained as in the original medium. The altered medium contains 15 g TJA and 5 g calcium lactate in water to make one liter.

The altered medium, tomato juice agar, containing 0.5% calcium lactate (TJAC½%), was tested against WACCA½% by plate-count enumerations of bacteria in known cultures. Three skimmilk cultures were used: A, a 20-hr

[1] Bacto Tomato Juice Agar Dehydrated, Difco Laboratories, Detroit 1, Michigan.

TABLE 1

Mean colony count per milliliter for 10^{-7} dilutions of three cultures in four agar media

Culture	Plating	Media			
		WACA[a]	WACCA½%	TJA[a]	TJAC½%
A	1	310	*	308	*
	2	254	*	249	*
	3	284	*	267	*
	4	168	*	170	*
	5	357	*	321	*
	6	343	*	342	*
B	1	38	39	43	42
	2	38	38	33	34
	3	32	27	34	32
	4	39	39	38	42
	5	35	34	27	29
	6	36	41	38	39
C	1	171	21	164	20
	2	168	20	162	20
	3	170	17	145	18
	4	171	22	189	23
	5	189	20	172	15
	6	185	15	170	21

* Colonies developed but were not counted, because no typical clear zone surrounded them.
[a] Without the aroma bacteria indicator.

culture of *Streptococcus lactis;* B, a four-day culture of citrate-fermenting bacteria from a lactic culture; and C, a mixture of 25 ml each of A and B. Cultures A and B were held in an ice bath for 1 hr before Culture C was made, then all remained in the ice bath until plated. Each culture was plated in four media: whey agar with casaminoacids (WACA), WACCA-½%, TJA, and TJAC½%. A 10^{-7} dilution of each culture was plated in triplicate. The diluent was water containing 0.1% peptone. The plates were incubated 72 ± 2 hr at 25 ± 1 C, then colonies were counted using a Quebec colony counter. The bacterial enumerations were made six times, and the order of culture plating was varied thus: ABC, ACB, BAC, BCA, CAB, and CBA. At each enumeration, the plates were poured within 1 hr.

Table 1 gives the data obtained. "T"-test analyses showed no significant differences ($P > 0.05$) between mean counts in WACA and TJA for either Culture A or Culture B. An analysis of variance showed no significant differences ($P > 0.05$) among the mean counts of Culture B in the four media. Chi-square tests showed no significant differences ($x^2 < 3.81$) between the mean counts for Culture C in WACA and WACCA½% or in TJA and TJAC½%. Predicted counts for Culture C in TJA and TJAC½% were calculated, based on the counts obtained for Culture A in TJA and Culture B in TJAC½%. Chi-square tests showed no significant differences ($x^2 < 3.81$) between the predicted counts and actual counts.

Therefore, we conclude that TJAC½%, which is much easier to prepare, is as useful as WACCA½% to enumerate citrate-fermenting bacteria in lactic cultures.

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EFFECT OF ORZAN IN PELLETED ALFALFA UPON PRODUCTION AND QUALITY OF MILK [1]

Pelleting as a method of preparing hay and other feeds for ruminants has developed rapidly, and continued expansion will depend largely upon the development of improved processing methods and equipment which will offer greater economy of production. The use of binder materials in pelleted feeds, particularly in roughages, has been suggested as a means of ensuring better compression, less pellet breakdown (hence, less fines) during handling in storage, and greater mill output. It is axiomatic, however, that use of such materials should not detract from the final animal product. This study was undertaken to test one such material, Orzan (ammonium lignin sulfonate) in pelleted alfalfa fed to dairy cattle, with special emphasis on the quantity and quality of milk produced.

EXPERIMENTAL PROCEDURE

Alfalfa hay from a uniform source was inspected in the bale, made into ⅜-inch pellets with and without 2% Orzan, and fed as the sole roughage to 12 dairy cows paired according to milk yield, stage of lactation and gestation, age, and general health. Six cows (Group A controls) were fed pelleted alfalfa hay without Orzan and the remaining six (Group B) received pelleted alfalfa hay containing 2% Orzan. A four-day feed adjustment period preceded the 28-day experimental test period. Both groups were fed twice daily, and consumption and refusal of the pellets were recorded daily. Concentrates were fed according to milk and milk fat production (7) and were adjusted at seven-day intervals. Chemical composition of the concentrates and two test feeds was determined by proximate analyses.

Milk yields were recorded twice daily after each milking. Milk fat and solids-not-fat (SNF) were determined weekly from 24-hr composite samples by the Babcock test and Golding bead method (5, 6), respectively. Body weight changes were determined by averaging three successive daily weighings. All data were treated statistically by analysis of variance.

RESULTS AND DISCUSSION

Chemical analyses of the pellets as given in Table 1 indicate a hay of good quality, the small differences being no more than expected with single samples. The analysis of concentrates fed to both experimental groups is also shown.

A study of the mean daily yields of 4% FCM and SNF (Table 2) shows no significant difference ($P > 0.05$) between the two groups. Initial production of 4% FCM for Group A was 38.7 lb per cow daily, with a mean of 33.5 lb

[1] Orzan (ammonium lignin sulfonate) is a product of Crown-Zellerbach Co., Camas, Washington.

TABLE 1

Chemical composition of experimental feeds

Description	Dry matter	Crude protein	Ether extract	Crude fiber	Ash	N.F.E.
	(%)					
Concentrates	89.83	15.66	2.40	8.62	9.16	64.16
Alfalfa pellets (control)	87.04	16.39	1.87	24.65	8.96	35.17
Alfalfa pellets (with Orzan)	86.91	15.57	1.76	24.74	9.12	35.72

TABLE 2

Mean daily milking performance, feed consumption, and body weight gains

Group	4% FCM	SNF	Dry matter (DM) consumption			Wt gains
			Pellets	Concentrates	Total/ 100 lb body wt	
	(lb)					
A (control)	33.50	2.91	22.79	8.61	3.17	1.28
B (Orzan)	33.29	2.96	20.03	8.27	3.00	0.90

during the test period. Group B produced an initial daily mean of 38.5 lb of 4% FCM, with a daily mean of 33.3 lb per cow during the test period. Milk fat percentages for Group A declined from a mean of 5.07 before the experiment, with weekly means of 4.67, 4.67, 4.48, and 4.39 for Weeks I, II, III, and IV, respectively. Group B, having a pre-experimental milk fat percentage of 4.65, declined to 4.20, 4.52, 3.93, and 4.27 for the same four weekly tests. Post-experimental milk fat percentages increased to 5.07 for Group A and 4.68 for Group B, indicating the effects of pelleted alfalfa hay on the depression of milk fat percentages which corresponds to the findings of other workers (1-4, 8-14). There were no apparent changes in the percentages of SNF.

Feed consumption for both groups is shown in Table 2. Dry matter (DM) consumed by Group A was higher for pellets and concentrates, either when calculated in total, or per 100 lb body weight. Group B, apparently more efficient in converting DM to 4% FCM, consumed 2.76, .34, and .17 lb less DM in pellets and concentrates, and per 100 lb body weight, respectively, than Group A. Group A had an initial average weight of 992 lb compared to 944 lb for Group B. This greater feed intake by Group A was undoubtedly utilized for the additional body maintenance requirement of the larger cows and for the greater gain in body weight, which averaged 1.28 lb daily for Group A and .90 lb for Group B animals. However, differences in both consumption and body gains were statistically nonsignificant ($P > 0.05$).

In summary, this study showed no significant differences ($P > 0.05$) in milk yields, SNF yields, consumption, and weight gains between cows fed pelleted alfalfa hay with or without Orzan. Consumption was higher among the control group, owing to their greater body size and perhaps less efficiency in feed conversion to milk. The depression of milk fat percentages by pelleted roughages was demonstrated. Decline and stoppage in rumination was observed among some of the cows with a craving for more fibrous diet, as evidenced from the gnawing of wood partitioning between stalls. No toxic effects were observed among the animals as a result of feeding the Orzan pelleted alfalfa hay as herein described.

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SOURCES OF ERROR IN ENERGY UTILIZATION STUDIES WITH LACTATING DAIRY CATTLE [1]

True differences of 5% or less in the utilizable energy content of rations by dairy cattle may be extremely important in large dairy farming operations. Yet, the coefficient of variation (C.V.) of milk production data collected by using a common experimental design, the continuous type trial, may be expected to range from 4 to 15% (8). In an experiment involving four treatments, with a true difference of 5% between rations and with a true C.V. of 4%, 15 replications would be required per treatment to give a 90% probability of detecting the difference (two-tailed test) with a test of significance at the 5% level (3), as illustrated in Figure 1. An increase in the C.V. to 7% would require 42 replications to detect a true treatment difference of 5% with the aforementioned probability and significance levels. Body weight change data are extremely variable (1), and the C.V. for weight changes from means of three weights taken at the begining and end of an experiment may exceed 100% (6). Thus, with the limited number of cows, say six to 15, that may be available in experiment station herds for each treatment, the probability of detecting true differences as small as 5% is low. For these reasons, it appeared desirable to evaluate techniques that might increase the precision of energy utilization studies and,

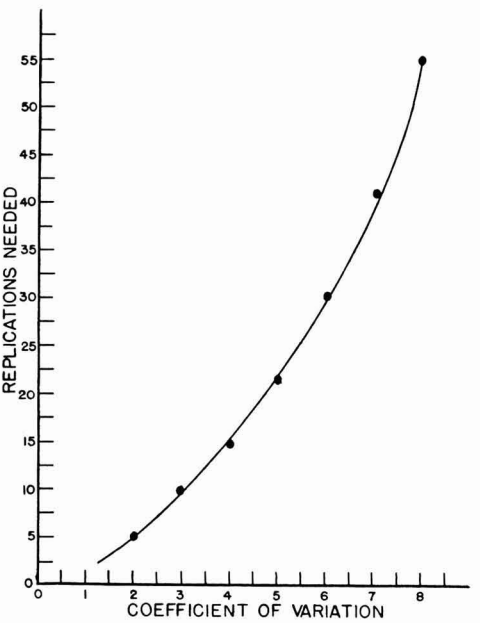

FIG. 1. Relationship between C.V. of an experiment and replications needed for 90% probability of detecting a true treatment difference of 5% with a test of significance at the 5% level (two-tailed test) and with four treatments (3). With only two treatments add one replication to these values.

[1] The author acknowledges with thanks the helpful suggestions and criticisms of Dr. A. E. Drake, Research Data Analysis Laboratory, in the analysis of the data and in the preparation of the manuscript.

thereby, reduce the number of replications required per treatment.

To increase precision of the energy utilization data, maintenance energy, milk energy, and body weight change energy were combined into one value, which shall be referred to as therms of performance energy (P.E.) per 100 lb of air-dry ration.

Twenty-four dairy cows producing between 30.8 and 70.2 lb of milk daily and weighing between 931 and 1,547 lb were the experimental subjects. They were assigned at random to four groups, with six replications per group. Allowance of the ration, which consisted of 50% alfalfa hay and 50% of a concentrate mixture (on air-dry basis), for individual cows was determined from FCM production and body weight at the beginning of the 14-day standardization period. Management of cows in each of the four groups was similar. Half of the ration allowance was fed in the AM and the remainder in the PM. Refused feed was determined and recorded. Aliquots of milk produced at each milking was composited over seven-day periods and the milk fat percentage determined by the Babcock test. In addition, a 14-day composite of aliquots from each milking was prepared for measurement of milk energy by oxygen bomb calorimetry. All cows were weighed between 11 AM and 12 NOON daily throughout the 14-day standardization period.

Daily body weight change was determined in two ways, i.e., from means of three-day weights taken at the beginning and at the end of the 14-day period, and from the 14-day weights by linear regression. Brody (2) found that the average energy stored per pound of gain in body weight was 909 Cal. This factor was used to convert weight gain or loss into weight-change energy.

Milk energy was determined by multiplying mean daily milk production times milk energy per pound from bomb calorimetry measurements or was estimated by multiplying mean daily FCM production (5) times 329.5 Cal, which was the average energy content per pound of FCM from cows in the Auburn University herd (7).

The maintenance energy requirement per cow was estimated from the maintenance allowances recommended by Morrison (9).

To estimate the error variance associated with method of measuring weight change and with methods of determining milk energy production, therms of P.E. per 100 lb of ration were calculated by four procedures, as follows: (a) milk energy by bomb calorimetry + weight-change energy by linear regression + maintenance energy allowance; (b) milk energy by FCM $\times$ 329.5 + weight change energy from means of three-day weights at the beginning and at the end of the period + maintenance energy allowance; (c) milk energy as in (a) + weight change energy as in (b) + maintenance energy allowance; and (d) milk energy as in (b) + weight change energy as in (a) + maintenance energy allowance. Since the cows did not consume their entire allowance of hay or concentrate, the percentage of the ration consumed as hay and as concentrate varied from the 50% allowance of each. This was a potential source of variance in the P.E. data; hence, the per cent of total ration consumed as concentrate was calculated and used as a covariate (10) in the statistical analysis.

The C.V. for the P.E. values calculated by the four processes and the relative precision of each are given in Table 1. Procedure (a), which measured milk energy by bomb calorimetry and weight-change energy by linear regression, gave the lowest C.V. for the P.E. data and was arbitrarily assigned a relative precision of 100%. In contrast, Procedure (b), which includes the traditional methods of evaluating milk energy and weight-change energy, gave the highest C.V. (7.41%) and, consequently, the lowest relative efficiency (73%). These analyses indicate that most of the differences in the C.V. for the procedures used to determine P.E. was the result of differences in precision of measuring weight-change energy, with the linear regression procedure being more accurate than the three-day weight method. In this evaluation, milk energy measured by FCM $\times$ 329.5 or by bomb calorimetry was almost equal in precision.

TABLE 1

Coefficients of variation (C.V.) and relative precisions of procedures used to determine performance energy

Process	C.V.	Relative efficiency [a]
	(%)	(%)
(a)	5.41	100.0
(b)	7.41	73.0
(c)	7.31	74.0
(d)	5.50	98.4

[a] Compared to Process (a).

With a test of significance at the 5% level, 25 replications would be required to give a 90% probability of detecting a true difference of 5% with a C.V. of 5.41% as in Procedure (a). In contrast, with the same criteria, 47 replications would be required with a C.V. of 7.41%, as in Procedure (b). Thus, the extra accuracy obtained by taking daily body weights and calculating mean daily weight change by linear regression reduced the number of replications needed for the foregoing criteria by about 47%.

Following the standardization period, which yielded the data presented in Table 1, the four groups were randomly assigned to two experimental concentrate mixtures, each being pelleted and nonpelleted to give four concentrates.

Each of the four concentrates were fed as 50 and 70% of the air-dry ration, with alfalfa hay representing the remainder. Thus, the experimental design was a 2 × 2 × 2 factorial. The experimental period was 28 days. Except for differences in rations and length of period, the procedures for the experimental period were the same as those used during the standardization period.

The C.V. of P.E. calculated by four procedures, described previously, are given in Table 2. The lowest C.V. was obtained by the process that utilized bomb calorimetry to measure milk energy and linear regression to evaluate weight change energy. Nevertheless, the C.V. of P. E. determined by combining a precise and a conventional measure as in Procedures (c) and (d) were higher than the C.V. obtained by Procedure (b), which made use of conventional measures.

The C.V. obtained for P.E. during the 28-day experiment by each procedure was greater than that obtained during the standardization period. Further perusal and a test for homogeneity of variance (10) of the P.E. data for the 28-day experiment indicated that the relatively high C.V.'s were the result of heterogeneity of variance within the animals receiving 70% concentrates. For this reason, the P.E. data for the two levels of concentrates were

TABLE 2

Coefficients of variation (C.V.) of performance energy during the experimental period as related to procedure used to determine performance energy

Process	C.V.	Relative efficiency [a]
	(%)	(%)
(a)	8.02	100.0
(b)	8.74	91.8
(c)	9.61	83.5
(d)	10.06	79.7

[a] Compared to Process (a).

analyzed subsequently as separate experiments. Performance energy data of cows fed 50% of their ration as concentrates had a low C.V. by each procedure of determining P.E., with that of Procedure (a) being the lowest, Table 3. The C.V. for P.E. of cows fed 70% of their ration as concentrates was 2.13 to 2.92 times larger than that of cows fed 50% of their ration as concentrates, Table 3.

Heterogeneity of variance of P.E. data for cows fed 70% of their ration as concentrates probably resulted from low precision of measuring true changes in energy retention as body flesh. This low precision probably is attributable to changes in body fill.

In the experiments reported in Tables 1 and 3, the C.V. determined by Procedures (a) and (b) differed (approximate $P = 0.15, 0.32$, and 0.18) as determined by a "t"-test (4). Although the significance level was greater than 5%, the C.V. for Procedure (a) consistently was lower than that for other procedures. This consistency, even with the probability levels observed, appears to be meaningful.

TABLE 3

Coefficients of variation (C.V.) of performance energy during the experimental period as related to per cent concentrate in ration fed and to procedure used to measure performance energy

Process	C.V. by concentrate levels	
	50%	70%
(a)	3.16	6.74
(b)	4.19	10.44
(c)	3.69	10.79
(d)	4.34	10.95

Among data with homogeneous variance, use of concentrate intake as a per cent of total ration as a covariate with P.E. reduced the C.V. of the data by as much as 58.3%. A relationship similar to this is to be expected as concentrates provide more utilizable energy per pound than hays.

SUMMARY

A study was made to evaluate the effect of: (a) hay-to-concentrate ratio in the ration, (b) method of measuring milk energy yields, and (c) method of assessing energy gain or loss due to body-weight change on the coefficient of variation (C.V.) of performance energy (P.E.) data in dairy cattle experiments.

The C.V. of P.E. data obtained by combining (a) milk energy, (b) weight-change energy, and (c) maintenance energy allowance was lowest when milk energy secreted was measured by bomb calorimetry and weight-change energy was determined by linear regression from daily body weights.

In P. E. data with homogeneous variance as in the standardization period, the C.V. was always reduced when the per cent of concentrate in the ration was used as a covariate in the statistical analysis.

To have a high probability of true treatment differences of 5% or less, either the C.V. must be held under 4% or the number of replications employed must be large. Thus, it appears that refinement in experimental procedure that will reduce the C.V. is justified. Use of bomb calorimetry to measure milk energy yield, determining weight change energy by linear regression on daily body weights, and using the per cent concentrate in the ration as a covariable with P.E. data in statistical analysis were effective in reducing the C.V.

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CALF RESPONSE TO STARTERS OF VARYING ZINC CONTENTS[1, 2]

While zinc has been shown to be an essential nutrient for ruminants (8), and deficiencies have been reported under field conditions (7), the level required in cattle rations has not been established. Whether the zinc requirement of cattle is greatly affected by some of the factors which have major influences in simple-stomached animals, also has not been determined. Lease et al. (6) found that chicks fed sesame meal as the only protein source developed severe zinc deficiency symptoms, even though the diet contained a normal level of zinc. Soybean meal increases the zinc requirement of chicks and swine when compared to casein (13, 16).

The present study was conducted to determine whether the addition of zinc improved calf performance under selected practical conditions when soybean or sesame meals were used as protein sources. It was also designed in such a way as to place narrower limits of estimate on the zinc requirement of cattle than were previously known.

EXPERIMENTAL PROCEDURE

Five replications of Holstein females, three of Holstein males and two of Jersey females, were randomly assigned to four experimental starters on a within replication basis. With this design and one missing plot the total number of calves was 39. All starters were fed ad libitum. Ration 1 contained the following percentage composition: soybean meal (44% protein), 30.0; citrus pulp, 39.0; corn meal, 27.5; defluorinated rock phosphate, 2.0; salt mixture,[3] 1.0; and vitamin and antibiotic mixture,[4] 0.5. Ration 3 differed from Ration 1 in that 32.5% of sesame meal (41% protein) replaced 30% of soybean meal, 1.5% of corn meal, and 1.0% of defluorinated rock phosphate. These changes were made to equalize the protein content and to more nearly equalize the phosphorus and calcium contents. Rations 2 and 4 were identical with Rations 1 and 3, respectively, except for the addition of 5.5 g of ZnO per 100 lb.

Chopped Coastal bermudagrass hay containing 24 ppm of zinc was fed, with consumption limited to 0.5 lb per calf daily. All calves stayed with their dams for the first day and were then taught to drink colostrum and whole milk from plastic pails. For the remainder of the study they were maintained in separate wooden pens with concrete floors bedded with shavings. Beginning the sixth day they were fed a commercial milk replacer containing 60 ppm of zinc on the powder basis. Each Holstein calf received a total of 28.8 lb, and each Jersey 26.6 lb of dry milk replacer (10) during 32 and 38 days, respectively, mixed in the proportion of 1 lb of dry material to 9 lb of water. The calves were given water from the city supply which contained very little zinc (0.02 to 0.06 ppm), as described previously (9). The experimental starters were fed ad libitum from the sixth day of age for a period of 15 wk. Blood samples were taken the last week of the study and analyzed for zinc (18). The zinc content of the various feeds was determined by the same method.

RESULTS AND DISCUSSION

Differences among the four groups in weight gains and feed consumption were small and nonsignificant (Table 1). While the average blood zinc values were higher for those fed the supplemental zinc starters, the differences were not statistically significant. All calves remained in good health, and none of the zinc deficiency symptoms reported earlier (8, 9) were observed. The sesame meal was more than twice

[1] Journal Paper No. 215 of the College Experiment Station, University of Georgia.

[2] This report is taken from a dissertation submitted to the Graduate Faculty of the University of Georgia in partial fulfillment of the requirements for the Ph.D. degree.

[3] NaCl, 100 lb; $MnSO_4$, 316 g; $FeSO_4$, 200 g; $CuSO_4$, 38.5 g; $CoCl_2$, 10.1 g; and KI, 5.1 g.

[4] Vitamin A (20,000 IU/g), 125 g; vitamin D (8,810 IU D_3/g), 50 g; and chlortetracycline hydrochloride (10 g/lb), 50 g.

TABLE 1

Starter consumption, hay consumption, and weight gains for 15-wk period and blood and starter zinc content [a]

	Ration 1 (soy)	Ration 2 (soy + Zn)	Ration 3 (sesame)	Ration 4 (sesame + Zn)
Starter consumption *(lb/calf/day)*	4.27	4.21	4.14	4.08
Hay consumption *(lb/calf/day)*	0.23	0.24	0.27	0.23
Weight gains *(lb/calf/day)*	1.62	1.58	1.57	1.53
Blood zinc *(ppm)*	2.3	2.7	2.0	2.3
Zinc content of starter *(ppm)*	25	123	48	144

[a] Limited amount of milk replacer containing 60 ppm zinc, on the powder basis, was fed until the calves were 6 wk old.

as high in zinc (104 ppm) as the soybean meal (45 ppm), resulting in a higher zinc content in the rations containing this product. Sesame meal contains about 1.6% phosphorus (11), much of which is probably phytin (4). Phytic acid has been shown to increase the zinc requirements of chicks (13) and swine (12). Apparently, phytin phosphorus is poorly utilized by these species (2, 5), but is essentially as available to ruminants as that supplied by monocalcium phosphate (17). Raun et al. (15) reported that rumen microorganisms were able to hydrolyze appreciable amounts of calcium phytate.

Failure to obtain a response to supplemental zinc indicates that under the conditions of this study the critical level of zinc for calves is lower than that used. It also indicates that if sesame meal or soybean meal increase the zinc requirement of calves, as they do with chicks (3, 6), the effect was not sufficient to elevate the required level above that used in this study. Thus, tentatively, it could be concluded that the zinc requirement of calves, 7-15 wk of age, which had been fed a limited amount of milk replacer containing 60 ppm zinc, is not substantially above 25 ppm under the conditions of the study. A very drastic deficiency has been produced in calves, previously fed limited whole milk, on a diet containing 3.6 ppm, with definite changes observed by the 8th wk of age (9).

In a popular report, Beeson et al. (1) observed a response in rate of gain and feed per pound of gain with steers when a high concentrate diet containing 24 ppm of zinc was supplemented with 100 ppm of zinc over a 12-wk period. The apparent discrepancy between this report (1) and our results is not surprising, as wide differences have been observed in the zinc requirements of other species due to several factors, including a very pronounced location effect (14).

In future studies relative to the zinc requirements of cattle, it would probably be desirable to use a basal ration containing a lower level of zinc. A study of longer duration or a depletion phase, or both, at the beginning of such studies should be desirable.

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FEEDING POTENTIAL OF RECLAIMED FECAL RESIDUE [1]

Present-day trends are for both dairy and beef cattle to be managed in confinement. Furthermore, developments in recent years have caused an increase in the use of concentrate feeds for both dairy and beef cattle.

Formerly, swine were used to salvage grain voided in the feces of cattle fattened on high grain rations. Changes brought by mechanization and specialization in livestock enterprises, coupled with the fact that grains are now ground for feeding, perhaps are major factors responsible for the decline in interest in the following cattle with pigs. Nevertheless, feces from full-fed cattle contain appreciable amounts of undigested feed residue. Not only does fecal grain represent an appreciable loss of feeding value but fecal matter creates a serious disposal problem. Odors and flies caused by large-volume cattle feeding operations are presently creating urban health problems.

The objectives of this research were to (1) recover some of the fecal feed and (2) develop an effective means of disposing of organic residues voided by confined cattle.

EXPERIMENTAL PROCEDURE

Yearling steers were confined on concrete and fed a high grain feed mixture. The manure was collected daily in 30-gal garbage cans. Water was added to the cans and the manure was thoroughly stirred. Solid material was allowed to settle and the aqueous layer was poured off. The water washing was repeated and the fecal residue remaining was stored at approximately 33 F until needed for feeding. The wet fecal residue was mixed with the basal feed (Table 1) in the ratio of 40 parts of wet residue to 60 parts of basal feed. Feed was mixed daily and at the time of mixing dried yeast was added at the rate of 1 lb per 100 lb of feed. The final mixture was held in burlap bags about 12 hr before feeding. Three yearling steers were used in the feeding test. These cattle performed satisfactorily on a steer-fattening feed for 7 wk prior to the time they were placed on test. A 14-day preliminary feeding period on the test ration preceded the experimental period.

TABLE 1
Basal mixture

Ingredient	Amount
	(%)
Soybean meal	16.00
Ground ear corn	64.20
Cane molasses	16.70
Salt	1.67
Dicalcium phosphate	0.83
Vitamin A	Trace
Stilbestrol	Trace

RESULTS AND DISCUSSION

The performance data for the cattle are summarized in Table 2. No difficulty was experienced in getting the cattle to consume the experimental feed. No outward symptoms of harm resulted to the cattle as a consequence of

TABLE 2
Performance data for cattle fed washed fecal residue

Animal no.	Initial weight	Final weight
	(lb)	*(lb)*
1	720	880
2	760	970
3	595	775
Avg weight	692	875
Gain *(lb)*		183
Days on test		54
Daily gain *(lb)*		3.39
Feed dry matter per hundredweight gain *(lb)*		643

[1] This research was supported in part by Hatch Project No. 132.

consuming the mixture containing fecal residue. Daily live weight gain and feed efficiency of the cattle were excellent for steers of this weight, age, and condition.

These data are inconclusive with respect to the over-all benefit to be derived from reclaiming fecal residue for use as a feed. Nevertheless, the results of the test do show conclusively that cattle will eat mixtures in which wet fecal residue constitutes an appreciable part. The efficient feed conversion (643 lb per 100 lb of gain) needs confirmation. The results obtained justify systematic testing of many ramifications of the feeding procedure.

The results of this test suggest the feasibility of washing floors daily where slaughter cattle are housed and fed, in a manner similar to the procedure used with many dairy herds (1). For both dairy and beef herds, the relevance of this study is the potential to derive more than manure value for undigested feed and microbial residues.

SUMMARY

A new use for manure from dairies and feedlots is presented.

Cattle consumed a feed mixture containing washed wet fecal residue in amount equal to approximately 40% by weight of the mixture.

Cattle fed the fecal residue mixture gained over 3 lb daily and required less than 700 lb of dry matter per 100 lb of gain.

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EFFECT OF WHEY AND LACTOSE IN BEEF CATTLE RATIONS[1, 2]

The feeding of lactose to animals and humans has been reviewed by Atkinson et al. (1). Lactose is the preferred carbohydrate in the diet of the very young mammal. It is not only well utilized as a source of energy but has other beneficial effects, particularly in calcium metabolism and vitamin synthesis in the small intestines. However, in the animal with a functional rumen, data are lacking on the effect of feeding lactose. Lactate is converted to volatile fatty acids by rumen bacteria, with the majority being propionic acid (2). It has been suggested by Klosterman et al. (3) that the feeding value of corn silage was improved by treating it to improve its acid content. This raises the question concerning the possible value of including lactose in ruminant rations. Whey is a commercial source of lactose and could prove beneficial in beef cattle rations. The objective of this research was to study the value of added whey[3] and lactose in rations for beef cattle.

Four feeding trials with beef steers were conducted, to determine the value of added whey and lactose in growing and fattening rations. The initial trial was a study of the effect of adding 0.5 lb of whey per animal daily to a fattening ration composed of ground ear corn, high moisture corn, ground corncobs, and supplement. The rations fed were similar, except for the addition of the whey. Two lots of five steers each were fed each of the two rations for a 70-day feeding period.

In Trials 2 and 3, the fattening ration was composed of a full feed of ground shelled corn, limited alfalfa hay, and 1 lb of supplement per animal per day. In Trial 2, the whey treatments were control, control + 0.25 lb whey, control + 0.50 lb whey, and control + 1.0 lb whey per steer per day. In Trial 3, the addition of 0.35 lb of lactose (edible grade) per steer per day replaced the highest level of whey treatment in Trial 2. In Trial 2, one lot of 20 steers each was fed each treatment for 135 days. In Trial 3, two lots of eight head each were fed each treatment for 138 days. Carcass measurements were taken on each steer, including yield, grade, area of the rib eye (*longissimus dorsi*), and fat covering over the 12th rib. The area of rib eye was determined by tracing the outline of the 12th rib section on acetate paper. The area was then determined by aid of a planimeter. The thickness of the fat covering was an average of three measurements taken at the 12th rib section, as suggested by Nauman (4).

In Trial 4, the treatments in Trial 3 were repeated with steer calves fed wilted silage rations. Two lots of eight calves were fed each treatment for a 52-day period. They were

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[2] Supported in part by Midwest Dried Milk Co., Dundee, Illinois.

[3] The whey contained about 70% lactose.

TABLE 1

Response of steers to whey and lactose supplementation

	Type of ration	No. steers	Days fed	Initial weight	Daily gain	Daily feed	Feed/ cwt gain	Carcass [a] grade score	Dress-ing	Rib eye area	Fat covering
					Trial 1				*(%)* [b]	*(sq inch)*	*(inch)*
Control	Fattening	10	70	935	3.07	21.9	709				
0.50 lb whey	Fattening	10	70	925	3.27	24.0	734				
					Trial 2						
Control	Fattening	20	135	768	2.70	26.4	978	6.1	57.2	10.8	.76
0.25 lb whey	Fattening	20	135	781	2.77	26.6	960	6.6	57.2	11.6	.73
0.50 lb whey	Fattening	20	135	772	2.86	26.9	940	6.6	58.1	11.4	.85
1.00 lb whey	Fattening	20	135	776	2.76	27.2	985	6.3	58.7	11.1	.83
					Trial 3						
Control	Fattening	16	138	756	3.00	26.7	870	6.4	59.3	12.2	.70
0.25 lb whey	Fattening	16	138	752	3.11	26.4	850	6.8	58.5	11.7	.81
0.50 lb whey	Fattening	16	138	760	3.14	26.7	850	6.5	59.2	12.3	.84
0.35 lb lactose	Fattening	16	138	748	3.08	26.7	870	6.9	58.5	11.2	.84
					Trial 4						
Control	Growing	16	52	463	2.02	26.3	1,306				
0.25 lb whey	Growing	16	52	461	2.33	26.4	1,131				
0.50 lb whey	Growing	16	52	456	2.32	26.6	1,148				
0.35 lb lactose	Growing	16	52	462	2.25	26.5	1,175				

[a] Federal carcass grade score: 6 = high good, 7 = low choice.

[b] Dressing per cent based on full weight off experiment and hot carcass weight shrunk 2½%.

given a full feed of wilted silage, 2 lb of corn, and 0.5 lb of supplement. The silage contained, on the average, 61% dry matter.

The initial and final weights were the average of weights taken on two consecutive days, at the beginning and ending of the trial. Calves were weighed at 28-day intervals during the progress of the trials. Feed consumption was recorded on a lot basis.

Results of the four trials are shown in Table 1. In Trial 1, the addition of 0.5 lb whey per day increased gains by 0.20 lb per day. However, the cattle fed whey consumed more feed (24.0 vs. 21.9 lb per day) and required slightly more feed per unit of gain.

In Trials 2 and 3, the pattern was similar for each trial. The differences found due to the whey were not statistically significant, but they paralleled the findings in the previous trial. Substituting the equivalent amount of lactose as contained in 0.5 lb whey did not increase gains to the same extent as whey. The feed required per unit of gain was less for the steers fed whey. Although the differences on these limited numbers were not statistically different, the fat covering tended to be higher for the whey- and lactose-fed animals. No consistent pattern was evident for grade, dressing per cent, or rib eye area.

In Trial 4, the addition of whey or lactose to a growing ration increased ($P < .10$) rate of gain. There was no difference in the 0.25 or 0.50 lb whey levels. The feeding of lactose was not equivalent to feeding whey, but lactose did stimulate liveweight gains.

The data for the four trials were pooled for the control and the 0.50 lb level of whey. When the data were pooled, the increase in rate of gain was significant ($P < 0.5$).

The results of these trials would indicate that whey addition to both fattening and growing rations is beneficial in increasing animal performance. It has been shown in vitro that lactate yields a higher level of propionate than other sources of carbohydrate on fermentation by rumen microorganisms (2). Since lactose would be readily fermented to propionic acid, it is possible that the benefits are mediated in this manner.

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EXCRETION OF PROGESTERONE AND ITS METABOLITES IN MILK, URINE, AND FECES [1]

The excretion of C^{14} in urine and feces, after the administration of C^{14}-labeled progesterone, has been studied in a number of species. Riegel et al. (3) reported in 1950 that in a mouse approximately 50% of the injected progesterone-21-C^{14} activity was excreted in the feces in 48 hr. The order of magnitude of this fecal excretion in rodents has been amply confirmed. Gallagher et al. (1) and Sandberg and Slaunwhite (4) have demonstrated such excretion in the human and have shown in the human that relatively more excretion occurs via urine than feces. Gassner et al. (2), in a study in cattle using a labeled progesterone derivative, 17-hydroxyprogesterone-4-C^{14}, found 40–68% of the activity excreted in the feces and 1.2–5.4% excreted in the urine in somewhat over 100 days. In all such studies a considerable amount of activity has remained unrecovered. Autoradiographic studies (5) in rabbits and goats have shown a marked uptake of progesterone-4-C^{14} or its metabolites in lactating mammary glands. This finding suggested that milk might serve as a route of excretion for progesterone or its metabolites and the present study was conducted to test this possibility.

A Holstein cow in moderate lactation (fifth month) and pregnant (third month) was injected through a jugular catheter with approximately 65 μc of progesterone-4-C^{14} in 6.6 ml of propylene glycol during a period of 1 hr and 40 min (9:30–11:10 AM). Complete collections of urine and feces were made separately and each milking was collected separately. Total milk and urine volumes were recorded and 500-ml aliquots of milk and 1,000-ml aliquots

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of urine were taken for extraction. Total feces for a collection period were slurried with water, weighed, and an aliquot taken for extraction.

All sample aliquots were taken to approximately pH 1 with concentrated HCl. An equal volume of methanol was added to the milk aliquots and all acidified aliquots were refluxed 3 hr. After cooling, each sample was extracted four times with 200-ml volumes of $CHCl_3$ and three times with 250-ml volumes of ether. The combined extracts were taken to dryness in a rotary evaporator and taken up in counting solution to a known volume. The samples were counted in a liquid scintillation system and quench-corrected with an internal standard.

The percentage of injected dose recovered in each collection of urine, feces, and milk is shown in Table 1. It is immediately seen that these results with progesterone-4-C^{14} are similar to the values for urine and feces found in rodents with progesterone and in cattle with 17-hydroxyprogesterone. The extremely small recovery in milk indicates that milk is of no importance in the excretion of progesterone or its metabolites. This is an important observation, since cattle are usually pregnant and lactating simultaneously. These data also indicate that the mammary gland uptake of progesterone observed is not the result of excretory processes.

TABLE 1

Per cent of dose recovered in milk, urine, and feces after I.V. injection of Progesterone-4-C^{14}

Hours post-injection	Per cent dose recovered		
	Milk	Urine	Feces
0-7	0.03	.....	.....
0-12	....	1.68	45.06
7-21	0.01	.....	....
12-24	....	0.96	4.09
21-31	< 0.01	.....	.....
24-36	....	0.37	0.54
31-45	< 0.01	.....	....
36-48	.....	0.15	0.15
Total	< 0.06	3.16	49.84

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CONTROLLING RADIOACTIVE FALLOUT CONTAMINATION[1]

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FALLOUT FROM NUCLEAR TESTING

Many people are concerned about possible contamination of the Nation's food and milk supply with radioactive fallout from nuclear tests.

Our milk and food are not in danger today from such contamination. Radiation levels are still below the point of any serious concern. Conservative estimates place the daily consumption of Strontium^{90} in our entire diet at the lower levels of Range II of the Federal Radiation Council's recommended standards and guides—considerably below the levels at which special countermeasures would be considered.

Milk represents one of our Nation's most important foods. It forms a major part of the diet of infants and growing children. Its consumption by teen-agers and pregnant mothers is highly desirable. Its nutritional elements, as the Food and Nutrition Board of the National Academy of Sciences pointed out, would be difficult to replace without considerable added expense.

Even with the abundant food supplies available in this country, the White House Conference on Youth in 1960 reported that over 30% of our teenage girls lack sufficient calcium in their diet. The diets of 20% of the boys in the same age group were likewise deficient. These deficiencies could cause more serious consequences than present levels of radioactive contamination.

Medical and public health authorities have been concerned for some time that many people might give up drinking milk or eating essential foods for fear they may be contaminated with radioactivity. That kind of reaction could do far more harm to health than the infinitesimal amounts of radioactivity found in some foods. This concern has been expressed by such authorities as Dr. Luther L. Terry, Surgeon General of the Public Health Service; Dr. Donald R. Chadwick, Chief, Division of Radiological Health, Public Health Service; Dr. Herman E. Hilleboe, Commissioner of Health, State of New York; and Dr. Cyril L. Comar, Biologist, Cornell University.

The National Advisory Committee on Radiation, composed of authorities in the fields of medicine, public health, and health physics, has stated that there is a tendency of certain groups of the population to make their own interpretations of published levels of radiocontamination and to urge the application of those countermeasures which seem appropriate. The Committee urges the avoidance of independent countermeasure action. Not infrequently, such action involves the use of countermeasures associated with risks approaching or exceeding those of the contaminant. Often, such action is ineffective in reaching the objectives sought.

The Public Health Service is keeping a continual watch on the levels of radioactivity showing up in vegetables and dairy products. It is their conclusion that at the present levels we will all be much better off drinking milk and eating vegetables as usual, rather than reducing our consumption of these essential foods.

Much of the public discussion of fallout hazards has centered about the radioisotope Strontium^{90}. A review of available research studies indicates that detrimental effects of Strontium^{90} have never been observed in humans. There is no evidence at this time of Strontium^{90} damage to humans, and specifically there are no reported cases of bone cancer or leukemia in man caused by Strontium^{90}. Experiments with small laboratory animals have shown, however, that Strontium^{90} can produce injury when consumed in sufficiently large amounts. It is, therefore, prudent to assume that people might also be injured if exposed to significantly large quantities.

In the current concern for the consumption of radioactive materials in our food supply, emphasis must be placed on the serious limitation that exists in our knowledge of measuring the effects of radiation on the biological system. In fact, this limitation also applies to measurement of all causes and effects on living organisms.

In most cases, we can measure and know the effects of relatively large doses of radiation on a mammal. Experimentally, we can reduce these doses and observe that the reaction decreases. But we usually find that we can reduce the dose to a level where our present experimental techniques are not capable of revealing the results.

Doses of radiation that people may be receiving from fallout are far below the level that produces effects we can measure or observe at this time. Therefore, exact answers cannot be given to questions on the effects of present fallout levels. How can we conclude that an extremely low level of radiation may or may not be harmful to, say, one person out of a hundred thousand or one person in a million? We just cannot measure such minute effects.

Can we, or should we, accept the minute possibility of damage—the unmeasured possibility

[1] Presented at the 57th Annual Meeting of the American Dairy Science Association, University of Maryland, College Park, June 20, 1962.

of harmful effects associated with fallout at current levels, or even with considerably higher levels? We certainly have precedents galore for learning to live with hazards of this magnitude in our environment.

What about the countless highway and traffic accidents and the resultant mortality?

Do we know the effects of smog, or the excessive use of tobacco or alcohol, or the extended use of reducing diets, mixtures, and fashionable food fads?

None of these seems to produce the public concern or excite the interest of newspapers and popular magazines the way radioactive fallout does. The current reaction toward fallout is not consistent with reaction to other hazards in our environment. There may be special explanations for the attitudes that some people seem to have developed toward radioactive fallout, but some of them are extremely difficult to define.

Much of the concern with the radioactive fallout is due to a mystery psychology—a fear of the unknown. People know only that this something is quietly and invisibly dropping from the sky.

It is well to remember that radiation has always been with us. Life on earth has developed to its present state (good or bad) amid continuous natural radiation from rocks, soil, and outer space. Dr. Edward Teller, atomic scientist of the University of California, and known as father of the H-bomb, points out that the average American gets an annual dose of cosmic rays averaging .034 roentgen per year at sea level. People at higher altitudes, specifically Denver, get a larger dose of .05 roentgen per year. Compare this continual exposure with that from world-wide radiation from fallout. Dr. Teller points out that the bones of humans average .003 roentgen per year from Strontium^{90}. He estimates that at these levels, a person is not apt to receive over a 1-roentgen dose from this source during his lifetime. This indicates that we get about ten to 20 times the amount of radiation from cosmic rays that we do from Strontium^{90}. Dr. Teller calculates that people living in brick houses may receive more radiation from the radioactive material contained in the bricks than from current levels of Strontium^{90}.

FALLOUT FROM A NUCLEAR ATTACK

A nuclear attack on this country would produce fallout and radiation problems much different and many times more serious than those resulting from nuclear testing.

A conscientious historian has found that 278 wars have been waged by man from the time of Columbus' discovery of America until the beginning of World War II. Since then, small and large conflicts have been numerous in all parts of the world. War appears as the most common means of accomplishing man's lust for power or imposing his own desires or philosophy on a people.

Each age has faced the terror of the ultimate weapon—from the wooden club and the crossbow to cavalry, gunpowder, tanks, and planes. Now we face the threat of nuclear weapons. War in the 20th Century with rockets and nuclear devices no longer confines its threat to the individual, the family, the village, or even the nation. Today, war is a threat to the world.

In the meantime, however, we must find ways of defending ourselves against an all-out nuclear attack and means of minimizing its effects. Such a nuclear attack against this country would create many difficulties. Our agriculture and food industry would be faced with some special problems—including food production, food processing, and food distribution. Certainly, the maintenance and safety of our food supply is one of the most vital considerations in any national emergency.

Among our major concerns in food production following a nuclear attack would be the provision of a safe, adequate food supply. We would want to do everything we could to save our important food resources from the effects of radioactive fallout.

What is radioactive fallout? It is the dust-like material created when a nuclear bomb explodes close to the ground. Thousands of tons of earth and other materials rise in the mushroom cloud and combine with radioactive debris from the bomb. Fallout is a mixture of melted and unmelted grains of soil. Radioactive atoms produced during the explosion condense into and onto these particles to form the radioactive fallout.

The danger is the nuclear radiation emitted from this fallout. The predominating nuclear radiation hazard from fallout is the gamma radiation which travels through air from particles lying on surrounding exposed surfaces and ground areas. This radiation can pass into and through matter. As it does, it can change, damage, or destroy living cells through a phenomenon known as ionization.

Fortunately, although ionization does change living cells and other materials, it does not make the affected cells or materials radioactive. Once the source of the radiation—the radioactive fallout—has been removed, the irradiated materials are safe to handle or to eat.

The primary concern should always be to keep the (gamma) radiation exposures to humans as low as possible—for example, to protect the dairy farmer and his family from this hazard. This should be followed by efforts to protect the livestock from this hazard.

On the dairy farm following a nuclear attack, the dairyman would initially be concerned with the effects on milk production. Since most livestock would be outside the range of the initial effects of the weapons, the main question would

be how much radiation the animals had been exposed to.

Many of the dairy animals would likely be in the open. They would receive radiation damage from external sources. They would also ingest radioactive materials of fallout that would contaminate pastures and other growing crops.

Other animals would be in a barn or under some cover and would be fed from reserve food supplies that were uncontaminated. In this case, our only concern would be how well the cover had protected the animals from external gamma radiation.

And finally, there would be animals under similar cover but fed from contaminated feed supplies. Here again, the possibility of damage from both external and internal radiation would have to be considered.

You can see that we would face two sources of radiation hazards—an external source accompanying the arrival of fresh fallout in an area, and an internal source from contaminated feed and water consumed.

The major external hazard is due to gamma rays. These are similar to X-rays—penetrating and capable of traveling some distance from the fallout where they originate. Our major problem with fresh fallout would be protection, through adequate shelter, against these rays. They are produced in large part by the shorter-lived isotopes; the intensity of gamma radiation decreases in a relatively short time through the process of radioactive decay.

The gamma-producing isotopes can also cause an internal hazard by contaminating feed and water.

But the internal hazard arises also from beta particles, or rays, emitted from the longer-lived isotopes. These rays travel only a short distance in air and only a few millimeters in tissue. Protection against their external effects is relatively simple. However, once beta-particle emitters are ingested, they can, while inside the body, continue to emit radiation which damages the surrounding cells.

Thus, in our efforts to protect livestock, we must be prepared to deal with two needs:

First, we must protect against the immediate hazard from gamma radiation long enough to allow the fallout to decay to less hazardous levels.

Second, we must protect against the persistent hazard from consumption of contaminated feed and water.

Let us look more closely at these two threats from radiation and the means we have for dealing with them.

First, the external hazard.

Tolerance to radiation varies among species of animals, as well as among animals of the same species. All domestic animals, however, have a similar response to total body irradiation. Few, if any, will die following exposure up to 250 roentgens and few, if any, will survive after brief doses of as high as 1,000 roentgens. The smaller the dose and the slower the rate, the better radiation can be tolerated. Body size seems to have little to do with survival, although very young or very old animals may be more radiosensitive.

In the case of livestock, acute total body radiation exposures of 500 to 600 roentgens provide a mid-lethal dose—that is, the amount that would be expected to kill half of a large group of exposed animals within 30 days.

Poultry have more resistance. Their mid-lethal dose is about 900 roentgens. Incidentally, in addition to being more resistant, poultry are usually raised under shelter and fed stored feed. So they would offer one of the more dependable sources of fresh foods of animal origin after a nuclear attack.

There would be some danger of external radiation damage to animals from the principal internal hazard—the beta particles. If they come into actual contact with the skin and remain for an appreciable time, a form of radiation damage referred to as beta burns will result. The outer layers of the skin could receive a large radiation dose from the beta particles and in some circumstances this might cause serious burns.

Limited experimental evidence and field testing indicate that animals that fail to develop beta burns will ordinarily escape serious external radiation injury. Animals that sustain exposure intense enough to produce beta burns but live longer than 3 wk or a month fall into the same category as those without burns.

TABLE 1

Percentage of mortality of unsheltered animals after 24-hr exposure to various radiation doses

	Mortality				
	100%	80%	50%	20%	0
Species	Exposure dose (roentgens)[a]				
Cattle	650	600	550	450	300
Sheep	700	600	525	450	350
Swine	800	700	600	450	350
Poultry	1,200	1,100	900	600	400

[a] Exposure dose in area where livestock and building are located.

Initially, the greatest fallout hazard to animals would be the gamma rays.

The most valuable protection against gamma rays would come from keeping livestock under adequate cover at least during the first critical 24 to 48 hr—and longer if possible. With sufficient mass of shielding materials between the animals and the fallout, only a little of the radiation from gamma rays would penetrate into the sheltered area.

Distance would also afford some protection. The farther animals were from the source of radiation—the radioactive fallout—the less the exposure would be.

The value of shielding in preventing death and sickness among animals would be greatest in areas exposed to acute radiation doses about equal to the average mid-lethal dose. (See Table 2.) Even at low radiation intensities, however, there would be some beneficial effect from shelter. It would help to prevent fallout from contaminating the animal's coat and would minimize the hazard of contaminating herdsmen and livestock handlers.

An effective barn for fallout protection is the multi-story building with a loft full of hay or straw, and soil or other shielding material banked against the sides of the building. Ten feet of baled hay will give as much shielding against gamma rays as 1 ft of earth. About one-fourth of the new dairy barns being built today in the large northern dairy states are of the high-roof type (Gambrel or Gothic) with overhead haymows. The most effective protection would be found in underground basements such as we find in bank barns. Milking cows should be given the most protected location in the center of the barn. This would be the safest place for them, as well as attending personnel who do the milking.

Protection against light fallout would be provided by open hay storage buildings and pole structures for livestock shelter and feeding. Even a shed without sides would give some protection.

If there are enough buildings to house only some of the animals, the others should be put in a yard, near farm buildings. Large protected self-feeders and automatic waterers could be an important source of uncontaminated feed and water under these conditions.

Construction plans are available through State Extension agricultural engineers for an elaborate dairy barn and family fallout shelter. Although construction of this type is costly and does not lend itself to efficient operation, such a facility might be considered for protection of highly valued breeding stock. It is de-

TABLE 2

Effect of shelter on the mortality rate of livestock [a]

Kind of livestock and radiation exposure—unsheltered dose (no. of roentgens—one day)	Mortality rate by nature of shelter			
	No shelter	Tight wooden barn (protection factor of two)	Two-story barn with loft full of hay (protection factor of five)	Basement-type barn with loft full of hay (protection factor of ten or more)
	(%)			
Cattle				
500	30	0	0	0
1,000	100	30	0	0
3,000	100	100	80	0
Hogs				
500	30	0	0	0
1,000	100	30	0	0
3,000	100	100	50	0
Sheep				
500	38	0	0	0
1,000	100	38	0	0
3,000	100	100	80	0
Poultry				
500	10	0	0	0
1,000	64	10	0	0
3,000	100	100	20	0

[a] The reduction of radiation by shelter is described as the protection factor. For example, if the protection factor of any given structure is two, then the intensity of outside radiation is reduced by one-half. In fallout areas, one-half or more of the radiation would be released after the end of the first day.

signed in accordance with milk production ordinances. Here are some of the features of this plan:

1. A year-round production unit requires minimum change for emergency use.
2. A built-in family shelter allows the operator to care for animals during a fallout emergency.
3. All stored feed is manually accessible inside the barn.
4. Stored hay and straw are used for shielding.
5. Other livestock could be temporarily housed, fed, and watered inside.
6. An auxiliary generator assures electric power.
7. The water supply pump is inside the barn.

Such a facility would not be practical for most farmers, but, as we have seen in areas of moderate fallout, good use of the usual facilities found on farms would do much to provide protection against the external hazard from radiation.

Let us turn now to the problem of protecting livestock from the internal hazard.

Many different radioisotopes are created by a nuclear explosion. Most of them are of minor importance, because the amounts are small, their half-life is extremely short, or they do not find their way into the food chain where they could affect man and animals.

But four of these isotopes are capable of entering the food chain and contaminating crops as well as foods of animal origin. The four are Cesium^{137}, Iodine^{131}, Strontium^{89}, and Strontium^{90}. Each presents a different problem.

Cesium^{137} is chemically similar to the essential element potassium. When consumed and absorbed, this radioisotope is found primarily in the soft tissues. However, like potassium, it is not retained long in the body and is rapidly secreted in the milk or excreted in the wastes.

Iodine^{131} is the radionuclide of primary concern when one considers the short-lived nuclides of public health significance. Because of its similarity to ordinary iodine, this isotope can accumulate in the thyroid gland and is also secreted in the milk from dairy cows. Iodine^{131} has a relatively short half-life of eight days and would be the major internal hazard during the first 60 days following a nuclear attack.

There are considerable day-to-day variations in the average level of Iodine^{131} in milk. Values have varied on occasions by a factor of about two. This is not unexpected, however, as Iodine^{131} is rapidly transferred to milk (within 24 hr). Shortly after detonation, the Iodine^{131} content of both air and rainwater may vary rapidly. Uncontaminated rain could cause rapid reduction in the Iodine^{131} content of growing forage crops.

There are no practical ways at the moment of preventing a cow from secreting radioactive iodine in her milk if she is allowed to consume feed contaminated with this radionuclide. In fact, up to now, this has not been a fruitful avenue for research. The answer is to prevent the cow from consuming radioactive materials—or at least minimize the amount ingested. Limited studies have been made on comparing radioiodine in the milk of barn-fed with pasture-fed dairy herds. One such study indicated that the levels of radioactivity from worldwide fallout in milk from barn-fed animals were essentially nondetectable, whereas the secretions and excretions of pasture-fed cows contained several radioactive isotopes, including Iodine^{131}.

Studies have further shown only negligible quantities of Iodine^{131} in milk after herds have been on iodine-free feed for as a short a period as five to seven days.

COUNTERMEASURES

Countermeasures against fallout contaminants include those actions and procedures that would result in reducing or eliminating the exposure of the population to the hazards of radioactive materials. These procedures should be directed to the source of the contamination, to the mechanism that transmits it, or to the substance in which it gains access.

Countermeasures would be taken only after responsible authorities had carefully evaluated the situation and declared a state of emergency. The decision would not be an easy one. Medical assessment of the probable damage from radiation would have to be balanced against the cost of the decontamination measures, the resulting reduction in available food supplies, and the economic and social dislocations resulting from the action.

Countermeasures could be drastic, or they could involve changes in generally accepted farming practices. Some measures could be simply an improvement over local conditions and procedures. Some countermeasures could result in reducing the contaminant by only a small factor, but a combination of several of these measures could provide the necessary reduction.

Protecting feed and water from fallout. The principle of protecting stored feed and water from fresh fallout is simple: prevent the fallout from getting mixed into these materials. They may have been irradiated—but if the fallout did not come into actual contact with them, or if the fallout were removed—they would not be radioactive. They would be safe to eat or drink.

Since early radioactive fallout is dustlike in character, initially it provides a surface contamination of those things upon which it falls. We can keep it out of feed and water in much the same way that we would keep out ordinary dust—by placing a cover over them. And the fallout could be removed from some food products just as we get rid of dirt or dust—by me-

chanical removal, such as washing, paring, vacuum cleaning, and brushing.

Grain stored in a permanent bin and ensilage in a covered silo would be adequately protected against fallout. The contents could be used as soon as a farmer could safely get into the area to handle them.

Livestock creep feeders, self-feeders, and covered feeders could protect essential feeds from fallout. Bunker and trench silos of all types—if adequately covered—could also provide protection.

A haystack in an open field could be protected by a cover such as a tarpaulin. When it is safe for the farmer to leave his shelter, he can carefully remove the cover and remove the fallout.

Many materials, such as uncovered haystacks and piles of farm produce, could be safely used if the contaminated outer portions were removed.

Water stored outside—in stock water troughs, for example— should be covered with any material that will normally keep out dust. Larger farm ponds and lakes would, of course, be difficult, if not impossible, to protect.

As time passes following an attack, contamination of ponds and lakes would become less and less of a problem. The dilution of the radioactive fallout in the water and its adsorption by clay on the sides and bottom of the lake would be effective in reducing the hazard below that of the surrounding land.

Water from covered sources such as springs and wells would be essentially free of contamination, even where the fallout was heavy, and could be used with confidence for man and animals. A good protected water supply from a well or spring, distributed through troughs and automatic livestock water fountains, would provide an excellent continuing source of safe water for animals. This would be especially useful during the initial emergency when the livestock owner might be confined to a fallout shelter and would not be able to attend his livestock.

After the first critical 24 to 48 hr following an attack—or when outdoor work periods could be safely scheduled—livestock could be given short periods of exercise in areas or yeards that did not contain excessively contaminated vegetation or water. When it was no longer practical to keep animals off contaminated pastures, supplemental feeding with uncontaminated feed should be provided as far as possible.

Iodine^{131}. Iodine^{131} is present in relatively large quantities in fresh fallout. In dealing with the problem of Iodine^{131} contamination in milk, the ideal approach is the use of preventive or protective measures at the farm or production level—to try to keep it from entering the milk. A dairy farmer, therefore, should confine his milking animals to the barn before fallout appears in the area and provide uncontaminated forage and feed. Since radioactivity from Iodine^{131} largely disappears from materials after a 60-day period, the dairyman could use forage and feed that had been stored for at least 60 days after exposure to fallout. Freshly contaminated forage could be fed to nonmilking stock. Concentrate mixtures prepared on the farm should be made up of grains harvested before the fallout or stored for 60 days or longer if fallout is extremely heavy.

The problems associated with this requirement would vary by season, geographical location, dairy practices, and available facilities.

Iodine^{131} contamination of milk would be minimal in the northern states from fall to early spring when dairy animals are not usually on pasture but are normally housed and barn-fed.

In those areas where dry lot feeding is practiced, there should be little contamination of milk. Also, in those sections where a large part of the dairy ration is imported from other parts of the country, the delay in feed movement would result in feed of extremely low contamination.

Increased storage facilities would be required on some farms.

If, despite all precautions, fluid milk supplies should become contaminated, a number of steps could be taken to avoid danger to consumers. Since Iodine^{131} decays rapidly, delayed marketing is a key to safe usage of contaminated milk and dairy products.

FLUID MILK WITH HIGH CONTENT OF IODINE^{131}

Suppose the contamination of milk supplies with Iodine^{131} was localized. Milk from other areas could be brought in for immediate consumption, and the contaminated milk could be processed and stored for later use. This measure would require a large, efficient field organization with a monitoring capability to assess the Iodine^{131} content of milk at production level and the levels of contamination in local areas. If contamination was widespread, however, this procedure would be impractical. Consumption of fresh fluid milk would have to be deferred until radioactivity was reduced to safe levels.

FLUID MILK WITH RELATIVELY LIGHT CONTENT OF IODINE^{131}

If contamination was relatively light, storage for eight days might be adequate. (Eight days of storage would reduce the radioactivity by 50%.) Today's pasteurized homogenized milk should maintain its quality for this period at usual refrigeration temperatures. It is preferable that this storage take place before delivery to the consumer. Normally, three to four days are required for milk to reach the consumer. Few plants today have facilities to store milk for eight days. To do so would generally exceed available plant tank capacity and refrigerator facilities. A few plants could hold

milk for six days. In general, industry would have to prepare specifically for the application of this countermeasure.

FROZEN FLUID MILK

For high rates of contamination, storage for 30 to 60 days might be necessary before Iodine^{131} was reduced to safe levels. Storage for 60 days would reduce the radioactivity to less than 1%. Freezing of packaged milk in paper cartons for storage prior to delivery would be one way of handling the problem. The frozen product would be thawed before delivery to the consumer and the consumer would have no responsibility for keeping track of storage time. Storing frozen milk for 2 to 3 wk would provide a decrease in the Iodine ^{131} by 70 to 85%. During the period when fresh milk supplies were not available, reconstituted dry milk or evaporated milk could be safely used.

At the present time there are few freezing facilities at fresh milk plants. It is estimated that unused freezer space in public warehouses is available to store about one billion quarts of milk. This indicates the feasibility of storing single-strength milk as a possible countermeasure for Iodine^{131}. This space is quite well distributed by population density and at least three-fourths of the available space could be effectively utilized. The use of home freezers could also be considered.

Any of these marketing modifications would be difficult; nevertheless, they could become necessary. There should be no destruction of milk contaminated with Iodine^{131}. Since its half-life is short, the milk could be processed into products such as butter, cheese, powdered milk, and canned milk, and stored long enough to allow decay to take place.

PROCESSED MILK

The use of powdered whole milk, powdered skimmilk, and canned evaporated milk would provide safe products.

About 3 to 4% of our total fluid milk production is processed into evaporated milk. Of this, about 10% is consumed by infants up to six months of age, and the balance is utilized in home use. Industry maintains an even distribution across the country, and evaporated milk is sold in every state. The in-transit time to reach consumers is about two months. This would provide nearly 100% reduction in the intake of Iodine^{131}.

It is estimated that of the infant group that is bottle-fed on milk, about 60% are on evaporated milk. About 35 to 37% use commercial liquid or powdered products, which are about 20% powdered milk. About 2 to 5% are on fluid milk.

POWDERED MILK

The production of powdered whole milk is extremely limited. Powdered skimmilk production in the United States annually runs about two billion pounds, of which over 200 million pounds are packaged for consumer use. The rest is used in baked goods, other dairy products, prepared mixes, meat products, candy, for government purchase, and other miscellaneous uses. Here again, because powdered milk can be stored for a considerable time, the reduction of Iodine^{131} intake could be considered to be almost 100%.

Strontium^{90}. As the first few months passed following an attack, Strontium^{90} would take on new significance. Decay of the shorter-lived isotopes would leave Strontium^{90} as the principal material of radioactive contamination. In addition, the extremely small particles containing Strontium^{90} that had been carried into the stratosphere would be gradually reaching the earth.

In contrast to Iodine^{131}, which has a half-life of eight days, Strontium^{90} has a half-life of 28 yr. It would be present in our environment for a long time.

Furthermore, strontium gains entrance into the food chain, where it behaves much like calcium in soils, plants, man, and animals. Some of the strontium in fallout collects on plants and is absorbed. Some washes into the soil and remains in the top several inches of uncultivated lands almost indefinitely. From here, it is taken up into the plants along with calcium.

A close relationship between rainfall and the mean Strontium^{90} content of milk has been demonstrated in both this country and England. Rainfall is the principal mechanism for delivery of Strontium^{90} to the ground from the upper levels of air.

When dairy animals eat contaminated plants, a small part of the radiostrontium—only about 1%—is secreted in milk. A very small part goes into muscles, and a part collects in the bones, where it remains for a number of years. So far, strontium does not seem to be a problem in relation to our water supply.

Strontium^{89} is similar to Strontium^{90}, except for a shorter half-life of 53 days.

Naturally, it is as important to protect a cow's feed supply from strontium as from iodine, but the problem must be attacked differently.

Some protection against strontium could be gained by adjusting crop production practices. For example, adding lime or gypsum to highly acid soils and fertilizer or organic matter to infertile soils could reduce strontium uptake by plants as much as 50%.

ALTERING DAIRY CATTLE RATIONS

Research studies clearly show that the level of Strontium^{90} contamination of milk will depend upon the amount of calcium and strontium in the ration.

In an environment contaminated with Strontium^{90}, rations for dairy cattle can influence

the Strontium^{90} content of milk. For maximum effect, one should, in principle, reduce the plant calcium to a minimum and increase the mineral calcium to a maximum. For example, a diet of grass hay, corn, and inorganic calcium would provide less strontium for the animal than a diet of legumes, clover, lespedeza, or alfalfa, which are good sources of calcium but which would contribute a greater amount of strontium.

There would seem, however, to be little to gain in simply adding mineral calcium to an otherwise adequate diet. First, any dietary modifications would have to be carried out continuously over a reasonably long period of time to permit adaptation of the animal. Secondly, it would be difficult to get a dairy cow to eat more than 200 to 300 g of calcium per day, and higher levels might be inadvisable from the standpoint of the health of the animal.

By adding 2 or 3% $CaCO_3$ or other suitable calcium compounds to concentrate mixtures fed to dairy animals, the average calcium intake would be just about double. As an alternative practice an equivalent amount might be added by the farmer at the manger. This amount of extra calcium should not cause adverse long-time effects, provided enough phosphorus is available in the ration.

Limited field studies indicate that there is a relationship between the economic status of the farm and the amount of worldwide fallout in the milk produced thereon. For example: dairy animals maintained on farms with fertile lands, good pastures, under good animal husbandry and farming practices, will produce milk with less radioactive iodine and Strontium^{90} than the animals reared on farms of a low economic level with poor land and undesirable farming practices.

These studies also show that improving the dairy animal's ration to increase its milk production will also tend to reduce the radioactive iodine content of the milk.

SOIL DECONTAMINATION

There are further countermeasures that could be used against Strontium^{90} if it was present in the soil in amounts that constituted a serious hazard to the health of man. But these countermeasures are drastic—for example, deep plowing to place the strontium below the root zone, removal of ground cover such as mulch or sod, or scraping fields to remove the top several inches of contaminated soil.

DISCRIMINATION

Fortunately, the metabolic processes of both man and animals act to reduce substantially the amount of strontium deposited in the bones of man, compared with the amount originally present in the vegetation and in the soil where it grows.

This protection mechanism is measured by the term discrimination factor and refers to the natural preference that a biological system has for calcium over strontium. Relatively more calcium than strontium is carried along as these minerals move together through the food chain from the soil to the plant, then through the body to their resting place in the bones.

Strontium^{90} and calcium are very similar in behavior, but Strontium^{90} moves more slowly in metabolic processes and across membranes in man and animals. The magnitude of discrimination may be small in a single metabolic process but, by a succession of such processes, each one magnifying the preceding one, substantial discrimination does result.

In milk, the discrimination factor operates twice. The biological system of the cow screens out over 90% of the strontium from entering the milk, and the biological system of man screens out still more of the strontium from entering the bones.

Although 70 to 80% of the calcium in the average diet in this country comes from milk and cheese, calcium from these sources carries less than 50% of the Strontium^{90} associated with our foods. Plant foods—grains, vegetables, fruits, etc.—furnish about 15% of the calcium but, because they are consumed directly, they furnish over 50% of the total soil-derived Strontium^{90}.

The Food Protection Committee of the Food and Nutrition Board, National Academy of Sciences, has recently reported that "Milk has been the single food item most often used for analysis as an indicator of environmental radiocontamination. This is because milk is produced regularly year-round; is convenient to handle, bulk or aliquot; can be obtained so as to represent small or large areas; and does contain the most important radiocontaminants. It must be emphasized, however, that the most important parameter is the level of contamination of the total diet. The use of milk as an indicator food does not imply that a decrease in the consumption of milk would result in a decrease of the total Strontium^{90} intake. Foods substituted for milk would probably result in higher intake of Strontium^{90} because of the higher Strontium^{90}/calcium ratio in such foods."

This point is dramatically emphasized in a study by Kulp and Schulert (Science, May 18, 1962), showing that in 1960 human bones from persons in cities in South America contained levels of Strontium^{90} about one-half as great as those analyzed from cities in the Northern Hemisphere, yet the fallout in the Southern Hemisphere is only about one-fourth that in the Northern Hemisphere. This is attributed to the difference in diet, with a higher milk component in the Northern Hemisphere.

Research on animals indicates that a body well nourished with respect to calcium does not retain as much strontium as the body deficient in calcium.

STRONTIUM^{90} REMOVAL FROM MILK

A pilot plant research project for removing radioactivity in milk is being conducted cooperatively by the Atomic Energy Commission, the Public Health Service, and the U. S. Department of Agriculture. This study was initiated in the fall of 1959. It was justified by the work of Dr. Migicovsky, Canada Department of Agriculture, and scientists of England and the University of Tennessee, Oak Ridge, where radioactivity was removed from milk on a laboratory scale. The present work is being conducted in the Dairy Products Laboratory at the Agricultural Research Center in Beltsville, Maryland, and at the Public Health Engineering Center in Cincinnati, Ohio.

We are justifiably pleased with the accomplishments of this work to date. It was only a couple of years ago that some of the country's leading dairy research scientists were extremely pessimistic about the practicality of removing Strontium^{90} from milk without major changes in the milk composition.

In the newly developed process, milk is passed over an ion exchange resin. This procedure is much like that accomplished in the water softener found in many homes today. A similar process has been used in the past in the production of low-sodium milk.

It was found that the removal of strontium from milk could be increased from 60 to over 90% by first acidifying the milk from the normal pH of 6.6 to 5.4. After its flow through the ion-exchange resin, the milk is then neutralized back to its original pH of 6.6. This is followed by high-temperature, short-time pasteurization, and flash condensation in a vacuum pan to remove the excess water added during the acidification and neutralization. Then the milk is homogenized. Tests show no appreciable change in the chemical composition or in the taste of milk processed by this technique.

The pilot plant studies at Beltsville include a fixed bed resin column installation and a moving bed resin contactor.

The fixed bed resin column installation was designed to process approximately 100 gal of milk an hour. The columns must be regenerated when their capacity to remove radiostrontium falls off. Therefore, any fixed bed installation must necessarily contain a number of columns so that as one column is removing radiostrontium another is being charged or regenerated.

The moving-bed resin contactor handles the problems of resin regeneration, cleaning, sterilizing, and strontium removal in a continuous manner.

The process has been designed so that there is a minimum change in the major components of milk. This gives reasonable assurance that the ion balance in milk will not be affected to any appreciable degree. The process does increase the citrate, potassium, and sodium ions, and removes the stable strontium ions.

More laboratory research is required on several important aspects of the process. While this work is being undertaken, scientists also plan to study the commercial application of this promising procedure.

A study is now in progress to determine the nutritional qualities of the processed milk and the effects of possible changes in composition.

The microbiological problems that might be associated with the resin treatment process are also being investigated.

Determinations must be made on finding out what possible additives the resin could convey to the milk.

Rulings must also be made on necessary changes, if any, in milk regulations, if the dairy product composition is changed by the radionuclide removal process.

Intensive research will continue for modifications and improvements in the removal process that would reduce its cost or simplify its ease of operation.

SUMMARY

In a large measure, responsibility for protecting the Nation's food supply in a nuclear emergency rests on farmers themselves. Since no one knows where bombs might be exploded or where winds might carry the fallout, it is up to farmers everywhere to be prepared to protect their livestock and other agricultural resources.

A farmer needs to know the requirements for safeguarding animals and for producing safe animal food products. Then he can review his buildings and farming practices and be better prepared for protection if an attack comes.

It was pointed out that there are already available on most farms a number of facilities that could help protect livestock and feed. Except in highly contaminated areas, if these facilities were used to advantage following a nuclear attack, farmers would experience much less loss and would be in a better position to produce adequate supplies of healthful food. There are also procedures and practices that the producer and processor can take to provide safe dairy products following an emergency.

There are, of course, many questions that we still cannot answer. Further studies are continuing and should provide increased understanding in this field.

Research is being carried out by the U. S. Department of Agriculture to develop more knowledge about protection from fallout, whether it is created by nuclear attack or extended bomb testing. In addition to the work on the removal of radioactivity from milk, there are four other major research areas, including:

1. The study of the movement of isotopes through the soil and into the plants, and the means by which this movement can be altered or minimized.

2. The study of the movement of radioactive isotopes from contaminated feed to milk, and the means of altering this movement.
3. The effects of both the external and internal emitters on the biological system of the dairy animal.
4. The decontamination of soil.

Other research in both public and private institutions is also aimed at developing methods of protecting against radioactive contamination in our foods. As we have seen, there are a number of methods that can be considered for use under emergency conditions. As the research continues, the fund of dependable knowledge in this field is growing.

Emphasis is made of the importance of making a determined effort to gain new knowledge and to see that good use is made of what we have. This will provide a protective shield that is vital to our dairy industry, to our food resources, and to our national defense.

EFFECTS OF RADIOACTIVE CONTAMINATION OF THE ENVIRONMENT ON PUBLIC HEALTH

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The development of nuclear energy has created a new and uncertain element in man's world. Rapid progress is being made in peacetime uses of this form of energy. On the other hand, there is the possibility of destruction with nuclear weapons if world peace cannot be maintained. Between these extremes, the best and worst possible uses of nuclear energy must be considered.

Since all radioactive materials emit energy which has the power to damage living tissue, it is obvious that attention must be directed toward preventing undue radiation exposure in man. The energy emitted by radioactive materials can affect man in two ways: By radiation originating from a source outside the body, and by exposure resulting from radioactive materials taken into the body. The external radiation hazard is much the easier to control, since we have instruments which can measure the hazard at the time it exists. The internal radiation exposure problem, however, is a much more complicated one.

Most of the radioactive material gets into the body either through breathing or swallowing. A large part of these materials gets into our bodies through the food we eat and, for this reason, internal radiation is a matter of concern to agriculture. The dairy industry is especially interested, since milk is one of the main foods through which certain radioactive substances are ingested into the body. At this point we should consider the sources of radioactivity in milk and other foods.

Sources of radioactivity. Our environment has always contained some radioactivity. Radioactive potassium, radium, and other trace materials occur in varying concentrations in different foods in different areas. In recent years there has been added radioactivity because of man-made radioactive materials. Products of the fissioning of uranium and other materials have been appearing in food.

The fragments that result from the fissioning of uranium atoms are unstable. They achieve stability by giving off excess energy through a process of radioactive decay. Some of these substances quickly lose their radioactivity; others much more slowly. The term half-life is used to relate different radioactive materials, a half-life being the length of time during which any amount of a specific radioactive material loses one-half its radioactivity. Thus, Iodine^{131} has a half-life of eight days, whereas the half-life of Strontium^{90} is 28 yr.

In peaceful applications of nuclear energy, fission products are largely contained. However, these materials are released to the general environment when nuclear weapons are tested above ground.

In detonations of nuclear weapons above ground, large quantities of radioactive materials are deposited in the atmosphere. This material is carried by the air currents and eventually deposited as fallout on the earth, often brought down by rain. These particles can enter man and animals through air inhaled or through drinking water, as well as with food. However, investigations by the Public Health Service have shown, for example, in Strontium^{90}, that less than 10% of the total intake from fallout results from air and water. In general, foods, including milk, are the principal source of intake of fallout radioactivity.

Foods may be contaminated directly by the fallout particles being deposited on the edible parts, or certain of the materials may be absorbed from the soil through the root system of the plant. This radioactivity may reach man either by direct consumption of contaminated

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crops or, indirectly, through the consumption of contaminated forage by cattle, the secretion of these materials in milk, then consumption by man.

The relative contribution of different items in the diet to the total intake of radioactivity by man depends upon the specific radionuclide. Iodine^{131} tends to occur early in fallout and probably reaches man almost exclusively through liquid milk. This is especially true of the critical age-group, infants and children. Other foods do not appear to be important sources of radioiodine, probably because of the period of time which elapses between the contamination and the time the food is actually consumed. Since milk is an essential food for infants and other young children, and the weight of milk consumed by them is high in relation to their body weights, the contamination of milk with radioiodine during the first 60 days after nuclear attack would be primarily a problem of thyroid injury to young children.

Strontium^{90}, on the other hand, can reach man through a variety of items in the diet. Some studies by the Public Health Service and others indicate that milk and other dairy products may account for approximately 50% of man's intake of this radionuclide. Strontium^{90} is potentially the most dangerous nuclide to man that is formed in the fission reaction. Studies on the geographical distribution of the Strontium^{90} released into the atmosphere indicate that the majority has remained in the temparate zone of the northern hemisphere. Strontium^{90} has a long physical and biological half-life and is produced in relatively high yields in the fission reaction.

Chemically, strontium and calcium are similar, since they are in the same group of the periodic table, but physically they differ, since strontium is about two and one-half times as heavy as calcium. There are several discrimination steps against the uptake of Strontium^{90} in its passage from the atmosphere to the bones in man and, apparently, this is due to the difference in physical properties of the two elements.

PHS monitoring programs. As fission products such as Iodine^{131} and Strontium^{90} began to appear in foods in small quantities, it soon became apparent that a system was needed for widespread monitoring of radioactivity. Since April, 1956, the Public Health Service, in cooperation with state and local health agencies, has conducted a nationwide air-monitoring network to provide early information on potential radiation exposure of the population. The system now consists of 67 permanent sampling stations, at which daily samples are collected for a check on gross radioactivity of the air. In addition, a number of special stations monitor radioactivity levels near reactor sites.

In 1957, the Public Health Service undertook to measure the amounts of radioactive fission products in food. Milk was the first item selected to be surveyed. At present the Service maintains a network of over 60 stations representing milk consumed by about 34% of the population. Each sample is a composite of plants supplying not less than 90% of the milk supply of the city where the sample is taken.

Recently, the Service initiated a total diet sampling program at 20 boarding schools throughout the country. It has also contracted for the collection of teenage and infant total diet samples in 30 cities, to be analyzed for radioactive strontium content. In addition, the Public Health Service has a network of 343 stations monitoring general air and water pollution. The Food and Drug Administration has also expanded its program of monitoring levels of radioactivity in food.

All pertinent quantitative and qualitative data obtained from Federal surveillance systems are published regularly by the Public Health Service in the monthly technical report, Radiological Health Data, compiled by the Division of Radiological Health. To reduce the time lag, processed tabulations of gross beta activity in air, water, and precipitation and tabulations of Iodine^{131}, Strontium^{90}, and Strontium^{89} levels in milk are sent to health officers each week. Because of widespread public interest, gross beta activity in air is made available to the public press on a daily basis, and radioactivity levels in milk on a monthly basis. With each monthly release, special effort is made to provide simple, objective interpretations of the data. Many state and municipal health agencies are adopting similar practices.

As we have seen, milk has been used from the very beginning as an index food in the radiation surveillance program. Milk is an important source of information on human intake of many significant radionuclides from the environment. There are several reasons for this: (1) Many of the radionuclides considered to be of principal health interest occur in milk. Indeed, milk is often the most important source of the radioactive material in the diet. (2) Milk and milk products represent a significant part of the diet for all age-groups, and a very large portion of the total diet in infants and children. (3) The production of milk throughout the country at all seasons permits a continuous surveillance program showing both geographic and time variations.

The Public Health Service is fully aware of the difficulties being experienced by the milk industry and realizes that part of this may be due to concern created by the release to the public of data from its pasteurized milk radiation surveillance network. To help give the public a better understanding of the over-all situation, the Service issues frequent statements and press releases, and utilizes radio and television in its efforts to allay unwarranted anxiety about the fallout situation, as it affects

food. Some state and large municipal health departments are following similar practices. The Service is also intensifying its efforts to determine whether a food index system can be devised and developed which would supplement measurements in specific foods. This would depend on development of data which would permit a meaningful estimation of total intake of radioactive materials from analysis of index foods, including milk.

The levels currently present in agricultural products and food are very low; they are, indeed, measurable only because of remarkable developments in instrumentation. In most cases, measurement depends on the character and amount of radiation emitted, and prior chemical separation may not be necessary or possible. Since the analytical procedures are expensive in man-hours and equipment, routine analysis or monitoring of all foods is not currently feasible.

Biological effects of radiation. As we have stated previously, man is now being exposed to increasing amounts of artificially produced radiation. In addition, he has always been subject to some radiation from natural sources. We are constantly being bombarded by cosmic rays from outer space and rays from radioactive materials in the structure of our buildings, in our bodies, and in the food we eat. Man and lower forms of life have developed in the presence of such natural sources in spite of any radiation damage that may have been present.

On the other hand, we know that excessive radiation exposure can bring about sufficient damage to cause death. Our knowledge of radiation effects comes from observations on individuals over-exposed to radiation in the early years following the discovery of X-rays and radioactivity, from observations on patients treated with radiation for various disease conditions, and from animal research.

From these observations we have learned that individuals who recover from the acute effects of large acute doses of radiation, or who have accumulated relatively large doses of radiation, may after many years suffer certain long-term effects. These effects may occur either in the exposed individual, the so-called somatic effects, or in the offspring of individuals exposed prior to the birth of the children, the so-called genetic effects.

Studies have shown that relatively large radiation exposure increases the incidence of certain types of cancer in man. It is also known from experimental evidence that radiation exposure of the reproductive organs prior to reproduction will be accompanied by an increase in genetic mutations. These genetic mutations are in the main harmful, and result in an increase in stillbirths, fetal deaths, and in congenital malformations.

These effects have been noted after relatively large doses. The effects of small doses are less clear. The evidence on genetic effects indicates that even small doses of radiation result in an increased risk of genetic mutations.

Any biological effects from the particular problem of internal radiation, mostly through the ingestion of foods, probably will depend largely upon the radioisotope ingested. Some of these radioisotopes tend to concentrate in certain areas or organs of the body. Although control activities are applicable to all radionuclides occuring in the environment, particular attention is devoted to Iodine^{131}, Strontium^{90}, and Strontium^{89}. Each of these has an organ-selective characteristic: the thyroid gland for iodine; bone for Strontium^{90} and Strontium^{89}. Cesium^{137} is another important product of nuclear testing, but it does not localize in any particular portion of the body. Radium^{226} is a naturally occurring radionuclide, with a selectivity for bone.

The potential hazard of these radioisotopes depends largely upon their metabolic behavior in the body. Iodine^{131} is well absorbed, collects in the thyroid gland, and is fairly rapidly removed by physical decay and biological action. The two radioisotopes of strontium are only partially absorbed, but most of that which is absorbed is deposited in the skeleton. Because it has a half-life of only 53 days, Strontium^{89} soon disappears through physical decay. Strontium^{90} is removed more slowly by physical and biological processes, and substantial amounts of any Strontium^{90}, once deposited, will be present throughout the life of the individual. The rate of removal would be faster in a growing individual than in a mature one. Cesium^{137} is well absorbed into the body, collects in muscle and other soft tissues, and enters cells in a manner similar to that of potassium. It is removed from the body by biological action, with a half-life of removal that has been estimated at about 140 days in man.

Among the short-life isotopes, Iodine^{131} stands out as the most potentially hazardous. Iodine^{131} is a high-yield radionuclide and is so selectively concentrated in the thyroid that it will generally result in the highest tissue dose in early fallout. It poses a particular problem with respect to children, since it enters the body largely through fresh milk. Furthermore, children are not only more sensitive to the effects of radiation on the thyroid than adults, but the radiation has greater effect because of the relative size of the glands. The infant thyroid weighs only about 2 g, as compared to the adult thyroid, which weighs around 20 g.

The radioisotopes of greatest long-term concern are, of course, Strontium^{90} and Cesium^{137}, which closely resemble in chemical properties the physiologically vital elements, calcium and potassium, respectively. However, since Cesium^{137} has the relatively short biological half-life of 140 days, Strontium^{90} remains as the fission product of greater long-term concern.

Radioactive isotopes of strontium when deposited in the bone in very large amounts have been shown to produce serious consequences. Animals given large amounts of radiostrontium have been observed to develop bone cancer. This isotope is deposited in the bones of children at a faster rate than in those of adults, since the turnover of bone is greater in the growing skeleton. But since radiostrontium is assimilated in the bones, it constitutes essentially no genetic hazard, for its radiations do not reach the reproductive organs.

Radiation protection standards. Once it was understood that excessive radiation could have adverse effects on the body, standards for acceptable exposure were sought. In the early years these standards were based upon the concept of a tolerance dose, defined as a dose which, when received by exposed individuals, would produce no deleterious biological effects. In more recent years, however, evidence has been accumulating which casts doubt on the assumption that it is possible to determine a safe dose, particularly with respect to the genetic effects of radiation.

Thus, the establishment of radiation protection standards now involves the acceptance of a certain risk of deleterious biological effect with any dose as a standard. Acceptance of such a risk is based upon benefits to be derived. The benefits, such as better health through the use of medical and dental X-rays or sources of power from atomic energy, must outweigh the potential risk of radiation exposure. Basic radiation protection standards involve scientific data, but the final decisions are matters of judgment. Such decisions involve passing judgment on the extent of possible health hazards society is willing to accept in order to realize the known benefits of radiation. Man cannot entirely dispense with the use of ionizing radiations and, therefore, the problem in practice is to limit the radiation dose to that which involves a risk that is not unacceptable to the individual and to the population at large.

The Federal Radiation Council was established in 1959 to advise the President on radiation health matters, and on the policy decisions involved in basic radiation protection standards. Members of the Council are the heads of the agencies most significantly involved with radiation, the Department of Health, Education, and Welfare; the Department of Defense; the Department of Commerce; the Department of Labor; and the Atomic Energy Commission.

Recent recommendations by the FRC provide guidance in connection with environmental contamination with radioactive materials. The guidance is in terms of three ranges of transient rates of daily intake, of radioactive materials, and the appropriate actions which should be taken within these ranges. This system of ranges is intended to replace the use of a single numerical value. A continuous, or average daily intake, can be calculated which is estimated to result under specified conditions in whole body or organ doses equal to specified radiation protection guides or radiation dose levels. However, actual daily values fluctuate and the graded average intake concept is more practical.

The Federal Radiation Council has recommended that measures limiting intake of radioactive materials should be considered when indications are that levels averaged over a year will be within Range III. For Strontium^{90} this is 200 to 2,000 $\mu\mu c$ total daily intake. Range III for Iodine^{131} intake is 100 to 1,000 $\mu\mu c$ per day, averaged for a year.*

Some efforts to lower intake. Governmental and other research groups for several years have been studying methods for reducing the intake of radioactive substances. Some of these show promise, especially for use in localized situations such as in nuclear reactor accidents or near nuclear test areas.

Much of this research is aimed at future imponderables which may increase environmental radiation many times over its present levels. Although fallout from the Russian tests has increased slightly this spring and more will be added as nuclear testing continues, values of Strontium^{90} are not expected to be high enough to justify serious consideration of measures to reduce the intake of this radioactive substance. Nevertheless, the present situation calls for a program of constant surveillance and continual research to extend the boundaries of our knowledge of the biological effects of radiation and of measures which might be taken to reduce radioactivity intake by the population, if necessary.

Of the two radionuclides, Iodine^{131} and Strontium^{90}, the former is the most easily controlled, since it decays rapidly and within 35 days will be reduced to a small fraction of its original value. Storing of foods such as occurs in the processing and marketing of dry or condensed milk results in essentially complete decay of the radioactive iodine. Thus, by switching from fresh milk to dry or evaporated milk the physician has a simple method for lowering intake

* Author's Note: According to a statement released September 17, 1962, by the Federal Radiation Council, the Radiation Protection Guides established for peaceful application of nuclear energy were not intended to set a limit at which protective action against nuclear test fallout should be taken. When applied to fallout, the guides can be used as an indication of when there is a need for detailed evaluation of possible exposure risks and when there is a need to consider whether any protective action should be taken under all relevant circumstances. In the statement, the Council advised that individual fallout situations require individual evaluation before specific action is taken and that, on request, FRC would provide advice to local authorities on specific situations.

of radioiodine by young children. Other methods for lowering intake of this radionuclide are under investigation.

Because of its long half-life, radiostrontium presents a much more difficult problem in finding suitable countermeasures. Fortunately, there is a protective mechanism termed the discrimination factor. As the strontium and calcium move through the food chain from the soil to the plant, through the body of the animal to the milk, and then through the body of man to its resting place in the bone, relatively more calcium than strontium is left. The cow's biological system plays a large part in screening out the strontium present in vegetation.

In addition to this natural discrimination between calcium and strontium, ways are being sought to further lower the intake of this radionuclide. For example, there is evidence, not yet fully accepted, that under usual feeding practice increased levels of stable calcium will decrease the retention of ingested radiostrontium. The addition of calcium to the soil may help lower the uptake of strontium by plants, since it has been found that in regions where soil and water are low in calcium, both calcium and strontium will be more readily taken up.

These measures need much additional study before they could be considered for use. However, among possible methods studied for use in emergency situations, one may soon be at a stage of development where it could be adopted. This is the ion-exchange process for the removal of strontium from milk.

Development of the ion-exchange process for removing radioactive strontium from milk was undertaken as a standby measure more than 2 yr ago, in a cooperative project participated in by the Public Health Service, the Department of Agriculture, and the Atomic Energy Commission.

Since the initiation of the project, substantial progress has been made. A pilot plant was put into experimental operation at Beltsville, Maryland, late last year. Milk contaminated with radiostrontium is passed through a bed of synthetic resin charged with a mixed solution of ions—calcium, potassium, sodium, and magnesium—present in milk. As the milk passes through the column, the strontium ions in the milk change places with the ions on the resin.

An important feature of the process is that the acidity of the milk is adjusted, before passing it through the resin column, from its normal pH of 6.6 down to 5.4 or 5.3. At the normal pH of milk, most of the strontium is bound by other milk constituents and is slow to be exchanged. At the low pH, strontium is largely converted to a soluble and more readily exchangeable form.

About 90% of the radioactive strontium in milk can be removed. The process has been developed to secure maximum removal of Strontium^{90} without inducing significant adverse changes in the milk's chemical composition, physical stability, or flavor. The ion exchange process must now be evaluated in a commercial scale installation and additional research conducted on nutritional and bacteriological aspects.

Although the possible consequences of any countermeasure must be carefully weighed against the possible gains, it is felt that the ion-exchange process shows great promise as one of several stand-by measures that could be used if there were a clear need. It is believed that such a process would become important in the event of a nuclear attack. In addition, it might be used in case of an accident in an atomic energy installation resulting in release of large amounts of radioactivity, or a vastly increased contamination of the atmosphere as the result of a nuclear testing program of far greater magnitude than any country has undertaken to date.

However, at its present stage of development and at present and foreseeable levels of radioactive strontium, use of the process would not provide sufficient benefit to counterbalance the health risks inherent in a major disruption of milk production and distribution.

Countermeasures, however, should not be a purely governmental concern. Industry, too, should be giving thought to development of procedures that could be invoked within its structure in the event of emergency. The technical and organization resources of the dairy industry, for example, could be of considerable assistance in framing standby measures that would have the merits of feasibility and enforceability.

It is important to emphasize that drastic measures to control the air, water, and food supplies of large population groups might hold threats to health more immediate and serious than the increased risk from the radiation exposure accompanying nuclear tests. Because of this fact, federal, state, and local health authorities, and other appropriate officials and scientific groups, are concentrating their attention on particular problems that may develop in particular areas. Before any action is taken, the consequences of such actions would be weighed carefully against the risk of radiation exposure.

Special caution should be exercised in placing any restrictions on the use of milk. Accusing fingers have been pointed at milk without proper regard to the implications involved, with the result that the position of milk as an essential foodstuff, in the opinion of the American public, has been questioned. The Public Health Service views with concern the actions of well-meaning but unqualified individuals or groups who are urging curtailment of milk consumption. Any disruption of dietary patterns, particularly in children and infants, may have serious health effects.

Milk should continue to be the outstanding source of calcium in the diet because the calcium it supplies has had much of the strontium present in vegetation screened out by the biological system of the cow. Furthermore, results of research with animals indicate that a body well nourished with respect to calcium does not retain as much strontium as a body deficient in calcium. It is apparent, therefore, that if the major source of dietary calcium of the United States were shifted away from milk to cereal or vegetable products, the population might actually be increasing the relative level of Strontium[90] in the diet.

The atomic revolution has created many new and complex problems, including problems in public health. A desirable balance between radiation hazards and radiation benefits can be maintained in the future through a rapidly strengthening system of nationwide control. However, our best protection is a well-informed public, with federal, state, and local governments alert to the problem. Vigilance is still our best protection.

OUR INDUSTRY TODAY

ROLE OF STATISTICAL INFORMATION IN CHANGING DAIRY INDUSTRY [1]

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If you were given the choice of one and only one statistical series indicative of supply in the dairy industry, what would it be? Doubtless, different responses would be forthcoming, in light of the varied interests represented in this group. Some would think of cow numbers, others production per cow, total milk produced, or possibly feed supplies, pasture conditions, maybe butter or cheese output. Consideration of the question may impress you with the fact that no single indicator could possibly satisfy the current need for information. One might also be impressed with the variety of relevant statistical series available and surmise why or how they come to be. A conclusion might be reached that they are so numerous as to be confusing.

A first consideration in an evaluation is to visualize the purposes the statistics are intended to serve. Dairy statistics, like crop and livestock estimates generally, serve a varied clientele with diverse primary and secondary uses, so the statistics must be regarded as multipurpose data. Among the purposes are to enable farmers to bargain intelligently for prices or to plan production changes; to enable businessmen to manage procurement, stocks, transportation, distribution, and pricing on the basis of informed decisions; to enable legislators to exercise judgment in establishing agricultural policies; to provide administrators with statistics needed to conduct governmental programs; and to give economic analysts the data required to diagnose the ills of an industry and suggest corrective measures, as well as to prepare outlook information regarding what to expect in the way of changes in supplies and prices. Multipurpose data run the risk of not being completely satisfactory for any single use. This situation confronts the user with a problem of becoming acquainted with the different series and selecting those most suited to the need at hand.

Another consideration is the feasibility of gathering data that can be summarized with an acceptable degree of accuracy and timeliness. At one time the ability to get any data at all was a determining factor, and this probably accounts for the fact that the oldest continuing series on dairying is on the numbers and values of milk cows and heifers 2 yr old or older, which was started in 1867. Currently, this problem might be better stated as the ability to acquire the data at reasonable costs; and costs must be measured in terms of the ability and willingness of those supplying the original data, as well as the monetary expenses required to assemble the data.

A third consideration in evaluating a new or proposed series is the net gain in information obtainable in light of the already available series. One facet of this point is the degree to which the new data can contribute to the confirmation of accuracy or the internal consistency of a total estimating program. Another facet is whether the additional data improve the ability of users to forecast. A single statistic indicative of a current supply situation, no matter how accurate, is rarely useful in and of itself. The user almost invariably needs comparable data for past periods, in order to judge the current situation, and he is most likely to want supporting data to permit him to judge what is ahead and project his plans accordingly.

ORIENTATION OF DAIRY STATISTICS

With these background points in mind, a strong case can be made that milk production estimates might be regarded as a central focal point for judging dairy supply. A report entitled Milk Production, issued on the 11th or 12th of each month, gives estimates of milk production during the preceding month for 36 states and for mainland United States. Annual estimates of milk production for the United States have been issued since 1930 but, as you may note, separate milk production estimates by months are still unavailable for 14 states.

Before passing judgment on whether this is a good record for such an important statistic, it may be well to consider how the estimates are derived. Estimates of monthly milk production are the product of estimated numbers of milk cows multiplied by estimated production per cow. For current estimates, the rate per cow is based on the average rate reported by farmers voluntarily in mail surveys each month. This indication is adjusted for the effect of sample selectivity resulting from over-representation of reports from high-producing herds.

The estimates of milk cow numbers are based on semiannual surveys of a sample of farms from which data are obtained on various classes of livestock on hand. By classifying dairy animals into milk heifers under 1 yr of age and

[1] Presented at the 57th Annual Meeting of the American Dairy Science Association, University of Maryland, June, 1962.

1 to 2 yr old, the data also give some idea of future expansion or contraction in the milk cow population. In some states data from a State Farm Census or Assessment of Livestock are important indicators of annual changes in milk cow numbers.

The milk production estimate also needs confirmation on a short-term basis and still further verification as benchmarks can be established. Data for these purposes are obtained from looking back toward the farm, to examine estimates of factors of production, and from looking forward to the markets, to examine evidences of disposition and utilization of milk.

SUPPORTING PRODUCTION DATA

Accordingly, you may note that the monthly production report called Milk Production includes related statistics such as data on pastures and feeding. Reports issued from April through November give pasture condition data expressed as a per cent of normal. In alternate months throughout the year the reports give data by states on the average quantity of grain and concentrates being fed daily to milk cows. Other relevant data include the estimated value of concentrate rations per hundred pounds computed by regions, and the milk-feed price ratio which measures the relationship between milk prices and feed costs, to give an indication of the favorable or unfavorable position of dairymen. In addition to these monthly data, certain issues of the report, Milk Production, include more detailed data by states on quantities, kinds, and value of grains, concentrates, and roughage fed to milk cows.

Behind these production data directly related to milk is a whole program of farm statistics that has a distinct bearing on current and prospective milk production. For crops, the cycle of estimates begins each year with intentions to plant, followed by crop acreages and yield forecasts during the growing season, and ends with harvested acres, production, and final utilization of the crop. A wide range of other estimates is made throughout the year, including livestock inventories, animals on feed, output of livestock products, stocks on hand, prices paid and received by farmers, and farm labor statistics. These estimates are released through the medium of about 700 reports per year.

For longer-term benchmark data for crop and livestock estimates, dependence is placed on the Census of Agriculture, which covers all farms. Important data relevant to milk production estimating that are revised every 5 yr, when necessary, to bring them in line with the Census, include numbers of farms keeping cows and numbers of cows on these farms classified by age groups.

MARKET DATA

A wealth of extremely pertinent data is collected from marketing agents. This is possible because most milk is delivered to processing plants, which are relatively few in number compared with farmers, and which usually keep good records. There are several other advantages that make it easier to collect data from milk processing plants than from dairy farmers. In most states there are central licensing agencies requiring dairy plants and establishments to be licensed before they can operate. These provide reliable sources of names and addresses to keep the lists current for mailing questionnaires. Also, in 33 states the U. S. Department of Agriculture has cooperative agreements with state agencies which are generally supported by a state law requiring dairy plants and establishments to report their operations at least once a year. In the market milk areas a large share of the milk is subject to regulation by Federal milk market administrators or State Milk Commissions, which also become valuable sources of data. As a result of these circumstances, and the persistence of dairy statisticians in urging plants to report, a high proportion of total milk marketed and manufactured is covered in the current estimating procedures.

Market data begin with receipts of milk and cream from farmers reported by dairy plants which become the most important check data for annual revisions of monthly production estimates by states. Dairy plant reports also provide the principal bases for estimates of quantities of milk and milk fat in cream sold by farmers, average prices received by producers, and cash receipts from these products. These estimates are published by states each April for the two preceding years in the report entitled Milk Production, Disposition and Income. This report also includes annual estimates of quantities of milk used for various purposes such as manufactured dairy products, fluid consumption, and farm uses.

MARKET MILK DATA

Of the 125 billion pounds of milk produced in the United States in 1961, about 46% was used for fluid purposes, such as bottled whole milk and cream. The basic publication for the fluid sector of the dairy industry is the monthly Fluid Milk and Cream Report. Developed in 1918, it has provided a permanent record of prices of fluid milk and cream items at both producer and consumer levels since that date. At the present time prices are reported for about 160 markets each month. The report carries tables on dealers' buying prices for milk for fluid use, as well as price quotations for gallons, half-gallons, and quarts at wholesale, at homes and at stores. An average of dealer-buying prices for all 160 markets and an average of single-quart home delivery prices in 25 markets are prepared each month. For 38 of

the markets, 17 other quotations, including price quotations of special grades of milk, cream, and Cottage cheese, are prepared monthly. In recent years, a special table has been prepared to show blends of class prices including premiums in Federal Order markets and blend prices in state and unregulated areas. The report also includes, on a monthly basis, sales of fluid milk and cream items in 66 Federal Order and 6 state marketing areas and receipts of milk from producers in 76 Federal Order and five state areas.

Supplementing the monthly publication is a Fluid Milk and Cream Consumption bulletin, which has been published every other year. The most recent issue was released last month as USDA Statistical Bulletin 312. Starting in 1942 with 19 markets in the Northeast, it has expanded to about 80 markets in all sections of the country. Annual per capita rates of consumption are prepared for 12 individual items by market for 63 Federal Order Markets, six areas under state milk control, and two other areas. Statistical Bulletin No. 312 carries a compilation for the years 1950-59; and the May, 1962, Fluid Milk and Cream Report includes the data for 1960 and 1961.

MANUFACTURED DAIRY PRODUCTS DATA

About 50% of the total milk production in 1961 was used in manufacturing dairy products. The collection of data on the production of manufactured dairy products was started in 1917, to give officials of the Federal Government concerned with wartime food problems an accurate picture of the supplies of dairy products available for consumption by civilians and our armed forces at home and in Europe. After the end of the war, this work was not only continued but expanded during the ensuing years to satisfy continuing demands for data on production, prices, sales, and stocks of dairy products in general. Today, manufacturing operations of one kind or another are carried on in over 7,000 plants in the United States. In addition, frozen products are made by over 33,000 counter-freezers or small retail establishments. Data are gathered each year from these 40,000 plants and establishments for approximately 49 different commodities, such as butter, various types of cheese, condensed and evaporated products, dry milk production, and frozen products. This information is published in the Department's annual bulletin, Production of Manufactured Dairy Products, in July of the year following the year to which the data relate.

Butter and cheese output has been reported monthly for major states and the United States since the mid-1920's in the publication Production of Creamery Butter and Cheese. These estimates are based on a large sample of plants drawn from the complete anual survey of manufactured dairy products and are published about the end of the month following the month to which the data apply. Evaporated, condensed, and dry milk products reports are also based on a large sample and published with about the same time lag as for creamery butter and cheese. The Evaporated, Condensed, and Dry Milk Report, issued in Washington, includes data on production, manufacturers' stocks, and manufacturers' prices, and also classifies dry whole milk prices by size of package. It also includes manufacturers' selling prices of evaporated milk per case, by regions, as well as prices paid farmers for milk by condenseries, by major states.

A monthly report entitled Production of Ice Cream and Related Frozen Products is issued from our Chicago Dairy Office. This includes the estimated production of ice cream for major states and the United States and production of ice milk, sherbet, Mellorine-type frozen desserts, and water ices for the United States. These estimates are based on sample reports from plants and represent substantial segments of the industry. A monthly report, Production of Cottage Cheese, is now being issued showing Cottage cheese curd and creamed cheese production for the United States.

Since both creamery butter and American cheese are traded on the Chicago Mercantile Exchange, weekly reports of production on these commodities were developed in Chicago. These show quantitative United States production each week for the week ending the previous Thursday night and are issued for butter every Tuesday morning and for American cheese every Wednesday morning. They also give percentage changes from the preceding week, the same week a year ago, and a 5-yr average for the major geographic divisions and for such important producing states as Wisconsin on American cheese, and Minnesota, Wisconsin, and Iowa on creamery butter.

Another weekly report issued from our Chicago office is the American Cheese Warehouse Report, which shows the receipts of American cheese according to styles in Wisconsin and in some of the major geographic regions, and also stocks, by styles, in Wisconsin. This report is issued on Wednesday and covers data for the preceding week ending on Saturday. One of the major uses of this report is to indicate any shift in the various styles of American cheese being manufactured.

All of the data indicative of milk utilization must have a logical consistency with the milk production data and also be internally consistent in accounting for the complete disposition of milk fat and skimmilk. This can be depicted schematically by the analyst. The necessity for maintaining a rational explanation for every substantial change in dairy product output, in terms of where the milk or milk fat came from or went to, imposes rigid dis-

cipline upon the dairy estimator. It also helps the dairy analyst to assess the validity of the estimates and forecasts.

Turning again to prices, we issue a monthly report from Chicago entitled Milk Prices Paid by Creameries and Cheese Plants. This is a report obtained from manufacturing milk plants showing prices paid for milk per hundredweight and the fat test of the milk. These data are shown by states for milk used for American cheese and butter and creamery byproducts and by the United States only for Swiss, Brick, Munster, Italian, and all other varieties of cheese. The price series on milk for creamery butter and byproducts, American cheese and milk for canning are combined, to arrive at a price which forms the basis of the parity equivalent for manufacturing milk. This combined price is then projected to the current month and used not only in the parity calculations on milk but also as a base for pricing surplus milk in the Federal Milk Market Orders along the East Coast. In addition, we issue a Minnesota-Wisconsin manufacturing milk price estimate which is used in some 40 markets in the Mid-West as a base for pricing both fluid milk and other classes of milk under the Federal Milk Marketing Orders.

Prices received by farmers for fluid market milk, manufactured grade milk, and all milk sold wholesale to plants and dealers during the previous month are published monthly in Agricultural Prices by states, geographic divisions, and for the United States. National estimates published on prices received for all milk sold by farmers at wholesale, and for milk fat sold in cream, are shown by states and regions for the current month.

STOCKS DATA

Except for the weekly American Cheese Warehouse Report, we have been looking mainly at the various reports dealing with the production, utilization, and prices of milk and manufactured dairy products. Another factor important to the dairy supply situation is the stocks of storable products on hand. The volume of holdings is closely related to current and prospective prices, so producers and distributors are anxious to know whether stocks of dairy products are larger this year compared with last year, and a 5-yr average. To help supply this information, the Statistical Reporting Service issues a Cold Storage Report each month. In it one can find statistics on the amount of food held under refrigeration for some 80 different food classifications. Of particular interest to the dairy industry are the statistics on fluid and plastic cream, butter, condensed milk (both sweetened and unsweetened), evaporated and condensed milk (case goods), American cheese, Swiss cheese, and other natural varieties of cheese. Not only are national stock figures shown but also their distribution according to regional locations and, in the case of butter and American cheese, a breakdown by States.

The first Cold Storage Report was released in 1914, but it was not until 2 yr later that dairy products were included in this national report. The purpose of the Cold Storage Report is to enable both buyer and seller to be equally informed in the market place. More recently, this reporting program has taken on another role; namely, providing information for certain defense planning programs assigned to the Department of Agriculture. Based on individual reports for approximately 3,000 plants, which include public, private, and semiprivate facilities, and reflecting the refrigerated capacities in each, the program gives us a fairly good idea of the vulnerability profile of the industry and the stock situation in metropolitan areas and rural areas as well.

Market data on milk and dairy products in the aggregate provide the means for keeping one's finger on the pulse of the dairy industry as milk flows through numerous channels from farms to consumers. The statistics quickly reveal seasonal and trend changes occurring in a dynamic industry influenced by new technologies in farm production, dairy processing, and distribution methods; and by changing habits of living and patterns of consumption. They are, therefore, essential to farmers and businessmen for gaining understanding of the reasons for the changes and for making decisions in response to them; and also to legislators and government administrators for devising policies and programs to facilitate adjustments to changing economic conditions.

This review of available data on the dairy industry has stressed the variety of statistical series, the relationships between them, and how the estimates are developed. They are not complete, entirely accurate, nor exhaustively used. The Statistical Reporting Service is continually striving to improve these conditions and solicits the help of extension specialists in these endeavors. Encouragement given to crop reporters and plant operators to fill out and return the mail questionnaires promptly will be of direct aid to SRS. Educating the farmers with respect to the data available and how they can be used to advantage is of indirect aid in getting the cooperation of reporters. This approach also contributes directly to the accomplishment of your own mission.

The first step is to become familiar with the reports yourself. To facilitate this, copies of each of the ones referred to are available here for examination. If any of them provides a statistic you can use in your work, arrangements can be made for you to receive it by completing one of the forms provided for your convenience.

WHAT SOLIDS SHOULD BE ADDED TO FLUID DAIRY PRODUCTS?

Theoretical Considerations

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The practice of adding extra milk solids to fluid dairy products is relatively new. The use of solids-not-fat in the formulation of nonfat and low-fat fluid milk products has received considerable attention (7, 9, 15, 17). In recent years, much interest has been shown in the possibility of standardizing regular fluid milk for SNF content. While fat standardization of fluid milk has been accepted for many years, this new concept of double standardization is receiving increasingly more favorable attention.

The decrease in per capita milk consumption in recent years has accelerated the need for a re-evaluation of our present system of products and product marketing. Increasing the palatability of our fluid milk products offers one means of encouraging more milk consumption. The need for uniformity in flavor is all-important. The addition of SNF offers an excellent means both for improving our product quality and also for lowering our surplus milk solids (17).

The addition of solids to nonfat and low-fat fluid milks will be emphasized in this discussion. The fortification of fluid milk with extra solids may well present problems from the standpoint of regulatory agencies and health department officials. These will not be discussed at this time. However, some of the theoretical considerations, where their usage is concerned, will be presented.

What solids should be added to fluid dairy products? Or perhaps we should say, "What solids are available for addition to fluid dairy products, if desired and permitted?" We have a choice of products we might use. They are all by-products of our normal dairy processing operations and are available in grade classifications for such a purpose. The main products are: (a) nonfat dry milk (NFDM), (b) lactose, (c) dried whey, and (d) dried buttermilk.

Nonfat dry milk has been used for increasing the solids content in ice cream mixes for many years. It has also been used to help build up the solids content of nonfat fluid milk. Lactose has received attention recently as a good additive to supplement and/or replace NFDM. This discussion will deal primarily with the use of these two products for the solids fortification of nonfat and low-fat fluid milks.

In discussing the theoretical considerations involved in the use of extra solids, let us briefly review some of their advantages. The over-all advantages for using NFDM may be listed as follows (1): (a) requires no refrigeration in storage, (b) requires a minimum of storage space, (c) economical to purchase, (d) uniform in composition, (e) uniform in flavor and texture, and (f) available year-round. Increasing the nonfat milk solids content in fluid dairy products improves nutritional value as well as appearance and palatability (1, 5, 7, 9, 16).

Advantages for using lactose as a source of extra solids may be listed as follows (17): (a) lower ingredient cost, (b) improvement of flavor, and (c) development of a fuller, smoother body. In addition to the above, items (a), (b), and (d), listed for NFDM, would also apply for lactose.

Flavor considerations and consumer acceptance. Flavor is one of the most important factors in the successful marketing of most any foodstuff. Milk is no exception. Milk fat plays a major role in giving milk the rich, full-bodied taste that we know so well. Solids other than fat also contribute to the flavor of milk. Variations in the SNF level of milk are not uncommon (6, 13, 16). A low SNF content is going to be more noticeable organoleptically in nonfat or low-fat milk than in whole milk because of the absence or low level of fat. The weak, insipid, watery, flat taste of such a milk can be improved considerably by the addition of milk solids.

The addition of NFDM to nonfat and low-fat milk has a very beneficial effect, both with respect to flavor and body (7, 16). Holland and Winder (9) found the addition of 2% NFDM or 3% condensed skim to nonfat fluid milk to be most desirable. The addition of 0.5% milk fat to a solids-fortified nonfat milk improved the product considerably. Their report confirms the feasibility of processing a fortified-modified skimmilk that is nutritious, delicious, and economical.

What is the potential for modified/fortified nonfat milk? Weckel (15) summarized his findings by saying that: (a) There is a market for fat-free milk, (b) this market is above and beyond that now being administered by the dairy industry, (c) this product is easily processed and acceptable to consumers, and (d) such a product can be enhanced nutritionally by fortification.

Results obtained at Clemson (4) suggest the following:

(a) Nonfat fluid milk without fortification lacks flavor. The product tends to be too thin or watery and tastes flat and insipid.

(b) The addition of NFDM is very beneficial. Approximately 1-1.5% seems best. If 2% solids or more are added, a strong milk powder

flavor becomes evident. Less than 1% added solids is better than none, but not as desirable as the optimum recommended.

(c) With 2% fat milk, the addition of 2% NFDM resulted in a fuller flavor and better body than either the 2% fat milk itself or the NFDM fortified nonfat fluid products.

Foust (7) reported that of 320 consumers, 296 preferred a modified product (fat 1.5%; lactose 7.8%; protein 5.8%) over regular homogenized (fat 3.8%; lactose 4.7%; protein 3.5%) and Golden Guernsey milk. Not one preferred the Guernsey milk; 16 picked the homogenized and eight picked a product that was fat-free with added solids.

Custer et al. (5) concluded that it was highly desirable to double-standardize milk (fat and SNF) to improve its nutritional value, uniformity of flavor, and acceptability. The consuming public is becoming increasingly conscious of the importance of proteins in nutrition—hence, SNF fortification should meet with favor. This should hold true especially where a corresponding decrease in the fat content is contemplated.

Stull and Hillman (13) made consumer acceptance observations on milk beverages of varying fat and SNF content. Threshold taste tests indicated that many people could 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.

Lactose addition to milk has been recommended (18) and practiced (7). Data presented by Foust (7) show that the lactose content was increased by about 3% and the protein content by 2% with good results. Published literature pertaining to pure lactose fortification is scarce. Studies at Clemson (4) have shown that the addition of about 1% lactose to nonfat milk or a 2% fat milk is optimal. When 2% lactose was added to these products, they were criticized as being too sweet.

Specific advantages claimed for the use of lactose in milk solids fortification are (18): (a) elimination of chalky, powdery taste, and staleness of ordinary modified skimmilk, (b) increased sweetness for greater consumer acceptance, (c) masking of the vitamin aftertaste of fortified skimmilk and the undesirable serum solids flavor. To attain these advantages, recommendations (18) have been made for the addition of 1% lactose and 1% NFDM to nonfat fluid milk. No specific data have been presented in substantiation of these recommendations.

Keeping quality. The keeping quality of nonfat or low-fat milk products can be maintained at a satisfactory level. The addition of extra solids usually necessitates departure from standard processing procedures. Use of higher pasteurization temperatures will result in putrefactive type organisms predominating in the final product. Hence, post-pasteurization contamination is extremely important.

Light can have a destructive effect on the vitamins in milk. This is particularly significant in vitamin-fortified milks because of its labelled potency and the fact that sales promotion and dietary use hinges on the presence of the declared factors. In addition to the effect of light on vitamin destruction, the flavor of milk is often impaired. The types of solar-induced flavors most commonly encountered are oxidized and sunlight (solar-activated). Flavor changes are much more readily detected in bland products such as plain nonfat milk. Solids fortified milks have the advantage of masking a lot of these flavors unless they are severe. Brown bottles have been recommended to preserve vitamins and flavor and used in certain markets. Use of milk cartons has helped considerably in preventing light-induced flavors in milk.

Holland and Winder (9) reported that modified skimmilks held up well in storage. They found a decrease in cooked flavor after seven days of storage at 35 F. The seven-day product was slightly superior in flavor to the one-day-old product.

Cultured buttermilk. The use of NFDM in the manufacture of cultured buttermilk has the following specific advantages (1): (a) the body and viscosity of the product can be controlled by varying the solids content of the reconstituted or fortified nonfat milk, and (b) reconstituted or fortified nonfat milk is uniform in composition and, hence, produces more uniform cultured buttermilk.

Cultured buttermilk with 11% nonfat milk solids will have more flavor and body than that with 8.5-9.0% nonfat milk solids. The addition of 1% or more of milk fat as homogenized cream will also improve the flavor of the cultured buttermilk. Flavor will usually improve during the first two days of storage in the bottle if held below 40 F.

The higher the heating temperature within the range of 185-195 F, and the longer the holding time, the heavier will be the body of the cultured buttermilk. Increased solids content will also help increase the viscosity. In addition, higher acidity development helps to increase the body. Increased solids content helps reduce wheying-off in buttermilk.

Use of lactose as a source for extra solids in buttermilk has received recent attention. Some of the advantages proposed may be listed as follows (18): (a) insures more uniform culture performance, (b) improves flavor, (c) develops smoother body, and (d) decreases wheying-off. In support of the first advantage, the argument is given that extra lactose promotes growth of both flavor- and acid-producing organisms. It is hard to conceive why the extra lactose should be so beneficial, especially when it is known

that only about 25% of the normal lactose content in milk is consumed by the ordinary lactic fermentation (8).

The citric acid content of milk is quite important for flavor-producing cultures. In flavorful cultures all the normal citric acid may be consumed (8). Hence, milk that is low in citric acid may cause lack of flavor and aroma in cultures. The addition of SNF would help to remedy this, whereas pure lactose would not be very beneficial. The addition of 0.2% of citrate is often practiced to help in the development of more flavor and aroma in cultures.

Much remains to be learned about the nutritional properties of milk and the requirements of specific cultures. Research in this field has implicated enzymatic digests (2) and trace minerals (8) as playing possible roles in bacterial metabolism. While average nutritional variations in milk may not directly account for culture failures, it is reasonable to assume that increasing the solids content with top-grade SNF might help improve culture activity in some instances. The addition of pure lactose would only increase an already over-abundant supply of this commodity.

Chocolate drink. Solids fortification of chocolate drink is highly desirable. A good-quality chocolate drink can be made by proper formulation of milk, milk solids, chocolate, sugar, and stabilizer. The chocolate-sugar-stabilizer formulation need not be discussed at this time, since it generally involves special formulas recommended by chocolate suppliers. The addition of 2% NFDM was found to be most desirable for a nonfat chocolate drink (4). A nonfat milk base of 11% solids has been recommended by ADMI (1). The addition of 1.0-2.0% milk fat helps to make the chocolate drink formulation more mellow and full-bodied. Nonfat chocolate drink with 2% NFDM was considered almost equal in flavor and body to a 2% fat chocolate drink with no extra NFDM (4). A 2% fat plus 2% NFDM chocolate drink was judged to be slightly rich and slightly heavy, but was described as very good.

Use of lactose for the solids fortification of chocolate drink has been proposed. The following advantages have been suggested (18): (a) it boosts milk fat and chocolate flavor, (b) it is economical—possible to reduce milk fat level, and (c) it improves body. To avoid excessive sweetness in chocolate drink with added lactose, it is desirable to decrease the amount of sucrose. It has been suggested (18) that to maintain the same sweetness level in the formula it is advisable to reduce the sucrose content 1 lb for every 5 lb of lactose added. This practice would minimize the possibility of over-sweetening the chocolate drink. Use of lactose would thus permit the addition of more solids than would otherwise be possible with its desirable effect on body.

Results obtained at Clemson (4) have shown that 2% lactose addition to nonfat chocolate drink results in a product that is thin, too sweet, and poor in color. The color of this product was similar to the nonfat chocolate drink without any solids addition. In the 2% fat series, the 2% added lactose sample was judged slightly inferior to the 2% fat having no added solids and the 2% fat plus 2% NFDM. Results obtained from limited formulations have shown that the addition of 2% NFDM or 2% fat to nonfat chocolate drink will enhance the color. The addition of lactose only to a nonfat chocolate drink will do very little for color improvement.

From our own observations and theoretical considerations it would appear that the addition of milk solids is highly desirable, but they should be NFDM. Nonfat dry milk solids are superior in their influence on the improvement of flavor, body characteristics, and color of the resulting chocolate drink, be it nonfat, low-fat, or high-fat.

SUMMARY

In summarizing, we can state that:

Nonfat and low-fat beverage milks fortified with NFDM and vitamins A and D are palatable products.

The addition of 2% NFDM to nonfat and low-fat milk seems to be optimal. Higher concentrations result in objectionable flavors. Lower concentrations fail to improve flavor and body characteristics to any significant extent.

The addition of 1-2% fat to NFDM fortified skimmilks will result in a fuller flavor and better body than the same milk without fat.

The addition of NFDM in preference to lactose seems to be indicated. Data concerning the use of lactose as a means of solids fortification are limited. However, since milk already contains adequate quantities of lactose, the need or desirability for lactose addition requires more justification.

Cultured buttermilk with 11% nonfat milk solids will have more flavor and body than that with 8.5-9.0% nonfat milk solids. The addition of 1% or more of milk fat will materially improve the flavor of such cultured buttermilk.

Use of lactose as a source for added solids in cultured buttermilk is questioned. The advantages claimed for its use can be achieved much better by the use of NFDM.

The flavor and body characteristics of chocolate drink can be improved by adding milk solids. Increasing the nonfat milk to 11% solids content seems justified. The addition of milk fat is optional, but 1-2% makes the beverage much more palatable.

Use of lactose as a source of extra solids for chocolate drink does not improve the product as much as does NFDM.

Nutritionally, nonfat milk solids are to be preferred as a source of extra solids as compared to lactose, because of their protein and mineral content.

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ASSOCIATION AFFAIRS

CHALLENGES AND OPPORTUNITIES IN THE DAIRY INDUSTRY [1]

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From the beginning of time, animals have been a part of man's environment. They have played an increasingly important role in his struggle to provide life's necessities. They have been, and in some parts of the world continue to be, an important means of transport and motive power. They enabled our forebears to span a continent and to link its parts together by road and railroad.

Out of this background has come an animal agriculture which is highly organized, intricate, complex, and marvelously integrated into the economic life of our nation. A most important part of this great complex is the dairy industry—an industry estimated to provide approximately 25% of the nutritive values in the American dietary and which has come to its present eminence largely as a result of the findings of science being applied to its several processes. This research continues to provide the new and extended knowledge which is one of the principal factors of the industry's continuing growth and development, while education provides the trained personnel required to operate its several parts.

Research and education have understandably grown and developed along disciplinary and subject matter lines. As a result, each scientific discipline has been, in considerable measure, isolated from the whole body of science. Likewise, each educational subject has been somewhat isolated from the whole body of education. There has been contact of only a limited nature between disciplines and between subject matter departments in educational institutions. While there is evidence that this situation is changing, the changes called for, because of the complex organization of our society, of industries within that society, and of productive units within those industries, are more far-reaching than those now visible.

A dairy farm is a production system. Problems encountered in its operation are bound together in what might be termed problem complexes. Solutions applied by management come from knowledge supplied by many disciplinary fields. Production of feed and forage may involve soil physics and chemistry, plant nutrition and physiology, pathology, entomology, and nematology, genetics, engineering, and water management. Herd management will involve all the disciplines associated with veterinary science. The operation of a logistical system to supply feed and handle products will involve the several disciplines supporting agricultural engineering. A means for evaluating the contribution of each animal to the whole operation, and for evaluating the cost of support in relation to value of output, requires application of economic measurements, as does evaluation of investment in relation to yield.

Are research and education so developed and organized as to be capable of regarding dairying as a system; of responding to any particular need of the system with understanding of its relation to the system as an entity? There seems to be no affirmative answer to this question, and it seems there is need to develop what might be termed systems research capability. To do so will require the formation of research teams from each of the applicable scientific disciplines. The competition among disciplines to improve and make more efficient the working of the system would add generative power to the further development of each.

Emphasis on further development within individual disciplines should not be reduced. Indeed, as the frontiers of knowledge are further extended, there is challenge to each disciplinary area to keep pace.

For example, on the production side of our industry, the average productive life of a cow is estimated to be less than 3 yr. Yet, at 5 yr of age the cow should be at her most productive period. Here is a costly defect in the productive systems collectively and individually. Wherein lies the defect? How is it to be overcome? Is the problem within the fields of genetics, nutrition, engineering, pathology, or is it a system problem?

The average annual production per cow in our national dairy herd, according to latest statistics, is about 7,400 lb of milk, and the average annual production of cows in testing associations, about 11,000 lb. Here is another kind of defect in many individual production systems—a defect in management—in economic analysis—in communication—in human motivation. Systems research capability necessarily reaches beyond the biological and physical sciences to the social and behavioral area—an area of growing importance as our economic and social systems become more involved.

The processing and manufacturing side of the dairy industry is no less comprised of unit

[1] Presented at the 57th Annual Meeting of the American Dairy Science Association, University of Maryland, CollegePark, June, 1962.

systems than is the production side. Functions involved are assembly, processing, packaging, merchandising, distribution, selling, and management. All of the scientific disciplines have contributed to the development of this side of our industry; they continue to do so in ever greater degree. But here, also, the contribution has been largely fragmented. Adaptation of new knowledge has been stimulated more by competitive pressures between system units than from understanding among the scientific disciplines of where their discoveries best fit the needs of either the industry or its unit systems. Here, too, is need for system research capability.

For example, two processors operate in the same market. Their supply problem is similar; they perform the same general functions; they employ the same number of people. One does twice the dollar volume of business of the other.

In another market two processors of comparative size compete with each other. Variance in efficiency measured by gallonage processed per man-hour is 35%. Is it not a proper function of research to determine what constitutes an efficient system? How can education develop people capable of operating the several parts of any system unless there is first understanding of what constitutes an effective system? How can this be determined except by the measurements provided by research?

Within our society, there has long been close association between the economic community and the educational community to the benefit of both. The educational community is dependent for its support upon an efficient, productive, and profitable economic community. Without the monetary support provided for education by a profitable economic community, education could not have reached nor could it maintain its present stature. In turn, the economic community is dependent upon the educational community to provide a continuing flow of people having the basic training enabling them to become effective workers in our economic structure. There is also dependence upon education to develop the scientists who will discover new knowledge or new applications for present knowledge.

So it is within the dairy industry. There is interdependence between education and the industry. They need each other: the people of America need both. It is, therefore, appropriate that industry and education understand each other's needs and capabilities. While associations in industry and education have been close and cordial, there can and should be improvement in their communications. Even now changes are being made in curriculums of a number of institutions which have in the past and will in the future make significant contributions to the improvement of the dairy industry. History, philosophy, economics, political science, and the social sciences are being recognized as having a proper place in education for a career within the scientific or technological area. At the same time, there appears also a realignment of subject matter in the science areas, so that the graduating student has had a broader base of exposure to the several areas of knowledge needed to become an understanding and useful part of his society.

There seems no general understanding within the dairy industry of the changes being made, nor of the reasons for them. The industry needs to take them into account in its recruitment program. In addition, it is a matter of concern that students seeking a career within the dairy industry are diminishing in number. This industry is neither dead, dying, nor decadent; it is alive, vital, and necessary. However, to achieve effectively its function of providing a major part of the American dietary it must have a continuing flow of able young men into its several parts. Here is a challenge to the industry and to education also.

We in the dairy industry have another area of deep interest. Basically, our industry is engaged in supplying flavor and nutrition to the American people. We do this through a highly complex and marvelously intricate logistical system, in the form of milk and its products. They are the most versatile of any food group. They have wider degree of public acceptability than does any other food group. Yet questions are now being raised as to the nutritional effect of the fat component of these products. Knowledge of nutrition is in such a state that the questions raised cannot be answered categorically and authoritatively. There is real need for a significant and immediate increase in nutrition research, to more conclusively determine the nutritional results obtained from the ingestion of milk and dairy products. There is also need for a wider and more effective distribution to our customers of nutrition knowledge.

There is also an opportunity to improve public awareness of the esthetic and social values attendant on the mealtime event. We are a mobile society. Family interests are wide and complex. Social forces at work on the family are centrifugal in nature. The mealtime event is centripetal; it brings the family together. It provides opportunity for family devotions, for discussion of family hopes and aspirations, for those many niceties of human relations which help the family to be a family rather than several individuals living in the same dwelling. It is within the family that character is shaped, nurtured, and developed; here are established the value concepts which, in the aggregate, shape society itself. In our kind of world we must, if we are to survive, have a society possessing the character necessary to resist both challenge from without and erosion from within. We of the dairy industry, by supplying a necessary and wanted food, can help make the mealtime

event an occasion for building both the physical and moral strength required for survival in a world now divided.

It is to this purpose that those in research and education and those of the great dairy industry find common cause. They are now challenged to find most effective means of keeping this great industry fully capable of making the adaptations necessary to its continued growth and development in, and for its continued service to, a society grown so complex and inter-related as to deny isolation of any of its facets. The record of the past gives hope of promise for the future.

STATISTICS ON ATTENDANCE AT TWO RECENT ANNUAL A.D.S.A. MEETINGS

H. F. Judkins, Secretary-Treasurer, A.D.S.A.
32 Ridgeway Circle, White Plains, New York

	1961 Wisconsin		1962 Maryland	
	No.	% of total	No.	% of total
Members from universities and USDA	475	42.8	508	45.0
Members from industry	260	23.4	263	23.0
Student affiliates	236	21.2	234	20.0
Nonmembers from universities or USDA	54	4.8	81	7.1
Nonmembers from industry	86	7.7	56	4.9
Total	1,111		1,142	
Number of women	311		438	
Number of family groups with or without children			426	
Number of family groups with children	130		213	50
Number of family groups with one child	42	32.3	55	26.3
Number of family groups with two children	56	43.0	84	39.3
Number of family groups with three children	23	17.6	48	22.5
Number of family groups with four children	9	6.9	24	11.2
Number of family groups with five children	0	0	2	0.94
Total number of children	259		473	
Avg no. children per family	2.00		2.00	

Comparisons of major interest:

1. Larger total attendance in 1962.
2. Percentage classification of members attending almost identical both years.
3. Many more women and family groups in 1962.
4. Many more children in 1962.
5. A few more families of five children should take care of our membership for years to come.

Occupational classification of members and nonmembers from industry

	1961	1962
Artificial breeding associations	28	22
Board of Health (state or local)		1
Breed associations	13	11
Chain store representatives	6	3
D.H.I.A.		1
Dairy processing companies	108	66
Dairy products broker		1
Dairy Products Improvement Institute		1
Farmers' cooperatives	6	12
Farming	1	3
Gov't laboratories or state agriculture department	7	13
Manufacturers of feed or feed supplements	35	20
Manufacturers of equipment and supplies	92	62
Milk producer associations	9	1
Miscellaneous or unknown	15	20
National Research Council		4
Private laboratories	10	9
Publishing	10	5
Trade associations	17	9
Wisconsin Alumni Research Foundation		1
	357	265

There were fewer persons attending from dairy processing companies, from equipment and supply manufacturers, and from feed manufacturing companies in 1962.

PROGRAM

ANNUAL MEETING SOUTHERN DIVISION, AMERICAN DAIRY SCIENCE ASSOCIATION MEMPHIS, TENNESSEE

C. W. REAVES, University of Florida, Gainesville

February 4-6, 1963

Monday AM

JOINT SESSION DAIRY PRODUCTION, DAIRY EXTENSION AND DAIRY MANUFACTURING

Presiding: B. E. GOODALE

8:30 AM

Riverview Room, King Cotton Hotel

Interrelation of the major milk constituents of farm deliveries in Virginia. W. K. Stone, M. C. Conner, and N. R. Thompson, Virginia Polytechnic Institute, Blacksburg.

Natural variation in the freezing point value of milk from a single herd. R. W. Henningson, South Carolina Agricultural Experiment Station, Clemson, S. C.

Influence of milk fractions on activities of lactic cultures. H. C. Olson and S. E. Gilliland, Oklahoma State University, Stillwater.

The relationship of phospholipid content and fatty acid composition to milk fat hydrolysis. W. R. Wallace, Louisiana State University, Baton Rouge.

Relationship of milk pipeline height to increases in acid degree values. J. H. Gholson, Louisiana State University, Baton Rouge.

Effect of feeding thyroxine on secretion of Iodine^{131} in milk. J. K. Miller, E. W. Swanson, and R. G. Cragle, UT-AEC Agricultural Research Laboratory, Oak Ridge, Tennessee.

Taste panel studies of a low-fat spread. J. B. Mickle, M. E. Leidigh, M. V. Malkus, R. D. Morrison, H. D. Baker, and R. L. Von Gunten, Oklahoma State University, Stillwater.

11:00 AM

General Convention Session, ASAW
Ballroom, Chisca-Plaza Hotel

Monday PM

DAIRY MANUFACTURING

Presiding: J. J. WILLINGHAM

2:00 PM

Colonial-Jefferson Room, King Cotton Hotel

Values for the forced convection heat transfer coefficients of several dairy products. M. L. Peeples, Texas Technological College, Lubbock.

The effect of certain refreezing techniques on the activity of mixed flora cultures. J. T. Cardwell, Mississippi State University, State College.

Crystallization temperatures of various fat-water mixtures cooled at different rates. J. B. Mickle, M. H. Beauford, and L. C. True, Oklahoma State University, Stillwater.

The persistence of cooked flavor in milk pasteurized at 194 F. B. J. Demott, University of Tennessee, Knoxville.

The relationship between soluble peptide nitrogen and flavor of Cottage cheese. D. M. Naff and W. K. Stone, Virginia Polytechnic Institute, Blacksburg.

Mechanical salting of Blue cheese curd at hooping. J. J. Janzen, South Carolina Agricultural Experiment Station, Clemson, S. C.

Response of lactic cultures to heat treatment of milk. H. C. Olson and S. E. Gilliland, Oklahoma State University, Stillwater.

Spoilage of milk by thermoduric psychrophiles. H. C. Olson, Oklahoma State University, Stillwater.

Selective plating technique for detecting concontamination of pasteurized milk. H. C. Olson, Oklahoma State University, Stillwater.

Monday PM

JOINT SESSION, DAIRY PRODUCTION AND DAIRY EXTENSION

Presiding: I. W. RUPEL

2:00 PM

Riverview Room, King Cotton Hotel

Effect of thermal stress on growth of newborn Holstein calves to 90 days of age. P. F. Randell and L. L. Rusoff, Louisiana State University, Baton Rouge.

Effect of certain management practices on milk production in Georgia. H. W. McKinney and O. T. Fosgate, University of Georgia, Athens.

Nutritional evaluation of permanent pastures during the summer months with lactating cows. A. S. Achacoso, L. L. Rusoff, Cecil Branton, B. R. Farthing, and J. L. Fletcher, Louisiana State University, Baton Rouge.

The value of forage species alone or in mixtures for lactating cows. W. R. Murley and H. D. Gross, North Carolina State College and Clark Walker, Piedmont Research Station, Salisbury, North Carolina.

Influence of feed additives on the utilization of Coastal Bermuda and oat hays by dairy heifers. W. A. King, C. C. Brannon, and H. J. Webb, South Carolina Agricultural Experiment Station, Clemson, South Carolina.

Effects of age at cutting and weathering on Coastal Bermuda grass hay. J. C. Johnson, Jr., D. W. Beardsley, G. W. Burton, F. E. Knox, and B. L. Southwell, Georgia Coastal Plain Experiment Station, Tifton.

Nutrient losses and chemical changes of Coastal Bermuda grass when ensiled at two stages of maturity. W. J. Miller, C. M. Clifton, and N. W. Cameron, University of Georgia, Athens.

The influence of stage of maturity on the nutritive value of Sudan grass. O. T. Stallcup and George V. Davis, University of Arkansas, Fayetteville.

Tuesday AM

JOINT SESSION, DAIRY PRODUCTION AND DAIRY EXTENSION

Presiding: B. E. Goodale

8:00 AM

Riverview Room, King Cotton Hotel

Crossbred dairy cattle compared to their purebred contemporaries for milk and fat production. G. W. Brandt, C. C. Brannon, South Carolina Agricultural Experiment Station, Clemson, South Carolina; and W. R. Harvey, Biometrical Services, and R. E. McDowell, Dairy Cattle Research Branch, USDA, Beltsville, Maryland.

Changes in feed calculations in the machine DHIA program. M. E. Senger, North Carolina State College, Raleigh.

Relationship of feeding patterns with production of North Carolina DHIA herds. Fred N. Knott, North Carolina State College, Raleigh.

9:30 AM

SOUTHERN DIVISION, A.D.S.A. BUSINESS MEETING

Riverview Room, King Cotton Hotel

11:00 AM

GENERAL CONVENTION SESSION, ASAW

Ballroom, Chisca-Plaza Hotel

Tuesday AM

DAIRY MANUFACTURING

Presiding: H. C. Olson

8:00 AM

Colonial-Jefferson Room, King Cotton Hotel

The enumeration of citrate-fermenting bacteria in commercial lactic cultures. W. W. Overcast and J. D. Skean, University of Tennessee, Knoxville.

The effect of inorganic nitrogen sources on the growth of various pseudomonads. C. Vanderzant and A. E. Bardin, Texas Agricultural Experiment Station, College Station.

Taxonomic studies on lipolytic aerobic spore-forming bacteria obtained from milk. Mary Anne Holdar and Earl R. Garrison, University of Arkansas, Fayetteville.

Oxalic acid as a buffer for dye milk protein determinations. Rupert Seals, Tennessee Agricultural and Industrial State University, Nashville.

Consumer preferences for corn syrup solids replacement levels in vanilla ice cream. John J. Sheuring and Eugene J. Finnegan, University of Georgia, Athens.

9:30 AM

SOUTHERN DIVISION, A.D.S.A. BUSINESS MEETING

Riverview Room, King Cotton Hotel

11:00 AM

GENERAL CONVENTION SESSION, ASAW

Ballroom, Chisca-Plaza Hotel

Tuesday PM

DAIRY MANUFACTURING

(Food Science and Technology Option)

1:30–5:00 PM

Peabody Hotel, Room 201

A program composed of three Dairy Manufacturing papers and six Food Science and Technology papers selected to interest dairy specialists.

The titles of the three Dairy Manufacturing papers are:

Rapid determination of the undenatured serum protein content of milk. J. W. Saunders, L. L. Koontz, and W. K. Stone, Virginia Polytechnic Institute, Blacksburg.

The influence of milk protein on the physical properties of low-fat frozen desserts. W. K. Stone, R. A. Smith, and R. L. Hills, Virginia Polytechnic Institute, Blacksburg.

Consumer preference for sugar levels in ice cream and frozen desserts. IV. Corn

syrup solids replacements levels in chocolate ice cream. John Sheuring and Eugene J. Finnegan, University of Georgia, Athens.

6:30 PM

ANNUAL BANQUET AND HONORS PROGRAM

(Place to be announced)

Tuesday PM

DAIRY PRODUCTION

Presiding: S. D. MUSGRAVE

1:45 PM

Riverview Room, King Cotton Hotel

Livability of bovine spermatozoa when diluted with different semen extenders. O. T. Fosgate and I. M. Khann, University of Georgia, Athens.

Postulation of gonadotropic complex in repeat breeder dairy cattle based on clinical treatment with gonadogen. N. Pati, The Agricultural and Technical College of North Carolina, Greensboro.

Rumen parakeratosis in dairy cattle. L. J. Bush, B. Harris, Jr., J. D. Friend, and S. D. Musgrave, Oklahoma State University, Stillwater.

Zinc content of some feed materials and ingredients. W. J. Miller and J. K. Miller, University of Georgia, Athens.

Dwarf corn as a silage crop. Dero S. Ramsey, Mississippi State College, State College.

Lactation response of cows to alternate day and night grazing of Coastal Bermuda and Gahi-1 millet. G. H. Rollins, Auburn University, Auburn, Alabama.

4:00 PM

MEET WITH DAIRY EXTENSION

Parlor B, King Cotton Hotel

Considerations in developing effective forage evaluation programs. (A swap-shop session involving Extension and Research personnel.) Discussion Leader: R. E. BURLESON, Federal Extension Dairyman, USDA, Washington, D. C.

6:30 PM

ANNUAL BANQUET AND HONORS PROGRAM

(Place to be announced)

Tuesday PM

DAIRY EXTENSION

Presiding: H. W. ANDERSON

1:45 PM

Parlor B, King Cotton Hotel

1:45 Business meeting.

2:00 Experiences with mastitis control programs. Panel discussion: M. F. Ellmore, Chairman (Va.); J. N. Maddux (Ga.); J. D. George (N.C.); Buck Green (La.).

3:00 Dairying in the South under prospective dairy programs. Stanton P. Parry, Department of Agricultural Economics, University of Tennessee, Knoxville.

3:40 Recess.

4:00 Considerations in developing effective forage evaluation programs. (A swap-shop session involving Extension and Research personnel.) Discussion Leader: R. E. BURLESON, Extension Dairyman, USDA, Washington, D. C.

6:30 PM

ANNUAL BANQUET AND HONORS PROGRAM

(Place to be announced)

Wednesday AM

JOINT SESSION, DAIRY PRODUCTION, DAIRY MANUFACTURING, AND DAIRY EXTENSION

Presiding: B. E. GOODALE

8:30 AM

Riverview Room, King Cotton Hotel

A comparison of methods of estimating regressions for use in the Orange-G method of milk protein determination. C. P. Breidenstein, A. J. Guidry, and J. E. Johnston, Louisiana State University, Baton Rouge.

The relationship between udder and teat measurements and milk flow. J. M. Rakes and Gordon L. Ford, University of Arkansas, Fayetteville.

The effects of grain feeding level on the rate of passage of food through the digestive tract of the cow. O. H. Horton and John W. Bell, University of Arkansas, Fayetteville.

Effect of supplemental feeding of cows on pasture on milk production and composition. J. T. Huber, G. C. Graf, and R. W. Engel, Virginia Polytechnic Institute, Blacksburg.

Effects on milk production of supplementary bulky concentrate feeding. J. M. Wing and C. J. Wilcox, Florida Agricultural Experiment Station, Gainesville.

Simple vs. complex grain mixtures for lactating cows. W. R. Murley, North Carolina State College, Raleigh.

ABSTRACTS OF PAPERS

PRESENTED AT MEETING OF EASTERN DIVISION OF THE AMERICAN DAIRY SCIENCE ASSOCIATION

UNIVERSITY OF MAINE, ORONO,

AUGUST 14, 15, 1962

Supply of and demand for dairy technology graduates. H. F. JUDKINS, Secretary-Treasurer, A.D.S.A., White Plains, N. Y.

Thirty-four state universities graduated only 133 students with B.S. degrees in Dairy Technology in 1961 and about half of them were located in nine states. Six states reported no Dairy Technology graduates and the others five or less.

Several Dairy Department heads reported the supply of graduates is below demand. Dairy Industry Supply Association and dairy processing companies are seeking men trained in engineering, liberal arts, and science.

Dairy Departmtent heads and leaders in industry give these causes for low enrollment: curriculum too vocational; rate of advancement in industry too slow; lack of industry training programs; salaries too low; the merging of processing plants, resulting in fewer opportunities. One large company, operating about one-third its original number of plants, will need only 18 new men to fill ten key positions in the next 5 yr; only two of them are directly in production. Efforts made to recruit students from high schools have not been fruitful. It is suggested that Dairy Department heads recruit from within the university and endeavor to interest good students in the colleges of business, science, and engineering in taking courses in Dairy Technology.

Removal of radiostrontium from milk with tricalcium phosphate and resulting changes in milk composition. L. F. EDMONDSON,* A. R. LANDGREBE, A. M. SADLER, AND H. E. WALTER, Eastern Utilization Research and Development Division, USDA, Washington, D. C.

The effectiveness of tricalcium phosphate in removing Sr^{85} from milk and the effects of the procedure on milk composition were studied. The phosphate was added to Sr^{85} in vivo-labeled milk at the rate of 80 g per liter and the resulting precipitate, containing Sr^{85}, was removed by centrifugation. The effects of temperature and pH level of the milk and phosphate-milk contact time were determined. Heated milks were cooled before centrifugation.

Heating milk to 70 C for 30 min, immediately following addition of phosphate, resulted in removal of 85% of the Sr^{85}. When the pH of milk was adjusted to 5.4 with H_3PO_4 and then to 8.2 with KOH before adding phosphate, 93% removal of Sr^{85} was obtained by heating to 70 C for only 4 min. When phosphated milk (pH 6.6) was stirred at 5 C for 24 hr, only 60% of the Sr^{85} was removed. All procedures utilizing calcium phosphate precipitation reduced the milk nitrogen from 23 to 33%. Usually, more than 50% of the milk calcium, in addition to the added phosphate calcium, was precipitated. Also, a decrease in milk phosphorus occurred in some instances.

Regeneration of cation exchange resins used for the removal of radiostrontium from milk. J. Y. HARRIS, Division of Radiological Health, U. S. Public Health Service, Department of Health, Education and Welfare, AND D. G. EASTERLY,* J. K. AVANTS, AND L. F. EDMONDSON, Eastern Utilization Research and Development Division, USDA, Washington 25, D. C.

Effects of regenerant concentration, temperature, contact time, and re-use of regenerant on regeneration efficiency of cation exchange resin columns following use in removing Sr^{85} from milk were studied. Resins were not considered regenerated until 99.7% of Sr^{85} had been removed. From 25 to 30 bed volumes of salt solution containing 1,310 milliequivalents (meq) of Ca, Mg, Na, and K per liter were required when completely fresh solution was used in the normal downflow procedure.

Utilization of salts was slightly more efficient when the concentration was 655 meq than when it was either 131 or 1310 meq per liter, and at 85 C than at 20 or 5 C. Contact time had a pronounced effect. The relative distribution coefficient (K_D) was 1, 3.9, and 6.2 at 10, 100, and 1,000 sec, respectively.

Laboratory data show that efficient regeneration of resins was accomplished by reuse of salt solution and addition of eight or less bed volumes of completely fresh regenerant. However, calculations show that resins may be regenerated by passing used regenerant through them until it is completely exhausted, followed by only four completely fresh bed

* Person who delivered paper.

volumes. Feasibility will depend on cost of more complex equipment relative to savings in salt solution.

Bovine metabolism of Sr^{85}, Ba^{133}, Cs^{134}, and I^{131}. J. Y. HARRIS,* Division of Radiological Health, U. S. Public Health Service, Department of Health, Education and Welfare, Washington 25, D. C.

The excretion of Sr^{85}, Ba^{133}, Cs^{134}, and I^{131} by Holstein cows was measured daily for a week following one oral dosage.

During seven days, 0.92, 1.64, and 80.50% of the oral dose (2 mc) of Sr^{85} were detected in the milk, urine, and feces, respectively, by a cow producing 16 lb of milk daily.

A second cow, producing 44 lb of milk daily, received simultaneously Cs^{134} (0.1 mc) and Ba^{133} (0.17 mc). During seven days, 10.7, 13.9, and 53.2% of the Cs^{134} and 0.5, 1.4, and 78% of the Ba^{133} were detected in the milk, urine, and feces, respectively.

A third cow, producting 11 lb of milk daily, received I^{131} (0.18 mc). During six days, 14, 21, and 15% of the dose were detected in the milk, urine, and feces, respectively. The accumulation of I^{131} in the thyroid reached a peak of 20% of the dose at 48 hr. The effective half-life for I^{131} in the milk, urine, feces, and thyroid during an initial six-day period was calculated as 0.84, 0.97, 1.00, and 6.00 days, respectively.

Survey of the time to flush sanitizing solutions from processing equipment with milk. A. C. SMITH,* University of Connecticut, Storrs, AND R. F. ANDERSON, R. W. WALDO, AND C. W. CHAFFEE, Department of Agriculture and Natural Resources, Hartford, Connecticut.

This study included 28 processing trials in 15 dairy plants. Freezing points were determined on milk samples from storage tanks and on samples taken (at 15-sec intervals for 17.5 min) after clarification, from the balance tank, and after the cooling section of the high temperature–short time (HTST) pasteurizer. The sampling period began when a milky appearance first showed in the discharge lines, except for the balance tank, which started with the first addition of milk from the clarifier.

Minutes of flushing-time variations among processing runs were clarifier, 1¼-10; conventional balance tank, 4¼-12½; pasteurizer with conventional balance tank, 2½-13; balance tank with sump, 1-3½; and pasteurizer using balance tank with sump, 2-5½. Recommendations to avoid adulteration and minimize flushing time include (a) education of plant personnel, (b) determination of flushing time for each dairy plant, (c) use of a balance tank with sump, (d) inclusion of the clarifier within the HTST system, (e) avoidance of diversion during start-up procedures, (f) removal of as much water as practical from all equipment before flushing with milk, and (g) flushing of all by-pass circuits with milk during start-up.

Cleaning and sanitizing a portable milk transfer system. W. S. MUELLER,* University of Massachusetts, Amherst.

The scarcity and high cost of labor is stimulating dairy farmers to buy portable milk transfer systems. An approval by the state or municipal sanitary authorities, or both, is necessary before such equipment can be used legally. While some states have approved these transfer units, others have accepted them on a temporary basis pending additional data on whether these units can be maintained in a sanitary condition. The purpose of this study was to furnish such information. Tests were run at the University of Massachusetts dairy barn, at a dairy farm near Amherst, and in the University dairy plant. Effects of type and temperature of cleaning solution, and type of sanitizer used, are included in the study. The chief criteria for the determination of the sanitary condition of the milk transfer unit were the membrane filter method and the bacterial count of the milk produced. Results obtained indicate that a portable milk transfer unit, including 100 ft of plastic tubing, can be readily cleaned and sanitized so as not to impair the quality of the milk supply.

In-package oxygen scavenging with catalysts applicable for dry milk. A. TAMSMA* AND M. J. PALLANSCH, Dairy Products Laboratory, Eastern Utilization Research and Development Division, USDA, Washington 25, D. C.

Residual oxygen in hermetically sealed cans containing spray-dried whole milk foam was scavenged by combination with hydrogen to form water. Commercially available pellets of supported films of palladium and platinum were used to catalyze the reaction. Rates of reaction were such that within 24 hr residual oxygen levels in packs containing nitrogen plus 10% hydrogen dropped from an average of 1% to less than 0.003%. These low levels of oxygen were maintained in the packs during extended periods of storage, with a resultant improvement in flavor stability of the product. The described procedure for scavenging in-package oxygen was found to be simple, inexpensive, and effective. It may also improve flavor of stored products by the catalytic reduction of volatile carbonyls formed in the packaged material.

Recovery of off-flavors from whole milk powder. F. E. KURTZ,* Eastern Utilization Research and Development Division, USDA, Washington, D. C.

Off-flavors are recovered directly from whole milk powder by a short-path migration, at

1 μ pressure, from the powder to a liquid nitrogen-cooled condenser. This process has been examined for possible flavor contributions by equipment or artifacts originating in the powder. Lubricants and gaskets were held 3 hr at 25 degrees and the condensate combined with whole milk. Powders were held at 25 or 40 degrees with and without prehydration to increase the free fat and the condensate combined with milk reconstituted from the residual powder. Comparisons were made by a taste panel between these samples and, respectively, untreated milks and milks reconstituted from untreated powders. Condensates from silicone and Neoprene gaskets lowered milk scores four to five points; those from silicone and Apiezon N greases, Teflon gaskets, and Viton O rings were without effect. The procedure has been developed to where, using the most favorable combination of conditions for recovery of off-flavors (40 degrees and prehydration of powder), the score is lowered one point or less.

Determining the bacteriological quality of farm water supplies using 2,3,5 triphenyltetrazolium chloride. E. P. MERRILL,* H. V. ATHERTON, AND D. KLEIN, University of Vermont, Burlington.

Potable water by Public Health Service standards must not contain more than two coliforms/100 ml. Many other organisms causing spoilage in dairy products may be present in treated water supplies. The need for a simple convenient method of assessing such contamination is evident.

The reduction test employing TTC was modified for this purpose.

To test the method and to provide survey data on bacteriological quality, 104 samples of water were collected from the cold-water taps in farm milkhouses.

Examination of the limited data suggests the following:

1. No reliable relationship exists between reduction as determined by comparison with Munsell colors standards and the numbers of organisms in any bacterial group.
2. Positive reduction at 24 hr incubation generally reflected the presence of even small numbers of coliforms or psychrophilic bacteria in the samples of water.
3. Samples showing negative reduction in 24 hr but positive reduction in 48 hr had high Standard Plate Counts.

Gas chromatographic determinations of the flavor compounds of commercial starter culture organisms. G. W. KURTZ* AND J. J. JAGGARD, Dalare Associates, Philadelphia, Pennsylvania.

Pure cultures of *Streptococcus cremoris, Streptococcus diacetylactis, Streptococcus faecalis, Streptococcus lactis, Streptococcus thermophilus,* and *Lactobacillus bulgaricus,* used in the preparation of commercial starter cultures, have been subject to gas chromatographic analysis for flavor compounds.

Differences were shown to exist in the kind and amount of flavor compounds present in various organisms. In addition, a variation was shown in the amounts of flavor constituents produced by different strains of the same organism.

Changes in percentage of protein, milk fat, and solids-not-fat during the milking process. H. C. GILMORE* AND S. N. GAUNT, University of Massachusetts, Amherst.

The milk produced during the first, second, third, and over 3 min of milking at night and morning milkings was sampled from ten cows from each of four breeds: Ayrshire, Guernsey, Holstein, and Jersey. Samples were tested for protein by the Orange G dye binding method, solids-not-fat by the Golding plastic beads, and milk fat by the Babcock procedure.

Mean percentages of the milk constituents for Periods 1, 2, 3, and 4 were, respectively, for protein 3.49, 3.46, 3.40, and 3.34; for solids-not-fat 8.97, 9.07, 8.98, and 8.89; for milk fat 2.46, 3.62, 4.90, and 6.30. Evening and morning mean percentages were 3.46 and 3.38 for protein, 9.06 and 8.90 for solids-not-fat, and 4.70 and 3.94 for milk fat. Statistical analysis shows the effect of breed and time of day were highly significant for all components. The stage of milking was highly significant for solids-not-fat and milk fat but not for protein percentage.

On a fat-free basis, there was little change in protein but solids-not-fat increased as milking progressed. Larger differences occurred, on a fat-free basis, between night and morning samples for protein and solids-not-fat.

Variations in protein, solids-not-fat, and fat percentages of herd samples. A. R. CORWIN* AND S. N. GAUNT, University of Massachusetts, Amherst.

Milk shipments, 2,112, from 35 herds from September 21, 1961, to April 25, 1962, were sampled and tested twice weekly for protein, SNF and fat. Herds included cattle of all breeds but were predominantly Holstein. Protein determinations were made by Orange G dye, SNF by Golding, and fat by Babcock.

Means and standard deviations were: for fat 4.0%, 0.48; SNF 8.64%, 0.27; protein 3.27%, 0.26; SNF less protein 5.37%, 0.23; and total solids (T.S.) 12.68%, 0.71. Correlations between components were: protein and SNF 0.64; protein and fat 0.65; SNF and fat 0.79; SNF less protein to protein −0.39; SNF less protein to SNF 0.47; SNF less protein to fat 0.19; TS to protein 0.68; TS to SNF 0.91; TS to fat 0.96, and TS to SNF less

protein 0.30. Confidence limits (99%) were computed. Regressions and their standard errors were computed for all combinations. Charts were prepared to show the distribution and relationship of the tests.

The very high correlation of TS with fat indicates that it is not necessary to test for TS in herd milk. The lower correlations of protein with the other components indicates that protein is somewhat more independent than SNF or fat.

Recovery of several cholinesterase-inhibiting pesticides from milk. J. E. BEAM* AND D. J. HANKINSON, University of Massachusetts, Amherst.

Known amounts of several cholinesterase-inhibiting pesticides were added to raw and pasteurized homogenized samples of mixed herd milk. Pesticides included in the study were: Sevin, Trithion, Parathion, Malathion, Guthion, Dipterex, and Ronnel.

Fifty-gram aliquots of the treated milks were extracted using the acetonitrile-chloroform method of Timmerman et al. (J. Econ. Ent., 4:441. 1961). The conversion of the extracted pesticides to potent in vitro cholinesterase inhibitors was accomplished by the H_2O_2–acetic acid oxidation procedure developed by Patchett and Batchelder (J. Agr. Food Chem., 8:54. 1960), for the determination of Trithion. In vitro cholinesterase assays were carried out using the direct colorimetric method of Archer and Zweig (J. Agr. Food Chem., 7:178. 1959). The action of red blood-cell cholinesterase upon indophenyl acetate produces a color change measurable at 625 mμ. The presence of a cholinesterase inhibiting pesticide is reflected by a decrease in color development. Standard curves were prepared for each pesticide. Recovery of each pesticide from milk was determined by comparison with its standard curve.

Detection levels expressed in ppm for Sevin, Trithion, Parathion, Malathion, Guthion, Dipterex, and Ronnel, respectively, were 0.3, 0.04, 0.008, 0.04, 0.04, not recovered, and 0.2.

Effect of vacuum fluctuations in the teat cup on certain measures of udder health. D. E. STANLEY, E. M. KESLER,* AND A. L. BORTREE, Pennsylvania State University, University Park.

A motor-driven nylon petcock was substituted for the original one on a floor-type milking machine. Thereby the vacuum in the teat cups was made to vary between 7 and 15 in Hg about 53 times/min. Two trials, each utilizing 12 cows, were run. Test periods were of 21 and 26 days' duration; each followed preliminary standardization periods. In each trial two cases of clinical mastitis developed among the six cows milked with the altered machine. Milk production was not affected by treatment; it averaged 39.5 and 47.4 lb/day in Trials I and II, respectively. California Mastitis Test scores on milk from experimental cows rose significantly, as compared with those from controls. Direct leucocyte count of the milk also increased, due mainly to the mastitic cows. Hotis tests, run on milks produced in Trial II, indicated little change in Hotis-reactive organisms. The number of milks showing alkaline pH rose in the experimental group. All members of this group exhibited an increase in alkalinity of the milk secreted by one or more quarters. The average time required to milk the cows in the experimental group increased from 5.8 to 7.6 min/cow.

Effects of nitrogen fertilization and date of harvest on the acceptabiliy of timothy forage. A. W. MAHONEY* AND B. R. POULTON, University of Maine, Orono.

Two plots of Climax timothy were fertilized at 40 and 120 lb of nitrogen per acre. Each plot was harvested at two dates—June 12 and June 27, 1961—resulting in four treatments. These forages were individually fed to eight wethers divided into two groups of four. Each group remained on its respective nitrogen-fertilized forage throughout the trials. Each forage was fed on a calculated maintenance intake digestion trial. Following this, an ad libitum intake digestion trial was conducted. Daily intake data were recorded at ad libitum feeding. The double-digestion trial made it possible to study the effects of level of forage intake on the apparent digestibility of protein and energy.

The date of harvest and the rate of nitrogen fertilization had a highly significant ($P = 0.01$) effect on the digestible protein value of these forages. The date of harvest had a highly significant ($P = 0.01$) effect on the digestible energy content, the relative intake, and the nutritive value index, each decreasing as the forage became more mature. The rate of nitrogen fertilization did not have a significant effect on the digestible energy content, the relative intake, or on the nutritive value index of these forages.

Substrate level and nitrogen supplementation influence upon in vitro forage evaluation. J. L. EVANS,* J. ARROYO AGUILU, W. V. CHALUPA, M. C. STILLIONS, AND M. W. TAYLOR, Rutgers—the State University, New Brunswick, New Jersey.

Four forage samples plus Avicel were ground and fermented for 12 and 24 hr at 39 C in 100-ml beakers, each containing 20 ml of fresh rumen inoculum (bovine), 30 ml of buffer-nutrient solution, and varying amounts of cellulose or nitrogen, or both. The addition of 15 mg of nitrogen as urea to the different fermentation mixtures (cellulose sources contained 0.0, < 2.0 or $> 3.5\%$ nitrogen) in-

creased cellulose decomposition uniformly by about seven percentage units. As fermentation time was increased from 12 to 24 hr, forage cellulose decomposition rate decreased, but Avicel decomposition rate increased. As substrate level for the cellulose sources studied was increased, mg cellulose decomposition increased at a decreasing rate. The percentage cellulose decomposition decreased with increasing substrate levels between 0.16 and 0.45 g. The equation which expresses this relationship may be stated as $Y = y - 74.1\ (X - x)$ with Y, y, X, and x equal to adjusted percentage cellulose decomposition, actual percentage cellulose decomposition, g substrate to be corrected to, and g substrate in the fermentation mixture, respectively. The standard error of estimate was 0.56, and the significant correlation coefficient was −.98.

Appetite for concentrates in mid-lactation compared with appetite for forage. A. A. Rimm and R. E. Mather,* New Jersey Agricultural Experiment Station, Sussex.

Approximately 100 days after calving, 36 cows were placed on excellent alfalfa hay (ad lib.), corn silage (limited), and concentrates (approximately Morrison Standard requirements) for 3 wk (I). The following three weeks both hay and concentrates were fed ad lib. (II). Ten cows were continued an additional 3 wk with no hay, and with concentrates and silage ad lib. (III). From I to II the following changes were observed (feeds on D.M. basis): concentrate intake +17.3 lb (13.6 to 30.9), forage intake −11.6 lb (24.8 to 13.2), TDN +8.4 lb (26.7 to 35.1), FCM +2.5 lb (42.1 to 44.6), body weight +38 lb, milk fat −0.05%, protein +0.16% and SNF +0.11%. Regression of forage on concentrates was −0.65 lb.

Among-cow partial correlations (adjusted for size and FCM) for 36 cows were: forage intake, I with II, 0.45**; forage (I) with concentrate (II) 0.25; total intake, I with II, 0.39* (unadjusted, 0.85**). Simple correlation between concentrate intake (II) and weight (I) was only 0.21. For ten cows the partial correlation of concentrate intake in II and III was 0.49.

Appetite for concentrates of cows in mid-lactation was not closely related to appetite for forages nor to weight or production.

Effects of soluble, mixed sodium and calcium lactate salts for lactating dairy cows. H. P. Adams* and R. E. Ward, Eastern States Farmers' Exchange, Inc., West Springfield, Massachusetts.

Mixed sodium and calcium lactate salts have been used as a treatment and preventive of ketosis. To test the effect of lactates on milk production, 12 cows were divided into two nearly equal groups for a double-reversal trial of three- to 60-day periods in which a typical 16% protein commercial dairy ration was compared to a similar ration containing 6% mixed lactate salts. Concentrate, hay, and silage were weighed and that not consumed was weighed back. Concentrate feeding was adjusted bimonthly, based on production.

The average daily differences in favor of the lactate containing concentrate in milk production, milk fat production, fat test, FCM, and change in body weight were, respectively, +1.43 lb, −0.005 lb, −0.15%, +0.46 lb, and +0.24 lb. The fat test was significantly lower when lactate salts were fed.

Influence of calcium and phosphorus levels and ratios upon the digestibility of various nutrients. A. M. Smith* and G. L. Holck, University of Vermont, Burlington.

Twenty-one four-month-old Holstein steers were randomly assigned to diets with a calcium-to-phosphorus ratio of 1:1, 2:1, 4:1, 8:1, 8:2, 8:4, or 8:8. The animals received their assigned diet until they weighed 600 lb. Duplicate digestion trials were conducted on two calves from each of the treatment groups. No significant difference was detected between treatments as regards the digestibility of organic matter, protein, nitrogen-free extract, and ether extract. A highly significant depression in crude fiber digestibility was noted with the calves receiving the 1:1 ratio, when compared with the calves on the other treatments. As the calcium-to-phosphorus ratio was widened, a depression in the digestibility occurred in the organic matter and nitrogen-free extract. This depression in the digestibility of these two components was reversed when additional phosphorus was added, so that the ratio was 8:2. However, the further addition of phosphorus failed to reveal this counteracting effect. The apparent digestibility of the ether extract increased considerably as the ratio of calcium to phosphorus was widened. The significance of these figures and their application to the methods available for fat detection were discussed in detail.

Carbon sources for in vitro protein synthesis by rumen microorganisms. W. H. Hoover,* E. M. Kesler, and R. J. Flipse, The Pennsylvania State University, University Park.

Clarified rumen fluid from a fistulated steer on a semipurified diet was used as the incubation medium for several in vitro tracer investigations. Carboxyl-labeled acetate, propionate, butyrate, valerate and glucose-U-C^{14} were studied individually using unlabeled glucose as an energy source.

From the decline in specific activity of the labeled volatile fatty acids (VFA) during incubation, the rate at which each VFA was utilized by rumen microorganisms was estimated. The average rates calculated for ace-

tate, propionate, butyrate, and valerate in mg/liter/hr were 131, 18.2, 13.2, and 0.6, respectively. Glucose was utilized in proportion to the amount added. At 10 and 20 mg/ml levels, 556 and 1,155 mg/liter/hr were utilized, respectively.

After incubation, some cellular components of the microorganisms were examined for C^{14} content. Nucleic acids contained label from acetate and glucose but little or no activity from propionate, butyrate, or valerate. Activity from all labeled metabolites was found in the proteins. Of the activity that disappeared from the labeled acetate, propionate, butyrate, valerate, and glucose during incubation, the average amounts incorporated into proteins were 2.5, 2.1, 1.6, 2.6, and 2.9%, respectively. Cellular polysaccharides also became labeled from each source.

Heritability of resistance to bovine mastitis. W. S. GAUNYA,* University of Connecticut, Storrs, and R. E. MATHER, New Jersey Agricultural Experiment Station, Sussex.

Data were collected from four herds to study the heritability of resistance to bovine mastitis. These data included 1,001 mastitis histories from cows which had at least one positive test or had completed two lactations. Mastitis was diagnosed quarterly by leucocyte and bacterial examination. The degree of resistance was expressed as: lactation-age at which cows became mastitic (Y_1), lactation-age at which the first positive test was observed (Y_2), and as resistant or susceptible, in which cows not mastitic through two lactations were designated as resistant (Y_3).

On the basis of within-herd intraclass correlations between paternal sisters, the heritability estimates of Y_1 and Y_2 were 0.07 and 0.18, respectively. The within-herd intraclass correlations between maternal sisters yielded heritability estimates of 0.11 and zero, respectively. The within-herd regression of daughter on dam led to heritability estimates of 0.12, 0.01, and 0.14 for the Y_1 and Y_2, and Y_3 variables, respectively. Within-herd full-sister correlations led to heritability estimates of 0.34 and 0.34 for the Y_1 and Y_2 variables, respectively. When these estimates were combined, the pooled estimate of heritability for the Y_1 and Y_2 variables was 0.13 ± 0.06 ($P < 0.05$) and 0.03 ± 0.02, respectively.

Phage-typing staphylococci of bovine origin. M. GERSHMAN* AND J. F. WITTER, University of Maine, Orono.

In Maine we are in the process of typing cultures with different sets of phages to ascertain the effectiveness of the various phages on staphylococci indigenous to this region. We are as present employing in our studies Phage 42D of the international group and the Seto-Wilson series. To date 85 hemolytic, coagulase-positive bovine staphylococci have been studied. Sixty-three, or 74.1%, were found to be sensitive to one or more of the phages employed. The phage-sensitive cultures were divided into six types, or patterns. Of the 85 cultures from ten herds, 39, or 45.3%, were sensitive to only one phage; 26 to S6, 12 to S2, and 1 to 42D. Twenty-four, or 28%, exhibited a phage pattern; 2 to S2/42D, 21 to S1/S3, and 1 to S4/S6. Twenty-seven, or 31.8%, of the cultures were sensitive to phage S6, 21 or 24.7% to S1, 21 or 24.7% to S3, 14 or 16.5% to S2, and 3 or 3.5% to 42D. None of the cultures was sensitive to Phage S5.

Growth response in heifer calves injected with normal bovine globulin. D. L. MACFADDEN, University of Massachusetts, Amherst.

Sixteen heifer calves were used to study the effect of injected bovine globulin on growth rate.

Four Holstein, two Guernsey, and two Jersey calves were randomly assigned into groups. Group I received whole milk at 10% of the body weight plus hay and grain ad libitum; Group II received the basal diet plus a 10-ml injection of bovine globulin given at the start of the trial. Calves two days old were separated from dams and placed on experiment. Duration of trial was 6 wk.

Mean birth weights were: Group I, 75 lb; Group II, 77 lb. Mean weights at 6 wk were: control, 106 lb; treated, 121 lb. During the trial Group I gained 30 lb; Group II, 45 lb. Gain expressed as per cent birth weight: controls, 142%; treated, 159%. Average daily gain was: controls, 0.75 lb; treated, 1.06 lb.

The difference in growth rate was significant at 5% level when the data were analyzed by analysis of variance.

The consumption of grain and hay was greater in the treated group; this difference was not significant.

Estrous cycle synchronization in cattle. H. VAN BLAKE, M. R. BRUNNER, AND W. HANSEL, Cornell University, Ithaca.

Thirty-six Holstein heifers were used in two trials, testing the ability of an orally active progestational agent, 6-chloro-Δ-6-17-acetoxyprogesterone, to synchronize estrous cycles. In the first trial, four groups of four heifers each were fed the hormone at 0.3, 0.1, 0.05, and 0.025 mg per pound body weight per day. The higher levels were fed for 20 days and the lower levels for 15 days each. All levels were effective in inhibiting estrus during the feeding period. Two of the four heifers in the 0.3 mg level came in estrus 13 days after hormone withdrawal; those on the 0.1 mg level came in estrus six to eight days after hormone withdrawal. Those on the two lower levels came in estrus four to seven days after the hormone was withdrawn. No heifers conceived

when bred at the induced estrus in the 0.3-mg level group as compared to 2, 4, and 3 in the 0.1, 0.05, and 0.025 mg levels, respectively. In the second trial, 18 of 20 heifers fed 0.02 mg per pound body weight for 20 days came in estrus four to seven days after the hormone was withdrawn. The potency of this hormone may make it useful for estrous cycle synchronization.

Some factors affecting the motility and pH of frozen bovine semen. B. H. Bean,* B. W. Pickett, and R. C. Martig, University of Connecticut, Storrs.

The effects of two extenders, heated, whole homogenized milk (milk), and egg yolk–citrate (EYC), two freezing methods, addition of crushed DI to an alcohol bath, and a Linde Co. Model BF-1 liquid nitrogen freezer and three storage temperatures, −79, −92, and −196 C on the motility and pH of two ejaculates from each of seven Holstein bulls were measured throughout a nine-months' storage period.

Motility of samples extended in EYC were higher ($P < 0.01$) than samples extended in milk at all storage times when averaged across all other treatments. There was no significant difference between extenders when DI freezing and −79 and −196 C storage were used. Motility of sperm frozen in DI were significantly higher than comparable semen samples frozen with LN, regardless of extender or storage temperature. For EYC extender, storage temperatures of −92 or −196 C were not significantly different, with respect to motility; whereas, both were superior to storage at −79 C. Milk extended samples exhibited significantly higher motility when stored for three months at −196 C than when stored at either −92 or −79 C.

Low correlations between pH of extender and motility and between change in pH during storage and change in motility were found.

Some techniques for isolation of lipids from bovine semen. R. J. Komarek,* B. W. Pickett, R. W. Dietz, and R. G. Jensen, University of Connecticut, Storrs.

Lipids from semen have been extracted with 2:1 chloroform-methanol using the Folch et al. procedure (J. Biol. Chem., 226:497. 1957). Total lipids were separated into the various lipid classes by thin-layer chromatography.

An application of from 50 to 100 mg on a single plate is possible without evidence of overloading when plates with a silicic acid layer 750 μ thick are used. Separations of semen lipids were achieved by using a series of plates and rechromatographing the incomplete separations. Although further separation is possible, the following gross separation can be accomplished with as few as two plates; phospholipids, monoglycerides, diglycerides, and cholesterol, triglycerides, and cholesterol esters, and hydrocarbons; these groups include compounds of similar polarity. Tentative identifications were made with infrared spectrophotometry, by comparison with knowns on thin-layer plates and with specific tests for compounds or elements.

Effect of various methods of thawing on motility of frozen bovine spermatozoa. B. W. Pickett,* R. C. Martig, and B. H. Bean, University of Connecticut, Storrs.

In Experiment 1, ten semen samples were frozen in dry ice–alcohol (DI) and divided for storage at −79 C and −196 C. After 2 yr of storage, samples were thawed in water (39-40 C), ice water (1-2 C), air (21-23 C), and alcohol (1-2 C). After post-thaw motility estimates were made, samples were incubated at 32 C and estimated at 30 min intervals until motility reached zero.

Post-thaw motility values averaged across storage temperatures were lower ($P < 0.01$) after thawing in air than by other methods. Post-thawing data, and data obtained after incubation at 32 C, indicated that method of thawing had little influence on livability of spermatozoa during incubation of semen frozen in DI.

Experiment 2 was conducted as outlined above, except that different bulls and a liquid nitrogen (LN) freezing method replaced dry ice–alcohol. Inappreciable differences in motility were obtained due to thawing method. Samples thawed in alcohol and in ice water were superior ($P < 0.05$), with respect to combined post-thaw and incubation motilities, to samples thawed in warm water, but only cold alcohol thawing was higher than thawing in air.

Effect of glycerol in a milk semen diluent on the fertility of liquid bull semen. J. P. Mixner,* S. H. Wiggin, J. W. Preston, K. O. Pfau, R. Van Skiver, and R. E. Mather, New Jersey Agricultural Experiment Station, Sussex.

From February 1, 1960, to April 30, 1961, with alternate semen ejaculates by bulls, glycerol was incorporated into a heated homogenized milk diluent at the rate of 10%, in fertility trials by the Sussex County Cooperative Breeding Association. The diluent included 1,000 units of penicillin and 1.0 mg of streptomycin per milliliter. For the glycerolated milk treatment, semen was added to one-half of the total diluent, cooled slowly to 5 C, and an equal quantity of 20% glycerol heated-milk diluent was added in three equal parts at 10-min intervals. Four Guernsey and 15 Holstein bulls (total of 561 pairs of ejaculates) were used. A total of 13,657 first service cows were bred, including 5,081 cows on day of semen collection (Day 0), 6,100 cows on

Day 1, 2,142 cows on Day 2, and 334 cows on Day 3. The mean per cent 60-90 nonreturns to first service for the milk and milk-glycerol diluents, respectively, were: Day 0, 76.6 and 77.8; Day 1, 76.7 and 75.3; Day 2, 72.6 and 71.8; Day 3, 71.8 and 68.0; Days 0-3, 76.0 and 75.5. The use of glycerol in a heated hilk diluent was not beneficial.

CALL FOR PAPERS FOR THE 1963 ANNUAL MEETING OF THE AMERICAN DAIRY SCIENCE ASSOCIATION

S. D. MUSGRAVE, Chairman, Program Committee, A.D.S.A.
Department of Dairying, Oklahoma State University, Stillwater

The 58th Annual Meeting of this Association will be held June 16-19, 1963, in the Memorial Center of Purdue University, Lafayette, Indiana.

All members of the Association, including graduate student affiliates, are entitled and encouraged to submit papers for consideration for presentation. Participation by members of industry and by senior members of the Association is especially encouraged. The Program Committee favors the general policy that an individual present only one paper and that his name should appear as author on no more than two. This committee, with the Association officers and membership, wishes to stimulate excellence in research and in presentation and realizes that these restrictions may penalize certain members engaged in full-time research. Therefore, a member may exceed these limits if he or his department ranks the affected abstracts in order of preference for oral presentation.

Papers submitted for the Annual Meeting must be confined to research that has not been reported elsewhere. Abstracts of papers accepted for publication by a scientific journal before the annual meeting are not acceptable for presentation at this meeting. If the total number of acceptable papers submitted by the membership for presentation in a section program is too great to include in the program, the committee will assign some papers to be read by title. In this event, consideration will be given to evident quality of research to be reported and number of abstracts per author and per department. Abstracts arriving late may be read by title or rejected.

Attention is called to the Dairy Manufacturing Extension Section program, and interested members are urged to participate. This is a subsection of the Manufacturing Section.

Mimeographed copies of pertinent details and data are desirable for distribution at the time of presentation. At least 300 copies should be made available. They should be numbered with the assigned program number in the upper right corner of the first page. This can be supplemented with slides for projection, provided the author can adhere to the assigned time of 12 to 14 min for presentation of each paper. For papers presented in joint sessions or in a symposium, it is suggested that 500 copies of the mimeographed material be made available.

ABSTRACT PREPARATION

The Program Committee continues to encourage improvement in quality of material to be presented and in the method of presentation. Good experimental design and interpretation are essential. Exact compliance with these instructions for preparation of abstracts will simplify the task of the Program Committee, improve the program, and ease your own work load. Careful editing of abstracts before submission is essential! Each year a number of abstracts must be returned for correction or clarification.

1. Submitted titles and abstracts must be in the hands of appropriate section officers by March 1. This deadline must be met to permit publication of titles with the complete program in the April JOURNAL and abstracts in the May or June issue.
2. Four copies of each abstract must be typed double-spaced on 8½- by 11-inch paper. The original (on bond paper) and the first carbon should be mailed to the chairman, the second carbon to the vice-chairman, and the third carbon to the secretary of the Section where the paper is to be presented. The original copy will be used for publication in the JOURNAL.

Student affiliates entering the Graduate Student Presentation Contest should check below for additional instructions on submitting abstracts.

3. Abstracts must not exceed 200 words by actual count. Those exceeding 200 words must be returned to the author for revision.
4. The style and abbreviations of the JOURNAL OF DAIRY SCIENCE must be used. Please refer to abstracts in the May, 1962 JOURNAL for guidance.
5. Only initials of authors should be used,

except in unusual cases, where it may be necessary to use the complete name.

6. When more than one author is listed, indicate who will present the paper by an asterisk after his name.
7. The title should indicate clearly the nature of the research. It should not be repeated in the text. The abstract should indicate, insofar as possible, the design and major results of the investigation. Only completed research should be reported. Brief, essential statistics will make the abstract more meaningful.
8. The following form with no caps for the title is correct: Utilization of carbohydrates posterior to the rumen-reticulum of the bovine. J. T. Huber and N. L. Jacobson, Iowa State University.
9. If the author lists an address for an experiment station other than the university, such as a USDA research branch or a commercial company, the complete address should be provided, as in the following example: A study of dye reduction methods as platform tests for the detection of antibiotics. Burdet Heinemann, Producers Creamery Co., Springfield, Missouri.
10. All symposium papers should be typed double-spaced and organized according to the acceptable style of the JOURNAL OF DAIRY SCIENCE. The author should send the first copy directly to the JOURNAL editor, E. O. Herreid, before the annual meeting.

GRADUATE STUDENT PRESENTATION CONTEST

This contest will be conducted in both the Production and Manufacturing Sections. A cash award will be given to the winner in each section. Each institution is entitled to enter one participant in each contest. Participants must be student affiliate members. Complete rules for the contest will be sent to department heads. Those wishing to enter the contest must submit copies of their abstracts to the section officers as outlined above. In addition they must include a letter indicating their desire to enter the contest. This letter must be signed by the major professor and the department head. A carbon copy of this letter and four additional copies of the abstract should be mailed to the contest representative so as to be received by March 1.

For Production Section Graduate Student Competition: Dr. J. L. Albright, Department of Dairy Science, University of Illinois, Urbana.

For Manufacturing Section Graduate Student Competition: Dr. A. V. Moore, Department of Dairy Science, The Agricultural and Mechanical College of Texas, College Station.

Names and addresses of officers of sections to whom titles and abstracts should be sent are:

EXTENSION SECTION

Chairman: C. D. McGrew, Department of Dairy Science, Ohio State University, Columbus 10.

Vice-Chairman: D. E. Voelker, Department of Animal Husbandry, Iowa State University, Ames.

Secretary: C. H. Parsons, Department of Dairy and Animal Science, University of Massachusetts, Amherst.

PRODUCTION SECTION

Chairman: L. H. Schultz, Department of Dairy Husbandry, University of Wisconsin, Madison.

Vice-Chairman: V. R. Smith, Department of Dairy Science, University of Arizona, Tucson.

Secretary: P. M. Reaves, Department of Dairy Science, Virginia Polytechnic Institute, Blacksburg.

MANUFACTURING SECTION

Chairman: D. M. Graham, Pet Milk Co., Research and Development Center, Greenville, Illinois.

Vice-Chairman: E. L. Thomas, Department of Dairy Industries, University of Minnesota, St. Paul.

Secretary: M. E. Ellertson, Carnation Research Laboratory, 8015 Van Nuys Boulevard, Van Nuys, California.

(The Dairy Manufacturing Extension Section is a subsection of the Manufacturing Section. A. L. Rippen, Department of Food Science, Michigan State University, East Lansing, is chairman, and J. C. White, Professor of Dairy and Food Science, Cornell University, Ithaca, New York, is secretary of this subsection. All abstracts for papers in this subsection should be submitted through the regular channels of the Manufacturing Section, and should be identified for presentation at the Manufacturing Extension Section meeting and a courtesy copy sent to A. L. Rippen, Chairman.)

PRICE SCHEDULE FOR REPRINTS OF PAPERS THAT APPEAR IN THE JOURNAL OF DAIRY SCIENCE

H. F. JUDKINS, Secretary-Treasurer
32 Ridgeway Circle, White Plains, New York

The Executive Board, at the time of the Annual Meeting of the American Dairy Science Association at the University of Wisconsin, increased the price of reprints 25%, effective July 1, 1961. The new reprint schedule follows:

No. of reprints	Number of pages							
	2	4	8	12	16	20	24	28	32
	(Cost in dollars)								
50	17.50	20.00	36.25	51.25	67.50	78.75	97.50	115.00	125.00
100	20.00	22.50	41.25	61.25	77.50	92.50	112.50	132.50	145.00
200	22.50	28.75	51.25	76.25	97.50	117.50	143.75	162.75	185.00
300	28.00	33.75	62.50	91.25	117.50	143.75	173.75	205.00	226.25
400	30.00	40.00	72.50	107.50	137.50	170.00	205.00	241.25	266.25
500	33.75	45.00	83.75	122.50	157.50	195.00	236.25	277.25	306.25
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It is hoped that the publication of this reprint schedule will make it easier for interested people to obtain reprints in any number desired and, at the same time, aid in disseminating useful information to the dairy and related industries.

BOOK REVIEW

AGRICULTURAL ENGINEERS' HANDBOOK, edited by C. B. Richey, Paul Jacobson, and Carl W. Hall. McGraw-Hill Book Company, New York. 1961.

This 880-page handbook contains 57 chapters divided into four sections: 1. Crop-Production Equipment. 2. Soil and Water Conservation. 3. Farmstead Structures and Equipment. 4. Basic Agricultural Data.

The book provides a comprehensive coverage of modern engineering practices relating to agricultural problems. Those wishing to pursue individual topics in greater detail will find a good bibliography.

It will meet the needs of those seeking one general reference on farm machines and their operation. Although details of machine design are not included, most types of farm machines are described, including expected performance rates and details of operation.

The Soil and Water Conservation section is well illustrated and documented. The explanations are snfficiently clear so that it is possible to use them in designing basic conversion structures. Farm managers will find this section useful in planning various practices and structures.

In the farmstead structures and basic data sections, dairymen will find useful information on housing requirements, construction materials, electrical equipment, and thermal environment. Although few structural design data are given, there are numerous data on wind, feed, and other load factors. Animal space and atmospheric environment requirements are discussed at length. Feeds and nutrition are touched on briefly. The electrical equipment section contains several contributions from well-known authorities.

Chapter 57 is a well-written discussion of climate and solar energy and their relation to agriculture. Included are effects of climate on crops, animals, and buildings. Since this subject has only recently received much attention, most of the references are from bulletins and technical papers. The information is up-to-date, accurate, and usable.

The handbook is well-illustrated and documented. It will not replace more comprehensive texts on a specific subject, but it is a good general reference on the field of engineering as applied to agriculture.

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INDEX OF AUTHORS OF PUBLISHED PAPERS IN VOLUME 45 OF THE JOURNAL AND ABSTRACTS OF PAPERS PRESENTED AT THE ANNUAL MEETING* AND AT THE EASTERN,[E] SOUTHERN,[S] AND WESTERN[W] DIVISIONAL MEETINGS OF THE AMERICAN DAIRY SCIENCE ASSOCIATION

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