Journal of

DAIRY SCIENCE

Vol. 45   January, 1962   Number 1

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(Continued from front cover)

ADMINISTRATIVE INFORMATION

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.

Papers should be organized in this order:

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. Specific and concise summary of 200 to 225 words in which the title should not be repeated. Summaries are used by abstracting journals. 3. Statement of the problem, pertinent investigations, and purpose of the study. 4. Procedures. 5. Results. 6. Discussions. 7. Conclusions. 8. Acknowledgments. 9. References— all must have name of periodical, volume and page number, and date published. If a book, publisher's name and address must be added.

Manuscripts must be typed double-space, including all tabular material, on 8½- by 11-inch bond paper. Lines on each page should be numbered from 1 to 26, to make it easier for the Editorial Board to write reviews. The side margins should be one inch wide. Clipped to, pasted on, and written insertions are not acceptable.

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 $\bigcirc$, $\odot$, $\bullet$, $\square$, $\blacksquare$, $\triangle$, $\blacktriangle$, $\blacktriangledown$, $+$, or $\times$ 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 and ordinate should be in upper case and be about 4 by 4 mm and about 0.5 mm thick, so as to be readable when graphs are reduced to column width. Titles for figures (graphs) must be on separate sheets.

Tables should be typed, each on a separate page and placed together at the end of the paper. They are costly and graphs should be used whenever possible.

Abbreviations for titles of periodicals and for botanical, chemical, mathematical, physical, and statistical terms should conform to those in the Style Manual for Biological Journals.

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.

Critical reading of papers by persons other than the author(s) will help to clarify statements and eliminate errors.

All manuscripts should be submitted to the Editor-in-Chief.

[1] American Institute for Biological Sciences, 2000 P Street, N. W., Washington, D. C. Price $3.00.
[2] J. Dairy Sci., 44: 1788. 1961.

PEOPLE AND EVENTS

MEMORIALS

H. O. Henderson

Dr. H. O. Henderson passed away unexpectedly at his home at Morgantown, West Virginia, December 11, 1961. Born at Elder's Ridge, Pa., in 1889, he received his Bachelor's and Master's degrees at Penn State (1915-1916). After serving as county agent in Crawford County, Pa. for a year, he accepted a position with the Dairy Department at West Virginia University, where he remained until his retirement. He was the department head for 28 years. Dr. Henderson earned his Doctorate in 1928 at the University of Minnesota. He was co-author of the well-known text, Dairy Cattle Feeding and Management, which has been widely used in this country and Latin America. He also contributed to numerous bulletins, circulars, and trade journal articles, and innovated many practices and short courses for the betterment of the dairy industry of West Virginia. He was active in the A.D.S.A., belonged to the Alpha Gamma Rho fraternity, and was affiliated with many state and national dairy and health organizations and academic honoraries. An elder in the Presbyterian Church, he was the secretary-treasurer of the West Virginia Westminster Foundation.

Dr. Henderson was given the 1958 A.D.S.A. Master Teacher Award, an honor which he deeply appreciated. His wife, Marian, of Morgantown and son, Robert E., of Haines, Alaska, and five grandchildren survive. At the time of his retirement from active teaching, his friends set up the H. O. Henderson Student Loan Fund at West Virginia University. At the present time, contributions to the fund total well over $3,000. Loans are made to deserving students without interest charges until after they graduate.

John W. Bartlett

J. W. Bartlett, former Chairman and Professor Emeritus of the Department of Dairy Science at the College of Agriculture, Rutgers University, passed away October 28, 1961, after an illness of several months.

He was born August 1, 1891, in Granville, New York. He attended school in Granville and received a B.S. degree from the University of Vermont, an M.A. from Columbia University, and a Ph.D. from New York University.

Dr. Bartlett was appointed to the staff of Rutgers University on 1916 as extension dairy specialist. He served in that capacity until 1920, when he became field secretary of the New Jersey Holstein-Friesian Association. He was appointed head of the Department of Dairy Science, Rutgers University, in 1922, a position he held until his retirement on June 30, 1961. In addition to his supervision of the dairy program of resident instruction and research at Rutgers University, Dr. Bartlett directed activities of the Agricultural Experiment Station's 1,300-acre dairy research farm in Sussex County, Beemerville, New Jersey.

Dr. Bartlett pioneered in the cooperative artificial breeding of cattle, the production of grass silage, and improvement of pasture methods for dairy cattle. He received greatest recognition for results obtained from a Holstein-Friesian breeding project established in 1932, aimed at improving the quality of Holstein-Friesian milk by a selection of bulls and methods of breeding. A large number of bulls from the breeding project have been used throughout the United States. Dr. Bartlett had a deep interest in dairy cattle breeding problems and many breeders sought his advice.

Dr. Bartlett served a three-year term on the advisory board of the American Feed Manufacturers' Association and served as chairman of the dairy section in 1954. He was active in the New York Farm Club and was chairman of the research council of the Pure Bred Dairy Cattle Association. In recognition of his many years of service to Rutgers University and the dairy industry, the Cooperative Inter-Breed Cattle Association of New Jersey

established a perpetual trophy in 1952, awarded annually to the best dairy student in short courses at the College of Agriculture. Two years ago he was given the New Jersey Award for Merit by the State Milk Industry Association and a distinguished service award was presented to him in July by the Garden State Milk Council.

He was a member of the American Dairy Science Association, the American Society of Animal Production, the American Genetic Association, Sigma Xi, Alpha Zeta, Sigma Nu, Alpha Kappa Pi, and Phi Delta Kappa.

He is survived by his widow, Abbie; a son, J. W. Bartlett, Jr. of Gloversville, N. Y.; a daughter, Mrs. Martha B. Smithcors of Santa Barbara, California; and a stepson, Leonard Walsh of Old Bridge, N. J.; and seven grandchildren.

J. L. Brence

J. L. BRENCE, professor in the Montana State College dairy department, died Monday at his home, 915 West Dickerson, Mill Iron, Montana. Professor Brence was born February 20, 1907, in Waukegan, Illinois. In 1913 the family moved to a homestead at Mill Iron. Brence was graduated from Carter County High School at Ekalaka in 1929. He then enrolled at Montana State College, from which he was graduated in 1934 with a Bachelor of Science degree. He was employed in industry until 1941, when he returned to M. S. C. as a staff member. He earned his Master's degree in 1944 and has continued as a staff member ever since. John will be remembered for his work with the dairy processing industry. Brence was a member of Gallatin Masonic Lodge No. 6 and was an officer of Zona Chapter No. 12 and Commander of St. John's Commandery No. 12. He was also a member of the Baptist church, the Royal Arch Masonic bodies, Yellowstone Council of Boy Scouts of America, the Merry Makers Club, and Lily of the Valley Chapter of Eastern Star; also a member of Alpha Gamma Rho social fraternity, Alpha Zeta and Sigma Xi honorary fraternities. He married Priscilla Y. Hauberg. Survivors include his parents of Mill Iron; two daughters, Phyllis Barnes of Bozeman and Mrs. Herbert (Betty) McAllister, Great Falls; a son, John David Brence of Bozeman; five brothers, Joseph T. Brence of Big Creek, California; Edwin F. Brence of Mill Iron; and Raymond J. and Harry T. Brence of Bozeman; two sisters, Mrs. Florence Blutt, Sioux City, Iowa, and Mrs. France M. Lewis of Salinas, California; two grandchildren and numerous nieces and nephews.

Professor Brence was highly respected in his field and will be greatly missed by his many associates and friends. He was revered by his students and they came to him for counsel whenever a problem arose.

G. B. Olsson

G. B. OLSSON, Extension Associate Professor of Dairy Science, University of New Hampshire, died suddenly on December 23, 1961.

Professor Olsson graduated from the University of New Hampshire in 1922 and had been a member of the Dairy Science staff at New Hampshire since 1944. He was Superintendent of Official Testing and had charge of the DHIA program in the state. In addition, he assisted with the 4-H dairy activities in New Hampshire. Professor Olsson was a member of the American Dairy Science Association and Epsilon Sigma Phi.

Leonard D. Brown Appointed on Michigan State Faculty

L. D. BROWN, who went to Michigan State University nearly three years ago as an instructor and has just completed the work for the doctorate, has been appointed Assistant Professor in the Dairy Department at that institution. His new assignment contemplates both teaching and research work. He assumes leadership of some of the projects that C. F. Huffmann conducted before his retirement July 1. One of these projects relates to the economies in the more liberal feeding of concentrates—increased energy intake—with the cows of superior inheritance.
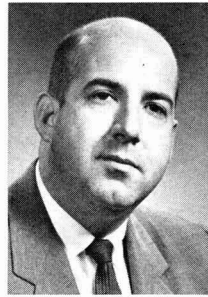
L. D. Brown

In Brown's research studies he will continue the work on calf feeding, several phases of which he has previously published. He also will lead the project on the use of organic solvents as forage preservatives. He will collaborate with other workers in the department on the rumen studies, factors that influence the production of volatile fatty acids, and urea utilization.

To date, Brown's numerous publications have proved to be contributions of high value.

He is a native of Kentucky. His Bachelor's degree was earned at Western Kentucky State College and his Master's at the University of Kentucky. He has had several years of experience in dairy herd operations and mangement.

Connecticut

A Symposium on Radioactive Fallout in Milk will be held on Dairy Manufacturing Day, April 4, 1962, at 10 AM., at the College of Agriculture Auditorium, University of Connecticut, Storrs.

Topics covered will be: (1) Fallout, nature, etc.; (2) public health tspects; (3) control

measures, including isotopic removal; (4) public relations. Although speakers have not yet been selected, it is hoped to have an outstanding group.

For information, contact: DR. R. G. JENSEN, Department of Animal Industries, University of Connecticut, Storrs, Connecticut.

New Hampshire

N. F. COLOVOS, Associate Professor of Dairy Science, University of New Hampshire, attended the European Zootechnic Association Symposium at Wageningen, Holland, in September, 1961.

Professor Colovos presented a paper on Energy Studies and Their Application to Dairy Cattle Feeds.

North Carolina

Max E. Gregory Appointed to New Department

M. E. GREGORY, who for the past three years has been Dairy Technology Extension specialist at Ohio State University, Columbus, Ohio, assumed the position of Assistant Professor in Food Science and Processing at North Carolina State College, Raleigh, as of January 1, 1962.
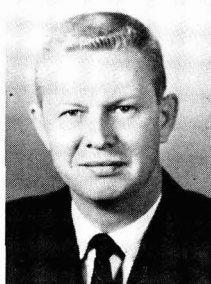
He received his B.S. degree from the University of Tennessee at Knoxville, and M.S. and Ph.D. degrees from North Carolina State College.

M. E. Gregory

New Appointments at Oregon State

DR. L. M. LIBBEY and DR. M. W. MONTGOMERY joined the staff of the Department of Food and Dairy Technology at Oregon State University last July as Research Associates. Montgomery is working with E. A. Day on oxidation of fats and Libbey is working with E. A. Day and J. O. Young on the chemistry of Cheddar cheese flavor. Both received their Ph.D. degrees from Washington State University last June.

Oregon Dairy Industries Short Course and Convention

Featured speakers at the Oregon Dairy Industries Short Course and Convention, to be held February 13-15 at Oregon State University, Corvallis, will be: F. V. KOSIKOWSKI, Cornell University; R. H. NORTH, Executive Director, International Association of Ice Cream Manufacturers; and E. S. HARRIS, Economist, United States Department of Agriculture.

Ohio

29th Annual Dairy Technology Conference

Plans are complete for the 29th Annual Ohio State University Dairy Technology Conference to be held at Columbus, February 13-15. The Conference will feature the theme Foundations for Progress: Men—Machines—Methods. The program will feature 30 speakers in its five main sections—Milk Supply, Engineering, Management, Quality Control and Cultured Products, and Ice Cream. The roster of speakers will include the following university representatives: W. S. ARBUCKLE, University of Maryland; C. W. HALL, Michigan State University; J. G. LEEDER, Rutgers University; J. C. OLSON, University of Minnesota; and C. E. PARMALEE, Purdue University.

The presentations in the Milk Supply Section will include: genetic aspects of SNF composition of milk milk contamination and adulteration; the mastitis situation; various outlooks on milk control; and bulk milk problems. Special programs will be held for fluid and manufacturing milk which emphasize problems of milk production specific to each of these areas.

Topics to be discussed in the Management and Plant Operations Section are: selection and development of men for higher responsibilities; obtaining production from men in a union atmosphere; management's responsibility to personnel; advances in milk sterilization and aseptic canning; fresh flavored sterile and concentrated milk products; UHT processing of fresh milk and flavor removal. One phase of this section will deal with trained manpower and will emphasize the use of engineering knowledge to integrate men and machines.

The Engineering Section will include: discussions on mechanization and automation with respect to the Ohio situation; developments of systems for the future; single service containers; cost analysis. Important considerations in converting to plastic-coated milk containers, and materials and methods for preventative maintenance also will be discussed.

The section devoted to Quality Control and Cultured Products will include: a critical appraisal of methods for fat and solids testing; control of milk quality problems; essentials of consumer package control; application of research facts to the production of cultured products, including culture care, phage control, and creaming and flavor problems in Cottage cheese; and advances in Cottage cheese manufacture.

Topics in the ice cream section will include: an evaluation of major nonfat ingredients for ice cream; ice cream flavor research; man-
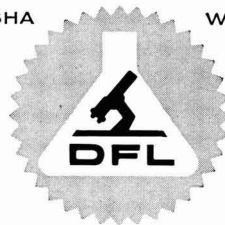
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Special clinics will feature new fluid milk products and special-flavored ice creams. The Conference will be highlighted by a banquet on February 14, held with the joint cooperation of the four Ohio Dairy Technology Societies.

The Ohio State University recently announced plans for an International Symposium on The Role of Food in World Peace, as a portion of its observance of the Land Grant Centennial, to be held April 30 through May 2, 1962. DR. R. M. KOTTMAN, Dean of the College of Agriculture and Home Economics, is Chairman of the International Symposium Council.

The principal objective of the proposed Symposium is to provide opportunities for a critical examination of the concept that helping food-deficit countries to produce more adequate food supplies will enhance the prospect for world peace. A secondary objective is to bring international attention to the magnitude and complexity of the problems inherent in the concept that food and peace are sequential. In pursuing these objectives, the implications of the export of the Land-Grant Idea to food-deficit nations will be explored thoroughly.

The Symposium will be centered around panels made of the present and former Directors-General of the Food and Agriculture Organization of the United Nations, and of other established authorities in international affairs.

The University's role in sponsoring this Symposium is evidenced by its sustained interest in world affairs, education for foreign students, and by its encouragement of staff members to work and study in universities overseas. Within the past decade, the University has entered into contract with foreign governments, notably the government of India, to aid in the establishment of Land-Grant College prototypes in overseas areas. Further evidence of the University's interest in world affairs can be found in the programs sponsored by its Mershon Committee on Education in National Security. This Committee supports a wide variety of research and educational projects designed to promote international peace and understanding.

DR. O. F. GARRETT, Director of Research, M & R Dietetic Laboratories, Columbus, was the featured speaker at the December meeting of the Columbus Section, American Chemical Society. His address described the three areas of research at M & R: general research and development; nutritional research; and clinical studies. A tour of the M & R production and laboratory facilities preceded the meeting.

The Department of Dairy Technology, Ohio State University, and the Ohio Department of Public Health will co-sponsor a Seminar for Public Health Administrators and Supervisors January 17-19 at Columbus. The Seminar will deal with public health problems on the administrative level, with participation being by invitation only. Later in 1962, the Ohio Department of Agriculture will join with these two agencies in sponsoring the Sanitarian's Short Course, March 19 through 23.

Market Milk and Ice Cream Conferences To Be Held at Purdue

F. N. Andrews, Head of the Animal Sciences Department at Purdue University, and F. J. Babel, professor in charge of the Dairy Manufacturing Section, have announced two, one-day meetings to be held in March 1962. The Market Milk Conference will be held on March 14 and the Ice Cream Conference on March 15, in the Memorial Center at Purdue University. The conferences are an annual affair sponsored in cooperation with the Indiana Dairy Products Association.

Wyoming News

DR. H. S. WILLARD has recently retired after serving nearly 40 years on the staff in the Dairy Section of the Division of Animal Science of the University of Wyoming. Dr. Willard returned last spring from his second foreign assignment. For the past two years, he was in Afghanistan as a member of the Wyoming University team.

DR. W. R. THOMAS has returned to the staff of the Dairy Section of the Division of Animal Science after a two-year leave of absence. During his leave of absence, Dr. Thomas earned his Ph.D. degree in Dairy Bacteriology at Iowa State University.

USDA
Federal Interagency Briefing of Dairy Industry on Strontium[90]

The U. S. Department of Agriculture, the Atomic Energy Commission, and the U. S. Public Health Service jointly sponsored two technical briefing sessions at Beltsville, Maryland, December 19 and 21, to review the status of fallout in relation to milk and to present a progress report of current research on means for removing Strontium[90] from milk. The first session, for the dairy processing industry, was attended by 150 persons, and the second, for dairy equipment manufacturers, had 50 persons in attendance.

The research was initiated in 1960 on a cooperative basis by the three agencies. The facilities and personnel are located in the Dairy Products Laboratory building at Beltsville. The work is being carried out on laboratory and pilot-plant scales by a new unit, Isotope Removal Investigations, which has a staff of chemists and food technologists with

DR. L. F. EDMONDSON as head. The removal process being developed is based on the ion exchange principle and is intended to be a standby method.

Six papers dealing with various aspects of radioactive fallout and progress of research on the removal of radiostrontium from milk were presented by representatives of the Atomic Energy Commission, the U. S. Public Health Service, and the U. S. Department of Agriculture. The laboratories and pilot plant of the Isotope Removal Unit were open to inspection.

N. D. Bayley Appointed Assistant Director

DR. N. D. BAYLEY was appointed Assistant Director, Animal Husbandry Research Division, Agricultural Research Center, Beltsville, Maryland, effective December 11, 1961.

Dr. Bayley is well known in the Division and in animal breeding circles throughout the country. Recently he was on special assignment to the office of the Division Director developing 5-year research plans. He is a native of Michigan and received his B.S. degree in animal husbandry at Michigan State. He did graduate work in animal genetics at the University of Minnesota and received a Ph.D. degree in Dairy Husbandry at Wisconsin. He was on the staff of the Dairy Husbandry departments at Wisconsin and Minnesota during the period 1948-55, and in 1955 was appointed as Assistant Head, Breeding and Management Section, Dairy Husbandry Research Branch, at Beltsville. Prior to his appointment as Assistant Director of the Division, he has been Leader, Breeding and Management Investigations, Dairy Cattle Research Branch.

J. G. Fransen Promoted at Beatrice Foods

J. G. FRANSEN has been promoted to administrative assistant to the executive vice-president of Beatrice Foods Company, Chicago.
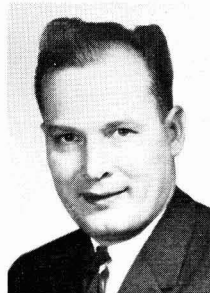
A veteran of more than 20 years with Beatrice Foods, Fransen started as a clerk in the cream procurement department of the company's Chicago plant in 1939. He advanced through various clerical and accounting positions and was named assistant general auditor in 1954.

J. G. Fransen

M. G. Korb Promoted at Borden's

M. G. KORB has been appointed assistant production manager for milk-based products of the Borden Foods Company. Formerly assistant superintendent of the company's powdered milk plant in Arcade, N. Y., Korb will assist E. C. HASKELL, production manager for evaporated and condensed milks, dehydrated

milks, malted milks, Instant Dutch Chocolate, and Hemo.

Cherry-Burrell Corporation Makes New Appointments

R. N. BAKER and G. F. COLVIN have been elected Vice-Presidents-sales of Cherry-Burrell Corporation for the Central and Eastern Sales Regions, respectively.

Baker, who has been with Cherry-Burrell for twenty-four years, was formerly Manager of the Company's Chicago Sales Branch. He will continue to have his headquarters in Chicago, and will have general jurisdiction over all sales, service, and warehousing operations of the sales branches in Chicago, St. Paul, Detroit, Pittsburgh, and Cincinnati.

Colvin was formerly Vice-President-Sales of the Pump Division of the Waukesha Foundry Company at Waukesha, Wisconsin. Prior to that he had been with Chrysler Corporation and then with Stainless Products Corporation of New York. In his new position as Vice-President-Sales Eastern Region, he will have jurisdiction over all sales, service, and warehousing operations of the Cherry-Burrell branches at Boston, New York, Philadelphia, and Charlotte, and the new Cherry-Burrell Service Center at Mountainside, New Jersey.

Formost Dairies Inc. Operating in Venezuela

A new Foremost Dairies plant producing whole powdered milk is now in full operation near Maracaibo, Venezuela. Foremost joined with the Venezuelan milk producers in the formation of Industrias Lacteas de Perija, C. A. (Ilapeca) to establish the plant. The new company will supply a substantial part of all powdered milk sold in Venezuela.

Lyle S. Turnbow Heads Foremost's Western Region

L. S. TURNBOW has been appointed Manager of the newly formed Western Region of Foremost Dairies, Inc. The new Region comprises all of Foremost's Fresh Milk and Ice Cream operations in California, Oregon, Washington, and Arizona.

Turnbow began his dairy industry career as a dairy science major at the University of California at Davis. In 1941 he joined Hage's Ltd. at San Diego and was its Vice-President and General Manager at the time this company was acquired by Foremost.

Completed Theses
M.S. Degree

M. G. CARRANCEDO—Stability of antifungal agents in Cottage cheese and their effect upon spoilage organisms. University of Nebraska.

S. K. DE—The physiological individuality of rectal temperature, pulse rate and respiration rate in calves with and without adrenaline administration. University of Nebraska.

J. W. KUHLMAN—Effect of stage of maturity on preservation and nutritive value. University of Nebraska.

DONALD PAUL STAHLY—Heat related factors affecting the rate of germination and subsequent growth of *Bacillus cereus* and *Bacillus licheniformis* spores in milk. The Ohio State University.

Ph.D. Degree

GORDON CHARLES KRESHECK—Changes in milk proteins at elevated temperatures as determined by light scattering. The Ohio State Univesrity.

Dairy Technology Societies

Central Illinois—Dr. L. D. Witter, Department of Food Technology, University of Illinois, was the speaker at the January meeting. His subject: Do You Know . . . ? "Mike" Hales of Paul-Lewis Laboratories will speak at the February session of this group.

Chicago—Dr. G. P. Gundlach, founder and president of G. P. Gundlach & Company, addressed the January meeting at the Furniture Mart. Dr. F. A. Kummerow, Food Technology Department, University of Illinois, spoke at the December meeting on the subject: The Experimental Lack of Evidence for a Role of Butterfat in Heart Disease.

Kansas—January 8 meeting of this group featured J. O. Hall as speaker. Mr. Hall is plant manager of Safeway Stores' Oklahoma City plant and president of the Oklahoma Dairy Technology Society. His subject was: Engineering the Dairy Plant for Efficiency.

Metropolitan—R. Hopkinson of Cherry-Burrell's Research and Development program at Cedar Rapids, Iowa, was featured as speaker at the January 9 meeting, with the topic Automation in Dairy Plants.

North Carolina—Topic of January meeting was Trends in Corn Syrup Usage, with L. G. Drusendahl of Les Drusendahl Company addressing the group.

Ohio—Christmas parties were the order of the day in December for all four groups comprising the Ohio Dairy Technology Society: Maumee Valley at the Pikeview Restaurant, Wauseon; Central Ohio, Desert Inn, Columbus; Northeastern Ohio, Greenbrier Restaurant, Parma Heights; and Cincinnati, Fort Mitchel Country Club, Fort Mitchel, Kentucky.

Tri-State—Topic of January was A Look at Our Laboratory, emphasizing the laboratory as a focal point of product control. Speakers: Elwood F. Schaffer, Pennsylvania Department of Agriculture, Harrisburg, Pennsylvania, and Joseph Sarandria, Laboratory Chief, Allegheny County Health Department, Pittsburgh, Pennsylvania.

Western Michigan—Program of the month (January) was devoted to expanding sales of dairy products. A. J. Brennan, Regional Merchandising Director, American Dairy Association, was the speaker of the evening.

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> —Russell Roberts
> Trainee Sealtest Foods
> Southern Division
> Atlanta, Georgia

CONTENTS

SECTION I states the objectives and requirements of the program.

SECTION II contains training schedules for all phases of plant operation for all types of plants. Many questions are asked for the trainee to answer before passing from one phase of training to the next, and progress reports and rating forms are provided.

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

The Appendix contains a list of professional and trade organizations and reference reading publications.

Copies will be needed by Plant Management, Plant Supervision, Plant Trainees, Teachers, for a reference text.

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Certain numbers of the Journal of Dairy Science are needed to maintain an inventory and to take care of orders. If any of the numbers listed are available and of no further use to the owners, please send them to the Garrard Press, 510 North Hickory Street, Champaign, Illinois. Fifty cents plus postage will be paid for each number.

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1925—September

1926—January
1932—September
1943—April
1959—January, April
1960—October

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1921—January, May, November
1922—January
1923—September, November
1924—January, May, July
1925—January, July
1926—March, May, November

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COMMONWEALTH AGRICULTURAL BUREAUX,
FARNHAM ROYAL, SLOUGH, ENGLAND

ADVERTISERS' INDEX

RESEARCH PAPERS

CHARACTERIZATION OF K-CASEIN OBTAINED BY FRACTIONATION WITH TRICHLOROACETIC ACID IN A CONCENTRATED UREA SOLUTION [1,2]

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SUMMARY

κ-casein-rich preparations were isolated from both isoelectric whole casein and crude α-casein by fractionation with trichloroacetic acid (12%) in urea solution (6.6 M). Preparations fractionated from isoelectric whole casein at 4 C and at room temperatures (20-24 C) contained $\cong 77\%$ and 55% of κ-casein, respectively. The latter preparations were nonstabilizing to α_s-casein. The preparation fractionated from crude α-casein contained $\cong 92\%$ κ-casein. The residual proteins consisted principally of β-casein and a small amount of λ-casein.

Sedimentation coefficients for κ-casein in the 92% preparation were $S_{20} = 12.9$ (polymer) in phosphate buffer at pH 7.0, $\Gamma/2 = 0.1$ and $S_{20}^{c=0} = 1.4$ (monomer) in phosphate:KOH buffer at pH 12.2, $\Gamma/2 = 0.19$. A weight average molecular weight of $\cong 24,000$ was calculated for the monomeric species by Archibald's approach-to-equilibrium method. An isoelectric point at pH 4.1 was determined by free-boundary electrophoresis. This preparation stabilized 90% of α_s-casein in a 1:10, κ-/α_s-casein mixture in the presence of 0.02 M CaCl$_2$. Following a treatment with rennin, 25.7% of the protein nitrogen was recovered as a soluble protein fraction.

The term κ-casein was used first by Waugh and von Hippel (16) to describe a calcium-insensitive fraction of casein. Linderstrøm-Lang (6), as early as 1925, recognized the calcium-insensitivity of a casein fraction which they called Z-casein. McMeekin (9) preferred this terminology to describe the fraction, while others refer to it as the calcium-insensitive or calcium-soluble fraction (1, 3, 18), α_3-casein (5), or merely as κ-casein (13, 16). Presumably, these investigators were reporting the same casein fraction obtained in various degrees of purity.

κ-Casein plays a major role in the stabilization of the casein micelle in its natural environment (15) and in the clotting phenomenon induced by the action of rennin (10, 12). Under the usual conditions of electrophoresis in alkaline buffers it migrates as a component of the leading electrophoretic peak, a characteristic which served to mask its earlier recognition.

κ-Caseins have been isolated by a variety of procedures. Waugh and von Hippel (16) treated a dialyzed casein sol with 0.25 M CaCl$_2$ at pH 7.0 and 37 C. The centrifuged supernatant, Fraction S, contained a relatively large concentration of κ-casein ($\cong 70\%$). Hipp et al. (5) added CaCl$_2$ (0.2 M) to a solution of α-casein at pH 7.0. The centrifuged supernatant contained a calcium-insensitive fraction which was concentrated further into a pellet of α_s-casein by centrifugation at 100,000 $\times$ G. Fox (3) treated a sodium caseinate sol with 0.15 M CaCl$_2$ at pH 11.0 and 5 C, lowered the pH to 8.3, and removed the aggregated fraction. The supernatant, clarified at 30 C, was adjusted to pH 4.7 to precipitate a kappa-rich fraction. Wake (13) isolated a fraction containing 98% κ-casein from Waugh and von Hippel's Fraction S by reprecipitation at pH 4.4 and 2 C.

The observations made by Hipp et al. (4), that isoelectric casein was soluble in concentrated urea solutions, and by Wake (12) and by Nitschmann and Beeby (10), that the primary scission product of the action of rennin on κ-casein—Glyco-macropeptide—was soluble

in 12% trichloroacetic acid (TCA) solutions, stimulated the authors to postulate that κ-casein would remain soluble in concentrated urea solutions upon the addition of 12% TCA. The highly dissociated state of casein micelles in concentrated urea solutions and the apparently strong hydrophilic nature of κ-casein support this postulation.

The data reported herein resulted from a study of the isolation and characterization of κ-casein-rich fractions isolated under various conditions as the soluble protein in concentrated TCA-urea solutions.

EXPERIMENTAL PROCEDURES

Testing the postulate. Experiments were performed to determine the suitability of trichloroacetic acid (TCA) for selective precipitation of non-κ-casein fractions from concentrated urea solutions of isoelectric casein. Isoelectric casein (wet casein containing ≅50% dry matter) was dissolved in concentrated urea solutions (ranging from 3 to 8 M) to give casein concentration in the order of 2 to 4% at 20-24 C. These solutions were divided into lots to which crystalline TCA was added in varying concentrations ranging from 6 to 20%. The protein fraction not precipitated by the TCA and recovered in the supernatant from these centrifuged mixtures was assayed both electrophoretically (in veronal buffer, pH 8.6) and as a substrate for rennin. Thus, the most effective combination of urea and TCA to remove the non-κ-casein fraction was found to be achieved through the combination of 12% TCA in a 6.6 M solution of urea containing approximately 2% casein. Higher concentrations of urea and TCA were ineffective as a means of achieving further resolution of the two fractions. Lower concentrations of TCA resulted in a less complete precipitation of the non-kappa fraction. Lower concentrations of urea produced lower yields of crude κ-casein in the supernatant.

Results of these exploratory experiments indicated clearly the practicability of the principles employed as a means for isolating κ-casein-rich preparations from casein.

Isolation of κ-casein-rich preparations. Pooled, raw milk was separarted at 40 C, diluted with an equal volume of water at 40 C, and adjusted to pH 4.6 with N HCl to precipitate the total casein fraction. The precipitated casein was dissolved with NaOH at pH 8.0, reprecipitated and washed with several volumes of a 1:1 (v/v) mixture of ethyl ether and ethanol. The washed casein was dispersed and reprecipitated several times to assure the

complete elimination of serum proteins. κ-Casein-rich fractions were isolated directly from isoelectric whole casein or a crude α-casein preparation, as indicated by the isolation flow diagram, Figure 1.

By starting with isoelectric casein in Step 3 and proceeding at a low temperature (3 C) through Step 4, a kappa-rich fraction was recovered from the supernatant which was similar in composition to Waugh and von Hippel's (16) Fraction S and was identified as Preparation No. 1. In the isolation of Preparation No. 2, TCA was added to the casein-urea solution at room temperature (20-24 C). This κ-casein preparation was characterized by a lower concentration of κ-casein (measured from area on sedimentation diagram), exhibiting lower electrophoretic mobilities and a higher sedimentation coefficient than observed for the κ-casein of Preparation No. 1. A corresponding increase in the concentration of the slow-sedimenting component, presumed to be β- and/or λ-casein, was noted. Preparation No. 2 was incapable of stabilizing α_s-casein in the presence of 0.02 M CaCl₂. Consequently, the practice of adding TCA to the casein-urea solution at room temperature was abandoned.

For the purpose of eliminating the relatively high carry-over of β-casein from the TCA precipitation step, the procedure was modified by introducing crude α-casein in Step 3 in place of isoelectric casein. α-Casein was prepared by modifying the Hipp et al. (4) procedure as outlined in Steps 1 and 2. The α-casein cut was obtained from a 3.3 M urea solutioon instead of from the recommended 4.63 M solution. Purification was pursued without the incorporation of sodium chloride. The resulting α-casein contained a small amount of electrrophoretically discernible β-casein which was carried into the crude κ-casein fraction. Further purification, adapted from a procedure reported by Wake (13), Step 6, was required to remove additional quantities of the β-casein. β-Casein responded to the preparative scheme as though it had formed a fairly stable complex with κ-casein. The purified kappa-preparation was identified as Preparation No. 3.

Studies currently in progress in this laboratory have shown that by repeating the α-casein purification procedure, Step 2, three to four times a β-casein-free, α-casein fraction was obtained from which a β-free, κ-casein was isolated in Step 3.

Interestingly, when salt was used in the purification of α-casein, as recommended by Hipp et al. (4), κ-casein was not recovered in the TCA-urea supernatant (Step 3). Instead, λ-

ISOELECTRIC CASEIN (Step 1, 20-24 C)

Dissolve in urea (6.6 M)
Dilute to 3.3 M by adding distilled
water dropwise
Adjust to pH 4.8 and allow to
stand for 1-2 hr
Decant most of the supernatant and
centrifuge remaining mixture

PRECIPITATE (Step 2, 20-24 C)   SUPERNATANT (Discard)

Redissolve in 6.6 M urea
Dilute to 4.0 M urea
Centrifuge at 1,000 × G for 20 min
Discard the supernatant and repeat Step 2

SUPERNATANT (Discard)   CRUDE-α-CASEIN [a] (Step 3, 3 C)

Redissolve in 6.6 M urea
(2-4% protein soln.)

[a] Isoelectric casein was substituted   Add solid TCA at 3 C
for Preparation No. 1 and 2   (12% w/w)

Allow to settle for 30 min and centri-
fuge at 1,000 × G for 30 min

SUPERNATANT (Step 4, 3 C)   PRECIPITATE

Raise to pH 7 with solid NaOH   (Principally α_s-casein, void of
Dialyze   calcium-insensitive fraction)
Pervaporate to 1-2% protein conc.
Adjust to pH 8
Add 2 M CaCl₂ to 0.25 M at 3 C
Allow to stand for 12-16 hr
Centrifuge (1,000 × G; 30 min at 3 C)

PRECIPITATE (Discard)   SUPERNATANT (Step 5, 30-35 C)

Centrifuge at 25,000 × G for 10 min

SUPERNATANT (Step 6, 3 C)   PRECIPITATE (Discard)

Dialyze
Pervaporate to 1-2% protein conc.
Adjust to pH 11.3 momentarily
Add cold 0.2 N HCl to pH 4.4
Centrifuge at 1,000 × G for 10 min

SUPERNATANT (Discard)   PRECIPITATE (κ-casein)

Dissolve with NaOH
Dialyze
Freeze-dry

FIG. 1. Procedure followed for obtaining κ-casein-rich fractions from a concentrated urea-TCA solution of isoelectric casein or crude α-casein.

casein was obtained in an almost pure state
(unpublished). Apparently, κ-casein is not nec-
essarily a component of the NaCl-purified α-ca-
sein. Zittle et al. (18) reported that a calcium-
sensitive, α-casein fraction was the primary
constituent of a precipitate recovered when a
6.6 M urea solution of α-casein containing
3.18 g of NaCl per 150 ml of solution was
diluted to 3.3 M urea and that a calcium-insen-
sitive fraction was the major constituent of
the supernatant.

ANALYTICAL METHODS

Electrophoresis. Free-boundary electropho-
retic analyses were performed in a Perkin-
Elmer, 38-A electrophoresis apparatus employ-
ing the 2-ml Tiselius cell at 2 C. Mobility
measurements were made from the position of
the initial boundary on the descending and
ascending patterns. The ascending patterns
were used principally as an aid in discerning
electrophoretic homogeneity. The buffers em-
ployed were made to 0.1 ionic strength and
pH values were measured at 24 C.

*Ultracentrifugal and diffusion characteris-
tics.* Sedimentation-velocity studies were con-
ducted in a Spinco Model E ultracentrifuge
equipped with phase plate and analytical ac-
cessories. Temperatures were controlled to
within 0.1 C for the duration of the analytical
runs. Boundary sedimentation distances were
measured from the maximum ordinate of the
Schlieren patterns.

Molecular weight determinations were made
by employing the Archibald approach-to-equi-
librium technique as described by Schachman
(11), but modified in the following ways:
First, the approach-to-equilibrium was made
in the synthetic boundary cell, as suggested by
Ehrenberg (2), whereas Schachman used the
synthetic boundary cell only as a refractometric
technique to determine the initial protein con-
centration (Co). Thus, following the approach-
to-equilibrium run, the cell contents were mixed
and solvent was added to the upper chamber
of the synthetic boundary cell in preparation
for a second centrifugation to determine the
initial protein concentration. Secondly, refrac-
tive area measurements were made from the
Schlieren pattern by placing fine-ruled, graph
paper on a fivefold enlargement of the photo-
graphic plate instead of from a fivefold en-
largement of a photographic tracing.

Diffusion characteristics were studied by two
methods: First, the Schlieren lens technique
was employed, utilizing a 2-ml Tiselius cell in
the Perkin-Elmer electrophoresis apparatus
maintained at an accurately controlled tempera-

ture of 20 C. Diffusion patterns were recorded
over a period of 24 hr from which peak areas
and heights were measured $(A^2/4\pi H^2)$ and
plotted as a function of time. The value of
the slope of the line connecting these experi-
mental points represented the diffusion coeffi-
cient. The second method utilized the Schlieren
diagrams recorded from a low-speed centrifuga-
tion ($\cong$15,000 rev/min) in a synthetic boundary
cell, according to the method of Ehrenberg (2).

Stabilizing factor. The technique of Zittle
(17) was employed to determine the ability of
the κ-casein preparations to stabilize α_s-casein
(calcium-sensitive fraction) in the presence of
calcium ions. Ten-milliliter test solutions con-
taining 100 mg of α_s-casein, various quantities
of κ-casein, and $CaCl_2$ in an equivalent concen-
tration of 0.02 M were held at 30 C for 30 min.
These mixtures were centrifuged at 3,000 × G
for 5 min. The amount of protein remaining
in the supernatant was determined spectropho-
tometrically at 280 mμ and represented the
stabilized protein system.

RESULTS AND DISCUSSION

*Comparative characteristics of the κ-casein-
rich preparations.* Electrophoretic, ultracen-
trifugal, and yield characteristics of the κ-
casein preparations described are recorded in
Table 1 and Figure 2. These data demonstrate
obvious differences in composition and prop-
erties between Preparations No. 1 and 2, ob-
tained directly from isoelectric casein, and
No. 3, fractionated from crude α-casein. Elec-
trophoretic resolution in neutral or alkaline
buffers showed that Preparation No. 3 con-
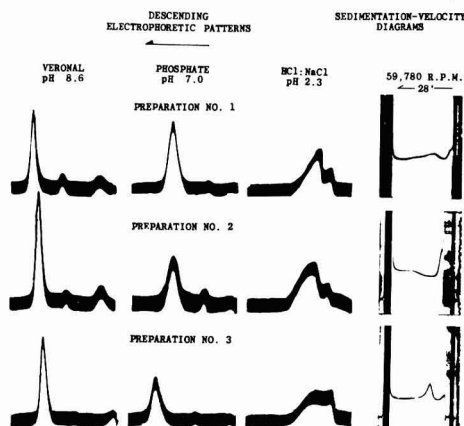
FIG. 2. Electrophoretic and ultracentrifugal
sedimentation-velocity patterns of the κ-casein
preparations reported in Table 1. Udtracentrifu-
gation was conducted in phosphate buffer (pH
7.0) at 3 C.

TABLE 1

Comparison of the electrophoretic, ultracentrifugal, and yield characteristics of κ-casein preparations

Identification of κ-casein preparation	Properties of the electrophoretic peaks [a]						Sedimentation-velocity[c] values for leading boundary		
	Buffer [b]						S_{20}	Concentration (A%)	Yield[d] (%)
	Veronal pH 8.6		Phosphate pH 7.0		HCl:NaCl pH 2.4				
	Peak 1	Peak 2	Peak 1	Peak 2	Peak 1	Peak 2			
Preparation No. 1 TCA added to solution of urea and isoelectric casein at 2-4 C	$\mu^{e}=-7.4$ $A\%^{f}=90$	-4.6 10	-6.6 96	-3.7 4	$+3.4$ 83	$+1.4$ 17	12.7	77	8.9
Preparation No. 2 TCA added to solution of urea and isoelectric casein at 21-24 C	$\mu=-6.2$ $A\%=90$	-3.6 10	-6.2 89	-2.6 11	$+3.0$ 85	$+1.2$ 15	20.6	58	6.0
Preparation No. 3 TCA added to solution of urea and crude α-casein at 2-4 C	$\mu=-6.7$ $A\%=99$	-3.4 1	-7.5 97	-3.2 5	$+3.1$ 76	$+1.2$ 24	12.9	92	8.3

[a] Fast component was presumed to be the κ-casein fraction.
[b] Concentration of protein was 1.0%.
[c] Protein was carried in phosphate buffer at pH 7.0, $\Gamma/2 = 0.1$, at 3 C.
[d] Expressed as the percentage of total isoelectric casein.
[e] Mobilities were measured from descending pattern.
[f] Relative areas were measured from descending pattern.

tained the least amount of electrophoretically discernible β-casein. Resolution in the HCl: NaCl buffer system became less definitive as the concentration of β-casein in the preparations decreased. Possibly, small amounts of β-casein were associated with κ-casein under these experimental conditions.

The sedimentation diagrams appeared to be more descriptive of the composition of the protein preparations. These data showed quite clearly that Preparation No. 3 contained the highest concentration of κ-casein. Presumably, the contaminating material in these preparations, as represented by the slow-sedimenting boundary, was a mixture of β-casein and λ-casein. Because of information obtained from the electrophoretic studies, it would be difficult to reconcile the presence of large quantities of β-casein unless this component migrated with κ-casein in the electrophoretic analyses. More logically, and in harmony with the interpretation of the authors, a large portion of the slow-sedimenting boundary recorded for Preparations 1 and 2 was attributed to the presence of λ-casein, which appeared to accompany the

isolation of κ-casein from isoelectric casein. The κ-casein of Preparation No. 2—room temperature fractionation—appeared to be considerably more poly-dispersed and possessed a higher sedimentation-coefficient than in either of the cold preparations.

Characterization of κ-casein. Preparation No. 3 was selected as being most typically κ-casein and was characterized more rigorously. The anhydrous protein was composed of approximately 0.35% phosphorus and 13.5% nitrogen. It was soluble to the extent of 15% in distilled water at 5 C, pH 7.0. In a 1:10 ratio with α_{s}-casein at 30 C, 90% of the α_{s}-casein was incorporated into a soluble, calcium-stable complex (Figure 3), a value similar to that reported by Zittle (17). The ability of κ-casein to associate with α_{s}-casein to forrm calcium-stable complexes is one of its basic characteristics. Waugh and Gillespie (15) stated that the calcium-caseinate micelles of milk contained α_{s}-, β-, κ-, and m-caseins.[4] Under appropriate

[4] m-Casein may be similar to what is more frequently referred to as λ-casein.
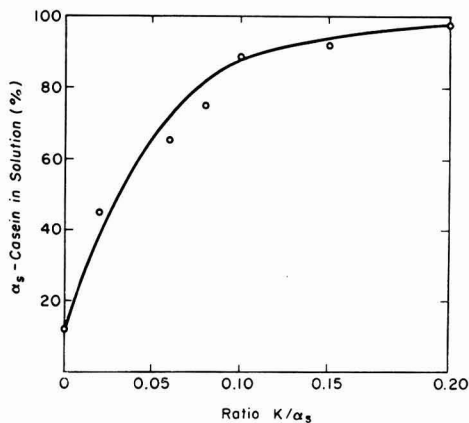
FIG. 3. The stabilization of α_s-casein by various amounts of κ-casein Preparation No. 3.

conditions, all of these components were capable of interacting and the most important interaction involved three molecules of α_s-casein and one molecule of κ-casein.

Electrophoretic and sedimentation characteristics of α-casein were compared with those for two κ-/α_s-casein complexes formed by two different treatments of a 1:4 mixture of κ- and α_s-caseins. In the first method, the protein mixture in a 1% solution was adjusted to pH 12 for 30 min at 24 C, followed by neutralization to pH 7.0. The adjusted solution was equilibrated against phosphate buffer (pH 7.0) prior to analysis. The second procedure consisted of dissolving the protein mixture in 6 6 M urea solution and adjusting to pH 4.8. When this solution was diluted to 3.0 M with respect to urea, a precipitate was obtained which was taken up in water and adjusted to pH 8.5 and dialyzed to remove urea. The urea-free solution was equilibrated against phosphate buffer (pH 7.0) prior to analysis (results shown in Figure 4).

Electrophoretic differences between the α-casein fraction and the induced complexes were minor. Apparently, the small quantity of β-casein present in the kappa preparation was either closely associated with the complex formed out of the urea solution or eliminated completely from participation in the complex structure, a possibility which seems most likely to have occurred.

The sedimentation-velocity diagram showed more discernible differences between these complexes. Obviously, the κ-casein must have entered into the complex with α_s-casein, since no boundary attributable to κ-casein was observed.

Sedimentation coefficients (S_{20}) for α-casein (4.3), the complex obtained following alkaline treatment (6.2), and the complex obtained out of the urea solution (4.7), were somewhat different. These values may refect differences in the structural details of the respective complexes. Waugh and von Hippel (16) reported an S_{20} of $\cong 6.5$ for a complex formed by using an alkaline treatment similar to the one described herein. A difference in the shape of the sedimenting boundary of the induced complexes was apparent. Diagrams for the alkaline-induced complex reflected a greater degree of homogeneity in the molecular species constituting the complex, an observation which might be explained on the basis of specific differences in the manner in which monomeric κ- and α_s-caseins reoriented under these experimental differences.

Sedimentation-velocity diagrams of an untreated κ-/α_s-casein mixture revealed three sedimenting boundaries: $S_{20} = 12.6$ (κ-casein), $S_{20} = 5.0$ (α_s-casein), and $S_{20} = 2.0$ (β- and/or λ-casein introduced as components of the kappa preparation). Waugh and von Hippel (16), and later Waugh (14), reported that 1:4 mixtures of κ-/α_s-casein would complex spontaneously at pH 7.0. Our data showed that spontaneous complex formation at pH 7.0 did not occur. Possibly this apparent difference in
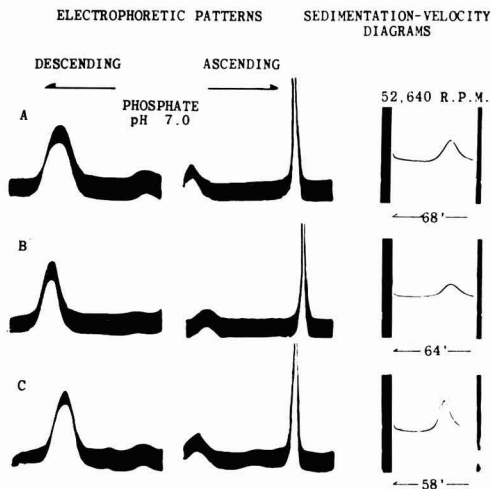
FIG. 4. Electrophoretic patterns and sedimentation-velocity diagrams of A) Hipp et al. (4) α-casein and κ-/α_s-casein complexes induced by B) precipitation from 3.0 M urea and by C) momentarily adjusting a solution of the mixture to pH 12.0. Ultracentrifugation was conducted in phosphate buffer (pH 7.0) at 20 C.

TABLE 2

Electrophoretic properties of κ-casein (Preparation No. 3) in buffers from pH 2.3 to 8.6

Buffer System ($\Gamma/2 = 0.1$)	pH	Protein concentration (%)	Mobility ($\mu = $ cm^2, sec^{-1}, v^{-1}, $\times 10^{-5}$) Descending	Ascending	Refractive area [a] (%) Fast peak	Slow peak
HCl:NaCl	2.3[b]	2.0	+2.4[c]	+3.2[c]	100[e]	0
			+0.8[d]	+2.2[d]		
Acetate	4.5[b]	0.6	−3.0	−3.1	......	....
Acetate	4.6	0.6	−4.4	−4.4	92.7	7.3
Acetate	4.7	0.6	−4.6	−4.7	......	....
Acetate	5.1	0.6	−6.3	−6.5	......	....
Acetate	5.3	0.6	−6.2	−6.6	......	....
Acetate	5.5[b]	0.6	−6.4	−6.4[d]	96.2	3.8
				−5.9[e]		
Phosphate	6.0[b]	0.6	−6.8	−7.6[d]	95.1	4.9
				−7.1[e]		
Phosphate	6.5	0.6	−7.2	−7.4	92.5	7.5
Phosphate	6.7	0.6	−7.7	−8.1	93.7	6.3
Phosphate	7.0[b]	0.6	−8.2	−8.5	94.7	5.3
Phosphate	7.1	0.6	−7.6	−8.1	97.7	2.3
Phosphate	7.5	0.6	−8.6	−8.9	......	....
Veronal:HCl	8.3	0.6	−7.5	−7.6	95.0	5.0
Veronal	8.6[b]	1.0	−6.7	−6.8	99.0	1.0

[a] Measured from the descending patterns.
[b] Electrophoretic patterns for these analyses are shown in Figure 5. Higher concentrations of protein are required at pH 2.3 to give comparable refractive areas.
[c] Mobility of the leading portion of the divided peak.
[d] Mobility of the trailing portiion of the divided peak.
[e] Combined area of divided peak.

complex formation can be attributed to the manner in which the original casein was obtained. Our casein was collected by isoelectric precipitation from skimmilk, whereas Waugh's procedure employed ultracentrifugal fractionation in the presence of calcium ions.

The data obtained from a detailed electrophoretic study of Preparation No. 3 are presented in Table 2. Descending and ascending patterns selected from these data are shown in Figure 5. A divided peak was observed in HCl:NaCl buffer at pH 2.3 both in ascending and in descending patterns. To a lesser extent, and restricted to the ascending patterns, a similar phenomenon was observed in acetate buffers at pH 5.5 and in phosphate buffer at pH 6.0. The nature of the split observed in the HCl:NaCl buffer was interesting from the standpoint that sedimentation-diagrams recorded under the same conditions showed a similar split in the boundary attributed to κ-casein. Although these observations seem to illustrate the heterogeneity of κ-casein, the authors feel that they manifest the existence of a polymeric species of κ-casein induced by the experimental environment.

The electrophoretic data showed that the principal peak, attributed to κ-casein, moved as a single boundary. A closer inspection of the Schlieren patterns revealed the presence of a small trailing peak with a mobility corresponding to that of β-casein. The refractive area of this peak varied from 7.5% in phosphate as pH 6.5 to 1.0% in veronal at pH 8.6 (Table 2). In HCl:NaCl at pH 2.3 the presence of a peak corresponding to free-migrating β-casein was not established, possibly because of the overlapping of the divided, major peak area or the association of β-casein with κ-casein. These observations form the basis for the conjecture that small amounts of β-casein are capable of interacting or associating with κ-casein in buffer solutions alkaline (pH 5.0 to 8.6) or acidic to the isoelectric point of κ-casein. The split-peaks observed in the ascending patterns of the mildly alkaline buffers (pH 5.5 and pH 6.0) could have been a manifestation of a less stable protein-protein associations.

The descending mobilities for the principal electrophoretic component recorrded in Table 2 were plotted as a function of the pH of the buffer, Figure 6. The solid line connects the

ELECTROPHORETIC PATTERNS

DESCENDING   ASCENDING

HCl:NaCl
pH 2.3

5000 sec.; F = 6.94 v. cm.$^{-2}$

ACETATE
pH 4.5

5000 sec.; F = 8.13 v. cm.$^{-2}$

PHOSPHATE
pH 5.5

5000 sec.; F = 8.24 v. cm.$^{-2}$

PHOSPHATE
pH 6.0

3600 sec.; F = 9.41 v. cm.$^{-2}$

PHOSPHATE
pH 7.0

3600 sec.; F = 10.09 v. cm.$^{-2}$

VERONAL
pH 8.6

3800 sec.; 10.96 v. cm.$^{-2}$
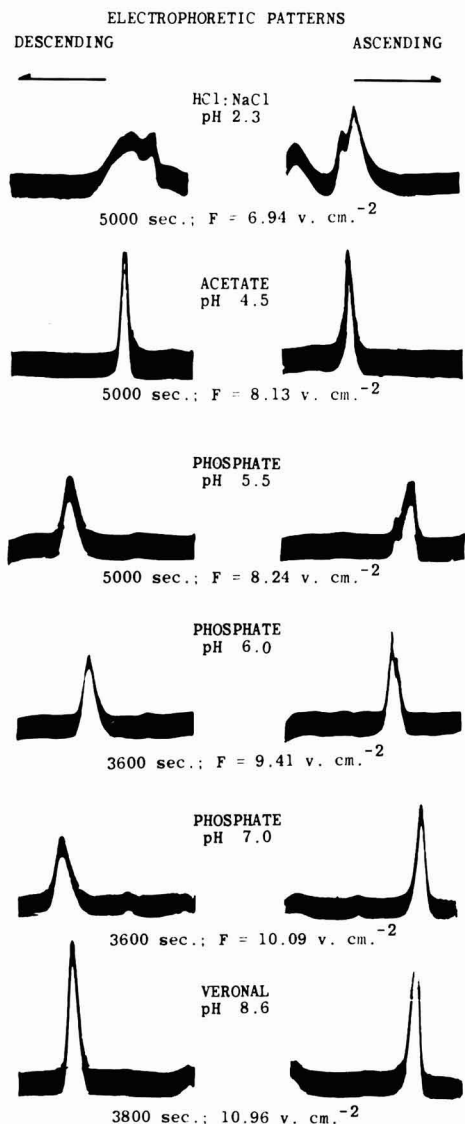
FIG. 5. Electrophoretic patterns of κ-casein Preparation No. 3 in buffers ranging from pH 2.3 to 8.6, Γ/2 = 0.1 (see Table 2).

experimental values and intercepts the point of zero mobility (isoelectric point) at approximately pH 3.8. The increases in mobility under experimental conditions represented by both extremities of the curve were not proportionate to changes in the buffer pH. These results support the previously held conjecture that κ- and β-caseins associate in alkaline or acidic media. The broken line connecting mobility

values of κ-casein presumably unassociated with β-casein was extrapolated to an isoelectric point at pH 4.1.

To identify further the isoelectric range of κ-casein at 0.1 ionic strength, the pH of minimum solubility was determined. Ten milliliters of 0.04 M acetate buffers containing 0.16 M sodium chloride and ranging in pH values from 3.6 to 4.5 were prepared. Five milliliters of each were added to 5-ml portions of a 2% κ-casein solution at 5 C. Following an equilibration period of 24 hr, the solutions were centrifuged at 3,000 × G for 10 min. The quantity of protein remaining in the supernatant demonstrated a minimum solubility in the range of from pH 3.8 to 4.2 (pH of mixture).

Ultracentrifugal data for Preparation No. 3 are recorded in Table 3. In phosphate buffer at pH 7.0, Γ/2 = 0.1, κ-casein sedimented as an asymmetric boundary comprising 92% of the refractive area of the Schlieren pattern. The observed sedimentation-coefficient of (S_{20}) 12.9 agreed well with previously reported values of 13.3 (16), 13.0 (8), and 13.2 (7). The slow boundary showed characteristics similar to those reported for β- and λ-casein. An S_{20} of 14.5 was observed for the κ-casein boundary in an
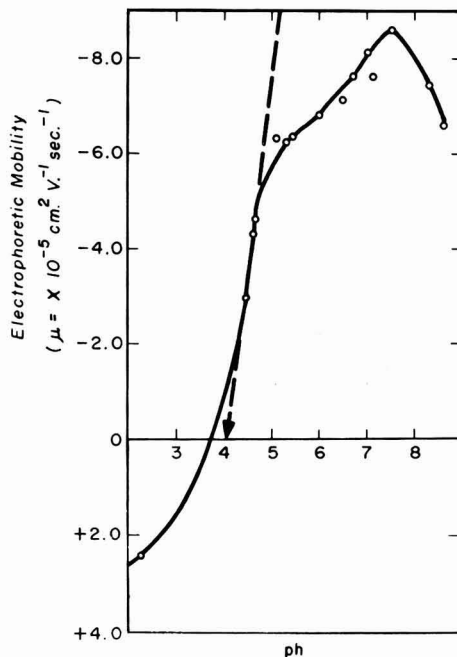
FIG. 6. A plot of the descending electrophoretic mobilities of κ-casein Preparation No. 3 at various pH values (see Table 2).

ultracentrifugally purified kappa preparation (96% κ-casein). Whether the S_{20} would approach the value ($S_{20} = 23$) reported for α_s-casein by Hipp et al. (5), if the purity of the preparation was increased further, remains an unanswered question. The increase in the S_{20} that accompanied the increase in purity further supports the conjecture that κ-casein tends to complex with β- or λ-caseins if present in the same system. The formation of these complexes would preclude complete kappa-kappa interpolymerization and would result in lower values for S_{20}. Alternately, extended purification of κ-casein preparations by high-speed centrifugation might possibly result in an elimination of less dense polymeric species. Possibly, κ-casein exists in a continuous range of polymeric species at pH values in the neighborhood of neutrality and as such could be separated by differential sedimentation into fractions characterized by the presence of a specific polymeric species. Therefore, sedimentation-coeffi-

cients may not be a criterion of qualitative homogeneity so much as an indicator of the polymeric species.

Ultracentrifugal data for the monomeric species of κ-casein were obtained in phosphate: KOH buffer at pH 12.2, $\Gamma/2 = 0.19$. A single sedimenting boundary was observed for which the S_{20} value decreased as the concentratioon of protein in solution was increased. This is an expected observation for a monomeric species in the absence of association-dissociation equilibria. An extrapolation of these concentration-dependent S_{20} values to zero protein concentration resulted in a value for the $S_{20}^{c=0}$ of 1.43.

Diffusion coefficients and weight-averaging molecular weight determination for monomeric κ-casein were made in phosphate buffer at pH 12.2, $\Gamma/2 = 0.19$. Diffusion coefficients (D_{20}) of 5.78×10^{-7} and 5.84×10^{-7} cm²/sec were determined, respectively, from the Schlieren patterns recorded in the electrophoresis apparatus and from Ehrenberg's (2) interpretation

TABLE 3

Ultracentrifugal characteristics of κ-casein Preparation No. 3 in neutral and alkaline buffer solutions

Protein conc. (%)	Buffer system	Sedimentation-velocity characteristics [a]		
		S_{20}	Concentration [b] (%)	Diagrams
0.6	Phosphate; pH 7.0, $\Gamma/2 = 0.1$	12.92	92	36'
0.6 [c]	Phosphate; pH 7.0, $\Gamma/2 = 0.1$	14.50	96	36'
2.7	Phosphate; pH 12.2, $\Gamma/2 = 0.19$	0.77	....	96'
1.7	Phosphate; pH 12.2, $\Gamma/2 = 0.19$	1.00	....	112'
0.8	Phosphate; pH 12.2, $\Gamma/2 = 0.19$	1.23	....	110'
0.6 [d]	Phosphate; pH 7.0, $\Gamma/2 = 0.1$	14.8	93	34'

59,780 rev/min

[a] Centrifugation was conducted at low temperatures (3 C), except for the analyses made at pH 12.2 (20 C).
[b] Expressed as the relative percentage of refractive area.
[c] Centrifugally purified.
[d] Alkaline-treated.

of ultracentrifugal data obtained in a synthetic boundary cell. A molecular weight of 22,800 was calculated by the sedimentation-diffusion method. Calculations according to the Archibald's approach-to-equilibrium method, employing measurements from two frames of the sedimentation diagrams (60 and 92 min), as a check on homogeneity of particle size, resulted in molecular weight values of 23,900 and 24,000, respectively. A value of 0.73 was assumed as the partial specific volume for κ-casein. These molecular weights fall within the range of values previously reported: 16,300 (16) and 26,000 (8).

To study the momeric species, many of these determinations were performed in highly alkaline buffer systems. Therefore, an exercise was undertaken to determine the effect, if any, of high pH treatment on the properties of κ-casein when re-examined in a neutral buffer. Kappa-Preparation No. 3 was adjusted to pH 12.2, held for 1 hr at 23 C, and returned to pH 7.0. The solution turned slightly turbid at pH 12, but was clarified by low-speed centrifugation. Recent experiments in this laboratory have shown that λ-casein is partially unstable and forms a fine precipitate in solutions adjusted to high pH values. With much of the λ-casein removed from the kappa preparation, the refractive area attributed to κ-casein in the sedimentation-velocity diagrams was increased by 1% to a value of 93%. Presumably, the bulk of the remaining 7% of the refractive area represented the β-casein content of the preparation. A corresponding increase in the sedimentation-coefficient (S_{20}) from 12.9 (untreated) to 14.8 (alkaline-treated) was noted.

An examination of sedimentation diagrams of the untreated preparation revealed asymmetry and convective disturbances suggestive of slow interconversion of various polymeric species. Following the alkaline treatment, the sedimenting boundary was more symmetrical and void of convective disturbances, an observation indicating a greater uniformity in polymer size distribution.

A 1% solution of a centrifugally purified, kappa preparation (96% κ-casein) in phosphate buffer (pH 7.0) was treated with crystalline rennin at 26 C. After 30 min the reaction was stopped by heating the solution momentarily to 80 C. The soluble and insoluble fractions, representing the scission products of the action of rennin on κ-casein (10), were separated by centrifugation at 100,000 × G for 3 hr at 4 C. The recovery of the soluble fraction amounted to 29% by weight of the κ-casein substrate. Assuming that the soluble fraction contained 11.2% N (10), 25.7% of the total nitrogen was released by rennin, a value consistent with that reported by Wake (12).

Although the data presented herein represented single preparations, two to four isolations were made and characterized. In general, these preparations showed properties characteristic of those reported.

CONCLUSIONS

κ-Casein-rich preparations were obtained by fractionation of isoelectric casein or crude-α-casein in a concentrated solution of urea (6.6 M) and trichloroacetic acid (12%). κ-Casein could not be isolated by the same method from α-casein purified by the procedure of Hipp et al. (4). κ-Rich preparations obtained from crude α-casein contained approximately 92% κ-casein, 7% β-casein, and 1% λ-casein.

Kappa-preparations obtained by the addition of TCA to concentrated urea-casein solutions at 3 C (Preparation No. 1) and at room temperature (Preparation No. 2) possessed different electrophoretic and ultracentrifugal characteristics. Preparation No. 1 contained more κ-casein characterized by an S_{20} of 12.9, whereas the κ-casein component of Preparation No. 2 showed an S_{20} of 20.

The possibility of an interaction between κ- and β-caseins in buffer solutions at pH values above and below the isoelectric points of the proteins was suggested.

Properties for kappa-Preparation No. 3, fractionated at 4 C from crude α-casein, were as follows: $S_{20} = 12.92$ at pH 7.0, $\Gamma/2 = 0.1$; $S_{20}^{c=o}$ (monomer), 1.43 at pH 12.2, $\Gamma/2 = 0.19$; D_{20} (monomer), 8.54×10^{-7} at pH 12.2; molecular weight of the monomer, $\cong 24,000$; isoelectric point at pH 4.1; stabilized 90% of α_s-casein in a κ/α_s ratio of 1:10; and contains approximately 26% of rennin-releasable nitrogen.

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DEPHOSPHORIZATION OF CASEIN BY HEAT TREATMENT.
I. IN CASEINATE SOLUTIONS [1]

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SUMMARY

Heat treatment of 3% sodium α-, β-, and unfractionated caseinate solutions at 110 to 140 C progressively released the casein phosphate as inorganic orthophosphate. Under the conditions studied, all of the samples conformed to first-order kinetics. The energy of activation E was calculated to be 28-29 kcal per mole of phosphate, the same as for the dephosphorization of O-serine phosphate. Calculations of the entropy change $\triangle S^*$ for the activated complexes of casein phosphate and O-serine phosphate gave a value of -5 cal per mole per degree. Structurally, the molecules are considered to have been altered very slightly from the original. The data indicate that the hydrolysis of phosphorus from casein proceeds through an oxygen-phosphorus fission.

The dephosphorization of caseinate sols by enzymes (7, 10, 12, 13, 17) and alkali (2, 14, 15) has been investigated thoroughly; however, the hydrolysis of casein phosphorus by heating has received little attention. Howat and Wright (8) observed in 1934 that sodium whole caseinate solutions were completely dephosphorized when heated at 120 C for 5 hr and that approximately 15% of the nitrogenous material was simultaneously released as small molecules. On the other hand, only 85% of the total phosphorus of calcium whole caseinate solutions was released by heating at 120 C for 5 hr; the nitrogen was released at a rate similar to that with the sodium preparation. The effect of cations on the rate of hydrolysis of casein phosphate has also been demonstrated by Bamann et al. (3) with caseinate systems containing cesium and lanthanum salts.

In 1936, Howat and Wright (9) provided evidence that the dephosphorization is related to heat coagulation of casein. They observed that for each 10-degree rise in temperature the rate of dephosphorization increased threefold. These same authors (8) suggested from titration curves of dephosphorized caseins that the phosphorus cleavage proceeded in a fashion

similar to the alkaline hydrolysis of casein phosphorus. Anderson and Kelley (2) have shown by isotope studies that the hydrolysis of phosphates by alkali is accomplished through the splitting of -C-O-bond (probably from serine or threonine phosphate) by means of a β-elimination reaction.

The present paper reports the rates of cleavage of phosphate in α-, β-, and unfractionated caseins and in O-serine phosphate solutions.

MATERIALS AND METHODS

Caseinate solutions. Whole casein from skimmilk was isoelectrically precipitated at pH 4.6 with HCl. The precipitate was washed twice with water and then freeze-dried. The α- and β-casein fractions were prepared from a single lot of casein by the urea method of Hipp et al. (6). By Tiselius electrophoresis the α-casein revealed only one peak at pH 7.8 in veronal buffer of ionic strength 0.1, whereas the β-casein preparation at pH 8.6 showed a small amount (3-5%) of α-casein to be present. The 3% sodium caseinate solutions were prepared as follows: Whole casein (7.5 g) was mixed with about 175 ml of water and approximately 1.5 to 2.0 ml of 1.5 N NaOH. The mixture was ground in a Potter-Elvehjem homogenizer, the pH adjusted to 6.66 with 1.5 N NaOH, and the volume made to 250 ml with water. In a study of pH effect, the amount of sodium hydroxide was varied as to obtain sols with pH ranging from 6 to 7. The α- and β-caseinate sols were prepared in the same manner as the whole caseinate sols.

O-serine phosphate solution. DL-O-serine phosphate [2] (100 mg) was dissolved in water, adjusted to pH 6.66 with sodium hydroxide, and made up to 100-ml volume.

Deproteinization. Aliquots of approximately 10 ml of caseinate solutions were sealed in 16 × 150 mm Pyrex tubes and heated at temperatures ranging from 110 to 140 C ($\pm$ 0.5 C) in a silicone oil bath for predetermined periods of time up to 1 hr. After heating, the samples were cooled, the sealed tubes opened, and 5-ml aliquots pipetted into 25-ml volumetric flasks and made to volume with 15% trichloroacetic acid (TCA). The solutions were then filtered through Whatman No. 1 filter paper.

Aliquots (1.5 to 2.0 ml) of O-serine phosphate solution were pipetted into 7- × 75-mm Pyrex tubes, sealed and heated at 120, 130, 135, and 140 C for 10, 20, 30, and 60 min. The tubes were cooled immediately afterward, opened, and the contents analyzed directly for phosphorus.

Phosphorus determination. The filtrates were analyzed directly for phosphorus after it was found that the phosphorus released from casein was completely inorganic. The method of Sumner (16) was used throughout this investigation. The concentration was obtained by reference to known solutions (K_2HPO_4) containing 0 to 0.2 mg of phosphorus per 50 ml of blue solution. The absorbance was measured with a Beckman Model B Spectrophotometer at a wave length of 640 mμ.

Digestion with sulfuric acid and hydrogen peroxide. Whenever nitrogen analyses were sought, the TCA filtrate was first wet-digested with sulfuric acid and hydrogen peroxide (11). A measured volume of a sample was placed in a 30-ml Kjeldahl flask and digested on a uniformly heated sand bath as follows: 1 ml of 1:1 H_2SO_4 (equal volumes of water and concentrated sulfuric acid) was added to the sample and heated for 30 min. The flask was allowed to cool, 3 or 4 drops of 30% H_2O_2 (Baker Analyzed Reagent) were introduced, and it was heated for another 30 min. This step was repeated until the digest was clear. Then 2 ml of water were added to the digest and the flask heated until dense white fumes were evolved. This step destroyed excess H_2O_2 which interferes in the phosphorus and nitrogen determinations. An additional 2 ml of water were added and heated to boiling to decompose all of the pyrophosphates formed during the digestion process. Finally, the flask was al-

[2] Lot No. 090971, California Foundation for Biochemical Research, Los Angeles 63, California.

lowed to cool and the digest transferred quantitatively to a suitable volumetric flask.

Nitrogen determination. The nitrogen was determined by the Nessler's method (11) and the reagent prepared according to the ACS specifications (1). The absorbances of the sample and the blank were read in a Beckman Model B spectrophotometer at a wave length of 500 mμ. The concentration was obtained by reference to standard solutions of ammonium sulfate containing 0 to 0.08 mg N per 25 ml of the colored solution.

Calculations. The casein phosphorus was calculated by difference between total phosphorus (after a wet digestion) and the TCA soluble phosphorus. Similarly, the casein nitrogen was obtained by difference between total nitrogen and TCA soluble nitrogen. The volume of the protein precipitated with TCA was neglected in all calculations.

RESULTS

Dephosphorization of whole, α-, and β-caseins. The first point investigated was whether the phosphorus hydrolyzed from 3% sodium caseinate sols is inorganic. This was accomplished by comparing the inorganic phosphate content of TCA filtrates with and without the H_2SO_4–H_2O_2 digestion. The experiment was performed on 3% sodium whole, α-, and β-caseins heated at 135 C for 10, 20, 30, and 60
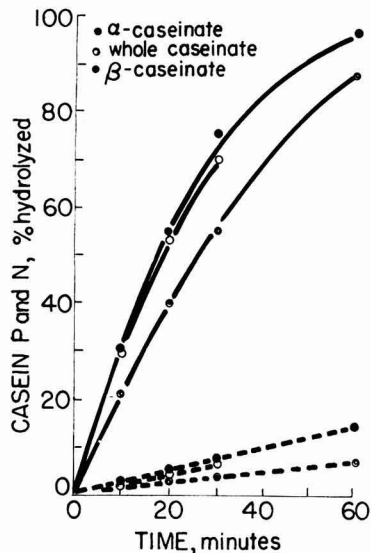
FIG. 1. Hydrolysis of casein phosphorus and nitrogen at 135 C.
Solid line—Casein P hydrolyzed
Dotted line—Casein N hydrolyzed.

TABLE 1

Comparison of inorganic phosphate content of TCA filtrates digested and undigested [a]

	Inorganic phosphates liberated from					
	Whole casein		a-casein		β-casein	
Time of heating	Digested	Undigested	Digested	Undigested	Digested	Undigested
(min)			(mg/100 ml)			
10	6.5	6.4	8.7	8.9	4.5	4.0
20	11.9	11.7	15.7	16.3	7.8	7.4
30	15.9	15.9	21.2	22.5	10.7	10.2
60	......	......	27.0	28.7	16.2	16.2

[a] All heated as 3% sodium caseinate solutions at 135 C.

min. Results shown in Table 1 indicate that the phosphorus released during heating is completely inorganic. This experiment also indicated that the light-tan color developing during heat treatment at 135 C for 30 or 60 min does not cause subsequent interference in the phosphorus determination.

The nitrogen rendered TCA-soluble during heating at 135 C was also estimated and compared with the phosphorus rendered TCA-soluble (Figure 1). The data expressed in per cent casein P and N hydrolyzed show that at the end of 1 hr whole and a-caseinate solutions have released approximately 95% of the total phosphorus and about 15% of the nitrogen. On the other hand, only 87% of the total phosphorus and only 7% of the nitrogen were released from β-caseinate in the same time. Of course, on an absolute basis two to three times as much nitrogen as phosphorus is rendered soluble in TCA. On the basis of this experiment no wet digestion was used in subsequent work to determine the phosphorus rendered TCA-soluble.

The rates of dephosphorization at various temperatures were determined with 3% sodium a-, β-, and whole caseinate solutions containing, respectively, 29.8, 18.4, and 22.5 mg of phosphorus per 100 ml. As shown in Figure 2, the a-caseinate was completely dephosphorized within 1 hr at 140 C. Whole casein behaved very similarly to a-casein in the rate of hydrolysis of phosphate. The amount of hydrolysis in 3% sodium β-caseinate solution was comparatively lower than that observed with whole and a-casein solutions. Approximately 95% of the total β-casein phosphorus was hydrolyzed at 140 C for 1 hr. Even at 110 C, 20% of the phosphorus from a-casein was released in 1 hr. At that temperature about 15% of the total phosphorus of β-casein was rendered TCA-soluble. The differences in the rates of dephosphorization of a- and β-caseins indicate that

probably the phosphate of a-casein is more labile than that of β-casein.
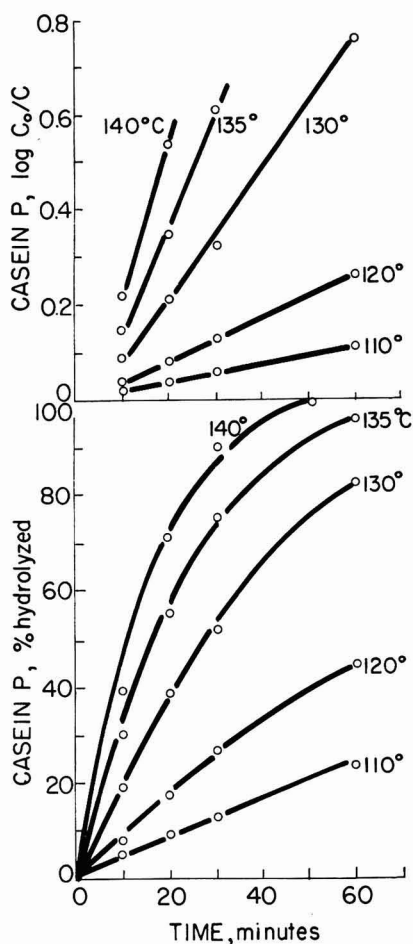
FIG. 2. Dephosphorization of 3% Na a-caseinate solution, pH 6.68.

The original pH's of 6.63, 6.68, and 6.72 of whole, α-, and β-caseins were virtually unaltered by the heat treatment. At 140 C for 1 hr the pH in the three solutions had decreased only by 0.2 pH unit. The slightly turbid solutions of α- and unfractionated caseins remained unchanged in appearance while heating at the low temperature; however, at 120 C for 30 min a pronounced turbidity was observed. At 135 C within 20 min, a slightly tan color had developed and minuscule coagulation of the particles became noticeable. A temperature of 140 C for 30 min caused the solution to become definitely tan in color and caseinate micelles had precipitated. On the other hand, the water-clear β-caseinate solutions, upon heating, were transformed to a milky-white turbid solution which reversed to

a water-clear solution upon cooling. Only samples heated at 135 C for 1 hr and at 140 C for 30 and 60 min acquired irreversible turbidity.

Effect of pH. Whole caseinate solutions containing 24.4, 24.6, and 24.8 mg of phosphorus per 100-ml solution were, respectively, adjusted with 1.5 N NaOH to pH 6.02, 6.46, and 6.97, and samples from each preparation were heat-treated at temperatures ranging from 110 to 140 C for periods of time up to 1 hr. In Table 2, the data on casein phosphorus revealed that the rate of hydrolysis is scarcely influenced by changes in acidity between pH 6 and 7. However, one might suggest that the rate appears somewhat slower in the solution at pH 6.02.

O-serine phosphate solution. Plots of per cent phosphorus hydrolyzed against time of heating showed that O-serine phosphate, containing 16.2 mg of phosphorus per 100 ml of solution, is hydrolyzed extensively during high heat treatment at near neutral pH (Figure 3).

Comparison of kinetic and thermodynamic data. The conformity to a first-order reaction is demonstrated by the linearity of the plot of log Co/C vs. time at any given temperature. Plots of α-casein phosphorus in log Co/C against time showed such a linear relationship within the range of temperatures used (Figure 2). This also applied for the β- and unfractionated caseins. Similarly, the O-serine phosphate solution conformed to the conditions of first-order reaction (Figure 3).

From these plots the rate constant k* was calculated according to the equation (5):

$$k^* = 2.303 \frac{(\log (Co/C)_2 - \log (Co/C)_1)}{t_2 - t_1}$$

where Co = original phosphorus concentration of the reactant

C = concentration of phosphorus of the product at any stage of hydrolysis

t = time in seconds

As shown in Table 3, whole casein at pH 6.02 has a slower rate of hydrolysis; however, it appears that from pH 6.5 to 7.0 the rate of dephosphorization is practically uninfluenced by changes in acidity. Moreover, the α-caseinate solution dephosphorizes more readily at a rate comparable to that of whole casein at pH 6.63. The β-caseinate at pH 6.72 compares closely to the rate of dephosphorization of whole casein at pH 6.02. At the same degree of acidity the phosphate hydrolysis in β-caseinate is slower than that observed with whole or α-caseins.
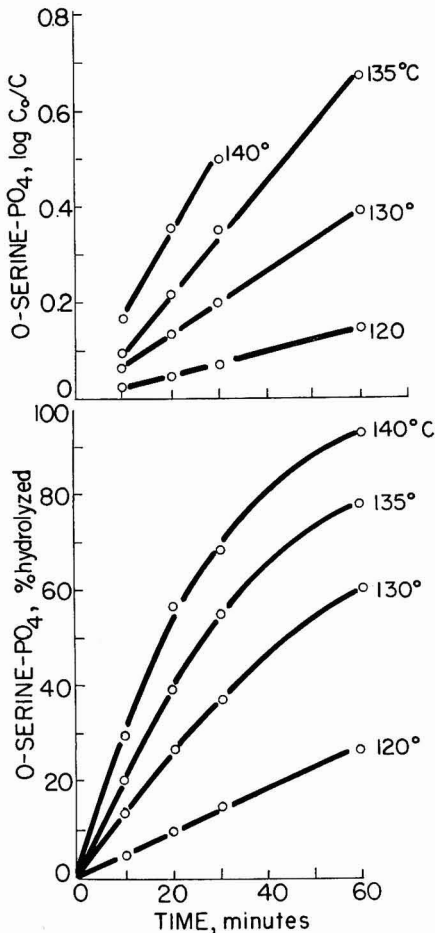
FIG. 3. Rates of hydrolysis of O-serine phosphate.

TABLE 2

Hydrolysis of casein P in 3% sodium whole caseinate solutions heated at various pH's

Heat treatment		pH 6.02 Casein P	pH 6.46 Casein P	pH 6.97 Casein P
			(mg/100 ml)	
None		24.4	24.8	24.6
Heated 110 C for	10 min	23.8	23.8	23.1
	20	23.3	23.1	22.2
	30	22.1	22.2	20.9
	60	20.8	20.6	19.9
120 C	10	22.8	23.0	22.8
	20	20.9	20.8	20.8
	30	19.5	18.8	19.3
	60	15.0	14.1	14.2
130 C	10	20.4	21.4	20.3
	20	16.3	17.0	17.9
	30	13.0	13.1	12.9
	60	6.1	5.8	6.6
135 C	10	19.4	18.8	18.0
	20	13.9	12.5	11.9
	30	8.2	7.0	7.4
	60	2.4	2.0	2.3
140 C	10	16.4	15.3	15.1
	20	8.6	7.3	7.5
	30	4.4	4.5	3.8
	60	1.1	0	0.4

TABLE 3

Rate constants $k^* \times 10^{-4}$ sec^{-1} for the casein dephosphorization in sodium caseinate solutions

		Whole casein				α-casein	β-casein	O-Serine-PO$_4$
pH		6.02	6.46	6.63	6.97	6.68	6.72	6.66
Heated at 110 C		0.43	0.58	0.67	0.86	0.71	0.44	......
120		1.26	1.51	1.52	1.51	1.61	1.27	0.95
130		3.24	3.22	4.33	3.59	4.08	2.90	2.51
135		4.72	5.18	6.26	6.26	6.33	4.44	4.17
140		8.52	9.40	9.63	9.98	9.60	7.78	6.31

TABLE 4

Thermodynamic data of the dephosphorization reaction of caseinate and O-serine phosphate, values obtained at 130 C

	E kcal mole^{-1}	$\triangle$ H* kcal mole^{-1}	$\triangle$ F* kcal mole^{-1}	$\triangle$ S* cal mole^{-1} degree^{-1}
Na whole caseinate solution	28.7	27.9	30.0	−5.0
Na α-caseinate solution	28.7	27.9	30.0	−5.0
Na β-caseinate solution	28.7	27.9	30.1	−5.0
Na O-serine phosphate solution	29.2	28.3	30.3	−4.9

O-serine phosphate is hydrolyzed more slowly than the caseinate solutions. It may be that in whole and α-caseins the phosphate is more labile than in β-casein and, in turn, more labile than in O-serine phosphate.

From the slope of a plot of log k* vs. 1/T (Arrhenius plot) the energy of activation was calculated according to the Equation (5):

$$E = 2.303 \, RS$$
where $R = 1.986$ cal degree^{-1} mole^{-1}
S = slope of the plot.

In Figure 4, the log k* of the phosphate hydrolysis of α-, β-, and unfractionated caseins is plotted against the inverse of the absolute temperature. Although the same slope is obtained for the three caseinate solutions, the plot for β-caseinate, due to its slower rate of hydrolysis, is displaced slightly lower than those for whole and α-caseins. In Figure 5, a similar graph relates the effect of pH on whole caseinate solutions. Again, it is observed that at pH 6.02 the plot, although of a slope identical to those of higher pH's, is displaced downward. The Arrhenius plot of the phosphate hydrolysis of O-serine phosphate solution (Figure 6) shows a slight difference in slope from those of the caseinate solutions. This is probably within experimental error.

The free energy change, the enthalpy change, and entropy change of the activated complex for the dephosphorization reaction were calculated from the following equations (5):

(a) $\triangle F^* = \dfrac{2.303 \, R_1T \, (\log \frac{R_2T}{Nh} - \log k^*)}{Nh}$

where k^* = rate constant sec^{-1}
R_1 = gas constant expressed as 1.986 cal degree^{-1} mole^{-1}
R_2 = gas constant expressed as 8.314 × 10^7 erg degree^{-1} mole^{-1}
T = absolute temperature
N = Avagadro's number 6.023 × 10^{23} mole^{-1}
h = Planck's constant 6.624 × 10^{-27} erg sec
$\triangle F^*$ = free energy change of the activated complex in calories per mole

(b) $\triangle H^* = E - RT$

(c) $\triangle S^* = \dfrac{\triangle H^* - \triangle F^*}{T}$

where $\triangle H^*$ = enthalpy change of the activated complex
E = energy of activation
$\triangle S^*$ = entropy change of the activated complex

From Table 4, the energy of activation for the hydrolysis of phosphorus from sodium α-, β-, and unfractionated caseinate solutions, regardless of pH, was found to be 28.7 kcal per mole. In the case of O-serine phosphate, the energy of activation was 29.2 kcal per mole. These two values are in good agreement with literature data on the hydrolysis of simple phosphomonoesters. The free energy change $\triangle F^*$ is approximately the same for all samples studied. The enthalpy change $\triangle H^*$ varied according to the temperature of the reaction. The entropy change $\triangle S^*$ of the activated complex was the same for all the systems studied. The low entropy change of −5 cal per mole per degree indicates that the activated complexes of sodium caseinate and O-serine phosphate are slightly more organized than the original molecules. It is assumed that structurally the casein is altered very slightly, when one considers that the change in entropy is much smaller than that of the heat denaturation of a typical globular protein as β-lactoglobulin.

DISCUSSION

The liberation of casein phosphorus in sodium caseinate solutions occurs when such systems are heated at high temperatures. Within 1 hr at 140 C, the casein in sodium caseinate is completely dephosphorized. This is in agreement with Howat and Wright's dephosphorization of sodium caseinate at 120 C for 5 hr. It appears from the dephosphorization of α- and β-caseinate solutions that the phosphorus of α-casein is more labile than that of β-casein.
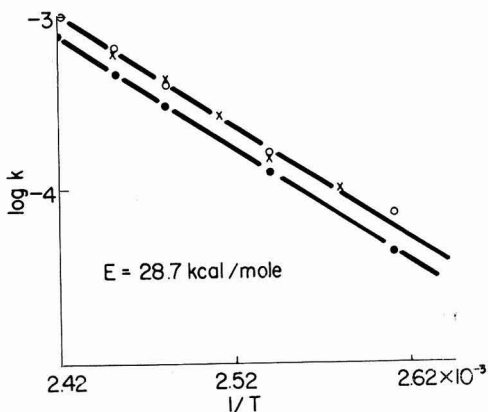
FIG. 4. Arrhenius plots for the dephosphorization of 3% Na whole, α-, and β-caseinate solutions.
×—Na whole caseinate, pH 6.63.
○—Na α-caseinate, pH 6.68.
●—Na β-caseinate, pH 6.72.

Whole casein dephosphorizes at approximately the same rate as α-casein. It may be concluded from the curves of the per cent of hydrolysis of casein phosphate that α- and β-caseins are hydrolyzed simultaneously when whole casein is heated.
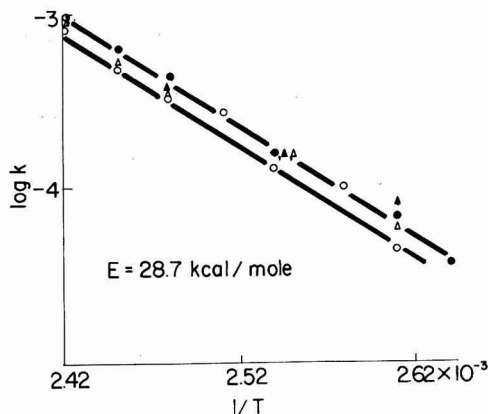
FIG. 5. Arrhenius plots for the dephosphorization of 3% Na whole caseinate solution at various pH's.

○—pH 6.02; △—pH 6.46; ●—pH 6.63; ▲—pH 6.97

The phosphorus removed from α-, β-, and unfractionated caseins is inorganic. In sodium caseinate solutions, the phosphorus hydrolyzed appears as dissolved inorganic phosphates. Results of Figure 1 on the release of nitrogen and phosphorus from heated caseinate solutions do not enable use to decide whether the phosphorus is hydrolyzed directly from casein in one step or is released first as a phosphopeptide or phosphoamino acid which is subsequently hydrolyzed. This might be ascertained by identifying serine and threonine in the TCA-soluble fraction and determining whether they are released in molar concentration equal to that of phosphate. However, further investigations will be required to prove this assumption.

The slightly lower rate of hydrolysis observed at pH 6.02 as compared to pH 6.97 is opposite to the effect encountered with methyl phosphate or β-naphthol phosphate (19, 20). These two phosphomonoesters have been shown to hydrolyze most rapidly at pH near 4, the rate decreasing to a minimum rate of hydrolysis at pH's 1 and 7.

Howat and Wright (8) suggested from titration curves that the hydrolysis of casein phosphate by heat is identical to that produced by alkali. Anderson et al. (2), in a study of the alkaline hydrolysis of casein, have proposed from isotopic studies that the dephosphorization of casein proceeds by a β-elimination reaction with a cleavage of the -C-O-linkage. This appears contrary to our findings with heat-induced dephosphorization. The energy of activation on the phosphate hydrolysis of casein was 28.7 kcal per mole. This value is in good agreement with the data of Swoboda and Crook (18), obtained from glycerol-1-phosphate, glycerol-2-phosphate, and ethanolamine phosphate. All of these compounds and a number of others (20) have been found by isotopic studies to cleave between the oxygen and phosphorus rather than oxygen and carbon. Butcher and Westheimer (4) support this theory by demonstrating the complete retention of configuration with the hydrolysis of phosphate from optically active 2-methoxypropyl-1-methyl ethyl phosphate at pH 4.0. Furthermore, Weil-Malherbe et al. (21) have shown that the energy of activation of hydrolysis of sodium pyrophosphate is 29 kcal per mole and in this case only an oxygen-phosphorus fission could have been involved. It appears, therefore, from the fact that sodium caseinate and sodium O-serine phosphate have about the same energy of activation, that the hydrolysis of casein phosphate at pH 6.6 also involves an oxygen-phosphorus fission.

The energy of activation data do not enable us to distinguish whether the phosphate exists in casein as phosphomonoester, as phosphodiester of the type -O-P-O, or as pyrophosphate. Phosphodiesters of the type -N-P-O-, as proposed by Perlmann (13), may be considered absent in casein. A critical study of the rate of hydrolysis of these various phos-
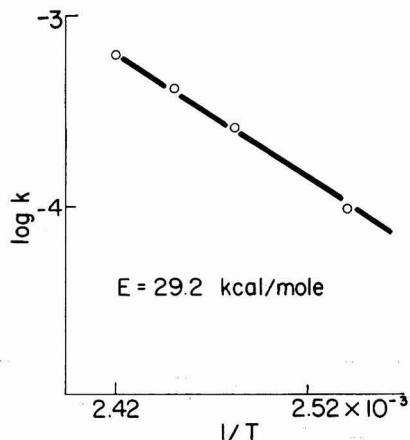
FIG. 6. Arrhenius plot of O-serine-PO₄ hydrolysis.

phoesters might enable us to decide whether more than one type of phosphoesters is actually present in casein.

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DEPHOSPHORIZATION OF CASEIN BY HEAT TREATMENT.
II. IN SKIMMILKS [1]

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SUMMARY

Heating skimmilks at 110 to 140 C caused extensive dephosphorization of the casein; the liberated phosphate accumulated in the inorganic colloidal fraction. Concentration of skimmilk to one-half of its original volume increased the rate of dephosphorization and preheating at 90 C for 10 min before concentrating decreased the rate in the 2:1 concentrate. At a given temperature, dephosphorization was slower in skimmilk than in sodium caseinate sols. The heat-induced dephosphorization of casein in skimmilk conformed to first-order kinetics with an energy of activation of 25 to 27 kcal per mole. The free energy change of the activated complex was about 30 kcal per mole and the entropy change −11 to −13 cal per degree.

The dephosphorization of casein by heat has been known since 1934, when Howat and Wright (3) showed that at 120 C for 5 hr a sodium caseinate solution was completely dephosphorized and only 85% of the total phosphorus was removed from a calcium caseinate solution. These same authors (4) suggested in 1936 that heat coagulation of the protein is correlated to the extent of dephosphorization. They also reported that the liberation of casein phosphorus was scarcely detectable during storage of commercial evaporated milk for 6 to 9 months at 20 C. However, they failed to emphasize possible dephosphorization during the sterilization treatment, or the relation of previous history of the milk to the rate of dephosphorization.

Torboli (10) reported that heating milk at 110, 120, and 130 C causes a progressive increase in nonprotein phosphorus and nitrogen. Of the total phosphorus in raw milk, 27 to 29% was found to be nonprotein phosphorus (NPP); the nonprotein nitrogen (NPN) was approximately 1% of the total. It was not stated how the samples for the phosphorus and nitrogen analyses were deproteinized; nevertheless, the per cent NPN is much lower than

usually observed with normal milks (7). Th phosphorus content reported for raw milk co responds closely to the concentration of di solved inorganic phosphorus and, therefore, would appear that the milk was not deprotei ized with an acid treatment. Torboli (10) sug gested that the heat coagulation of milk resulte from dephosphorization of the casein, whic made it more susceptible to precipitation b calcium salts. Heat treatments at 110 C fc 6 hr, 120 C for 2.5 hr, and 130 C for 1.25 h increased the nonprotein phosphorus to 67% of the total and caused coagulation. The 40% increase in NPP in heated milk could not hav arisen solely from phosphate released fror casein, because casein accounts for only abou 20% of the total phosphorus of milk. It ma have been due in part to solubilization of co loidal phosphate by the increased acidity pr duced in heated milk. Because it is not know how the milks were deproteinized, it is impos sible to deduce the significance of the data.

The results presented herein demonstrate tha heat treatment of milk causes casein dephos phorization. The rate of the hydrolysis o casein phosphate is compared in unconcentrate and concentrated raw and preheated skimmilks

METHODS AND MATERIALS

Milk Samples. Raw whole milk was obtaine from three different sources. An individua cow's (T-263) milk not stabilized against hea coagulation of the 2:1 concentrate by a pre heating treatment and the mixed milk of th University of Minnesota Dairy Herd stabilize by preheating (Table 1) were treated accord

TABLE 1

Casein phosphate contents, heat coagulation times and per cent casein phosphorus liberated at 135 C

	Unconcentrated			Concentrated 2:1		
Skimmilk	Casein P	Coag. time 135 C	Casein P liber- ated [a]	Casein P	Coag. time 135 C	Casein P liber- ated [a]
	(mg/100 ml)	*(min)*	*(%)*	*(mg/100 ml)*	*(min)*	*(%)*
A—Cow T-263						
Raw	25.6	22.5	38	51.6	2.8	10
Preheated 90 C—10 min	25.7	26.5	42	51.8	4.1	10
B—U. of M. herd						
Raw	23.4	26.8	49	46.9	6.5	17
Preheated 90 C—10 min	23.1	8.7	22	46.2	20.7	40

[a] Casein phosphorus liberated at the time of coagulation at 135 C, calculated from curves such as in Figures 3 and 4.

ing to the scheme presented in Figure 1. A third lot of milk, acquired from a local plant, was assumed to be heat-stable on the basis of previous experience with milk from this source. The samples from the individual cow, the University Dairy Herd, and the commercial plant are designated, respectively, as A, B, and C. All were separated at 35 C and the skimmilks cooled to 25 C. Preheating at 90 C for 10 min was performed in a laboratory stainless steel tubular recirculating heater. The heating time of 10 min is exclusive of a come-up time of approximately 3 min and a cooling time of approximately 1 min. Concentration was accomplished in a laboratory all-glass rotating evaporator at a temperature of about 40 C. Samples were concentrated slightly more than 2:1 and diluted with water to exactly one-half their original volume.

Deproteinization of milk samples. The same technique employed with caseinate solutions (2) was tentatively applied to unconcentrated and concentrated milk samples, but met with little success because of variability in the phos-

phorus analyses. It is presumed that this variability arose from trapping of dissolved phosphates in the protein coagula formed by the precipitation with trichloroacetic acid (TCA) and in some cases by the heat treatment.

The following method was, therefore, adopted throughout this study: Five-milliliter aliquots of unconcentrated and concentrated milk samples were pipetted into 16- × 150-mm Pyrex test tubes, sealed, heated, and cooled in a fashion similar to that for the caseinate solutions (2). The sealed tubes were then opened and the contents transferred quantitatively to glass-stoppered, graduated cylinders (25 ml for unconcentrated and 50 ml for concentrated). The transfer was accomplished by pouring the contents of the tubes into the cylinders and rinsing the walls of the test tubes with 2 to 5 ml of water. Small amounts of 20% TCA solution were used to rinse the last traces of sample from the tube. Finally, the mixture was made to volume with 20% TCA.

If the milk had been coagulated by heat treatment, the TCA preparations were then ground in a Potter-Elvehjem homogenizer until the coagulated mass was dispersed. All samples were filtered through Whatman No. 1 paper and aliquots of the filtrate were wet-digested for the phosphorus analyses, because organic phosphoesters are present in the TCA filtrates of milks.

METHODS

The phosphorus and nitrogen determinations and the wet digestion with sulfuric acid and hydrogen peroxide have been described previously (2). The casein phosphorus in milk was calculated as the difference between the total phosphorus and the sum of the TCA-soluble phosphorus and the lipide phosphorus.

Raw whole milk

separated at 35°C

Skimmilk

cooled to 25°C

raw   heated

90°C for 10 min.

concentrated   concentrated

1:1   2:1   1:1   2:1
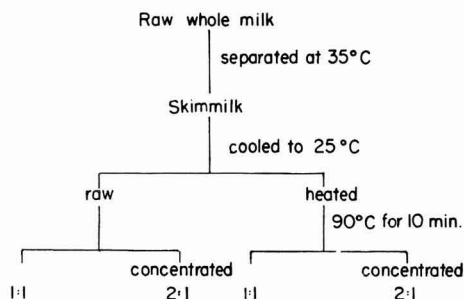
FIG. 1. Treatment of milk.

The lipide phosphorus was extracted by the Roese-Gottlieb method (8). The ethereal layers of two extracts were combined in a 250-ml beaker and evaporated to dryness over a steam bath. The residue was transferred to a 100-ml Kjeldahl flask with about 20 ml of chloroform and the solvent was evaporated again to dryness. Finally, the residue was submitted to the wet digestion.

Inorganic dissolved phosphorus was determined by analyzing without digestion ultrafiltrates obtained by the technique of Tessier and Rose (9). Phosphorus in the form of dissolved organic esters was calculated as the difference between the phosphorus analyses of digested and undigested aliquots of ultrafiltrate. Inorganic colloidal phosphorus was calculated as the difference between the phosphorus contents of the TCA filtrate and the digested ultrafiltrate.

Heat coagulation times were determined by rocking (19 sec per period) a 1-ml sample in a sealed pyrex tube (7 × 75 mm) submerged in a silicone oil bath at the desired temperature until the first visual coagulation was observed.

The kinetic and thermodynamic data were calculated from the equations presented in a previous paper (2).

RESULTS

Preliminary investigation. Initial studies were conducted to ascertain if dephosphorization of casein occurs in milk at a) the temperature and the time of usual preheating treatment (90 C for 10 min) and b) during sterilization at 120 C. Changes in the distribution of the other phosphorus-containing components in heated milk were also observed.

Bulk milk from the University Dairy Herd was separated and the skimmilk heated to 91 C for 10, 20, 40, and 60 min in the stainless steel tubular heater and the casein phosphorus determined. Table 2 shows that casein is not dephosphorized at 91 C for 10 min. Even after 1 hr of heating at this temperature, there is only a slight decrease in casein phosphorus. When skimmilk purchased from a local com-

mercial plant was autoclaved at 121-2 C for periods of time up to 1 hr, the various phosphorus fractions were altered greatly in the heated milk (Figure 2). The inorganic dissolved phosphorus decreased immediately in the first few minutes of heating and remained at a constant level thereafter. The organic phosphorus esters were hydrolyzed slightly as the time of heating progressed. Most important in this study was the rapid hydrolysis of casein phosphorus. In 1 hr, about 40% of the casein P was released.

Casein dephosphorization in skimmilk. Skimmilk samples A, B, and C were heated at temperatures from 110 to 140 C for times up to 1 hr and analyzed for casein phosphorus. The data for Skimmilk B are presented in Figures 3 and 4 in terms of per cent casein phosphorus released and also as log Co/C vs. time (Co = initial concentration and C = concentration at any time). The per cent of the casein phosphorus liberated at the time of coagulation was determined from the rate curves and is in-
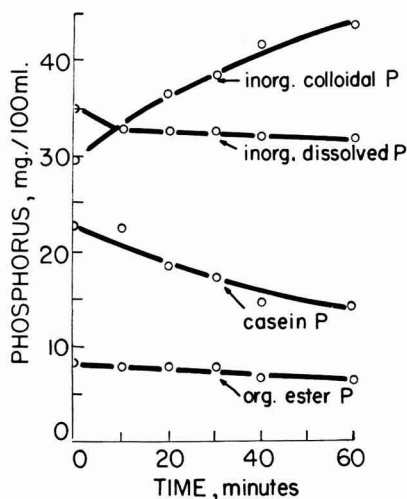
FIG. 2. Distribution of the phosphorus fractions of bulk skimmilk autoclaved at 121-2 C.

TABLE 2

Effect of preheating at 91 C on dephosphorization of casein in bulk skimmilk

Sample	Total P	TCA-sol. P	Lipide P	Casein P
		——(mg/100 ml)——		
Raw unconcentrated	109	85	1.5	22.5
Heated to 91 C for 10 min	109	85	1.5	22.5
Heated to 91 C for 20 min	109	83.3	1.5	23.2
Heated to 91 C for 40 min	109	85.4	1.5	22.1
Heated to 91 C for 60 min	109	85.8	1.5	21.7

TABLE 3

Rate constants for dephosphorization of casein in skimmilk

Sample	Rate constant k* $\times$ 10^{-4} sec^{-1}				
	110 C	120 C	130 C	135 C	140 C
Skimmilk A—					
Raw unconc.	0.47	1.17	2.21	3.41	5.32
Raw conc. 2:1	0.51	1.32	2.69	4.14	6.63
Preheated unconc.	0.53	1.48	2.21	3.62	5.30
Preheated conc. 2:1	0.41	0.99	2.11	3.34	4.90
Skimmilk B—					
Raw unconc.	......	1.08	2.40	4.03	5.94
Raw conc. 2:1	0.87	1.64	3.41	4.86	7.81
Preheated unconc.	0.59	1.34	2.76	4.79	6.75
Preheated conc. 2:1	0.47	1.32	2.88	4.38	6.19
Skimmilk C—					
Raw unconc.	0.36	1.08	2.02	3.57	5.25
3% Na Caseinate pH 6.63	0.67	1.52	4.33	6.26	9.63

cluded in Table 1. In all cases (concentrated or unconcentrated, raw or preheated) the plots of log Co/C vs. time were linear, thus conforming to kinetics of the first order. The rate constants calculated from these experiments are compiled in Table 3, together with constants for a sodium caseinate sol at pH 6.63 (2).

The dephosphorization of casein in skimmilk proceeds more slowly at any temperature than the dephosphorization of sodium caseinate. In the unconcentrated milks, preheating was virtually without effect on the rate of dephosphorization of casein in Milk A, but slightly increased the rate for Milk B. Twofold concentration of raw skimmilk increased the rate of dephosphorization in both A and B. Preheating before concentration markedly inhibited the rate of dephosphorization in the concentrated skimmilks.

Arrhenius plots of log k* vs. 1/T are given in Figure 5 for Milk B. The slopes are virtually identical for all treatments. The plot for the raw concentrated sample is displaced upward relative to that for the raw unconcentrated sample. Likewise, the preheating treatment displaced the plot for concentrated milk downward. Plots of nearly the same slope were obtained for unconcentrated Skimmilks A, B, and C; the energies of activation calculated therefrom are, respectively, 25.7, 26.4, and 26.7 kcal per mole.

Table 4 presents the values for free energy change ($\triangle F^*$), enthalpy change ($\triangle H^*$), and entropy change ($\triangle S^*$) of the activated complex calculated from these data. These functions for sodium caseinate at pH 6.63 (2) and for calcium caseinate [estimated from the data of Howat and Wright (4)] are also included in Table 4.

Since these experiments showed that temperatures (115-120 C) such as might be used commercially for sterilization of evaporated milk produce dephosphorization of the casein, a specimen of commercial evaporated milk was analyzed. It contained 242 mg of total phosphorus and 42.3 mg of casein phosphorus per

TABLE 4

Thermodynamic data of the casein dephosphorization reaction, values obtained at 130 C

	E [a] kcal $mole^{-1}$	$\triangle H^*$ kcal $mole^{-1}$	$\triangle F^*$ kcal $mole^{-1}$	$\triangle S^*$ cal $mole^{-1}$ $degree^{-1}$
Skimmilk A	25.7	25.0	30.3	−13.1
Skimmilk B	26.4	25.6	30.3	−11.6
Skimmilk C	26.7	25.8	30.4	−11.4
Ca whole caseinate solution [b]	26.6	26.2	30.5	−12.1
Na whole caseinate solution	28.7	27.9	30.0	− 5.0

[a] For milk samples calculated at temperatures ranging from 110 to 140 C. For calcium caseinate solution calculated at temperatures ranging from 95 to 115 C.

[b] Calculated from Howat and Wright's data at 115 C (4).

100 ml. If one estimates that the casein phosphorus should be at least 20% of the total in normal milk (5), this milk should have contained originally about 48 mg of casein P per 100 ml and thus about 12% of the casein P had been released in sterilization. This value is within the range expected from a sterilizing treatment (see Figure 4).

DISCUSSION

The dephosphorization of casein in milk has been shown to occur within 1 hr at heating temperatures ranging from 110 C to 140 C. Even at the sterilization temperatures employed by the milk industry, 15 to 20% of the casein P is released. However, complete removal of

FIG. 3. Rates of hydrolysis of casein phosphorus in unconcentrated skimmilk of University Dairy Herd. Solid line—preheated to 90 C for 10 min. Dotted line—raw sample.

the phosphorus from casein by heating milk is more difficult. This would also seem to be the case with the calcium caseinate solutions of Howat and Wright (3).

Dephosphorization occurs only after prolonged heating at lower temperatures; therefore, it is not surprising that preheating of milk at 90 C for 10 min does not produce any significant amount of dephosphorization.

Casein in milk is in the form of calcium caseinate micelles. The phosphorus removed from caesin during heating is inorganic and accumulates in the colloidal inorganic phosphorus fraction (Figure 2), probably combining with dissolved calcium in milk and calcium released from calcium caseinate, as was suggested by Howat and Wright (3, 4).

Under all conditions studied, the casein dephosphorization reaction followed first-order kinetics. Three factors: concentration, preheating, and metallic ions appear to influence the rate of this reaction. Twofold concentration of a milk, whether or not it was stabilizable by preheating, invariably increased the rate. Since concentration should not influence the rate constant of a first-order reaction, the rate increase observed upon concentration is probably due to a structural rearrangement or aggregation of the caseinate micelles.

Raw unconcentrated samples of the three milks studied had comparable rate constant for casein dephosphorization. However, preheating unconcentrated samples of Milk A caused little increase in the rate of hydrolysis of casein phosphate, but in Milk B the preheating definitely increased the rate of hydrolysis (Figure 3). In concentrated samples, the preheating inhibited the rate of dephosphorization in both cases, although concentrated Milk B was stabilized by the preheating, whereas concentrated Milk A was not. The preheating appears to prevent or counteract the effect of concentration; the raw unconcentrated and preheated concentrated samples were dephosphorized at nearly the same rates. Perhaps the preheating induces changes in the caseinate which prevent it from undergoing the structural rearrangements or aggregation which cause the higher rate of dephosphorization in concentrated raw milk. It might be speculated, for example, that an interaction between one or more components of casein and the β-lactoglobulin occurs during preheating. There is evidence for such an interaction and it has been suggested as the reason for the inhibition of rennet clottability by heat treatment (6).

The presence of metallic ions affects the rate of dephosphorization of casein. Polyvalent ions
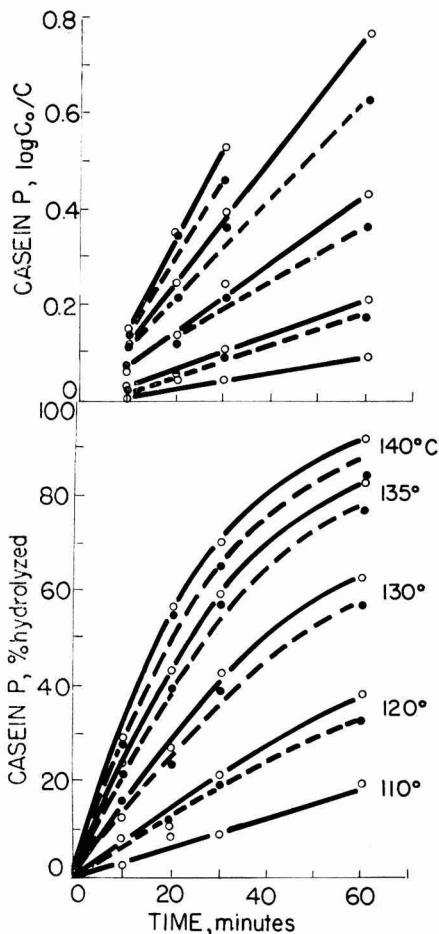
such as calcium (3) and lanthanum (1) decreased the rate of phosphorus hydrolysis when compared to caseinate solutions containing only sodium (2, 3). In milk the casein is in the form of calcium caseinate micelles and, consequently, the rate is slower. In milk the rate constant k* increases roughly twofold for each 10 C rise in temperature, whereas in sodium caseinate solution, it increases about threefold. The data in the literature provide no information for a possible relationship between the rate decrease of dephosphorization of casein and the valence of metallic ions. Further investigations with various metallic ions might prove interesting.

The negative entropy change of the activated complex calculated for the hydrolysis of casein

phosphate in milk and in calcium caseinate solution is greater than in sodium caseinate solution (2). This would indicate that in milk the caseinate micelles apparently acquire a more orderly pattern when activated by heat.

The present study does not provide a mechanism for the phenomenon of heat coagulation or for the stabilizing effect of preheating. Certainly, dephosphorization of the casein does not appear to play the key role in the coagulation phenomena. Dephosphorization proceeded at comparable rates in two milks which exhibited decidedly different coagulation behavior. Preheating inhibited the dephosphorization in the 2:1 concentrate of both samples, yet stabilized one concentrate against heat coagulation and did not stabilize the other. Dephosphorization of the casein may, indeed, be one of several factors which mutually influence the heat stability of milk, but at present it does not appear that dephosphorization can be related to the well-known differences among milks in heat stability and in response to heat treatment.

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FIG. 4. Rates of hydrolysis of casein phosphorus in concentrated skimmilk of University Dairy Herd. Solid line—preheated to 90 C for 10 min. Dotted line—raw sample.
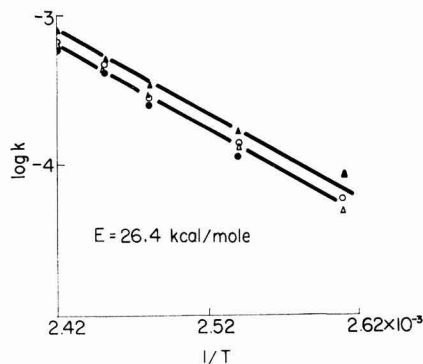
FIG. 5. Arrhenius plots for casein dephosphorization in milks of University Dairy Herd.

● raw unconcentrated
▲ raw concentrated
○ preheated unconcentrated
△ preheated concentrated

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PRODUCTION AND PROPERTIES OF SPRAY-DRIED
WHOLE MILK FOAM

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SUMMARY

Droplets of concentrated whole milk foam have been dried in a standard spray dryer. This has been done by incorporating nitrogen gas into the concentrate at high pressure prior to atomization. The resultant powder has excellent dispersibility properties and can be produced at rates in excess of those obtained by conventional operation. The method of foam-spray drying employed and the physical properties of the resultant powders are given in detail.

The dehydration of milk to a flavor-stable, easily reconstitutable powder is so obviously desirable that history extending back 500 yr records efforts to dry milk (3). In spite of repeated failures to obtain that ultimate product, a highly soluble powder reconstituting to a beverage virtually indistinguishable from fresh pasteurized milk, research has persisted in striving towards this goal. Progress has been made to the extent that at the present time approximately two billion pounds of milk powder are produced annually in the United States (8).

The bulk of this material is in the nonfat form, since dry whole milk produced by either roller or spray drying is difficult to disperse and has poor storage stability. Experiments reported in 1957 by Sinnamon et al. (5) indicated that drying foams of concentrated milk under high vacuum would produce a whole milk powder of excellent initial flavor and dispersibility. Morgan and his associates (4) demonstrated that this dispersibility could be maintained in foamed products even during drying at atmospheric pressure. Work carried out in our Laboratories has suggested that the unique feature of vacuum foam-dried milk, possibly contributing greatest to its high dispersibility, was the low density structure of the powder particles which allowed water uptake at rates which prevented destabilization of the protein systems during rehydration. To test this idea, and possibly produce an easily dispersible whole milk powder by modifications of a standard commercial drying technique, attempts were made to dry droplets of foamed milk concentrates in a conventional spray dryer. This paper describes the technique employed and some of the physical properties of the resultant powder.

EXPERIMENTAL PROCEDURES

All powders were made from milk obtained from a herd maintained under invariant husbandry at the Agricultural Research Center, Beltsville, Maryland. Milk was collected in bulk tanks over a three-day period before drying.

The milk was standardized to 3.3% fat, heated to 165 F for 15 sec in a Mallory heater,[1] homogenized at 2,500 lb and concentrated to 50% solids in a Wiegand[1] falling film evaporator of 100-gal-per-hour capacity.

The concentrate, held at 90 F, was dried using a modified 9-ft Swensen spray dryer[1] equipped with a pressure nozzle designed and manufactured by the Whiting Corporation.[1] The modification consisted of providing a means for injecting nitrogen gas under pressure into the feed line between pump and nozzle. This was accomplished by use of a stainless steel, modified T joint built as illustrated in Figure 1. This mixing device placed approximately 1½ ft from the outlet of a 100-gal/hr positive displacement pump, was supplied with nitrogen from standard pressure tanks. The nitrogen pressure was reduced to and held at 2,000 lb by a Victor regulator.[1] Rate of flow of nitrogen into the mixing device was determined by use of a Brooke's high-pressure flow meter[1]

[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.

and manually controlled by a needle valve in the line after the flow meter. The positive displacement pump was equipped with a spring-loaded by-pass valve which allowed the utilization of various nitrogen/concentrate ratios while maintaining a constant pressure of 1,800 lb on the spray head. The amount of concentrate dried per unit time was determined by subtracting the by-pass rate from the rating of the pump.
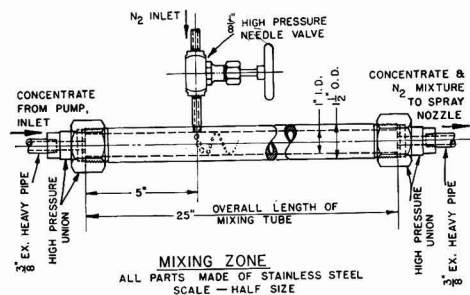
FIG. 1. Mixing device used for incorporation of nitrogen into milk concentrate prior to atomization.

Experimental powders were made by drying various nitrogen/concentrate mixtures using nozzles with orifice sizes ranging from .040 to .050 inch in diameter. A constant air inlet rate with an air temperature of 270 F was employed in all drying runs.

The moisture content of the resultant powders was determined by using the conventional toluene distillation technique. Bulk density was determined by measuring the volume occupied by a lightly tapped 10-g sample. Free fat and dispersibility in 34 F water were measured as previously described (7). Diameters of individual particles and clumps were measured using a microscope equipped with a calibrated eyepiece scale and above-stage lighting. Particle size distribution was ascertained by microscopic examination of random fields. Specific surface areas of the powder were determined using an adaptation of a standard permeametric method (1), which will be the subject of a later publication.

RESULTS

The blending of nitrogen gas and concentrated whole milk under pressure just before atomization in a conventional spray dryer produces material characterized by increased bulk and improved dispersibility. Viewed under a microscope the particles appear to be relatively

large spheres of dried milk foam, as shown in Figure 2. During drying a considerable number of the spheres coalesce and fuse into aggregates. The individual spheres and aggregates do not break or crush on handling and packaging. A distribution of particle sizes in conventional spray dried and foamed spray-dried powders is shown in Figure 3. Large aggregates found in the conventional material were due to clumping caused by the high moisture content of the powder.

The effects of incorporating increasing amounts of nitrogen in the concentrate before atomization on dryer operation and powder characteristics are tabulated in Table 1. From this it can be seen that as the amount of nitrogen in the feed is increased, the amount of concentrate being dried decreases along with the bulk density and moisture contents of the resultant powders. Increasing amounts of nitrogen in the concentrate increases the dispersibility, free fat, and particle diameters.

The increase in dispersibility is most marked between samples produced using zero levels of nitrogen and those produced by lowest level of nitrogen incorporation. Even though results from measuring the dispersibility of foamed samples are erratic, averages of all products produced by different-sized nozzles at each nitrogen injection level indicate an increase in dispersibility with increased N_2 incorporation. At constant atomization pressure, increasing the diameter of the nozzle orifice necessarily increases the amount of material being dried. A serious loss in the ability of the dryer to produce acceptably dried powder as nozzles of increasing orifice size are used is noted if nitrogen is not incorporated into the concentrate. This increase in moisture content of the powder with increasing nozzle orifice diameter is not so marked when nitrogen is blended into the dried feed. The increased efficiency of drying effected by the use of added nitrogen actually allows the dryer to successfully produce milk powder in quantities in excess of its rated capacity for conventional spray drying. Adequate demonstration of this can be obtained from comparisons of data in Columns 2 and 4 of Table 1. From the data therein, it can be seen that without gas incorporation none of the powders was dried to, or below, the acceptable 3.5% moisture level. Using the smallest nozzle, 669 lb of concentrate could be reduced to 3.6% moisture in 1 hr. By use of nitrogen incorporation, 793 lb of concentrate could be reduced to 3.2% moisture in a similar period of time. Part of this improved dryer performance may result from the increased buoyancy
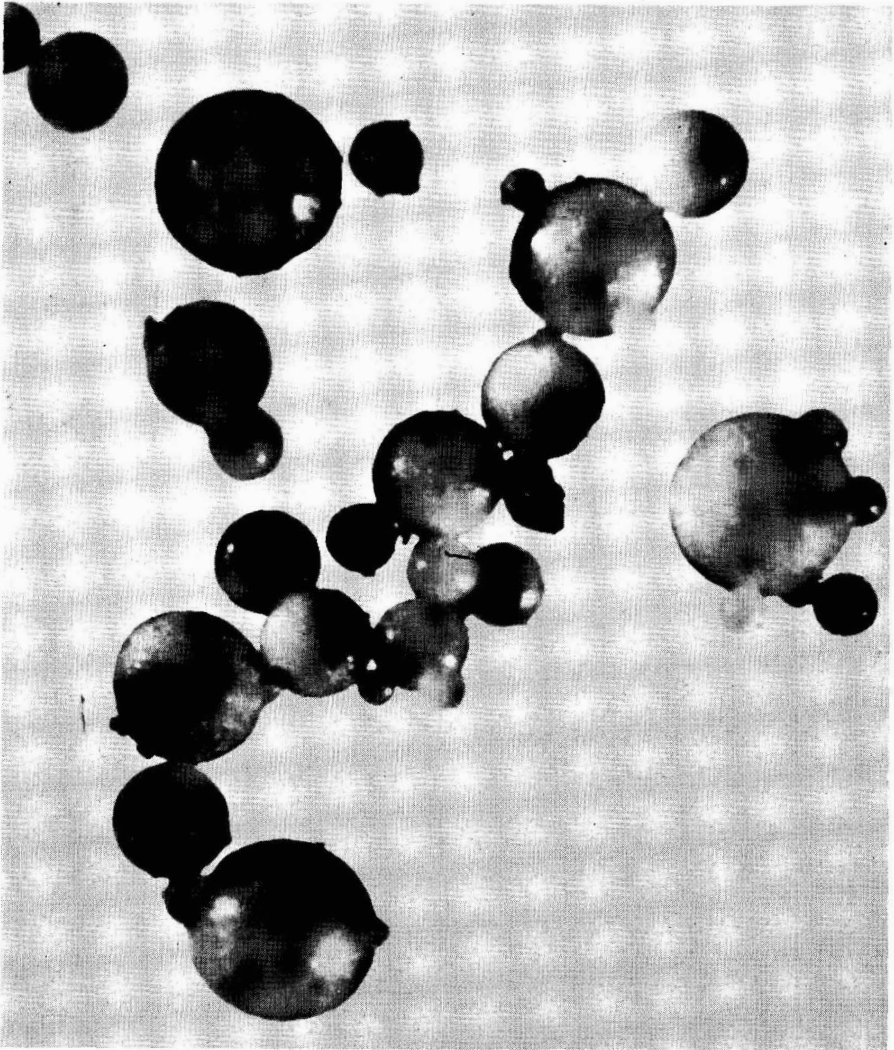
FIG. 2. Photomicrograph of foam-spray dried milk powder particles (285×).

of the foamed particles, which may slightly increase the time they remain in the dryer.

Since at least part of the increased particle size is brought about by the expansion of gas trapped in the spheres of milk foam formed during atomization, an increase in the specific surface areas of the powders should accompany the increased nitrogen content of the feed material.

Figure 4 graphically presents the relationships between surface area, nozzle orifice diameter, rate of nitrogen flow into the drier feed, and feed rate. From this it can be seen that the greatest increase in specific surface area with increasing nitrogen content is obtained using the smallest nozzle opening. The amount of surface expansion per unit nitrogen flowing into the concentrate falls off as the amount of nitrogen increases.

DISCUSSION

Even though the drying technique described in this paper necessitates the use of a gas under pressure, this inconvenience and cost may be compensated by improved dryer efficiency. This observed increase in drying rate with nitrogen

incorporation stems from the increased surface areas available for mass and heat transfer in the foamed particles. Resistance to diffusion of water vapor to the surface of the powder particles is also reduced by decreasing the density of the powder particles by bubble production.

Microscopic examination of powder particles suspended in glycerol reveals two types of bubbles in the particles. One type, extremely small and profuse, is located throughout the milk solids; the other, relatively large and fewer in number, occupies the interiors of the particles and the spherical protuberances on their surfaces.

It is reasonable that these two types of bubbles arise, respectively, from the dissolved gas and that dispersed in the liquid concentrate by the extreme turbulence in the mixing device and in the line to the atomizer. The pressure drop and temperature increase experienced on atomization may cause the dissolved gas to be released to form a fine-grained foam, trapping the larger bubbles of gas and some of the water vapor evolved during drying.

Some of the increase in particle size observed in powders produced by gas incorporation certainly results from the expansion of

the trapped nitrogen gas. However, by use of a micro-balance and microscopic dimension measurements, it was found that the individual powder particles had densities varying between .5 and .6 g/cc, which is much higher than would be anticipated from a simple expansion of a standard-sized spray droplet by trapped gas. Therefore, the increased numbers of large-sized particles observed in the foam-spray dried
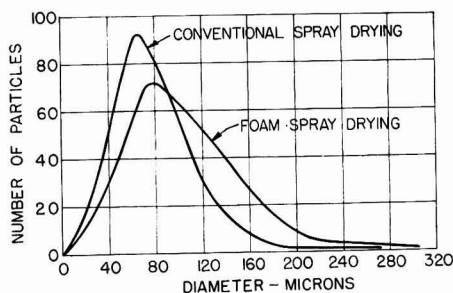
FIG. 3. Particle size distribution in spray-dried powders produced with and without gas incorporation. Both samples produced by spraying through .048-inch nozzle orifice. Foamed product made by introducing .15 standard cubic feet of N_2 into each pound of 50% concentrate before atomizing. Moisture content of powders: without gas, 4.9%; with gas, 3.2%.

TABLE 1

Physical properties of whole milk powder produced by incorporating pressurized gas into the concentrate prior to drying

Drying conditions			Physical properties of milk powder						
Nozzle orifice diameter	Dryer feed rate	Nitrogen injection rate	Residual moisture	Bulk density	Free fat	Dispersibility	Average individual sphere diameter	Average clump size
(inches)	(lb 50% conc/ hr)	(SCFN₂/ lb ᵃ conc)	(%)	(g/cc)	(%)	(%)	———(μ)———	
.040	538	.45	2.3	.21	25.1	85.5	94.86	147.56
.040	581	.21	2.5	.23	20.7	91.9	92.82	196.52
.040	634	.14	3.5	.30	8.0	89.2	84.32	195.84
.040	669	0	3.6	.53	2.8	69.8	68.34	280.50
.0465	709	.34	2.2	.24	19.5	93.0	104.04	269.62
.0465	739	.16	3.1	.26	13.2	80.8	96.56	153.00
.0465	779	.12	3.8	.32	7.0	85.5	92.14	250.24
.0465	802	0	4.5	.50	3.2	66.9	78.54	429.08
.048	743	.32	2.2	.24	18.2	93.8	93.84	140.76
.048	780	.15	3.2	.25	13.9	92.8	103.36	216.24
.048	811	.11	4.0	.32	7.9	86.2	87.04	259.08
.048	835	0	4.9	.45	4.3	55.3	62.90	330.14
.050	759	.31	2.6	.23	17.9	89.6	100.98	156.40
.050	793	.15	3.2	.25	11.7	88.5	93.84	200.60
.050	831	.10	4.0	.29	7.1	89.4	90.10	287.30
.050	855	0	5.6	.48	3.5	71.8	74.12	305.32

ᵃ Standard cubic feet N_2/lb 50% concentrate.

product must be due, in part, to a change in the atomization characteristics of the nozzle.

On reconstitution, the gas trapped in the interior of the spray-dried foams is released and rises to the surface to form a fine-grained froth. The persistence of this material has interfered with the dispersibility test used in this study. Even though the results are somewhat erratic, the improved dispersibility of the spray-dried whole milk foam powders is clearly demonstrated. These data tend to give some credence to the idea that, in whole milk powders, high bulk density with good dispersibility is not obtainable through manipulation of the drying technique alone.

The idea of changing the physical properties of a spray-dried powder by incorporating gas into the feed material is certainly not unique. A patent issued in 1957 to Standard Brands (6) describes the spray drying of foamed coffee extracts. The application of this principle to milk drying has undoubtedly been attempted in a number of laboratories. However, the methods and results of these experiments have never been published.

The technique of producing spray-dried foams as presented in this paper is relatively simple, easy to control, and can possibly be carried out in most existing driers with only minor modifications. The increased drying efficiency observed during the development of this technique has already been utilized to dry Cottage cheese whey (2). From preliminary work with other food products, it can be stated that this drying method will be most applicable to materials possessing high-foaming capacity and foam stability.

Studies of the stability of dried whole milks produced by this method are under way at present.

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FIG. 4. Relationships between some factors influencing specific surface area of spray-dried whole milk powder.

FAILURE IN THE PRODUCTION OF CITRATE PERMEASE BY
STREPTOCOCCUS DIACETILACTIS

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SUMMARY

Five strains of *Streptococcus diacetilactis* contained variants unable to form acetoin and gas from citrate. The variants differed from parent-type bacteria by not producing citrate permease, required for transporting citrate through the cell permeability barrier. Production of citritase by the acetoinless mutants distinguished them from cultures of *Streptococcus lactis* and *Streptococcus cremoris*.

The biochemical and serological properties of 35 strains of lactic streptococci producing acetoin, diacetyl, carbon dioxide, and acetic acid in milk, as well as large amounts of lactic acid, were studied by Swartling (11). He concluded that the name *Streptococcus diacetilactis*, originally given by Matuszewski et al. (9), should be applied. Sherman (10) suggested that decision regarding the giving of species distinction to *S. diacetilactis* should await clarification of certain reports indicating that some cultures of this organism may contain variants indistinguishable from cultures of *Streptococcus lactis* (7, 9, 11).

Recent reports clarify the initial steps involved in the formation of acetoin from citrate by cultures of *S. diacetilactis* and *Leuconostoc citrovorum*. Citrate is transported through the cell permeability barrier by a permease system (6) and split into acetate and oxalacetate by an enzyme citritase (5). Cultures of *S. lactis* and *S. cremoris* do not contain citritase (5).

Variants not producing acetoin and gas from citrate have been isolated from cultures of *S. diacetilactis* in this laboratory. Results reported here show that the variants contain citritase and differ from parent-type *S. diacetilactis* cells by not containing citrate permease. The findings clarify the taxonomic status of *S. diacetilactis*.

METHODS

Cultures. Five strains of *S. diacetilactis* were studied. Strains DRC1, DRC2, and DRC3, and bacteriophages active against them, were obtained from the Dairy Research Section of the Commonwealth Scientific and Industrial Research Organization, Australia. Strain NZ was obtained from the Dairy Research Institute of New Zealand, and Strain 957 from the Na-

tional Dairies Research Laboratories, England. One strain of *S. lactis* (C2) and one of *S. cremoris* (HP) were used for comparative purposes.

Cultural tests. Tests normally used for classifying streptococci and distinguishing *S. lactis* from *S. cremoris* were applied to cultures purified by colony isolation. Tests were run to determine: action on litmus milk at 22 C, growth in litmus milk at 40 and 45 C, growth in broth containing 4 and 6.5% sodium chloride, production of ammonia from arginine, fermentation of maltose, and fermentation of dextrin. Acetoin production was determined by the Voges-Proskauer test, and gas production determined by heating ripened culture in a test tube and observing for curd flotation.

Antibiotic production. Cultures were tested for the production of antibiotics by a method previously described (2), with two modifications. Lactic agar (3) replaced tryptone yeast phosphate agar and plates of agar were dried at 45 C.

Cell suspensions. Cell suspensions for use in determining citrate utilization were prepared as follows: Cultures were grown 40 hr at 26 C in flasks of citrate medium (5), harvested by centrifugation, washed twice with cold 0.05 M trishydroxyaminomethane chloride buffer at pH 7.0, and resuspended in the same buffer at a cell density of 40 mg/ml.

RESULTS

Five strains of *S. diacetilactis* had been propagated routinely for several weeks when colony isolation and selection resulted in some cultures that did not produce acetoin and gas. Eventually, one isolate that produced acetoin and gas and one that did not (subsequently referred to as the acetoinless isolate) were selected from each of the five. Acetoinless col-

onies were rare in Strain DRC1. Acetoin-producing colonies were not found in Strains 957 and NZ until after these strains had been grown at 40 C. Accurate estimation of the relative numbers of parental and variant type colonies was not accomplished, since attempts to devise a convenient scoring method were unsuccessful.

Action on litmus milk. Each of the five *S. diacetilactis* strains selected for study co-agulated skimmilk during overnight incubation at 22 C. With regard to this characteristic, there was no detectable difference between ace-toin-producing and acetoinless isolates.

Bacteriophage sensitivity. Strains DRC1, DRC2, and DRC3 had been found sensitive to their corresponding bacteriophages only. Strain 957 had been found sensitive to bacteriophage DRC1, and NZ to each of the three. Examination of the ten isolates revealed that each had the same bacteriophage sensitivity as its parent culture.

Antibiotic production. DRC1 and 957 produced an antibiotic (2). A check on the isolates showed that only those from Strains DRC1 and 957 produced the antibiotic.

Cultural test reactions. Table 1 shows the reactions of the ten isolates to the tests normally used for distinguishing *S. lactis* and *S. cremoris.* The acetoinless isolates reacted the same as corresponding normal isolates, except that they grew more slowly at 40 C. Growing acetoinless isolates at 40 C did not cause them to produce acetoin and gas.

Citrate utilization. Each of the cultures was tested for utilization of citrate with and without destruction of the cell permeability barrier with toluene (4). Two-tenths of a milliliter of intact-cell suspension or 0.2 ml of toluene-treated cells (removed from beneath the toluene layer with a pipette) was added to 0.8 ml of the following reaction mixture in a glass-stoppered test tube: 0.01 M Na citrate, 2.0 ml; 0.1 M MgSO$_4$, 1.0 ml; and 0.5 M phosphate buffer, 13 ml. The pH of the buffer was pH 6.5 (near the optimum for citritase) for toluene-treated cells and pH 5.0 (near the optimum for citrate permease) for intact cells. Tube contents were mixed, incubated 30 min or 1 hr at 30 C in a thermostatically controlled water bath, and tested for citrate by the pyridine acetic anhydride method of Marier and Boulet (8).

Results were the same for each of the acetoin-producing isolates. Intact and toluene-treated cells utilized all of the citrate in the reaction mixtures within the 30-min incubation period, indicating the presence of both citrate permease and citritase. Results were different for the acetoinless isolates. Toluene-treated cell suspensions utilized about 50% of the citrate in 30 min, and 100% in 1 hr, indicating the presence of citritase; but intact cells utilized none of the citrate, even after prolonged incubation, indicating absence of citrate permease.

Acetoin production by cell-free extracts. That the acetoinless variants possessed a complete and competent intracellular enzyme system for converting citrate to acetoin was confirmed for

TABLE 1

Reactions of *Streptococcus diacetilactis* isolates to cultural tests

Culture	Growth at		Growth in presence of		Production of acid		Hydrolysis of arginine
	40 C	45 C	4% NaCl	6.5% NaCl	Maltose	Dextrin	
DRC1	+	−	+	−	+	+	−
DRC1X [a]	+[b]	−	+	−	+	+	−
DRC2	+	−	+	−	+	+	−
DRC2X [a]	+[c]	−	+	−	+	+	−
DRC3	+	−	+	−	+	+	+
DRC3X [a]	+[c]	−	+	−	+	+	+
957	+	−	+	−	+	+	−
957X [a]	+[b]	−	+	−	+	+	−
NZ	+	−	+	−	−	−	−
NZX [a]	+[b]	−	+	−	−	−	−
S. lactis (C2)	+	−	+	−	+	+	+
S. cremoris (HP)	−	−	−	−	−	−	−

[a] This isolate did not produce acetoin and gas from citrate.
[b] Growth retarded.
[c] Growth slightly retarded.

the variant DRC1X. A cell-free extract was prepared from this culture, using the Mickle tissue disintegrator as previously described (5). A quantity of the extract (0.5 ml) was incubated at 30 C in a Warburg manometer with 50 μM of citrate in phosphate buffer at pH 6.5. The total volume was 2.7 ml; the final buffer concentration was 0.35 M. Magnesium and manganous ions (20 μM of each) and thiamine pyrophosphate (100 μg) were added as cofactors. In a similar control experiment, 0.5 ml of the original cell suspension was used in place of the cell-free extract. Reaction products were determined by previously given methods (5).

The cell-free extract had utilized 25.8 μM of citrate at the conclusion of reaction. Acetoin (5.0 μM), carbon dioxide (33.4 μM), pyruvate (9.3 μM), and oxalacetate (3.8 μM) were identified as reaction products. Acetate was not determined. In the same reaction time, the intact cells utilized only 0.3 μM of citrate. Detectable amounts of acetoin and the other reaction products were not formed.

DISCUSSION

Variants unable to produce acetoin and gas from citrate occur in cultures of S. diacetilactis. The proportion of these variants in the cultures may increase on continued daily propagation, eventually becoming so great that the ability to produce certain compounds desired in cultured dairy products may be seriously decreased or lost. Care should be used in purifying cultures of S. diacetilactis, to avoid selecting colonies of the variants. Incubation at 40 C was useful as an enrichment procedure for two cultures in which the acetoinless bacteria had dominated. On the other hand, growth in the citrate medium seemed to encourage predominance of the acetoinless type.

Results obtained in this laboratory show that cultures of S. diacetilactis have a permease system for transporting citrate into the cells (6). Failure in this transport system was the only major difference detected between the variants of this study and parent S. diacetilactis cultures. That the failure was loss of ability to produce citrate permease (1) is indicated by the fact that the mutants, though possessing a competent intracellular enzyme system for converting citrate to acetoin, were cryptic toward citrate until after destruction of the cell permeability barrier. Losses of ability to produce stereospecific permeases may result from spontaneous single-step mutations (1). That toluene-treated variant cells utilized citrate more slowly than parent-type cells

is attributable to the fact that constitutive enzymes not being used by an organism are normally present in comparatively lower concentrations. Citritase content has been found reduced in S. diacetilactis cells grown in the absence of citrate (5).

The S. diacetilactis cultures of this study, like those studied by Swartling (11), resembled S. lactis in their reactions to cultural tests used for classifying streptococci and distinguishing S. lactis from S. cremoris. Only DRC3 produced ammonia from arginine, and only NZ failed to produce acid from maltose and dextrin. The main difference from S. lactis is that S. diacetilactis produces acetoin, gas, and other compounds from citrate.

Possibilities that acetoinless variants occur in cultures of S. diacetilactis and that these variants are, in fact, S. lactis has resulted in hesitancy about giving species distinction to S. diacetilactis (10, 11). Particularly with regard to the latter possibility, the present results are of taxonomic importance. The acetoinless variants reported on here obviously are not S. lactis. They are S. diacetilactis mutants that can be readily distinguished from S. lactis and S. cremoris by their production of citritase.

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EFFECT OF CERTAIN VACUUM TREATMENTS ON FLAVOR AND PHYSICAL CHARACTERISTICS OF FLUID MILK [1]

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SUMMARY

Milks with garlic, silage, feed, and cooked flavors were treated under various conditions in a two-stage vacuum treatment unit with and without added steam. Vacua at four levels in the first chamber, with a constant rate of steam injection, reduced intensity of the off-flavors to a significant degree in most cases when judged by expert and consumer flavor panels. The additive effect of steam injection was not significant, except for garlic flavor as judged by the expert panel. Composition of the milk was controlled by maintaining a suitable temperature differential between the inlet of the milk to the equipment and discharge from it. Temperature at the discharge was regulated by controlling pressure (and thus boiling point) in the second chamber. A slight increase in homogenization index was noted, but this was judged to be of little practical importance.

Aeration as a method of removal of volatile off-flavors in milk has been used since 1897 (14). The simplest form of the process was the opening of vat lids during agitation and pasteurization. More complex forms of this ventilation of milk have developed to include the vacuum pasteurizer (Vacreator) (1, 9) and the more recently developed vacuum units designed to be used in conjunction with high-temperature short-time (HTST) pasteurizers. These latter methods may or may not employ steam injection as well as vacuum for flavor removal. The value of the Vacreator for flavor removal from cream and milk has been discussed by several investigators (1, 4, 9, 13, 16). Little has been done to evaluate the usefulness of the newer equipment for flavor removal and to learn whether such processing alters the physical characteristics of the milk. This study was designed to determine: 1) the effect of various vacuum and steam injection levels on the flavor quality of milk, 2) the effect of the process on the total solids content of the milk, and 3) the effect of the process on fat dispersion in homogenized milk.

METHODS

The vacuum treatment equipment used is well known commercially.[3] After passing through a commercial high-temperature short-time unit, the milk flowed from the flow-diversion valve through an atomizing valve into the first, or atomizing, chamber. Vacuum levels in this chamber were varied in four steps from 0 to 15 inches of mercury and steam injection held constant at 95 lb per hour. Steam, varying in amounts from 0 to 135 lb/hr, was mixed with the milk in this chamber for the trials on effect of steam injection. The treated milk then flowed through a vacuum differential valve and tangentially entered a second chamber where vacuum levels could be varied within the range of 18 to 26 inches of mercury. Milk was pumped from the bottom of this chamber to the hot (pasteurized milk) side of the regenerator section of the HTST unit. Vapors resulting at this stage were pumped from the top of this chamber by a vacuum pump and passed through a condenser and to the drain.

In the second chamber, vacuum levels were not recorded as such. Instead, adjustments were made to reduce the temperature at discharge from this chamber to a level below that of the milk at the flow diversion valve. This is referred to as temperature differential (TD). Inasmuch as there is a known temperature-

pressure relationship at the boiling point of milk, temperature measurements in the second chamber could be used for estimating vacuum levels. Temperature differentials of 0 to 22 F were used and maintained by a Taylor ratio controller. Steam flow was regulated by a specially calibrated valve. Curves furnished by the valve manufacturer were used to determine steam utilization, and took into consideration pressures on both sides of the valve.

Control samples for the flavor and fat dispersion studies were taken at the flow diversion valve, except for comparisons of different steam levels. For these trials, control samples were vacuum-treated milk without steam injection. Control samples for the total solids study were obtained from the raw milk holding tank. A period of at least 5 min was allowed for equipment and milk flow stabilization after each change in processing treatment.

When vacuum levels in the first chamber were being studied, steam flow was kept constant at 95 lb/hr (as recommended by the manufacturer), temperature differential maintained at 10 to 12 F, and pasteurization at 168 F for 19 sec. For the trials in which steam injection was the variable, the vacuum in the first chamber was maintained at 10 inches and all other conditions were kept constant. Trials involving the effect of temperature differential were made by holding steam flow at 95 lb/hr and vacuum in the first chamber at 10 inches. Cooked flavor was obtained by heating the milk to 175 F for 19 sec.

All samples were homogenized at 2,500-lb pressure by a two-stage homogenizer which also was used as a timing pump for the HTST unit. The homogenizer was located between the raw side of the regenerator section and the heating section of the pasteurizer.

The Gibson and Herreid (6) rapid method of determining fat emulsion stability was used as an index of homogenization. Fat dispersion also was studied by the microscopic method of Farrall, Walts, and Hanson (5). Total solids content was determined by the Mojonnier method (10).

Samples were analyzed organoleptically by means of two panels—a consumer panel and an expert panel. The consumer panel consisted of ten men and five women, ranging in age from 20 to 55. Members of this panel were selected from 35 individuals. Selection was based on the ability to detect varying intensities of off-flavors when presented in a triangular test pattern. A modified hedonic scale (11) was used for scoring purposes when the products

were evaluated by this panel. Panelists were instructed to rate the product according to their likes and dislikes and to make comparative ratings among the samples. Samples could be scored from a high of eight to a low of one.

The expert panel consisted of four experienced dairy products judges of the Dairy staff. The American Dairy Science Association student score-card was used in evaluation of the products. All samples were held 20-24 hr before judging. Both panels evaluated the products within a period of not over 2 hr.

Milk for use in these experiments was mixed herd milk from the University herd. With the cooperation of the herdsman, feeding programs were instituted to produce the off-flavors desired.

Because of a shortage of wild garlic during the 2 yr of this study, it was necessary to resort to special methods for obtaining garlic-flavored milk. A search of the literature (2, 18) made it evident that the flavor constituents of wild garlic and garlic concentrate (powder) were essentially the same. It was found, in preliminary feeding trials, that there was no discernible difference between the flavor imparted by the garlic concentrate and that produced by wild garlic. The addition of 2 lb of garlic concentrate to the feed concentrates of 60 cows resulted in a highly flavored milk.

RESULTS AND DISCUSSION

In preliminary trials, it was learned that only the vacuum level of the first or atomizing chamber could be varied without change in milk composition. For this reason effect of vacuum level on flavor removal was studied for this chamber alone.

Effect of vacuum level in first chamber on flavor removal. Garlic. It must be recognized that varying degrees of steam-flash in the first chamber will result with variations in the vacuum level. For example, when a five-inch vacuum is used the flash point or boiling point of the milk in this chamber will be 203 F. The temperature of the milk resulting from the addition of 95 lb of steam per hour was 192 F; therefore, under these conditions, boiling of the product in the first chamber did not take place. At 10 inches of vacuum the boiling point of the milk is reduced to 192 F, the approximate temperature of the milk and steam mixture. At 15 inches of vacuum, the theoretical boiling point is 179 F, which is below the temperature of the milk and steam mixture. It was noted in these experiments that the temperature did not fall to the theoretical level of 179 F, but dropped only five degrees to 187 F giving, in

TABLE 1

Effect of vacuum treatments on off-flavored milk

| Inches of vacuum | No. of trials | Type of flavor | Change in score | | | | | |
| | | | Consumer | | | Expert | | |
			Maximum [a]	Minimum [a]	Average [b]	Maximum [a]	Minimum [a]	Average [b]
5	13	Garlic	+2.27	+0.20	+1.05	+4.63	+1.00	+2.13
7.5	14	Garlic	+2.70	+0.49	+1.71	+4.75	+0.67	+2.23
10	14	Garlic	+3.23	+0.62	+1.83	+4.38	+1.00	+2.56
15	13	Garlic	+3.27	+0.15	+1.77	+4.50	+0.67	+2.48
5	8	Silage	+2.80	+0.10	+0.92	+3.02	0.00	+0.37
7.5	8	Silage	+2.00	0.00	+1.29	+3.02	−0.50	+1.39
10	8	Silage	+2.33	+0.10	+1.09	+3.00	0.00	+1.30
15	8	Silage	+2.40	−0.30	+1.24	+3.75	+1.00	+0.96
5	9 [c]	Feed	+1.34	+0.01	+0.22	+2.63	+0.34	+0.83
7.5	9 [c]	Feed	+2.08	−0.47	+0.65	+3.12	+0.67	+0.88
10	9 [c]	Feed	+2.34	−0.48	+0.74	+3.33	+0.13	+1.64
15	9 [c]	Feed	+1.75	0.00	+0.63	+3.33	+0.13	+1.75
5	8	Cooked	+2.16	−0.07	+0.37	+2.00	−0.25	+0.06
7.5	8	Cooked	+3.33	+0.19	+1.41	+2.25	+0.13	+0.91
10	8	Cooked	+2.28	+0.49	+1.26	+2.38	+1.17	+1.67
15	8	Cooked	+2.93	+0.29	+1.30	+2.13	+0.88	+1.69

[a] Average of all judges for a single trial.
[b] Average of all judges for all trials.
[c] Expert panel examined only seven trials.

effect, a superheating of the milk and steam mixture in the first chamber.

The effect of vacuum level on flavor removal is shown in Table 1. Because of the variation in scores of the control samples, it was thought that differences in score between the control and the treated products should be considered as well as score of the treated product. Average scores of the expert panel are shown in Figure 1.

Data from both the consumer and expert panel indicate that vacuum treatment materially reduced the intensity of garlic flavor in milk. When treated statistically by analysis of variance (17), the data thus obtained showed the results to be highly significant (P < 0.01). It will be noted from the data that the maximum improvement according to both panels was obtained at the 10-inch vacuum level. This would indicate that the superheating treatment that occurs at 15 inches of vacuum had little additional effect on flavor removal.

Greatest improvement in flavor was noted when the flavor defect was most pronounced. Since garlic-flavored milk automatically receives a low score on the score-card, the degree of intensity was recorded according to an arbitrary plan. The raw milks containing garlic flavor were divided into pronounced (35 or less) and slight (35.5 or above). Maximum improvement exhibited in the pronounced group was 4.75 points, the average being 3.23 points,

compared with a maximum improvement of 3.00 points and an average of 1.74 points in the slight group based on data from the expert panel. The trend of consumer scoring was in close agreement with that of the expert panel.

Silage. Greatest improvement in silage flavor was obtained at the 7.5-inch vacuum level, as judged by both panels. This would indicate that these flavors apparently are more volatile and that boiling is not essential for their re-
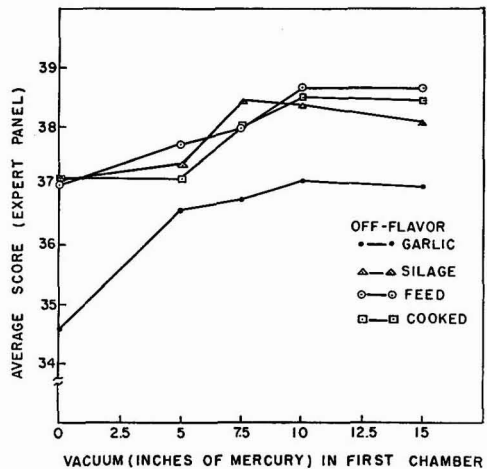
FIG. 1. Effect of vacuum treatment on flavor score of milk.

moval. There was some disagreement between the panels as to the value of additional vacuum. While the expert panel reported a statistically significant (P < 0.05) improvement only between the control and 7.5-inch level, the consumer panel reported a highly significant improvement at all levels of treatment.

Feed. Flavors resulting from grass, concentrates, or hay were classed in this study as feed flavors. The expert panel reported flavor improvement at all levels of vacuum, but little additional increase beyond the 10-inch or boiling level. The improvement in scores was statistically significant at the 5% level. The consumer panel reported flavor improvement at all vacuum treatment levels, but the differences in scores were not statistically significant. In fact, their judgment indicated less improvement in flavor in treatments above 10 inches of vacuum.

Cooked. A study of cooked flavor was not included in the original objective of this investigation. Observations made, principally by the expert panel, during the feed and weed flavor studies indicated that treatments affected the degree of cooked flavor. Trials were made, therefore, using raw milk of good flavor quality. It will be noted that in this case, because of the higher initial temperature at the flow diversion valve, the boiling occurred at a level between 7.5 and 10 inches of vacuum. From Table 1 and Figure 1 it will be noted that the

expert panel found a highly significant improvement as vacuum was increased up to 10 inches. The consumer panel, however, found maximum improvement to be at the 7.5-inch level. In other words, boiling brought about no further improvement.

Effect of steam injection on flavor removal. For these experiments, milk subjected to 10 inches of vacuum alone (no steam injection) was used as the control sample. Results of these experiments are shown in Table 2 and Figure 2.
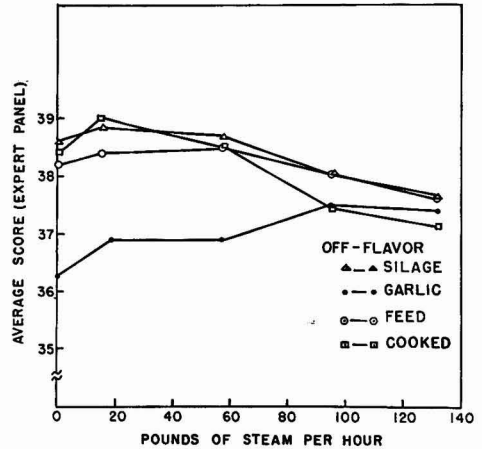
FIG. 2. Effect of steam injection on flavor score of milk.

TABLE 2

Effect of steam treatment on off-flavored milk [a]

| Pounds of steam per hour | No. of trials | Type of flavor | Change in score | | | | | |
| | | | Consumer | | | Expert | | |
			Maximum [b]	Minimum [b]	Average [c]	Maximum [b]	Minimum [b]	Average [c]
18.75	9	Garlic	+1.50	+0.18	+0.31	+1.13	+0.13	+0.71
57.00	9	Garlic	−0.70	0.00	−0.02	+1.83	+0.25	+0.78
95.00	9	Garlic	+0.60	0.00	+0.24	+2.33	±0.25	+1.30
132.50	9	Garlic	+0.88	0.00	+0.15	+2.33	−0.13	+1.26
18.75	9	Silage	+1.11	−0.19	+0.09	+1.16	0.00	+0.11
57.00	9	Silage	+1.64	0.00	+0.40	+2.16	−0.25	−0.04
95.00	8	Silage	−1.55	+0.33	−0.07	−2.42	0.00	−0.62
132.50	7	Silage	±1.60	+0.18	+0.01	−2.42	−0.67	−0.88
18.75	8	Feed	−0.83	+0.18	+0.21	+1.17	0.00	+0.14
57.00	8	Feed	+1.65	0.00	+0.36	+2.17	0.00	+0.38
95.00	8	Feed	−1.55	0.00	0.00	−1.25	0.00	−0.13
132.50	8	Feed	−1.64	0.00	−0.32	−1.75	0.00	−0.36
18.75	6	Cooked	−0.58	0.00	−0.04	+1.50	0.00	+0.62
57.00	6	Cooked	−1.00	−0.05	−0.24	−1.50	+0.13	+0.22
95.00	6	Cooked	−2.50	−0.55	−1.15	−2.00	−0.25	−0.81
132.50	6	Cooked	−2.33	−0.22	−1.13	−3.00	−0.25	−1.17

[a] Milk treated with vacuum but no steam was used as control.
[b] Average of all judges for a single trial.
[c] Average of all judges for all trials.

Garlic. There was little agreement between the consumer panel and expert panel as to the benefit of steam injection. The expert panel found a significant average improvement in flavor at the 95 lb and 132.5 lb per hour levels, with an average increase in score of 1.30 and 1.26 points, respectively. The higher level of steam injection, 132.5 lb per hour, apparently did not result in greater flavor improvement than did treatment at the 95-lb level. The consumer panel recorded no significant changes in flavor score which could be attributed to steam effect. From these data the value of the application of steam for garlic flavor removal would seem questionable, except for the fact that the average improvement in expert panel score attributed to steam was from 36.23 to 37.53. This probably is in the critical range of consumer acceptance.

Silage and feed. Neither panel found that steam injection improved the flavor to any significant degree when the raw milks contained silage or feed flavors. Both panels found some slight improvement, on the average, at the 18.75 to 57.00 lb/hr rate of steam injection, but this improvement was not statistically significant. There was an actual decrease in consumer panel acceptance when steam treatments above these levels were used.

Cooked. There was disagreement between the panels when steam treatment was applied to cooked milk. At all levels of steam treatment, the consumer panel considered the treated milk poorer than that subjected to vacuum treatment alone. As seen from Table 2 and Figure 2, the expert panel noted an improvement in score of milk at the lower levels of steam injection. These were not statistically significant, however. Further additions of steam above 18.75 lb per hour seemed to intensify the cooked flavor. The drop in score of heated milk as steam injection rate increased is obvious. The amount of heat to which milk was subjected following pasteurization was proportional to the amount of steam injected in the first chamber. At high levels of steam injection the intensity of the resulting cooked flavor was too great to be removed completely by vacuum treatment.

General. While milks with the greatest off-flavor in the control samples were improved most, many such milks were barely acceptable even after treatment. This is indicative of the fact that not all of the volatile off-flavors are removed even with optimum treatment. If the original intensity of the flavor defect is sufficiently great, enough will remain after treat-

ment to be objectionable. On the other hand, if the original intensity of the off-flavor is slight, removal of the same amount or proportion of off-flavor reduces its intensity below the flavor threshold, and thus the defect is considered to be completely eliminated.

It appears from these data that steam injection is of questionable benefit except in the case of a strong flavor such as garlic. This is at variance with Roberts (12) and with Cotner et al. (3). It must be noted, however, that the work of Roberts is based on observations of commercial samples from a number of plants using various types of equipment. Cotner et al. found that steam injection in laboratory apparatus was beneficial in reducing the intensity of rye and alfalfa flavors, but added little to vacuum treatment in dealing with silage flavor. The flavor from rye, and in some cases alfalfa, might well be classed with garlic.

Hedrick and Trout (7) and Smith et al. (15) reported that vacuum alone is effective in reducing off-flavors. The former specifically state that steam is of little additional benefit. Three of these reports (3, 7, 15) were published after work on this study was completed.

Effect of temperature differential and steam injection on total solids content of milk. Total solids determinations were made in preliminary trials involving changes in the vacuum level of the first chamber only. Changes in composition resulting from these vacuum level variations were not statistically significant and, on the average, varied from +0.11% to −0.10% total solids. As is pointed out by Lazar and Henningson (8), variations of this order can be found as a result of HTST treatment alone.

The change in total solids content of the milk (Table 3) brought about by varying the temperature differential was highly significant. For the type and capacity of equipment used, and atmospheric conditions prevailing in this study, a temperature differential of 10 to 14 F produced the smallest changes in total solids.

TABLE 3

Effect of temperature differential on the total solids content of fluid milk

| Temperature differential | No. of trials | Change in per cent total solids | | |
		Maximum	Minimum	Average
0	20	−0.42	−0.05	−0.21
4	22	−0.72	−0.01	−0.11
10	22	−0.28	0.00	−0.04
12	13	+0.10	0.00	−0.03
14	22	+0.21	−0.01	+0.05
22	22	+1.10	+0.03	+0.22

Theoretically, the rate of steam injection should not influence composition of milk, provided temperature differential is held constant. In these experiments, however, it will be noted (Table 4) that increased amounts of steam caused some dilution of the milk. This is undoubtedly due to the fact that higher steam injection rates increased radiation losses, requiring, therefore, a greater temperature differential to keep milk composition uniform.

TABLE 4

Effect of steam injections on the total solids content of fluid milk

Pounds of steam per hour	No. of trials	Change in per cent total solids		
		Maximum	Minimum	Average
0.0	10	+0.93	+0.03	+0.20
18.75	10	+0.20	−0.02	+0.12
57.00	11	+0.07	0.00	0.00
95.00	11	−0.50	−0.02	−0.10
132.50	11	−0.50	−0.11	−0.29

In the instruction manual accompanying the vacuum treatment unit, it is pointed out that a temperature differential is necessary to compensate for heat loss due to radiation from the surfaces of the unit. No attempt was made to calculate such heat losses in this study; however, it will be noted that a temperature differential of 12-14 F was necessary to keep the composition of the milk constant at constant rate of steam injection. This is not a fixed value and is directly dependent on length of lines, room temperature, amount of steam added, etc. For this reason, it is recommended that the appropriate temperature differential be established for each installation according to the conditions under which it is to be operated.

Effect of temperature differential and steam injection on milk fat dispersion. As can be seen in Tables 5 and 6, homogenization index apparently is increased by treatment within the equipment. The increase shown in Table 5 is statistically significant at all levels of temperature differential used, but apparently is not related directly to various levels. On the other hand, as steam injection rate is increased, there is an increase in homogenization index as determined by the Gibson and Herreid method. It must be pointed out, however, that at no time was the index over 3.6, compared with a United States Public Health Service permissible index of 10.

Microscopic examination of the samples by

TABLE 5

Effect of temperature differential on the fat dispersion of homogenized milk as measured by the Gibson and Herreid method

Temperature differential	No. of trials	Change in fat dispersion		
		Maximum	Minimum	Average
0	17	+1.25	0.00	+0.62
4	17	+1.00	0.00	+0.56
10	17	+1.00	0.00	+0.71
14	17	+1.00	0.00	+0.56
22	17	+1.00	0.00	+0.69

the method designed by Farrall et al. (5) indicated no globules or aggregation of globules of more than 2 μ in diameter in the milk. For this reason, it was impossible to establish a Farall index value.

One of the reasons for study of the effect of fat globule size and dispersion is that there have been several verbal reports that creamline has been observed in some instances on homogenized-vacuumized milk. Indeed, some of these reports state that the use of a homogenizer as a timing pump for the HTST unit and vacuum unit was impossible because of this dehomogenizing effect. In some plants, relocation of the homogenizer was deemed necessary. Results of the present study do not substantiate these reports. While there was a small increase in the homogenization index resulting from the vacuum treatment, there were no globules or clumps larger than 2 μ.

CONCLUSIONS

The vacuum treatment equipment used reduced the intensity of garlic, silage, feed, and cooked flavors in milk. Additional improvement resulting from steam injection appears to be of doubtful practical significance. By proper control of temperature differential, it was possible to prevent dilution or concentration of the milk. While homogenization index

TABLE 6

Effect of steam injection on the fat dispersion of homogenized milk as determined by the Gibson and Herreid method

Pounds of steam per hour	No. of trials	Change in fat dispersion		
		Maximum	Minimum	Average
0.00	8	+1.50	0.00	+0.08
18.75	9	+1.25	+0.25	+0.45
57.00	9	+1.50	0.00	+0.72
95.00	9	+1.50	+0.25	+0.75
132.50	9	+1.50	+0.25	+0.81

was increased slightly, the indexes for all milks were well below the limits established by U.S.P.H.S.

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SIGNIFICANCE OF PLASMA ULTRAFILTRABLE CA^{45} AND P^{32} IN MILK SYNTHESIS [1]

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SUMMARY

Three Jersey cows were injected intravenously with Ca^{45} and P^{32} simultaneously, and the ultrafiltrable as well as total Ca^{45} and P^{32} in milk and plasma were determined periodically to study the importance of these fractions in the formation of milk calcium and phosphorus. While most of the plasma P^{32} (85%) was still ultrafiltrable 1 hr after dosing, the activity appeared in both fractions of milk, indicating that plasma ultrafiltrable phosphorus is the precursor of milk phosphorus. After the first 5 hr post-dosing, the specific activity of plasma nonultrafiltrable P^{32} began to rise, whereas that of ultrafiltrable P^{32} continued to decline, and 43 hr were required for the ratio of plasma ultrafiltrable P^{32}/total P^{32} to reach a value (0.40) close to that of stable phosphorus (0.34).

One hour after dosing, 66% of the plasma total Ca^{45} was nonultrafiltrable, presumably protein-bound. Partitioning of Ca^{45} in milk took place also within 1 hr after dosing and the maximum concentration of milk ultrafiltrable Ca^{45} coincided in time (3-5 hr) with that of nonultrafiltrable Ca^{45}, suggesting that plasma ultrafiltrable calcium is the precursor of milk Ca. Milk ultrafiltrable/total Ca^{45} and P^{32} fell to values close to those of stable Ca and P between the third and fifth hours after dosing, which is the time required for the complete conversion of plasma Ca and P into all fractions of milk Ca and P.

Various approaches (1, 2, 6, 8, 11, 12) using radioactive tracer techniques showed that plasma inorganic phosphorus is rapidly utilized by the mammary gland for milk synthesis. Furthermore, some workers (6, 8, 11) concluded that the plasma inorganic phosphorus was the main precursor of milk phosphorus. On the other hand, limited information is available regarding the precursor-product relationship of milk calcium (3, 13, 15).

The inorganic phosphorus as determined in the above studies includes all forms of soluble and colloidal inorganic phosphorus besides some organic phosphorus liberated from its organic bonds by the acid treatment of plasma or milk during the analysis. Likewise, in the fractionation of plasma and milk calcium by electrophoretic technique, the direct current induced by electrophoresis is sufficiently strong to dissociate the calcium from the protein moiety. Ultrafiltration, however, does not encounter such problems.

The ultrafiltrable (UF) [3] fraction of plasma or milk contains calcium and phosphorus in ionized and soluble un-ionized forms. The amount of colloidal calcium and phosphorus, however, that passes through the cellulose tubing pores (< 4.8-mμ diameter) is too small to be of significance. Since the exchange of these elements between tissues and body fluid appears to take place in the UF form, the relation between plasma and milk UF Ca^{45} and P^{32} has been investigated in this study, following intravenous administration of the radioisotopes to dairy cows. The purpose was an attempt to clarify the significance of the plasma UF fraction in the secretion of milk calcium and phosphorus.

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[3] The abbreviations for ultrafiltrable and nonultrafiltrable are UF and non-UF, respectively.

METHODS

Two Jersey cows in a preliminary experiment and three in the main experiment were each injected intravenously into the jugular vein with a simultaneous dose of approximately 3.5 and 3.0 mc of Ca^{45} and P^{32}, respectively. Feed and water were provided twice a day.

Blood, milk, urine, and feces samples were collected at 1, 2, 3, 5, 7, 19, 27, and 43 hr after dosing. When milk-sampling periods were shorter than 5 hr, 10 IU of oxytocin were injected intravenously 1 min before milking to stimulate milk let-down. Blood samples were collected in 50-ml heparinized test tubes from the jugular vein opposite to the side of dosing and were immediately centrifuged to obtain the plasma.

Ultrafiltration of milk and plasma was carried out using the Prasad and Flink (10) method for blood serum. The CO_2 in blood plasma was allowed to equilibrate with the atmosphere and the pH was approximately 8.2 at the time of centrifugation. This pH change was allowed to occur due to the qualitative nature of the measurements taken. Plasma and milk aliquots of 10 ml were pipetted into the cellulose tubings and centrifuged at about 1,000 × g for 2 hr at 20 C. The concentration of the stable or radioactive element in the ultrafiltrate was multiplied by the moisture content of the milk and plasma to obtain the fraction of the UF element in the milk or plasma. The estimation was based on the experimental data shown in Table 1, which indicate that the concentration of the element in the ultrafiltrate is constant and independent of either ultrafiltrate volume, or ultrafiltration time. This conclusion is in agreement with the work of others (9, 14, 16), using either ultrafiltration or ultracentrifugation techniques.

Calcium was determined according to the method of Kamal (7), using 2-ml aliquots of plasma or milk ultrafiltrates. Phosphorus was determined by the method of Fiske and Sub-baRow (5). Differential counting was used for determining Ca^{45} and P^{32} activities, using 1.0 ml milk, plasma, ultrafiltrate, urine, or diluted HCl solution of feces ash, pipetted into stainless steel planchets (2.5-mm diameter, 2.0-ml capacity) and dried slowly in about 3 hr under infrared heat. Reference standards were prepared by diluting aliquots of Ca^{45} and P^{32} standard solutions with milk, plasma, ultrafiltrates, urine, or diluted HCl solution of feces ash which contained no radioactivity. The correction for self-absorption in this method was, therefore, unnecessary. Samples were counted with and without aluminum absorber (55.2 mg/cm^2), using a Geiger-Muller counter. P^{32} cpm were obtained by multiplying the cpm of the shielded sample by the attenuation factor (1.51) of the absorber. Ca^{45} cpm were obtained by subtracting the P^{32} cpm from the unshielded cpm of the sample. The individual variation in the chemical composition within a particular fluid, such as milk, apparently had insignificant effect on the self-absorption, since the coefficient of variations in samples obtained from different animals were 3.3 and 5.3% or less for P^{32} and Ca^{45}, respectively.

RESULTS AND DISCUSSION

Only the results of the main experiment which are in close agreement with those of the preliminary experiment are reported. The concentration and the rate of decline of plasma UF P^{32} (Figure 1) were close to those of total plasma P^{32} during the first 5 hr after dosing, indicating that during this period the injected P^{32} was still mainly in the soluble inorganic

TABLE 1

Effect of increasing ultrafiltration time on the concentration of stable and radioactive calcium and phosphorus in milk and plasma ultrafiltrates

Sample	Ultra-filtrate	Volume of ultra-filtrate (Succeeding 2-hr periods)	Ca^{45}	P^{32}	Calcium	Phosphorus
	(hr)	(ml)	(cpm/ml)		(mg/100 ml)	
Milk	2	2.17	2,180	620	38.3	22.9
Milk	4	1.45	1,990	600	42.8	22.8
Milk	6	0.96	2,030	550	39.6	22.6
Milk	8	0.68	2,140	550	43.0	23.0
Blood plasma	2	2.45	350	323	4.4	4.2
Blood plasma	4	1.31	352	323	5.1	4.1
Blood plasma	6	0.80	360	290	4.7	4.5
Blood plasma	8	0.51	336	293	5.3	4.4
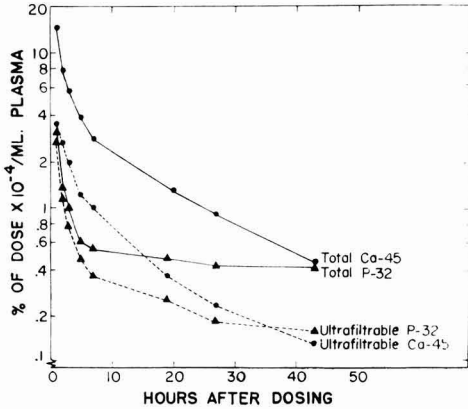
FIG. 1. Change in ultrafiltrable and total Ca^{45} and P^{32} concentrations in plasma with time after dosing.

form. It appears, however, that a small fraction of the plasma non-UF phosphorus exchanges with P^{32} 1 hr, or earlier, after dosing as shown by the relatively low specific activity of the plasma non-UF P^{32} (Figure 2). After the fifth hour post-dosing until the end of the experiment (43 hr), the specific activity of plasma non-UF P^{32} (Figure 2) rose gradually, whereas the plasma UF P^{32} specific activity declined with a diminishing rate. This change is attributed to the utilization of plasma UF P^{32} by tissues, with concomitant feed-back of P^{32} to the blood in various forms of UF and non-UF forms.

The concentration of plasma UF Ca^{45} was much lower than that of plasma total Ca^{45} 1 hr after dosing (Figure 1), implying an immediate binding of the major part of the injected

Ca^{45} with plasma proteins and/or its transformation to other non-UF forms. The Ca^{45} concentration declined rapidly in both fractions during the first 5 to 7 hr after dosing, then less rapidly thereafter, indicating a beginning of slow feed-back of Ca^{45} from tissues to blood. The injected Ca^{45} apparently exchanged with stable calcium at the same rate in both plasma fractions, since the specific activity of UF Ca^{45} was close to that of non-UF Ca^{45} (Figure 2).

The apparent large utilization of P^{32} and Ca^{45} by tissues is confirmed by the fact that only 20 and 40% of the dosed P^{32} and Ca^{45}, respectively, were eliminated outside the body in milk, urine, and feces during 43 hr of the experiment, leaving the great majority of the activity remaining in the body with very little activity in the plasma (0.7 and 0.8% of dose for P^{32} and Ca^{45}, respectively), as indicated in Table 2 and Figure 3. The P^{32} was being uti-

FIG. 2. Change in specific activities of Ca^{45} and P^{32} in milk and plasma with time after dosing.
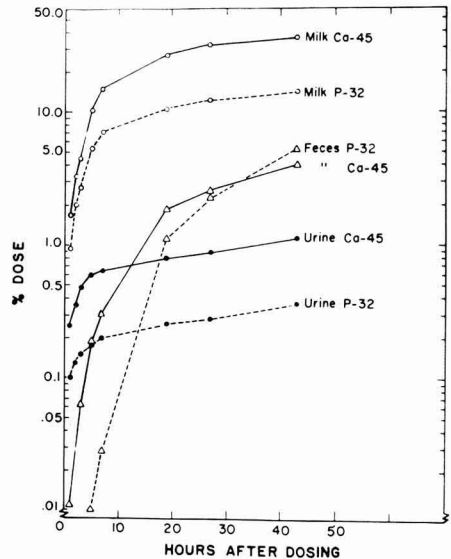
FIG. 3. Cumulative excretion of Ca^{45} and P^{32} in milk, urine, and feces.

lized at a faster rate by tissues and excreted at a slower rate in urine, feces, and milk during the first few hours after dosing, whereas the opposite was true afterwards where P^{32} began to feed back from tissues to plasma and consequently to urine, feces, and milk at a faster rate than Ca^{45}. Such an interpretation is substantiated by the declining segments in the curves of P^{32}/Ca^{45} of plasma, urine, feces, and milk during the first few hours after dosing

TABLE 2

Plasma and cumulative values of Ca^{45} and P^{32}

	Plasma Ca^{45} % of dose	Cumulative Ca^{45} % of dose			Plasma P^{32} % of dose	Cumulative P^{32} % of dose		
		Milk	Urine	Feces		Milk	Urine	Feces
43 hr after dosing	0.8	35.98	1.13	4.07	0.7	14.19	0.36	5.24
St.D.	0.14	6.70	0.18	1.20	0.07	3.90	0.32	1.10

and by the subsequent rising of the ratio afterwards (Figure 4).

While most of the plasma P^{32} was in the UF form during the first 5 hr after dosing, as previously indicated, milk P^{32} (Figure 5) was almost immediately partitioned to UF and non-UF fractions. The relationship between the two fractions remained relatively constant throughout the period. This indicates that the plasma UF phosphorus is the main precursor of both the UF and non-UF fractions of milk phosphorus.

The partition of milk Ca^{45}, similar to milk P^{32}, took place within 1 hr after dosing and thereafter (Figure 5). The time of attaining maximum concentration of milk UF Ca^{45} coincided with that of total Ca^{45} (3 to 5 hr after dosing), indicating that both UF and non-UF milk calcium are derived from a single fraction of plasma calcium, apparently the plasma UF calcium. The maximum concentrations of total and UF P^{32} in milk were also attained in 3 hr after dosing. These results imply that the

FIG. 5. Change in ultrafiltrable and total Ca^{45} and P^{32} concentrations in milk with time after dosing.

time required for incorporation of plasma calcium and phosphorus into all calcium and phosphorus containing phases of milk is from 3 to 5 hr, which agrees with earlier work (8, 15) in intact animals, but not with in vitro experiments (6) where 5 to 8 hr were required.

The ratio of UF P^{32}/total P^{32} in plasma was 0.85 at 1 hr after dosing (Figure 6), indicating that most of the injected P^{32} was still in the UF inorganic form. The rapid decline afterwards, which reached a value close to that of stable phosphorus in 43 hr (0.40 vs. 0.34), as shown in Figure 5, indicates that at least 43 hr are required by the tissue to distribute the plasma P^{32} in proportion similar to that of stable phosphorus in UF and non-UF forms. The close agreement between the values of the radioactive and stable ratios of UF Ca/total Ca in plasma 1 hr after dosing, which was identical at 43 hr (0.28 vs. 0.28), indicates a rapid exchange of Ca^{45} with stable Ca of the plasma UF and non-UF fractions. The ratios of UF/total P^{32} and Ca^{45} in milk were high for the first 3 hr after dosing and fell to values close to those of stable phosphorus (0.35 vs. 0.35) and calcium (0.22 vs. 0.24) in 5 hr after dosing, as shown in Table 3. These results

FIG. 4. Ratio of P^{32} to Ca^{45} in milk, plasma, urine, and feces.

TABLE 3

Plasma and milk averages of total and ultrafiltrable calcium and phosphorus

	Total calcium	UF calcium	UF Ca / Total Ca	Total Ca⁴⁵ (5 hr after dosing)	Total phosphorus	UF phosphorus	UF P / Total P	Total P³² (5 hr after dosing)
				UF Ca⁴⁵ / Total Ca⁴⁵				UF P³²
	(mg/100 ml)				(mg/100 ml)			
Blood plasma	10.4	2.9	0.28	0.32	13.8	4.6	0.34	0.76
C.V.	2.4	13.0	13.6	19.0	8.8	17.4	17.1	6.0
Milk	142.0	34.4	0.24	0.22	112.0	39.4	0.35	0.35
C.V.	9.8	9.4	0.1	12.5	9.7	12.5	11.0	16.0

indicate again that the complete conversion of plasma calcium and phosphorus to those of milk requires from 3 to 5 hr.

FIG. 6. Ratio of ultrafiltrable to total Ca⁴⁵ and P³² in milk and blood plasma.

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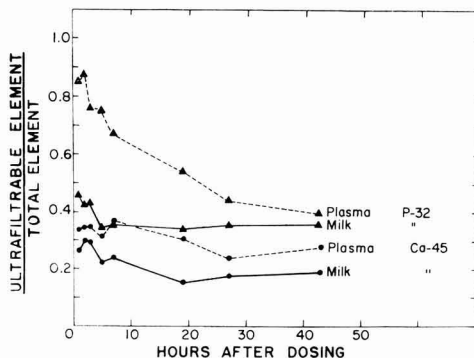
EFFECTS OF ADDING CONCENTRATES TO AD LIBITUM ROUGHAGE FEEDING IN THE DRY PERIOD

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SUMMARY

Two experiments were conducted to compare feeding dry cows for 6 wk before calving ad lib. roughage alone or ad lib. roughage plus 8 lb of concentrates daily. In one experiment, an equal amount of extra concentrates had been fed in the last part of the preliminary lactation to the cows not fed concentrates during the dry period. The cows used were paired on the basis of their previous lactation. A total of 75 pairs of cows was used in the two experiments. Feeding extra concentrates during the dry period caused a highly significant increase of 302 lb of FCM in the first 15 wk of the next lactation. Extra concentrates fed during the last of the lactation did not change body condition before calving and did not stimulate lactation after calving. Cows fed concentrates in the dry period gained 30.5 lb more in 35 days than pair-mates not fed grain. They also lost 20.3 lb more in 35 days after calving. These differences in weight change were highly significant. It is postulated that providing extra nutrients to the prepartum dry cow stimulates metabolism, resulting in high initial lactation levels, and that the high lactation level is partly maintained at the expense of the accumulated reserve body tissue.

The feed requirements of the dry cow for 2 months before calving have been established by a committee of the National Research Council (1) at from 60 to 100% above maintenance. This requirement may be met by ad lib. consumption of good quality roughage alone. Feeding grain in addition to ad lib. roughage to dry cows will result in greater weight gains and fattening, and it has been proposed as a method of increasing milk production in the next lactation (2-4, 9, 10). Results of some experiments in which a roughage ration equal to or slightly above maintenance was supplemented with concentrates during the dry period have shown significant benefits in the succeeding lactation (3, 9). Other comparisons of high concentrate and low concentrate or only roughage feeding in the dry period have not shown a significant difference in lactation due to dry period treatment (6, 8, 11). In the latter experiments high-quality roughage was fed and the cows were in very good body condition, between moderately fleshed and fat, when the different dry period treatments began.

The question of the relative efficiency of feeding a certain amount of additional concentrate in late lactation compared to feeding it in the dry period has been raised. Presumably, feeding extra grain during late lactation should

result in good condition of cows when they are turned dry, and would require less labor and attention during the dry period. Dry cows are usually separated from the milking herd and fed on pasture or other ad lib. roughage in such a way that concentrate feeding is not convenient. Because of these factors the relative values of feeding extra grain at different prepartum periods was determined under commercial herd management conditions.

EXPERIMENTAL PROCEDURE

Two separate experiments were conducted. In Experiment I the cows were treated alike in the lactation previous to the experimental dry periods. In Experiment II paired cows were fed equal amounts of extra concentrates in the prepartum period. One of each pair was fed concentrates in the dry period and the other was fed the same amount in addition to the regular concentrate ration in late lactation. The experiments were essentially alike in other respects.

Animals. Holstein and Jersey cows which had completed one or more lactations were selected from the University of Tennessee dairy herd. Cows were paired as closely as possible on the basis of breed, age, time of calving, and milk production characteristics (total yield for 30 wk and the lactation curve or persistency)

as expressed in previous lactations. The paired cows were assigned to two groups to give essentially equal fat-corrected-milk (FCM) yields in the preliminary lactations. Some cows were used in more than 1 yr's trial, but pairs were re-evaluated each year on the basis of the previous year's production.

Experimental periods. Each experiment was conducted with several pairs each year for three consecutive years. The numbers of completed pairs by years were 15, 14, and 11 in Experiment I, and 8, 9, and 18 in Experiment II. In addition to the preliminary lactation and dry period, each cow was under experimental observation for 30 wk of the succeeding lactation. Lactation yields were not used beyond 30 wk because of the possibility of varying stages of gestation or environmental effects other than the treatments altering the production responses in the later stages of lactation.

Feeding. The normal feeding of milking cows at the University of Tennessee during this period was a variety of roughages fed ad lib., along with liberal allowances of concentrates, about 1 lb per 2.5 lb of FCM. The concentrate mixture was about 18% crude protein, composed of corn, oats, wheat bran, soybean oil meal, and cotton-seed meal, plus salt and a phosphate supplement. Roughages fed consisted of a limited amount of grass-legume pasture, green chopped millet or Sudan grass, corn silage, oat silage, alfalfa silage, and a variety of hays. Most of the hay was of average quality, purchased locally, and consisted of alfalfa, lespedeza, red clover, orchard grass, and similar mixtures. Roughage was fed to both milking and dry cows in racks and bunks in amounts so that considerable refuse always resulted. Under these feeding conditions cows were usually in good body condition at the end of the lactation.

In Experiment I one of each pair of cows was fed only ad lib. roughage during the **dry** period. The other cow of each pair was **fed** roughage in the same way and, in addition, was also fed 8 lb of concentrates daily, beginning 42 days before expected parturition. In Experiment II one of each pair was fed 3 lb per day more concentrates than the pair-mate from 170 days before expected parturition to the drying-off date, 60 days before expected parturition. These cows, which had received 330 lb extra grain during the last 110 days of lactation, were fed only ad lib. roughage while dry. Their pair-mates were fed roughage ad lib. and, in addition, were each fed 8 lb of concentrates daily, beginning 42 days before expected parturition.

After parturition the cows in each pair were fed alike. The concentrate feeding schedule was prepared in advance on the basis of a liberal allowance for the expected production of each pair. The concentrate ration was increased at 2, 3, and 4 wk of lactation and then kept at the same level through 24 wk. From 25 to 30 wk, inclusive, the concentrate ration for each cow was adjusted on the basis of FCM production in the 21- to 24-wk period. Cows in Experiment II which had not been fed extra concentrates in the dry period were fed about 3 lb per day more concentrates than their pair-mates after 24 wk, which approximately duplicated the late lactation feeding of the preliminary lactation. This method of equalizing concentrate feeding was used to eliminate the possibility of the current lactation feeding overriding the possible effects of the dry period feeding. Roughages were fed ad lib. during lactation in the same manner as in the dry period and the amount fed was large enough to allow 10 to 15% refusal.

Weights. All cows were weighed at weekly intervals from 8 wk before expected parturition through 30 wk of lactation. Also, the cows of Experiment II were weighed for the two consecutive weeks nearest to each 24, 18, and 12 wk before expected parturition.

Milk records. Cows were milked twice daily at 12-hr intervals. All milk weights were recorded. Lactation records were started on the fourth day after calving. One day each week a composite sample of the AM and PM milkings of each cow was collected and tested for milk fat. These fat tests were used to construct 3-wk moving averages of tests, from which the weekly yields of FCM and milk fat were calculated. Occasional short periods of disturbed milk production due to mild mastitis or other sickness were corrected when possible by interpolation or extrapolation from the normal weekly yields of each cow. When the sickness was of long duration or correction was not reasonable the entire data for the affected pair were eliminated.

Analyses. Since the cows were carefully paired on the basis of production, the experimental data were treated as paired data, and significance of the treatment effects determined by analysis of variance (12).

RESULTS

Results of Experiments I and II are presented together in Tables 1 and 2.

Dry periods. It was planned that each cow have a 60-day dry period. In Experiment I,

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TABLE 1

Dry periods, dry period treatment, and body weight changes of paired cows in two experiments
comparing grain with no-grain feeding in the dry periods

	Experiment I		Experiment II	
Item	Concentrate and roughage in dry period	Only roughage in dry period	Concentrate and roughage in dry period	Only roughage in dry period
No. of cows	40	40	35	35
Dry period *(days)*	65.0	72.3	56.3	57.6
Concentrate fed *(days)*	38.4	0	37.6	0
Concentrate in dry period *(lb)*	307.	0	301.	0[a]
Concentrate fed in 30 wk lactation *(lb)*	2,561	2,567	3,016	3,093
Body weight changes				
−24 to −6 wk *(lb)*	....	....	98	92
− 6 to − 1 wk *(lb)*	51[b]	23	66[c]	31
Parturition, −1 to +1 wk *(lb)*	−129	−123	−109	−120
Lactation, 1 to 6 wk *(lb)*	− 34[b]	− 20	− 32[c]	− 6

[a] Each cow in this group was fed 330 lb more concentrates than its pair-mate in the last
110 days of lactation.
[b] Significantly greater than paired group average, $P = < .05$.
[c] Highly significantly greater than paired group, $P = < .01$.

TABLE 2

Production of milk, fat, and FCM in preliminary and experimental lactations of paired cows
in two experiments comparing grain with no-grain feeding in the dry period

	Experiment I		Experiment II	
Item	Concentrate and roughage in dry period	Only roughage in dry period	Concentrate and roughage in dry period	Only roughage in dry period
No. of cows	40	40	35	35
Preliminary lactation				
30-wk milk *(lb)*	7,417	7,396	7,606	7,449
30-wk fat *(lb)*	298	301	306	305
Avg fat test *(%)*	4.02	4.06	4.00	4.11
30-wk FCM *(lb)*	7,451	7,470	7,639	7,585
Experimental lactation				
30-wk milk *(lb)*	7,900	7,574	8,048	7,460
30-wk fat *(lb)*	313	300	325	302
Avg fat test *(%)*	3.96	3.96	4.04	4.05
30-wk FCM *(lb)*	7,860	7,531	8,096	7,523
1-15 wk FCM *(lb)*	4,626[a]	4,374	4,707[b]	4,348

[a] Significantly higher than paired group, $P = < .08$.
[b] Significantly higher than paired group, $P = < .05$.

several cows were used that were dry longer
than this. In both experiments, several cows
freshened earlier than expected. This resulted
in less than the planned dry period grain feed-
ing time. The average difference in dry period
length between paired groups was not sig-
nificant, and the differences in dry period grain
feeding between experiments were not signifi-
cant (Table 1).

Weight changes. The average weight change
from 24 to 6 wk prepartum was calculated for
cows in Experiment II, because the cows fed

only roughage in the dry period had received
extra concentrates in lactation during most of
this period. This extra feed failed to produce
any body weight advantage, as this group
gained 6 lb less than their paired mates (Table
1), which was a nonsignificant difference. Con-
centrate feeding in the dry period of one of
each pair produced significant differences in
weight gains from 6 to 1 wk prepartum in both
experiments. The gains in this period were not
as large as expected in any of the groups.
This may have been because all cows were

turned dry in relatively good body condition, and medium-quality roughage was fed. The weight gained by the cows fed only roughage
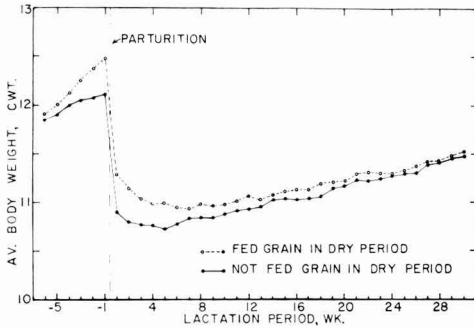
FIG. 1. Average body weight changes from 6 wk before to 30 wk after calving of 75 pairs of cows, comparing the feeding of concentrates and roughage with feeding only roughage in the dry period.

was about equal to the expected increase in weight of the fetus and uterus, so that practically no net gain in body weight resulted in this group during the last 5 wk of the dry period. The losses in weight during the week of parturition were nearly the same for both paired groups and the difference in loss between groups was not significant. During the first 6 to 10 wk of lactation, further weight losses usually occurred. It was noted that the groups fed only roughage while dry began to gain weight consistently at 5 to 6 wk of lactation, but those fed grain in the dry period lost weight or failed to gain for about 10 wk. This resulted in a significantly greater weight loss during early lactation for the cows fed extra grain while dry.

Milk and fat production. The average production data for the first 30 wk of the preliminary and experimental lactations are presented in Table 2. Since the cows were carefully paired, there were only very slight and insignificant production differences between paired groups in the preliminary lactations. In the first 15 wk after the differing dry period feeding, the cows which had been fed extra concentrates during the dry period produced 252 and 359 lb more FCM than their paired mates in Experiments I and II, respectively. These differences approached statistical significance. Although in each experiment this difference was maintained and increased slightly for another 15 wk, the average differences of milk, fat, and FCM for 30-wk due to dry period treatment were not statistically significant. This

occurred because of the variability in responses between pairs. In both experiments the fat tests of the groups fed grain in the dry period were maintained slightly better than were those of the cows fed only roughage in the dry period; however, the differences were not significant.

It is of special interest to note the comparisons between Experiment I and Experiment II, as an evaluation of feeding extra grain in late lactation rather than in the dry period. There is no indication that this treatment has altered the differential response to dry period treatment in any way. Also, the late lactation curves of the group fed extra grain in late lactation were not altered by this treatment in either the preliminary or experimental lactations (see Figure 2). This practice would seem to be rela-
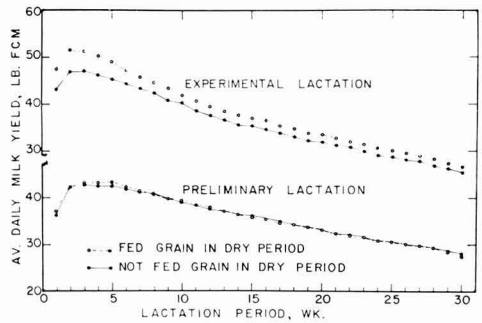
FIG. 2. Average preliminary and experimental lactation curves of 75 pairs of cows, comparing the feeding of concentrates and roughage with feeding only roughage in the dry period.

tively unimportant as a stimulus to milk production under the conditions of these trials.

Combined results. Since the responses in Experiments I and II were so similar, and the dry period and early lactation treatments were identical, the data for all 75 pairs in the two experiments were combined. The weekly body weight changes are presented in Figure 1. The weekly FCM production curves in the preliminary and experimental lactations are shown in Figure 2. These charts show that the groups were well paired on the basis of the preliminary lactation curve, and that the responses in both weight changes and production differed according to the dry period treatment. Those fed extra grain in the dry period gained weight faster before calving and lost more weight after calving, during lactation, than their mates. At the same time, they started milk production at a higher level and maintained it well above their mates for the first 10 wk of lactation, with

a lesser difference maintained for the next 20 wk. A stimulus to produce extra milk and to use body tissue in the process seemed to have been prompted by the extra dry period feeding.

A summary of the combined data is presented in Table 3. The 30.5-lb difference in body weight gain in the 5 wk before calving was highly significant, as was also the 20.3-lb difference in body weight loss in the first 5 wk after calving. In the combined data the 302-lb FCM difference in the first 15 wk of lactation was highly significant statistically, and the differences in milk, fat, and FCM for the first 30 wk of lactation were statistically significant. It should be noted that the mean differences in response were not large, being only 5.9% for the 30-wk period, and 6.9% for the 15-wk period. However, by using cows paired on the basis of previous lactations, it was possible to show that the response to dry period treatment was consistent enough to be significant.

Additional evidence of the lactation-stimulating effect of dry period feeding was the consecutive records of nine cows in each group used in the same treatment group two or more consecutive years. Those fed grain in the dry period averaged in 30 wk of lactation 8,074, 8,503, and 8,936 lb FCM, respectively, for the

first preliminary, and the first and second experimental lactations. Similar averages for the nine repeating cows which were not fed extra grain during the dry period were 7,974, 8,121, and 8,144 lb FCM.

The data presented are for only the 75 pairs which completed the experiments in normal condition. Seven additional pairs were cancelled because of death, serious illness, or other decided abnormality of one or both members of the pair. From the complete health records kept, it was recognized that other health difficulties undoubtedly did affect the responses of some of the remaining pairs. It is the opinion of the authors, however, that these effects did not measurably favor better lactations in either group. Examples of incidences in the concentrate and roughage groups, respectively, were: retained placenta, four and three; milk fever three and three; early calving (14 days or more), six and three; seriously congested udder after parturition, two and three; and serious digestive upsets, two and two.

DISCUSSION

Previously reported studies of the effect of prepartum feeding on the subsequent lactation have been reviewed by Burt (4) and Schmidt

TABLE 3

Dry period feeding, body weight changes, and milk and fat production of cows fed extra concentrates in the dry period compared to paired cows fed only roughage while dry [a]

| | Dry period feeding | | |
| | 8 lb concentrate/day and ad lib. roughage | Only ad lib. roughage | Mean difference |
Item			
No. of cows	75	75	....
Length of dry period (days)	60.9	65.4	−4.5
Conc. fed in dry period (days)	38.1	0	38.1 [b]
Conc. fed in dry period (lb)	304.	0	304. [b]
Body weight changes			
−6 wk to −1 wk (dry) (lb)	57.5	27.0	30.5 [b]
−1 wk to 1 wk (parturition) (lb)	−119.4	−121.3	−1.9
1 wk to 6 wk (lactation) (lb)	−33.4	−13.1	20.3 [b]
Preliminary lactation			
30-wk milk (lb)	7,505	7,421	84
30-wk fat (lb)	302.1	302.5	−0.4
Avg fat test (%)	4.03	4.08	−0.05
30-wk FCM (lb)	7,539	7,523	16
Experimental lactation			
30-wk milk (lb)	7,969	7,521	448 [c]
30-wk fat (lb)	320.1	300.9	19.2 [c]
Avg fat test (%)	4.02	4.00	0.02
30-wk FCM (lb)	7,970	7,527	443 [c]
15-wk FCM (lb)	4,664	4,362	302 [b]
Conc. fed 30-wk (lb)	2,773	2,812	−39

[a] Data from Experiments I and II.
[b] Highly significant statistically, P = < 0.01.
[c] Statistically significant, P = < 0.05.

and Schultz (11). Most such studies have shown a lactation increase due to better dry period feeding, especially when concentrates were fed. Many of the differences, however, have been so small that they were not statistically significant.

Results of this study agree with those of Blaxter (3), in showing a significant increase in early lactation FCM due to grain feeding in the dry period. Failure to agree completely with the reports of Greenhalgh and Gardner (8) and Schmidt and Schultz (11) is probably due to two factors. In the former study, only 36 cows and heifers were used and they were not paired on the basis of lactation potential. In the latter study, the level of feeding of the cows fed only roughage was high enough to make them equal in body condition to the medium-grain group, and it was stated that all cows were in high body condition when dried off. It is suspected that the quality of the roughage used in the Illinois and New York studies was superior to that of this experiment and to that found on most good dairy farms. The benefit from grain feeding in the dry period may depend largely upon the quality of roughage, which also affects the total nutrient intake under ad lib. feeding. With medium-quality roughage, as used here, benefits from dry period grain feeding may be slight; whereas, with poorer roughage dry period grain feeding may be even more beneficial than in this experiment.

It is also necessary to have large numbers of cows paired well on the basis of production to show statistical significance of short-term effects of no greater magnitude than that produced by different dry period treatments. Nine pairs (8) or 21 pairs (11) or 40 pairs (Experiment I) did not show significance, yet 75 pairs with about the same difference in production exhibited significant values. There are many factors which could affect a cow sufficiently to cause a 200- to 400-lb difference in milk yield, which would be large enough to mask the average difference observed in this study.

The body weight changes observed in this study agree with those of other similar studies from which weights were reported (5, 8, 9, 11, 13). It seems obvious that some stimulating change in lactational physiology results from liberal feeding of the cow in the 6 wk before calving. This may be due primarily to the increase in general metabolism, which may be expected when energy intake is elevated. It is difficult to explain what the relationship of weight loss and milk yield (Figures 1 and 2) may be in early lactation. If it is assumed

that the high body condition stimulates a higher rate of general metabolism and high lactation, the resulting energy losses may cause the weight loss observed. This would mean that the better condition a cow were in at calving, the surer would she lose a large amount of weight during lactation. These weight data seem to support this hypothesis. Assuming that this is true, the purpose of high precalving condition would be primarily to stimulate lactation, and secondarily to furnish a reserve of nutrients to support partially the stimulated lactation. Good feeding after calving may support established lactation, but it cannot stimulate initial lactation levels in the same way as extra prepartum feeding.

From economic considerations, these experiments have indicated that it is more important to feed a given amount (about 300 lb) of extra concentrates in the dry period than in late lactation. No harmful effects on the udders or health of the cows were indicated from such practice, which confirms the similar report of Fountaine et al. (7). The 300 lb of concentrates under the conditions of these experiments returned about 400 lb of FCM. To the value of the extra milk, less the cost of the concentrates, must also be added the cost of feeding the dry cow individually for 6 wk before calving. It is estimated that this may average 3 hr per cow; therefore, where labor costs are high, the economic benefit from extra dry period feeding may be doubtful or borderline.

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VALUE OF STERILE FORAGE SORGHUM HYBRIDS AS SILAGES FOR LACTATING COWS [1]

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SUMMARY

Two sterile forage sorghum hybrids (RS 303F and RS 301F) have been evaluated as roughages for lactating cows. These hybrids were male sterile and produced very few seed. Two experiments were conducted. In each experiment silage was fed ad libitum as the only roughage to 12 cows in a switch-back design. In the first experiment, using Jersey cows, RS 303F was compared with corn and Axtell sorgo. Corn silage was significantly superior in FCM produced and in amount consumed; however, consumption of silage dry matter was highest for Axtell. RS 303F and Axtell were found not to differ in other respects. Body weight change and milk fat percentage did not differ significantly among treatments. In the second experiment, using Holstein and Brown Swiss cows, RS 301F was compared with Tracy silage harvested at the early-dough stage and when the grain was mature. The silage from RS 301F was significantly superior to that of Tracy harvested at early-dough in silage consumed, FCM produced, and milk fat percentage. Silages from RS 303F and mature Tracy were not different in any respect. Dry matter consumption and weight changes were not measurably different for these treatments. Sterile sorghum silages were not inferior to other sorghum silages, which suggests that the seed content of these silages should not be used as a criterion for judging their quality.

Forage sorghums are an important silage crop in the Great Plains. A major reason for this popularity is the yield advantage of sorghums as compared with corn, especially under drought conditions (1, 10). Owen et al. (10) found that Sart, Tracy, and Texas seeded ribbon silages produced consistently higher yields than corn; however, cows fed corn silage produced more milk, consumed more silage, and gained more weight. Other reports (4, 9) also show corn silage to have a slightly greater value for milk production than sorghum silage. However, one experiment (11) resulted in similar milk yields for corn and sweet sorghum silage. A limitation of using sorghum for silage is the relative indigestibility of the seed. Fitch and Wolberg (2) reported that 42% of the seed in Kansas Orange silage and 36% of the seed in Atlas silage traversed the gastro-intestinal tract intact.

Received for publication October 11, 1961.

There appear to have been no reports on the feeding value of sterile forms of sorghums. Meyer et al. (7) compared a sorghum silage having a low grain-to-stalk ratio (Rex) and one with a higher ratio (Hegari). Weight gains of beef steers were practically equal for the two silages. Huffman and Duncan (5) compared an early-maturing corn variety containing about 17% grain with a later-maturing variety with about 1% grain. Since milk production was practically equal for cows fed the two different silages, they concluded that most of the grain-equivalent in immature corn silage was present in the vegetative parts of the plant.

The purpose of this investigation was to determine the relative nutritive value for lactating cows of silages made from recently developed sterile hybrid forage sorghums, compared to other common forage varieties and corn. It appeared desirable to learn whether a sorghum plant practically devoid of seed would be of higher nutrient quality than conventional sorghum having grain which is poorly utilized.

EXPERIMENTAL PROCEDURE

Experiment I. The silages compared in this experiment were Axtell, RS 303F, and corn.

Axtell is a recommended sorgo variety for eastern Nebraska and RS 303F is a male sterile forage sorghum hybrid which is sterile when grown in isolation from other sorghums. The silage crops were seeded in the same field, with the corn between the RS 303F and Axtell to minimize fertilization of the sterile sorghum. The corn crop was lost because of flooding following heavy rains, but the sorghums appeared undamaged. Corn from a nearby field was substituted. All silages produced good yields and were cut in the late-dough stage and stored in separate upright silos. There was approximately 5% seed set in the RS 303F field.

Twelve Jersey cows were selected for the experiment using two replications of a double-reversal for three treatments in a complete design (6). All cows used had been lactating at least 6 wk at the start of the first experimental period and none was more than four months pregnant at the conclusion of the experiment. The cows were maintained in stanchion stalls equipped with manger dividers and individual waterers. Cows were turned out daily for about 1 hr in a concrete lot for exercise.

During the first seven days of a 14-day preliminary period the cows were gradually changed from the usual herd feeding program (hay, silage, beet pulp, and concentrate mixture) to a ration consisting of a mixture of equal parts of the three experimental silages plus a concentrate mixture.[4] For the last seven days of the preliminary period this silage was fed as the sole roughage. The concentrate feeding level for each cow was held constant from the start of the preliminary period throughout the experiment. The levels fed were

[4] The concentrate mixture contained: ground yellow corn, 700 lb; ground oats, 600 lb; wheat bran, 300 lb; soybean oil meal, 150 lb; bone meal, 17.5 lb; and trace-mineralized salt, 17.5 lb.

those of Morrison (8) for usual roughage consumption and were based on average milk production and a one-day composite fat test during the week preceding the preliminary period. Cows were offered silage ad libitum three times daily (6 AM, 11 AM, and 5 PM) and all silage refused was weighed back and discarded prior to each subsequent feeding.

The feeding procedure described above was also followed during three 21-day experimental periods when the individual silages were fed. Milk weights were recorded daily and fat tests were made weekly on a one-day composite sample. All cows were weighed on the last two days of the preliminary and the experimental periods. Silage and concentrate consumption were recorded at each feeding. Proximate analyses of the silages used both in Experiment I and in Experiment II are presented in Table 1.

Experiment II. In 1958, another sterile forage sorghum hybrid, RS 301F, was compared with Tracy sorghum at both the early-dough and mature-seed stage as silages for lactating cows. The Tracy was planted at two different dates, permitting the harvest of the two stages during the same week (just following the first light frost of the year). The RS 301F was from a separate field in the same area and was harvested in the mid-dough stage. Growing conditions were favorable and crop production was considered good from both fields. The design and procedures for conducting this experiment were generally the same as for Experiment I. In this experiment, however, nine Holstein and three Brown Swiss cows were used. An equalized feeding plan was used with concentrate[5] reductions made weekly.

[5] The concentrate mixture contained: ground yellow corn, 700 lb; ground oats, 600 lb; wheat bran, 300 lb; soybean oil meal, 100 lb; linseed oil meal, 100 lb; bone meal, 18 lb; and trace-mineralized salt, 18 lb.

TABLE 1

Proximate analysis of silages

| | | Dry matter constituents | | | | |
	Dry matter	Crude protein	Ether extract	N-free extract	Crude fiber	Ash
				(%)		
Experiment I						
RS 303F (sterile)	75.13	8.64	2.33	49.18	30.56	9.29
Axtell	72.56	9.07	2.62	53.17	25.66	9.48
Corn	76.54	10.66	3.37	54.21	24.17	7.59
Experiment II						
RS 301F (sterile)	76.03	7.55	2.04	44.77	34.79	10.85
Tracy, mature	74.60	5.94	3.43	49.25	33.86	7.52
Tracy, early-dough	74.87	6.72	2.67	47.48	35.29	7.84

TABLE 2

Comparison of RS 303F and Axtell sorghum with corn as silages for lactating cows

Silage	Consumption of silage		Body weight gain [b]	FCM [c] (4%)	Avg milk fat [d]
	As fed [a]	D. M.[a]			
		(avg lb/day)			(%)
RS 303F (sterile)	41.5	10.3	0.17	29.2	5.34
Axtell	42.9	11.7	0.90	29.6	5.30
Corn	46.2	10.9	0.29	31.7	5.26
Standard error	±1.60	±0.35	......	±0.65	±0.093

[a] Significantly different, $P < 5\%$.
[b] Measured for Periods I and II only.
[c] Significantly different, $P < 1\%$.
[d] Not significantly different.

RESULTS AND DISCUSSION

Experiment I. Data on milk production, silage consumption, and weight changes are shown in Table 2. The production of fat-corrected-milk (FCM) for the three silages was significantly ($P < 1\%$) different. The superiority of corn in stimulating production of milk compared to the two sorghums is in agreement with previous work (4, 9, 10). Though there was a trend toward a higher percentage of fat for the silages producing the lower amounts of milk, these differences were not significant. The total intake of silage was greatest for corn, whereas the difference between Axtell and sterile silage was not significant. When the silage intakes were calculated on a dry matter basis Axtell consumption was significantly higher than the other silages.

On the tenth day of the final experimental period the supply of RS 303F silage was exhausted; therefore, data for the final period on RS 303F sorghum were based on milk production of the eighth, ninth, and tenth days and the fat test at the seventh day of this final 21-day period. The validity of using these three days for computing milk production data

was tested by another analysis of the entire production data, using the same three days' production for each period on all cows. Very similar results were obtained, although the standard error of the means was somewhat higher.

Average body weight changes were positive; however, several of the cows, especially the first-calf heifers, appeared to be in poor to fair condition at the conclusion of the experiment. The rate of weight changes for the full 21-day periods is presented in Table 2. Due to the variation in weight changes among cows and periods no conclusion on the effect of rations seems justified.

Experiment II. Performance data are presented in Table 3. Silages from the RS 301F and mature Tracy were significantly superior ($P < 1\%$) in milk-stimulating effects (FCM) to the early-dough Tracy. Silage from Tracy harvested at the early-dough stage appeared to be partially shredded and unevenly cut, in contrast to the other silages, which were much more uniformly clean-cut. The reason(s) for this difference is not known; however, it seems possible that this physical difference may have

TABLE 3

Comparison of silages from RS 301F (sterile) and Tracy harvested at two stages of maturity as roughages for lactating cows

Silage	Consumption of silage		Body weight change [b]	FCM [a]	Avg milk fat [c]
	As fed [a]	D. M.[b]			
		(avg lb/day)			(%)
RS 301F (sterile)	60.7	14.7	+0.42	32.6	4.05
Tracy, early-dough	52.1	13.2	−0.09	31.1	3.82
Tracy, mature	56.7	14.4	+0.27	33.8	3.97
Standard error	±2.12	±0.66	±0.64	±0.50	±0.077

[a] Significantly different, $P < 1\%$.
[b] Not significantly different.
[c] Significantly different, $P < 5\%$.

detracted from the palatability of this silage. The difference in dry matter intake for the early-dough silage and the other silages is sufficient to account for the disparity in milk yields. The reason for the higher milk fat test for the sterile compared to early-dough Tracy is unknown. Body weight changes were highly variable and, though favoring the RS 301F, were not significantly different among silage treatments. Helm and Leighton (3) reported that soft-dough Tracy silage was about 25% higher in TDN than mature Tracy.

These studies indicate that silages from the hybrids (sterile) are of approximately equal nutritional value to common sorghum varieties for lactating cows. These results suggest that among genetically different sorghums the grain content should not be used as a criterion of silage quality. The practicality of using sterile forms of sorghums appears to depend primarily on acreage yields and other agronomic qualities. Additional research is needed to clarify the contribution of sorghum seed to silage quality.

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EVALUATION OF FORAGES IN THE LABORATORY.
I. COMPARATIVE ACCURACY OF SEVERAL METHODS [1,2]

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SUMMARY

Eleven forages of known digestibility, as determined in conventional digestibility trials with animals, were used to compare several laboratory methods for the nutritive evaluation of forages.

An artificial rumen method, in which the fermentation of forage carbohydrate was measured, yielded estimates of TDN significantly correlated with the animal digestibility data. However, these TDN estimates were consistently low.

The values for digestible laboratory nutrients (DLN) were not significantly correlated with any of the animal data studied.

TDN values estimated from partial regression coefficients used in connection with the chemical composition of forages were significantly correlated with animal digestion coefficients for dry matter, organic matter, and energy, but were not significantly correlated with animal TDN.

Forage cellulose digestion in the artificial rumen was closely related to the in vivo digestibility, being significantly correlated with TDN as well as digestion coefficients for dry matter, organic matter, and energy.

During the past several years there has been an ever-increasing interest in the evaluation of forages with respect to quality. From this general interest, another line of endeavor has arisen; namely, the estimation of nutritive value from relatively simple in vitro techniques. Pigden and Bell (5) measured the per cent carbohydrate of forage fermented by rumen microorganisms in an artificial rumen. This in vitro digestibility value was used to predict TDN. A short laboratory method for determining digestible nutrients was reported by Thurman and Wehunt (8). The procedure involved autoclaving forage samples in dilute hydrochloric acid and weighing the dried insoluble residue. The weight loss, expressed as a per cent, was

referred to as digestible laboratory nutrients (DLN). These DLN values were reportedly related to published and determined TDN values for similar feedstuffs.

The information usually obtained for a given forage is its proximate chemical composition. Such information is frequently obtained in fertilization and other agronomic studies. Many attempts have been made to correlate a certain chemically determined nutrient with the over-all nutritive value of forages. In most instances, these correlations have not been very satisfactory, because the amounts and proportions of several nutrients in the forage affect its digestibility and nutritive value. This limitation was recognized by Schneider et al. (6,7), who developed partial regression equations for estimating either TDN or the digestibility of any specific nutrient from the proximate composition of the forage in question.

The purpose of the present study was to compare the relative accuracy of several in vitro techniques for use in estimating the nutritive value of forages.

MATERIALS AND GENERAL METHODS

During 1956-58, conventional digestibility trials were conducted using sheep and dairy heifers fed all-hay rations (1, 3). From these trials, 11 hays of known nutritive value were available for laboratory evaluation work, including three alfalfa, three bromegrass, two

orchardgrass, and three reed canarygrass hays. These hays ranged in crude protein content from 7.12 to 22.90% and in TDN from 52.23 to 64.66% on a dry matter basis. For the sake of brevity in this paper, these forages will subsequently be referred to as test forages.

The artificial rumen procedure of Pigden and Bell (5), for measuring carbohydrate fermentation, was used as proposed, except that the amounts of all constituents were double and the anthrone colorimetric reaction was measured at a wave-length of 625 mμ.

DLN was determined on the test forages, using the method of Thurman and Wehunt (8).

The second set of equations published by Schneider et al. (6), in 1952, based only on proximate composition, were used to estimate TDN on the test forages. Correlation analyses were performed according to Ostle (4).

RESULTS AND DISCUSSION

Carbohydrate fermentation. The method of Pigden and Bell (5) involved the determination of carbohydrate fermentation in vitro by rumen microorganisms. Regression equations, incorporating the per cent digestion of carbohydrate, were used to predict TDN. These workers found a close relationship between the predicted TDN and actual TDN determined by conventional digestion trials. However, the forages used to test the accuracy of their estimate were the same forages used to develop the in vitro–in vivo mathematical relationship.

It seemed desirable, therefore, to test this method, using other forages of known nutritive value. This was done with the 11 test forages.

Estimated TDN values calculated from in vitro digestibility data, using the original regression equations (5), were significantly cor-related with the several in vivo values selected (Table 1). However, these estimated TDN values were found to be considerably lower than the actual TDN values. Several factors tend to reduce the usefulness of the method. First of all, it was necessary to develop new prediction equations, using the same forages used to test the reliability of the estimate. When this was not the case, the estimates were lower than the actual TDN. Secondly, the carbohydrate analysis procedures are rather tedious and somewhat deficient in reproducibility, so that wide acceptance by those wishing a routine test is doubtful.

Digestible laboratory nutrients. Another laboratory method proposed for forage evaluation is the determination of DLN (8). Four determinations were made on each of the 11 test forages using this technique. As is shown in Table 1, the DLN values were not significantly correlated with TDN or any of the other animal digestibility data. These results are in agreement with a recent report by Johnson et al. (2). Studying 15 assorted feedstuffs, they found differences between TDN and DLN ranging from +3.55 to −20.23%, with an average difference of −8.95%. Although the DLN method is very simple, it lacks the necessary accuracy to be used as a reliable means of estimating nutritive value.

Chemical composition. Several mathematical relationships have been developed for the estimation of TDN from proximate chemical composition of forages. The equations of Schneider et al. (6) were used to estimate TDN of the 11 test forages. It is of interest to note in Table 1 that these TDN estimates were not significantly correlated with animal TDN. On the other hand, there was a significant correlation with

TABLE 1

Relationship between in vivo and in vitro measures of forage nutritive value
(Correlation coefficients, *r*)

In vivo measure	In vitro measure			
	TDN [a] from carbohydrate fermentation	DLN [b]	TDN [c] from proximate analysis	Cellulose digestibility
TDN *(%)*	0.725*	−0.124	0.494	0.669*
Digestible organic matter *(%)*	0.791**	0.145	0.726*	0.844**
Digestible dry matter *(%)*	0.738**	0.150	0.747**	0.811**
Digestible energy *(%)*	0.750**	0.189	0.695*	0.801**

* Statistically significant at P < 0.05.
** Statistically significant at P < 0.01.
[a] Method of Pigden and Bell (5).
[b] Method of Thurman and Wehunt (8).
[c] Method of Schneider et al. (6).

each of the other in vivo values used in the comparison.

Artificial rumen. Since none of the above methods was consistent in its ability to estimate the nutritive value of forages, an alternative approach was tested. Numerous studies have been carried out on the in vitro digestion of cellulose by rumen microorganisms. Realizing that digestibility is an important characteristic controlling the nutritive value of feeds in general, it was postulated that the measurement of in vitro cellulose digestion of roughages would give an indication of the nutritive value of that roughage. The validity of this postulate was tested, using the 11 test forages. Results of preliminary studies are shown in Table 1, where in vitro cellulose digestion is compared to certain in vivo digestibility data. The per cent cellulose digestion in the artificial rumen was significantly correlated with each of the in vivo values. The further development of an artificial rumen system, patterned after that used by Pigden and Bell (5), is described in detail in the following paper.

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EVALUATION OF FORAGES IN THE LABORATORY. II. SIMPLIFIED ARTIFICIAL RUMEN PROCEDURE FOR OBTAINING REPEATABLE ESTIMATES OF FORAGE NUTRITIVE VALUE [1, 2]

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SUMMARY

The usual artificial rumen procedure has been simplified and adapted to give repeatable estimates of forage nutritive value. A 1-g sample of the ground forage was fermented in a 125-ml Erlenmeyer flask containing 30 ml of McDougall's artificial sheep saliva adjusted to pH 6.7 and 25 ml of strained rumen fluid obtained from a fistulated cow fed and managed in a standardized manner. Immediately after the addition of all contents the flask was flushed with CO_2, capped with a Bunsen valve, and incubated at 39 C for 24 hr.

The per cent forage cellulose digested in this manner was significantly correlated with TDN, DDM, DE (calories per gram), and the digestion coefficient of energy determined in conventional animal digestibility trials. The artificial rumen method and prediction equation given in this paper yielded DE estimates on 19 forages which, when compared with the animal DE values, showed a coefficient of variation of 5.2%. The inclusion of a standard forage in each trial allowed adjustments to be made so that cellulose digestion obtained on two different days had a coefficient of variation of only 1.59%.

It was found that the crude protein and digestible protein content of hays are highly correlated ($r = 0.999$) and that the latter could be estimated from the former with a resultant standard error of 0.25 and coefficient of variation of 2.26%.

A simple, accurate, and repeatable method for estimating the nutritive value of forages would be a valuable tool for those concerned with forage production and utilization. In the preceding paper (4), several methods were compared, but no one of them satisfied all requi-

sites of a routine method in terms of accuracy, repeatability, or simplicity. Preliminary evidence was presented which indicated that per cent forage cellulose digestion in the artificial rumen could be used to estimate nutritive value of forages.

Use of the artificial rumen specifically for the quantitative estimation of nutritive value was first reported by Pigden and Bell in 1955 (18). At about the same time, Baumgardt and Hill (5) reported studies of the dry matter digestion of various forages in the artificial rumen. Forages of known animal digestibility were not available for the study, but the observed loss of dry matter during the in vitro fermentation appeared to be related to the quality of the forages. This work was extended by Clark and Mott (7), using forages of known nutritive value. Digestibility estimates obtained with the artificial rumen procedure during the spring of the year were significantly correlated ($r = 0.77$) with data obtained on the same forages in conventional digestion trials. However, when the artificial rumen digestion trials were repeated in the fall, the estimates

were low and no longer significantly correlated with the animal trial data. Variations in the diet of the fistulated, inoculum-donor cow could possibly account for these differences. Using a similar artificial rumen technique, Asplund et al. (1) reported a close correlation between dry matter digestibility in vivo and both dry matter loss and fatty acid production in vitro.

Recently, many workers have found forage cellulose digestion in vitro to be highly correlated with the digestibility of various forage constituents in vivo (2, 9, 11, 14, 19). There is little doubt that the artificial rumen or in vitro rumen fermentation technique offers a method of studying the digestibility of forages and, in fact, a method for estimating nutritive value.

Research presented in this paper deals with the development of an artificial rumen procedure specifically designed for evaluating forage quality. Special emphasis has been placed on simplicity, standardization, and repeatability. Each of these factors is highly important if a routine method is to give consistently reliable estimates of nutritive value.

EXPERIMENTAL PROCEDURE

Forages. For the development of the artificial rumen evaluation procedure it was important to have samples of forages on which the nutritive value had been determined in conventional digestion trials. Through research conducted at the New Jersey Agricultural Experiment Station (3, 15), 11 such forages were available for preliminary work. After tentative procedures were developed, several additional forages with known animal digestibility were obtained from other laboratories [6] and four additional samples became available from work at the New Jersey Station (6). Included were samples of alfalfa, birdsfoot trefoil, orchardgrass, bromegrass, timothy, and reed canary grass.

Artificial rumen system. Although the artificial rumen system itself was one of the variables in some of the experiments, the basic procedure used was as follows: A 1-g sample of the ground (40-mesh), air-dry forage was fermented for 24 hr at 39 C, using 30 ml of buffer-mineral solution and 25 ml of rumen fluid. The container used was either a 100-ml tall-form

[6] Forage samples were made available from the Pasture Research Laboratory, University Park, Pa., through the courtesy of Dr. J. T. Sullivan, and from the Pennsylvania State University, University Park, through the courtesy of Dr. J. W. Bratzler.

Pyrex beaker or, more generally, a 125-ml Erlenmeyer flask. Either system was satisfactory, but use of the flask resulted in a larger surface area and less splashing on the sides. Immediately after placing all components in the flask (or beaker), CO_2 was flushed through for about 15 sec and the flask was immediately capped with a rubber stopper equipped with a Bunsen valve. When this all-glass system was compared to the semipermeable membrane, no appreciable differences were found in cellulose digestion or volatile fatty acid patterns. This is in agreement with a more comprehensive study by El-Shazly et al. (10).

At the end of the fermentation period, microbial activity was stopped by the addition of 1 ml of 4 N H_2SO_4. The contents were quantitatively transferred into a 100-ml Pyrex beaker (in the case of the flask method) with the aid of a wash bottle. The fermentation residue was dried in a forced-draft oven and the entire sample was analyzed for cellulose by the method of Crampton and Maynard (8), as modified by Matrone (16). With this modification, no further transfers were involved, since the acid digestion, washing, and ashing are all carried out in the Pyrex beaker.

Rumen fluid inoculum. Rumen fluid was collected from a cow fitted with a permanent rumen cannula. This cow was maintained on a uniform, all-hay diet, supplemented only with trace-mineralized salt, bonemeal, and water. On the day of collection, hay and water were removed at 8 AM and the rumen fluid was collected at 1 PM, placed in a previously warmed vacuum jug, and taken to the laboratory. As soon as possible the fluid was squeezed through four layers of cheese cloth and 25.0 ml of the strained rumen fluid used directly to inoculate each flask.

Buffer-mineral solution. A solution was prepared similar to the composition of ruminant saliva as given by McDougall (17), and had the following composition in grams per liter: $NaHCO_3$, 9.8; $Na_2HPO_4 \cdot 7 H_2O$, 7.0; KCl, 0.57; NaCl, 0.47; $CaCl_2$, 0.04; and $MgSO_4 \cdot 7 H_2O$, 0.12. This solution was bubbled vigorously with CO_2 until the pH was approximately 6.7 and the bubbling was then continued more slowly while the solution was being added to the fermentation flasks. To avoid excessive loss of CO_2 and resultant rise in pH, 12 flasks were assembled at one time, capped with Bunsen valves, and placed in the incubator. The process was repeated until the total experiment was assembled. With this buffering system the pH in the fermentation flasks usually was still

above 6.6 at the end of 24 hr of fermentation.

The latest modification was the addition of urea and glucose solutions in an amount to supply 0.05% of each in the total volume in the fermentation flask.

Calculating per cent cellulose digestion in vitro. The usual method of calculating digestion coefficients is applied to the in vitro system by equating the starting and ending fermentation media to the feed and feces, respectively. Since the rumen fluid inoculum used in this study also contributed a small amount of cellulose, the equation used to calculate per cent cellulose digestion in vitro was:

$$\frac{\begin{array}{c}\text{(amount of cellulose in hay and} \\ \text{inoculum)} - \text{(amount of cellulose} \\ \text{at end)}\end{array}}{\begin{array}{c}\text{(amount of cellulose in hay and} \\ \text{inoculum)}\end{array}} \times 100$$

Between 5 and 35 mg of cellulose was usually present in 25.0 ml of the strained rumen fluid inoculum.

In addition to the hays to be tested in each artificial rumen trial, several flasks containing a standard or known value forage(s) were also fermented. This provided a means of correcting for any day-to-day variation due to different lots of inoculum. In any one trial the values for the unknown hays were adjusted on a unit basis in accord with that day's result with the standard forage.

RESULTS AND DISCUSSION

Amount of substrate. The method used for the determination of cellulose (8, 16) was simple, highly reproducible, and adaptable to routine analysis. However, the method was designed for cellulose estimations on 1.0-g samples of forage. Although the starting sample in the artificial rumen studies was 1.0 g, there was only a fraction of this amount remaining for analysis after fermentation. To determine the effect of varying amounts of substrate on cellulose recovery, analyses were carried out on 0.50, 0.75, 1.00, and 1.25 g of forage. By comparing the per cent cellulose in Table 1, it can be noted that there was a slight increase in the amount of cellulose recovered as the sample size increased from 0.5 to 1.25 g. However, the difference was so slight that it would have a negligible effect upon the accuracy of artificial rumen studies in which cellulose was being determined on samples within this range.

While the above data indicate that the amount of cellulose or forage substrate did not significantly affect the cellulose determination, there was still the possibility that the per cent

TABLE 1

Effect of forage sample size on the accuracy of the cellulose determination

Forage sample size	Cellulose	
(g)	(g)	(%)
0.50	0.111	22.13
0.75	0.166	22.18
1.00	0.225	22.47
1.25	0.284	22.72

cellulose digested by rumen microorganisms might be altered. This is important, since forages may vary in cellulose content from 15 to 35% or more. Thus, if a constant amount of forage is supplied to each artificial rumen without regard to the cellulose content, there would be a wide difference in the actual amount of cellulose supplied as substrate for rumen microorganisms.

To test the effect of forage and cellulose concentration, a high-quality alfalfa hay (low cellulose) and a low-quality orchardgrass hay (high cellulose) were digested in vitro, each at forage substrate levels of 0.5, 1.0, and 1.5 g. The amount of cellulose digested increased in proportion to the amount added, so that the per cent digested remained nearly the same (Table 2). Similar results have been reported by Kamstra et al. (13), with forage levels supplying 0.30 to 0.80 g of cellulose per flask. Also, Quicke et al. (19) found nearly the same per cent cellulose digestion for forage levels from 0.44 to 2.50 g (cellulose levels from 0.08 to 0.48 g).

It was concluded that 1.0 g of forage would offer a suitable substrate concentration since, even with extremes in cellulose content, this would supply from 0.15 to 0.35 g of cellulose.

Day-to-day variation. In using the artificial rumen procedure to estimate forage quality, it is necessary that the method yield repeatable results from day to day. Since any variation

TABLE 2

Effect of amount of forage substrate on cellulose digestion

Forage substrate		Cellulose digested in 24 hr	
Kind	Amount		
	(g)	(g)	(%)
	0.5	.065	48.2
Alfalfa	1.0	.136	50.4
	1.5	.210	50.4
	0.5	.052	27.1
Orchardgrass	1.0	.105	27.3
	1.5	.156	27.7

in the rumen fluid inoculum could cause variation in the in vitro fermentation, careful attention was given to controlling the timing of feed and water intake and to provide the animal(s) with a uniform diet.

A fistulated Holstein cow in the Rutgers University herd was maintained on an all-alfalfa hay ration. Rumen fluid was collected at 8 AM after feed and water had been withheld for 12 hr. In vitro cellulose digestion was measured with a single forage substrate and samples of inoculum collected on four days during a 2-month period. The resultant digestion values had a standard deviation(s) of 0.713 and a coefficient of variation (CV) of 1.68%.

At the Wisconsin Station, a fistulated, ovariectomized Jersey cow was maintained on an all-alfalfa hay ration and given uniform care and management. Sufficient first-cutting Vernal alfalfa hay to feed this cow for 1 yr was harvested on June 7, 1960, in the late-bud stage, and artificially dried. Fasting and collection were carried out as described in the Procedures. Typical results are presented in Table 3. The within-day, within-forage repeatability can best be appreciated by noting the standard deviations and coefficients of variation for the standard forage.

The in vitro cellulose digestibility of the standard forage was assumed to be 46.0%, the average of many trials. Cellulose digestibilities of the test forages (alfalfa and Ladino clover) were adjusted by the number of percentage units that the standard forage digestibility differed from 46.0%. To compare the day-to-day repeatability, the average adjusted values were compared by the method of intra-class correlation, with a resultant correlation coefficient (r_I) of 0.991. From this comparison the between-day standard deviation and coefficient of variation were 1.02 and 1.59%, respectively.

Although this might be considered acceptable repeatability for this type of worrk, studies are continuing to improve methods of standardization so as to obtain still greater precision. Forages to be evaluated are routinely digested in duplicate on each of two days and, after adjustment in relation to the standard forage, the average value is used for comparison.

Length of fermentation period. To determine the optimum time for the in vitro fermentation, the digestion of forage cellulose was determined over a 48-hr period. Since rates might be expected to vary with forage quality, a high-quality alfalfa hay and a low-nitrogen, more mature orchardgrass hay were used as substrates. From Figure 1 it can be seen that the

TABLE 3

Typical results and repeatability of the artificial rumen procedure

Forage		Per cent cellulose digestion					
		September 8, 1960			September 22, 1960		
		As is	Adjusted [a]	Avg	As is	Adjusted [a]	Avg
Alfalfa	A	64.4	61.7	62.0	59.8	59.3	59.6
		64.9	62.2		60.3	59.8	
	B	58.7	56.0	56.0	56.5	56.0	56.0
		58.7	56.0		56.5	56.0	
	C	55.1	52.4	51.9	52.4	51.9	51.6
		54.1	51.4		51.7	51.2	
	D	51.4	48.7	49.6	51.7	51.2	51.4
		53.2	50.5		52.0	51.5	
Ladino clover	E	77.1	74.4	74.6	75.5	75.0	75.0
		77.6	74.9		75.5	75.0	
	F	79.4	76.7	76.2	74.3	73.8	74.0
		78.5	75.8		74.8	74.3	
	G	76.2	73.5	74.1	73.8	73.3	73.0
		77.4	74.7		73.3	72.8	
	H	71.7	69.0	69.2	71.2	70.7	70.3
		72.0	69.3		70.4	69.9	
Standard forage		48.3	$\bar{x} = 48.7$		47.5	$\bar{x} = 46.5$	
		48.6	$s = 0.32$		46.1	$s = 0.79$	
		49.0	$CV = 0.66\%$		45.7	$CV = 1.70\%$	
		49.0			46.8		

[a] Adjusted to the long-time average cellulose digestion of the standard forage, 46.0%.
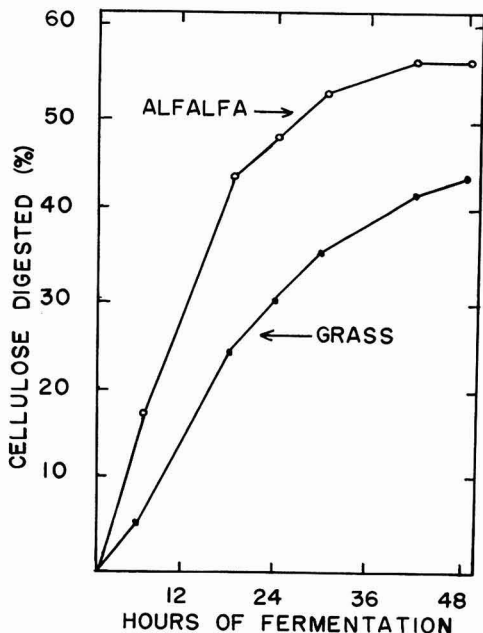
FIG. 1. Rate of in vitro digestion of the cellulose of alfalfa and low-nitrogen orchardgrass hays.

initial rate of digestion of the alfalfa was greater and the maximum was obtained sooner (42 hr) than for the grass. Apparently, the maximum cellulose digestion of the grass had not yet been obtained at 48 hr. This is in agreement with the results reported by Quicke et al. (1959), in which a longer fermentation period was necessary to obtain maximum cellulose digestion of more mature forages.

In further work, 31 forages with known animal digestibility data were fermented in vitro for 24 and 48 hr. The average cellulose digestion values were 47.6 and 60.0%, respectively. Thus, the increase in digestion of cellulose, an average of 12.4 percentage points, was due to the longer fermentation period. The correlation between in vitro cellulose digestion and in vivo digestible energy (DE) was highly significant for both fermentation times ($r =$ 0.854, 24 hr; $r = 0.748$, 48 hr). It was concluded that there is no advantage in extending the fermentation period to 48 hr, insofar as the estimation of DE is concerned.

Predicting nutritive value. The efficacy of the previously described artificial rumen procedure as a predictor of forage nutritive value was tested, using samples of 31 hays of known nutritive value. Of the 31 hays, TDN had been determined on 15, animal digestible dry matter (DDM) on 27, and animal DE (per cent and calories per gram) values on all 31. The reliability (SE, CV) of the regression equation for predicting DE is quite similar to that reported by Hershberger et al. (11).

If urea and glucose are added to the fermentation media, the in vitro cellulose digestion values are increased. In this case, the equations in Table 4 were found acceptable if the intercept values or constants were changed to: TDN, 20.8; DDM, 29.0; DE(%), 26.5; DE (calories per gram), 1,162. Since conditions will vary from laboratory to laboratory, each should develop its own in vitro–in vivo relationship, using forages of known animal digestibility.

When checking the accuracy of prediction equations, it has been common to use the same forages as were used to derive the equations in

TABLE 4

Regression equations and reliability for estimating forage nutritive value from per cent forage cellulose digestion in vitro (X)

In vivo value (Y)	No. of samples	Correlation coefficient (r)	Regression equation	Standard error of estimate	Coefficient of variation
					(%)
Total digestible nutrients (%)	15	+0.621[a]	$Y = 23.9 + 0.782X$	2.89	4.85
Digestible dry matter (%)	27	+0.776[b]	$Y = 31.8 + 0.711X$	3.41	5.13
Energy digestibility (%)	31	+0.777[b]	$Y = 29.3 + 0.711X$	3.30	5.28
Digestible energy (calories per gram)	31	+0.854[b]	$Y = 1295 + 33.16X$	116	4.20

[a] Significant at the 5% level of probability.
[b] Significant at the 1% level of probability.

TABLE 5

Accuracy of a prediction equation for estimating the digestible energy content of different forages from per cent cellulose digestion in vitro

For- age	Digestible energy		
	Pre- dicted	Actual	Devi- ation
		—(cal/g)—	
A	2,939	2,919	+ 20
B	2,740	2,745	− 5
C	2,581	2,424	+157
D	2,900	2,962	− 62
E	2,890	2,806	+ 84
F	2,720	2,476	+244
G	2,929	2,969	− 40
H	2,886	2,821	+ 65
I	2,661	2,411	+250
J	3,171	2,997	+174
K	3,012	2,927	+ 85
L	2,803	2,574	+229
M	3,132	3,092	+ 40
N	3,059	2,887	+172
O	2,774	2,541	+233
P	2,906	3,009	−103
Q	2,654	2,678	− 24
R	2,452	2,494	− 42
S	2,833	2,805	+ 28
Average	2,844	2,765	+ 79
			SE 144
			CV = 5.2%

the first place. It seemed more logical to test this artificial rumen procedure, especially the applicability of the regression equations in Table 4, by using forages other than those used to develop the relationship. Samples of 19 forages from the Pennsylvania Station were fermented, using the artificial rumen procedure described, including the urea and glucose additions. The DE estimates obtained are compared with the animal values in Table 5. There was a tendency for the prediction equation to slightly overestimate the actual DE (average = 79 calories per gram). However, this accuracy was considered acceptable, particularly in light of the standard error and coefficient of variation, which are only slightly larger than those for the original expression (Table 4).

Estimation of digestible protein. Although available energy is the usual limiting nutritive factor of a forage for animal production, it is also important to know the available or apparently digestible protein. Many equations have been offered for estimation of the available protein from the crude protein content of forages, and these were recently reviewed by Holter and Reid (12).

Information was available from the forages studied at the New Jersey Station to allow

a statistical comparison of the concentrations of crude protein and apparently digestible protein. On 11 such forages the correlation between crude and digestible protein was highly significant ($r = +0.999$). The equation best fitting these data was $Y = 0.931\ X − 3.619$, where Y is the predicted digestible protein and X is the crude protein, each expressed as a per cent of the dry matter. The closeness of this relationship can clearly be seen in Table 6, where the predicted and actual digestible protein values are compared. These data lend further support to the concept that digestible protein can be predicted as accurately as it can be experimentally determined in a conventional digestion trial. It is suggested that equations based on a large number of observations, such as those of Holter and Reid (12), be used.

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TABLE 6

Comparison of digestible protein predicted from crude protein and actual digestible protein

For- age	Crude pro- tein	Pre- dicted digest- ible pro- tein	Actual digest- ible pro- tein	Devi- ation
		—(%)—		
1	19.35	14.39	14.16	+0.23
2	8.20	4.01	3.75	+0.26
3	13.76	9.19	9.16	+0.03
4	18.85	13.93	14.01	−0.08
5	18.31	13.43	13.26	+0.17
6	7.12	3.01	3.20	−0.19
7	16.80	12.02	11.88	+0.14
8	17.28	12.47	12.89	−0.42
9	14.00	9.41	9.58	−0.17
10	16.38	11.63	11.54	+0.09
11	22.90	17.70	17.78	−0.08
				SE = 0.25
				CV = 2.26%

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SMALL-SAMPLE IN VIVO CELLULOSE DIGESTION PROCEDURE FOR FORAGE EVALUATION [1]

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SUMMARY

Small samples of forage in nylon sacks were suspended in the rumen of fistulated cows to determine how the coefficients of cellulose digestibility obtained by this method compared with those obtained by conventional digestion trials.

The cellulose digestion coefficient of 63.7% determined for Coastal Bermuda in a conventional digestion trial was not significantly different from the coefficient of 61.0% at the end of 72 hr by the small-sample method. Cellulose digestibility of average-quality alfalfa hay was 56.5% by conventional methods. This was not significantly different from the digestion coefficient (55.2%) determined by the small-sample method.

Three fistulated cows were fed Coastal Bermuda hay and eight different hay samples were subjected to digestion by the small-sample method. A correlation of +0.83 was obtained when the 48-hr legume and 72-hr grass hay digestion coefficients were compared with results from conventional trials.

Significant positive correlations between cellulose digestion measured by the two methods indicate that the small-sample technique used with a regression equation might provide a valid estimate of cellulose digestion.

The yield and chemical analysis of a forage will give some information concerning its usefulness. However, the final evaluation of any forage must be based on its nutritive value. Conventional digestion trials with large animals are prohibitive for screening a number of forages or for routine forage-testing programs, because of the time and expense involved.

In recent years, a number of research workers have investigated means of obtaining an estimate of forage feeding values by small-sample methods. The majority of these small-sample procedures are based on the use of in vitro techniques (2-4, 7, 9, 10, 14, 18); however, several workers have studied the use of small-sample in vivo techniques.

Quin (19), in 1939, suspended silk bags in the rumen of fistulated sheep and observed rate of disintegration of feed. McAnally (15) studied the digestion of wheat and oat straw by placing the samples in silk squares and suspending them in the rumen of fistulated sheep. Erwin (11) placed nylon bags containing forage in tygon tubing and suspended them in the anterior dorsal sac of the rumen of cattle and was able to detect weight loss of forage. Cotton threads were suspended in the rumen of cows by Balch and Johnson (1), who found that the cotton was digested much faster in the ventral than in the dorsal area. Miles (16), using 20-g samples of forage in silk sacks, also obtained a significant increase in cellulose digestion in the ventral area as compared to the dorsal area of the rumen. He also washed silk sacks containing feed with water and failed to force out particles of material from within the sacks. Lambert and Jacobson (13) suspended empty nylon sacks in the rumen and got no appreciable passage of cellulose from the rumen into the sacks.

In an attempt to more nearly approach conditions that exist in the rumen of cattle, and to reduce some of the inherent limitations of an artificial rumen as reported by Burroughs et al. (6), studies at the Mississippi Station have been concentrated on the improvement of a small-sample in vivo digestion procedure using rumen-fistulated cows as outlined by Miles (16).

EXPERIMENTAL PROCEDURE

Conventional total collection digestion trials were conducted, using seven-day preliminary

and seven-day collection periods with ad lib. feeding. Staples and Dinusson (21) found that comparable digestion coefficients could be obtained by either seven- or ten-day collections. Fecal material was collected, using the harness technique as modified by Gorski et al. (12). Collection sacks were emptied two times a day and a well-mixed fecal sample removed and refrigerated. Duplicate aliquot samples were composited daily for moisture and chemical analysis. The daily portions for chemical analysis were frozen until the end of the collection period and then dried at 80 C in a forced-draft oven. Daily moisture determinations were made on the second portion of feces by oven-drying for 20 hr at 105 C (17). All forages were dried at 80 C to a constant weight in a forced-draft forage dryer.

A representative sample of forage was ground through a ten-mesh screen and triplicate 3-g samples of each forage were weighed into 2-by 4-inch nylon sacks for the small-sample in vivo procedure. The sacks were made from nylon parachute material with approximately 80 by 130 threads per inch. The pores between the threads averaged 6 by 11 μ. The tops of the sacks were tied with nylon line and anchored with metal washers to hold them down in the ventral portion of the rumen. Nylon cords about 2 ft long were used to attach the suspended sacks to the plastic fistula cap (Figure 1). The sacks were placed in the rumen for the various digestion periods. When the sacks were removed, they were placed in 75% ethanol to stop microbial activity.

In the laboratory, the sacks were washed free of rumen ingesta particles and each placed in a 150-ml beaker, to which 45 ml of a mixture of nitric and glacial acetic acids was added. Cellulose analyses were made by the Crampton and Maynard procedure (8), with the following
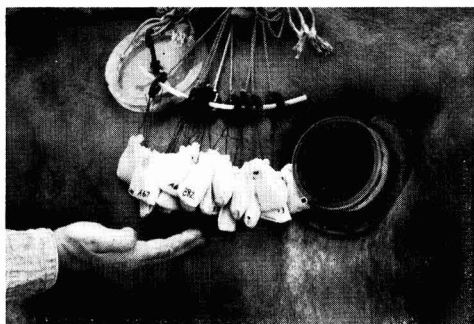
Fig. 1. Set of forage samples for small-sample in vivo technique ready to be placed in the ventral part of the rumen.

modifications. The beakers were heated on a hot plate and the contents gently boiled for 20 min. The samples were stirred occasionally with a glass rod. The material was rinsed from each beaker with 95% ethanol and transferred quantitatively to a No. 4 Gooch crucible, using suction flasks. The residue was washed with hot 95% ethanol.

The difference in the amount of cellulose at the start and that remaining after the period of digestion was considered to be the amount digested. To determine the amount of cellulose at the start, triplicate samples of each forage used in the digestion trials were placed in nylon sacks and analyzed for cellulose.

RESULTS AND DISCUSSION

The early studies conducted at the Mississippi Station used the procedure of suspending 20-g samples of forage in the rumen in silk sacks, as outlined by Miles (16). However, the use of smaller samples in nylon sacks, as suggested by Lambert and Jacobson (13), was found to reduce the amount of work and error and, therefore, this procedure was adopted. The trials reported herein were conducted, using 3-g samples of forage suspended in the rumen in nylon sacks.

Trial 1. Trial 1 consisted of studying the effect of nylon residues on the percentage cellulose obtained by the modified Crampton-Maynard technique and of determining the quantity of material lost through the nylon sacks. Cellulose contents were determined on four empty nylon sacks, resulting in an average residue of 2.9 mg indicated as cellulose. This would add about 0.1% to the cellulose values obtained on forage when analyzed in the sacks.

Eleven different grass forages and six legumes were ground through a ten-mesh screen and placed in nylon sacks. The samples were suspended in beakers of water and agitated vigorously with a shaking machine for 18 hr. Cellulose analyses were made on the water. The results indicated an average loss of 0.24% of the cellulose through the pores of nylon sacks. When Solka Floc materials were used, cellulose losses were from 15 to 30% with BW 40, and 2 to 6% with BW 20, indicating these materials were unsatisfactory for use in this small-sample in vivo technique.

Trial 2. The object of this trial was to determine the period of digestion necessary with the small-sample digestion method to obtain results comparable to those obtained by conventional methods. Three rumen-fistulated cows were fed average-quality Coastal Bermuda hay, and an average digestion coefficient of 63.7%

TABLE 1

Comparison of cellulose digestion of Coastal Bermuda grass hay by simultaneous small-sample in vivo and conventional methods [a]

Method	Avg cellulose in forage	Hours digested in nylon sack [b]			
		36	48	60	72
		(%)			
Small-sample in vivo [c]	36.0	51.6**	55.4**	60.1*	61.0
Total collection (avg three cows)		63.7	63.7	63.7	63.7
Difference		12.1	8.3	3.6	2.7

[a] In all tables conventional refers to a seven-day total collection trial with ad lib. feeding.
[b] Significantly different from other treatment mean by Student's "t"-test: ** .01 level, * .05 level.
[c] Average of two series of triplicate samples digested in three rumen-fistulated cows.

was obtained by conventional methods. Representative samples of the same hay were digested, using the small-sample in vivo technique in two series of 36-, 48-, 60-, and 72-hr periods in the fistulated cows during the conventional trial. Average cellulose digestion coefficients of 51.6, 55.4, 60.1, and 61.0% were obtained for the respective hours. The cellulose digestion coefficient obtained at the 72-hr digestion period was not significantly different from that obtained in the conventional digestion trial when compared by Student's "t" test (20). Results of this study are shown in Table 1.

A similar series was conducted using average-quality alfalfa hay. These data are presented in Table 2. The coefficient of digestibility for alfalfa hay cellulose in the total collection trial was 56.5%. The percentages obtained using the small-sample technique were 55.2, 54.9, 55.2, and 56.4 for 36-, 48-, 60-, and 72-hr digestion periods, respectively. There was no significant difference between the percentage digestion obtained by the conventional and small-sample methods. A correlation of +0.656 (significant at .05) was obtained when the small-sample digestion coefficients for Coastal Bermuda at 72

hr and alfalfa at 48 hr were compared with the coefficients from the total collection trials in this study.

Trial 3. To test the small-sample digestion technique as a screening tool, three fistulated cows were fed a standard ration of Coastal Bermuda hay and 3-g samples of eight different hays were digested for 48- and 72-hr periods. The small-sample cellulose digestion percentages were compared to the digestion coefficients obtained during other studies (5) with similar hays fed ad lib. in total collection digestion trials. The results of Trial 3 are presented in Table 3. All of the hays studied except alfalfa were lower in cellulose digestion by the small-sample technique at the 72-hr period than the digestion coefficients determined in the conventional digestion trials. Part of the difference in cellulose digestion, between the two methods, might be attributed to the fact that the cows were allowed to refuse a portion of the hay in the conventional digestion trials. Cellulose digestion obtained with the ad lib. feeding system in the conventional trials was on that portion consumed, whereas a composite of the complete

TABLE 2

Comparison of cellulose digestion of alfalfa hay by simultaneous small-sample in vivo and conventional methods

Method	Avg cellulose in forage	Hours digested in nylon sack			
		36	48	60	72
		(%)			
Small-sample in vivo [a]	32.0	55.2	54.9	55.2	56.4
Total collection (avg two cows)		56.5	56.5	56.5	56.5
Difference		1.3	1.6	1.3	0.1

[a] Average of two series of triplicate samples digested in two rumen-fistulated cows.

TABLE 3

Cellulose digestion of forages by small-sample in vivo technique and by unrelated conventional digestion trials

		Small-sample technique [a] Cellulose digestion		Total collection digestion trials (seven-day)	
	Avg cellulose in forage	48 hr	72 hr	Cellulose digestion	Hay refused daily
			(%)		
Alfalfa	29.1	52.2	55.6	53.2 [b]	10
Oat	37.5	59.0	66.6	71.8 [b]	27
Johnsongrass	35.9	58.0	64.0	73.4 [b]	26
Soybean	36.0	44.3	46.2	54.7 [b]	50
Coastal Bermuda—1st cutting high fert. [d]	32.2	50.9	56.7	68.2 [c]	13
Coastal Bermuda—1st cutting low fert. [e]	31.3	50.3	55.6	65.0 [c]	15
Coastal Bermuda—2nd cutting high fert. [d]	30.9	46.7	51.5	66.6 [c]	15
Coastal Bermuda—2nd cutting low fert. [e]	32.2	50.6	58.0	63.4 [b]	16

[a] Average of triplicate samples digested in three rumen-fistulated cows.
[b] Data from other digestion trials at the Mississippi Station (5).
[c] These digestion coefficients from unpublished data at the Mississippi Station.
[d] High fertility—300 lb N, 150 lb P_2O_5, and 300 lb K_2O/acre.
[e] Low fertility—50 lb N, 25 lb P_2O_5, and 50 lb K_2O/acre.

hay sample was used in the small-sample method. The conventional digestion trials were conducted to determine the maximum nutrient intake of the various forages and, as shown in Table 3, relatively large amounts of the stemmy portions of the hays were refused. A correlation of +0.83 (significant at .05) was obtained when the 48-hr legume and 72-hr grass hay small-sample cellulose digestion coefficients shown in Table 3 were compared to the conventional digestion figures.

A significant positive regression of $\hat{Y} = 4.86 + 0.7947\ X$ was calculated between cellulose digestion by the two methods. This regression is shown in Figure 2.

FIG. 2. Relationship between cellulose digestion coefficients obtained in small-sample in vivo trials and unrelated total collection digestion trials.

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METHOD FOR DETERMINING FLUORINE INTAKE OF DAIRY COWS UNDER FIELD CONDITIONS

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SUMMARY

This field method enables an investigator to determine if the fluorine intake of a herd is within safe limits for dairy cows. It consists of determining average weights by heart-girth measurements, average milk production, and consumption of hay, concentrates, and silage from Dairy Herd Improvement Association records, or by weighing at the farm. Pasture consumption is calculated by difference between the total digestible nutrients required and those supplied by other feeds. Water intake is based on Morrison's standard. The fluorine content of feeds and water is determined, and the amount of fluorine ingested is calculated in milligrams per kilogram of body weight. It is possible to determine fluorine ingestion for cows within an accuracy of ±20% at the 95% confidence limits. During 5 yr, 321 fluorine determinations were made in 60 herds.

The controlled feeding experiments of Newell and Schmidt (6), Suttie, Miller, and Phillips (10), and a research team at Utah State University (11), have provided information from which tolerance limits for fluorine ingestion have been established. An Advisory Committee, appointed by the National Research Council, reviewed the findings on this subject through 1960 and concluded that the tolerance level for fluorine from a soluble source for lactating dairy cows, over an indefinite period of time, lies between 30 and 50 ppm in the total ration, depending upon the level of milk production (8). The Advisory Committee also showed that for lactating cows, 30 ppm in the feed is equivalent to 0.82 mg/kg of body weight for a 1,200-lb cow, and 1.00 mg/kg for a 900-lb cow. Fifty (50) ppm is equivalent to 1.40 mg/kg and 1.70 mg/kg, respectively.

The literature does not report any method for determining the rate of ingestion of fluorine under field conditions. Herein described is a method developed to obtain the fluorine intake of dairy herds under field conditions. Using this method, 321 determinations of fluorine ingestion rates have been made in 60 dairy herds over a 5-yr period.

It is the purpose of this report to describe the procedures followed and show the reliability expected with this method.

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METHOD OF PROCEDURE

This method of measuring the fluorine ingestion rate of a herd involves the determination of the average body weight of cows in the herd, the amount of fluorine ingested in water and each type of feed in the ration, and the total amount of fluorine ingested per unit of body weight.

(1) *Body weight of dairy cows.* Body weights were determined from heart-girth measurements, as described by Peterson (7). To minimize the problem of inadequate farm facilities, and the reluctance of some owners to allow handling of every cow in the herd, the average weight per cow was obtained by measuring a representative number of cows, usually at least one-third of the animals in the herd.

To test the reliability of this procedure, five herds were selected and heart-girth measurements made on all cows in the herds. The mean weight was determined for the entire herd. In addition, the mean weight and its standard error were calculated from determinations made on the usual number of animals selected at random. Table 1 shows a comparison of these values.

These data show that the standard error for the random sample varied by from 3 to 6% of the mean determined by measuring the entire herd. They further show that the mean weight obtained from the random sample varied on the average by about 3% and, at the most, by about 9% from that determined by measuring every animal in the herd. The slightly increased accuracy obtained by meas-

TABLE 1

Average weight per cow in five herds according to heart-girth measurements of all cows versus a random sample [a]

Herd	No. of cows in entire herd	Mean weight entire herd	Standard error for random sample	Mean weight of random sample		
				1	2	3
				—(kg)—		
A	28	530	18	536	512	518
B	27	604	18	594	593	607
C	35	542	20	592	521	544
D	19	451	28	418	452	460
E	28	513	24	515	521	477

[a] The random-sampled portion varied with the size of the herd. When the herd consisted of 30 or more cows, at least one-third of the cows were measured. If the herd consisted of less than 30 cows, at least 50% of the cows were measured.

uring the entire herd is not worth the additional disturbance of the cows.

When determinations of fluorine intake were made periodically on a herd, it was not always possible to make individual heart-girth measurements each time; however, the average heart-girth measurement of a herd does not vary to any marked extent from time to time, unless a considerable number of animals has been replaced. When this replacement occurred, new heart-girth measurements were made. Examples of average cow-weights calculated from heart-girth measurements at intervals over a 2-yr period are shown in Table 2. These weights did not change appreciably over a 2-yr period of time; therefore, new measurements were not essential every time the rate of fluorine ingestion was determined.

(2) *Milligrams of fluorine ingested from each type of feed and water.* The determination of milligrams of fluorine ingested from each type of feed requires two values: (1) the average number of pounds of each feed consumed by

TABLE 2

Average weight per cow in five herds according to heart-girth measurements taken at intervals over a 2-yr period [a]

Herd	Interval		
	1	2	3
	—(kg)—		
F	516	534	505
G	604	620	584
H	517	487	501
I	537	542	515
J	588	565	583

[a] The random-sampled portion varied with the size of the herd. When the herd consisted of 30 or more cows, at least one-third of the cows were measured. If the herd consisted of less than 30 cows, at least 50% of the cows were measured.

the cows and (2) the fluorine content on a dry-weight-basis of the feed material. To get the total fluorine intake, it is also necessary to measure the amount obtained from water.

(A) Hay. The amount of hay fed was obtained from records of the Dairy Herd Improvement Association (DHIA) when available. These records were compiled under the supervision of the Extension Service, Utah State University, in cooperation with the U. S. Department of Agriculture. If DHIA records were not available, the herd operator established the type of hay and number of bales, or the amount of hay out of the stack consumed by the entire herd each day. A representative amount being ingested on that day was weighed. From this value, the total weight ingested by the herd daily and, hence, the average amount per cow, was determined.

Fluorine content of the hay was determined each time a herd study was made. From the amount of hay consumed and its fluorine content, the milligrams of fluorine contributed by the hay to the diet was calculated. Fluorine content of the samples of feed and water was determined according to standard analytical procedures of Willard and Winter (12), as modified by Remmert et al. (9).

(B) Silage. The amount of silage being fed to the herd was taken from DHIA records, if available. If not, the amount being consumed was weighed and the average amount per animal calculated. The fluorine added to the diet by silage was determined in a manner similar to that described for hay.

(C) Concentrate. The amount of concentrate fed to the herd, its fluorine content, and the average amount of fluorine added to the ration of the herd were determined in the same manner as for hay and silage.

(D) Pasture. The amount of forage consumed by animals on pasture cannot be measured directly as can the other types of feeds. It is possible, however, by knowing the total digestible nutrient (TDN) requirements for a herd of dairy cows, and the amount of TDN in other feeds ingested, to estimate the amount of forage obtained from pasture.

The TDN requirements of dairy cows and the TDN supplied by each individual feed were determined from the standard tables of Morrison (3). Milk yield was obtained at the time each herd was studied, either by weight records at the farm or from DHIA records when available. In each instance, the higher amount in the range of nutrients recommended in Morrison's standard for maintenance and milk production was used in establishing the herd TDN requirement.

The difference between the calculated TDN required by the herd and the TDN supplied by known amounts of other feeds such as hay, silage, and concentrates was assumed to come from pasture. The quantity of pasture forage required to furnish this amount of TDN was calculated from the Morrison standards (4). For example, if it was shown that 2 lb of TDN daily (dry weight) was required from pasture forage, each animal would have to consume about 11 to 14 lb of fresh material per day, depending on whether the pasture consisted of grass in poor to fair condition and closely grazed, or a fertile pasture with some legumes. If the pasture was composed entirely of one species, the factor for that particular species was used in calculating the consumption.

To determine the level of fluorine in pasture forage, a representative sample was taken from each pasture and analyzed in the same manner as other feeds. From the amount of pasture forage consumed and its fluorine content, the contribution of this feed to the total fluorine intake was calculated.

(E) Mineral mix. Mineral mix fed free-choice was estimated to be 1% of the total concentrate fed, according to the rate suggested by Henderson and Reeves (2). The fluorine contribution of minerals to the diet was determined as for other feeds. However, minerals were generally premixed with the concentrates and in that event their fluorine contribution was included in that of the concentrates.

(F) Water. The amount of water consumed by each animal per day under field conditions is impossible to measure. Therefore, a standard water intake of 110 lb daily was used according to Morrison (5). Samples of water

TABLE 3

Representative data sheet used in determining the average fluorine consumption of a dairy herd

Name: John Doe
Date feeds sampled: 6-25-57
Average weight per head: 1,285 lb or 584 kg
Average yield of milk per head per day: 42.9 lb testing 3.8% fat
Pounds TDN required for maintenance: 10.0
Pounds TDN required for production: 13.7
      Total TDN required: 23.7

Feed	Amount fed	TDN	TDN fed	Dry matter	Dry matter	Dry matter	Fluo-rine	Fluo-rine
	(lb)	(%)	(lb)	(%)	(lb)	(kg)	(ppm)	(mg)
Hay	10.0	50.3	5.0	92.0	9.2	4.2	22	92
Silage	25.0	18.1	4.5	22.1	5.5	2.5	17	43
Concentrate	10.3	74.0	7.6	89.0	9.2	4.2	4	17
Pasture	44.3	14.9	6.6	21.5	9.5	4.3	16	69
Mineral mix	......	......	....	......	....	....	....	....
Total in feed	89.6	......	23.7	......	33.4	15.2	....	221
Water	110	......	....	..... .	....	50.0	0.40	20
Total milligrams of F								241

241 mg F

$$\frac{241 \text{ mg F}}{584 \text{ kg of body weight}} = 0.41 \text{ mg F/kg body weight per head per day (average ppm F} = 16)[a]$$

[a] Average parts per million of fluorine in the total ration are calculated by dividing the total milligrams of F by the kilograms of dry matter, i.e., $\frac{241}{15.2} = 16$.

available to the herd on the date of study were taken and the fluorine content determined.

(3) *Total amount of fluorine ingested per unit of body weight.* The total amount of fluorine ingested per unit of body weight was obtained by totaling the milligrams of fluorine supplied by water and other components of the ration, as shown in Table 3.

RESULTS AND DISCUSSION

To ascertain the reliability of herd intake determinations, triplicate determinations were made in one day on each of five dairy herds. The entire procedure as outlined above was carefully repeated three times, assuring that each sampling was a true replicate. The total fluorine ingestion rate per unit of body weight for the triplicate determination is shown in Table 4. These results show that at the 95% confidence limit the values determined will be within 20% of the true mean, indicating that the method outlined above provides a fairly accurate and reliable estimate of the total milligrams of fluorine ingested per kilogram of body weight of dairy animals under field conditions.

This method enables an investigator to determine whether the average fluorine intake of a particular herd is above or below that considered safe for dairy cows, or is a borderline case. Each determination requires about one man-day of effort to collect the material and necessary information, prepare the samples for analysis, and make the necessary calculations. To the cost of this man-day must be added the cost of about four fluorine analyses. In actual practice, it has been found more practical to have two men work as a team in gathering the material and preparing the samples. Two men can cover two to three herds a day, depending on the type of feeding operation.

A refinement of this method allows increased nutrient requirements for animals in their last two to three months of pregnancy and for growth of heifers in their first and second lactations, as suggested in the most recent edition of Morrison's Feeds and Feeding (3). This slightly increases the total nutrient allowance of herds in those instances where feed consumption is based partially or wholly on calculated TDN requirements, e.g., those herds on pasture. It in no way affects those herds where the total intake is determined by weighing the feed.

The procedure just described was devised to fit a given set of conditions in a particular locality, and the results have been accepted as evidence in litigation involving fluorosis in dairy cattle, Erekson et al. vs. United States Steel Corporation (1). It is believed the method is adaptable to any set of conditions where field determinations of fluorine intake are desired, provided the types of feed and method of feeding, which differ from location to location, are given proper consideration. Employing this method, the level of fluorine ingestion by dairy cows can be determined on any given day. The average level of fluorine ingestion over a period of months or years can also be ascertained, if a determination is made when there is a major change in the source of feed, such as changing from dry-lot to pasture, and the length of time on each feed regime is properly weighted.

To date, 321 such fluorine intake determinations have been made on 60 different herds. These results have provided excellent background for evaluating individual cases of possible fluorine effects on dairy cows.

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TABLE 4

Triplicate determinations of the milligrams of fluorine ingested per kilogram of body weight of five different dairy herds

Herd	Determination number		
	1	2	3
	———(mg/kg)———		
A	.68	.56	.69
B	.59	.49	.61
C	.40	.50	.49
D	.37	.36	.35
E	1.04	.88	.96

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SECRETION OF HEPTACHLOR EPOXIDE IN THE MILK OF COWS FED FIELD-CURED HAY FROM SOILS TREATED WITH HEPTACHLOR

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SUMMARY

Alfalfa hays containing high, medium, and low residues of heptachlor and heptachlor epoxide were fed to dairy cows for a period of 30 days. Residue determinations were made on milk samples prior to feeding the contaminated hay, and at intervals during the treatment and post-treatment periods. The residues on the hay resulted from treating the alfalfa in the early spring with heptachlor either in granulated form or mixed with fertilizer.

Heptachlor epoxide was detected in the milk of cows fed on hay containing significant residues. The residues in the milk rose rapidly during the first ten days of feeding, and changed very little during the remainder of the 30-day feeding period. The magnitude of residues in the milk was generally proportional to that in the hay.

Residues in the milk returned to pretreatment levels at 15, 30, and 45 days after treatment stopped in the low, medium, and high treatment groups, respectively.

The consumption of the insecticide at the levels employed in this study had no apparent effect on milk production, weight, or general health of the animals.

Heptachlor has been recommended for control of the alfalfa weevil in many areas of the country. The conversion of this material to its epoxide form on alfalfa was reported by Gannon and Decker (4). Heptachlor epoxide has been shown to be excreted in milk of dairy cows fed heptachlor (2) or alfalfa sprayed with heptachlor (8). The epoxide has also been found in milk when technical heptachlor or the epoxide was introduced into the concentrate portion of dairy cow rations (1, 3).

The objectives of this investigation were (a) to determine if heptachlor epoxide was excreted in milk of dairy cows fed hay produced on soil treated with granulated heptachlor, (b) to study the accumulation of the epoxide residues in milk from cows fed hay containing known residues for an extended period, and (c) to observe the persistence of the epoxide in milk after contaminated hay was removed from the ration.

EXPERIMENTAL PROCEDURE

Three graded treatment levels were selected on the basis of total heptachlor and heptachlor epoxide residues found in field-cured, baled alfalfa hays (Table 1). These residues resulted

from application of heptachlor either as granules or mixed in fertilizer to young alfalfa in the spring. A check involving untreated hay was included in the experiment.

Eight lactating Holstein cows were divided into four groups, duplicating each treatment level and the check or untreated lot. All cows received a ration of 30 lb of timothy hay (grown on soil never treated with heptachlor) and 14 lb of concentrate per day for a 30-day pretreatment period. During the 30-day treat-

TABLE 1

Residues of heptachlor and heptachlor epoxide on hays (ppm)

Treatment group	Hay Lot	Hepta- chlor	Epox- ide	Total
Control	1 [a]	0.045	0.065	0.11
Low	2 [b]	0.11	0.17	0.28
Medium	3 [c]	0.18	0.28	0.46
High	4 [d]	0.45	0.50	0.95

[a] From college dairy farm on soil never treated with heptachlor.

[b] A 1:1 mixture of hay Lots 1 and 3.

[c] From college dairy farm treated with 2 lb of heptachlor per acre in fertilizer mixture on February 2, 1960.

[d] Obtained through the courtesy of the Virginia Department of Agriculture.

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TABLE 2

Summary of feeding experiment using alfalfa hays with known residues of heptachlor and heptachlor epoxide

Treatment group	Ani- mal	FCM/ day	Heptachlor epoxide in milk (ppm)[a]							% intake secreted into milk[b]
			Pre- treat- ment	Days after feeding started			Days post- treatment			
				10	20	30	15	30	45	
		(lb)								
Control	1	28.3	0.020	.024	.017	.026				
	1A	43.2	0.024	.020	.028	.036				
Low	2	32.9	0.038	.065	.042	.088	.035			22.1
	2A	32.6	0.040	.060	.054	.082	.037			20.0
Medium	3	31.3	0.026	.092	.080	.108	.050	.037		22.8
	3A	35.5	0.050	.075	.112	.138	.058	.033		29.0
High	4	36.0	0.036	.210	.230	.234	.10	.064	.040	28.6
	4A	36.8	0.047	.210	.185	.246	.12	.087	.054	28.3

[a] Values are adjusted to a 4% FCM basis.

[b] Calculations made on the averages of the figures given for ten, 20, and 30 days after feeding started, and corrected by subtracting values for control animals.

ment period each cow was offered a daily allotment of 30 lb of alfalfa-orchardgrass hay and 14 lb of concentrate. Hay was fed to cows twice daily and refused hay was weighed back and discarded just before the next feeding. A post-treatment period of 60 days followed in which the cows received timothy hay, corn silage, and concentrate. Cows were weighed for three consecutive days at the beginning and again at the end of the treatment period, and at weekly intervals throughout the treatment period.

Milk from each cow was mechanically separated and the cream (approximately 50% milk fat) was frozen until subsequent analysis. Treatment groups were milked in the following order: control, low, medium, high; and the separator was rinsed between cows and flushed with detergent between treatments. Cream samples collected six days prior to treatment; ten, 20, and 30 days after treatment started; and 15, 30, and 45 days post-treatment were analyzed for content of heptachlor epoxide according to the method of Meyer et al. (5).[1]

Heptachlor and heptachlor epoxide residues on hay Lots 1, 3, and 4 (Table 1) were determined by a revision of the method of Polen and Silverman (7, 9).[1] In addition, hay Lot 4 was analyzed in two laboratories by the method of Mills (6).[2] The figures given in Table 1

[1] Analyses made by the Velsicol Chemical Corp., Chicago, Ill.

[2] Analyses made by England Laboratory, Washington, D. C., and by the Division of Chemistry and Foods, Department of Agriculture and Immigration, Richmond, Virginia.

for Lot 4 are averages of figures reported from the three laboratories.

RESULTS AND DISCUSSION

A summary of the results of the feeding experiment is presented in Table 2. Heptachlor epoxide was present in the milk ten days after feeding of the insecticide-contaminated hay. Levels of epoxide residues in milk increased slightly between the tenth and 30th days of treatment. Residues had returned to pretreatment levels at 15, 30, and 45 days after treatment stopped in the low, medium, and high treatment groups, respectively. Amounts of heptachlor epoxide secreted in the milk were generally proportional to the content of residues in hays, regardless of level of intake. The ratio of heptachlor epoxide secreted in the milk of treated animals (corrected for controls) to the total residue taken in ranged from 20 to 29%. These figures are considerably higher than those reported by other workers (1, 3). A possible explanation of these higher percentages is that natural residues in cured hay resulting from treatment with granulated heptachlor were used in this investigation, whereas in other studies the insecticide was either added directly to the concentrate or sprayed upon the forage.

There was little refusal of hay during the treatment period, with daily intakes of hay for the high, medium, low, and control groups averaging 28.8, 28.7, 27.3, and 29.5 lb, respectively. Milk production (4% FCM) was maintained at a fairly constant level during the entire period. There were no marked changes

in appetite, body weight, or general health of the test animals.

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AGE AND HERD EFFECTS IN NEW ZEALAND DAIRY COW RECORDS

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SUMMARY

Age-correction factors related to the level of herd production have recently been shown to differ little from multiplicative factors in reducing the herd-by-age interaction variance component in age-corrected records. The herds initially available for studying these variance components were from New York data, averaging eight cows per herd. Larger herds than these are more suitable for such a study and results are reported here of investigating the same variances among New Zealand herds, averaging 58 cows each. Variance components were estimated by three different methods, and in all analyses the interaction component when using herd-level factors was approximately equal to that of the actual records, whereas that for the multiplicatively corrected records was larger. This suggests that multiplicative factors perhaps introduce interaction effects, or magnify those already present in uncorrected records, whereas herd-level factors may not.

Herd-level age correction factors, developed by Searle and Henderson (4), appeared by their design to take account of any interaction that may exist between herd environment and the effect of age on production. Records are corrected by adding to them a proportion of the age-corrected herd average, the exact proportion being dependent on the individual cow's age. Thus, all herd-mates of the same age receive the same correction to their records. This assumes that cows of differing genetic merit in the same herd increase in production by the same amount as they grow older, an assumption which is not necessarily true. On the other hand, the use of multiplicative factors assumes that cows of the same age in different herds increase in production in direct proportion to their own records, independently of the different herd effects, except as these have affected the records. This assumption is not necessarily true, either, and possibly the best procedure for age-correcting records lies somewhere between these two methods. Various ways of comparing them are discussed by Searle and Henderson (5), one being a study of herd-by-age interaction effects in records corrected by both methods. It was found that the variance component due to these effects was less when records were corrected by the herd-level factors than by the multiplicative factors. This was from a study of New York herds where, for analysis of variance purposes, the herd

classification was taken as herd-season, dividing each herd into cows that freshen in the fall and those that freshen in the spring. There was an average of 8.1 cows per herd in the study with the average number per herd-season less than this, and the average number per herd-season-age group less still. Since larger groups are more suitable for investigating these variances, a further study has been made using New Zealand data where the herd size is greater, approximately 60 cows per herd.

DATA AND METHODS

Production records (pounds of milk fat) were available for 17,123 cows in 293 pedigree Jersey herds in the 1959-1960 dairying year. Only cows aged 2, 3, 4, and 5-9 yr were used (the majority of most cows in the herds) and only herds with at least two cows in each of these age groups were included in the study. Very few herds were excluded on this account. The number of cows in each age group and their mean production are shown in Table 1. Ages are recorded to the nearest year in New Zealand, because of the seasonal pattern of calving, and records of cows aged less than 5 yr were corrected up to the level of mature cows (those aged 5-9 yr). Two sets of corrected records were obtained, one using multiplicative factors for multiplying the actual record by 1.30, 1.15, or 1.03, according to whether it was of a 2-, 3-, or 4-yr-old cow, and the other using herd-level factors which add to the actual record a proportion of the age-corrected herd av-

TABLE 1
Summary of data
(293 pedigree Jersey herds, 1959-60 dairying year)

Age	No. of cows	Mean no. of cows per herd	Mean production
(yr)			(lb fat)
2	4,018	14	306
3	3,157	11	333
4	2,611	9	371
5–9	7,337	25	383
Total	17,123	59	354

erage, 23, 13, and 3%. These are the factors given in (3), which also shows the method of obtaining the age-corrected herd average.

All records were corrected by both sets of factors and variance components estimated for the two sets of corrected records and the actual records. Three methods of variance component estimation were used: (i) Henderson's method I (1), where the effects due to age, herd, age-by-herd interactions, and error are all considered random. (ii) Method III of Henderson (1), using the computing procedures given in (6). The age effects are here considered fixed and the variance components due to the other three (random) effects are estimated free of age effects; and (iii) the analysis of subclass means, a direct Method I analysis of the age-herd subclass means, all of which were nonzero because all herds in the study had some cows in all age groups.

The error variance obtained as the mean square within the age-herd subclasses is the same for all three analyses. Its value for the records corrected by the herd-level factors is the same as for the actual records, because with

these factors the same amount is added to the records of similarly aged cows within a herd. It is larger, however, for the multiplicatively corrected records. The sampling variances of the variance component estimates were not obtained, but the data were divided into two groups of approximately equal size and analyses made of each group separately. The groups were of 8,847 cows in 144 herds and 8,636 cows in 149 other herds.

RESULTS AND DISCUSSION

Results are shown in Table 2, from which it is immediately noticeable that the error variances are similar for the two groups of herds; the age variances are also similar, with considerably smaller values for the corrected records than the actual records. Half of the second group of herds are from a region of the country above average in production, the remainder and all the first group being from an average region. This accounts for the herd variance estimates in the second group of herds exceeding those in the first.

Comparisons within each set of data analyzed reveal that both sets of age corrections reduce the age variance component to almost zero, compared to its value in the actual records. The multiplicative factors increase the error and herd components, compared to those of the actual records, but the herd-level factors increase only the herd component.

The interaction variance components for the herd-level corrected records are, in all analyses, approximately 70% of those for the multiplicatively corrected records. The difference is similar in both groups of herds and indicates that herd-level factors correct records so as to contain less herd-by-age interaction effects

TABLE 2
Variance components in a herd-by-age analysis of age-corrected records

Contribution to variance	8,487 cows in 144 herds			8,636 cows in 149 herds		
	Corrected records			Corrected records		
	Actual records	Multiplicative	Herd level	Actual records	Multiplicative	Herd level
Error	4,821	5,706	4,821	4,817	5,654	4,817
Age effects considered random (Method 1)						
Age	1,400	57	34	1,490	34	17
Herd	1,913	2,294	2,311	2,736	3,237	3,231
Age × herd	223	322	226	190	299	195
Age effects considered fixed (Method 3)						
Herd	2,844	3,454	3,413	3,964	4,743	4,667
Age × herd	169	238	166	149	228	150
Age effects considered fixed (analysis of subclass means)						
Herd	1,937	2,406	2,401	2,679	3,323	3,292
Age × herd	176	252	169	158	264	154

than multiplicatively corrected records. But what appears to be more important is that in all analyses the interaction component for herd-level corrected records is very close to its value for the actual records. The higher value for the multiplicatively corrected records may, therefore, suggest that multiplicative factors introduce interaction effects or magnify those that are present, whereas herd-level factors do not. If so, it could be a good reason for favoring the use of herd-level factors over multiplicative factors. One cannot say if the difference observed in Table 2 is significantly different from zero, but it occurs in all the analyses. Possibly, the general increase in the error and herd components of the multiplicatively corrected records accounts for the increase in the interaction component, but the lower values in the records corrected by the herd-level factors suggest that these may be the more preferable factors. On the other hand, the observed differences, while consistent among all the analyses, are small, and the ease with which multiplicative factors can be used in practice probably outweighs the merits of the herd-level factors with their slightly more complicated calculations. Herd-level factors shall continue to be used in New Zealand, however, one of their added advantages being that they avoid the over-inflationary effects of multiplicative factors on records of young stock given preferential feeding, cases of which occasionally occur and can give rise to misleading progeny-test results in natural proofs.

The different analyses present results of interest. The Method I analysis treats the age effects as random, whereas in the two other analyses they are taken as fixed. The herd components are generally greater and the interaction variance less in the latter case. Among the two age-effects-fixed analyses, however, the herd components are less in the subclass means analysis than when using the Method 3 analysis, but the interaction components are slightly greater. The subclass means analysis can only be made when all subclasses have observations in them, as was the case in these data.

EARLIER CALVING

The correction factors used in this study were calculated from data collected some 10 yr ago and since then there has been a trend in New Zealand toward earlier calving of 2-yr-olds. A study (2) made by the New Zealand Jersey Cattle Breeders' Association of 48,868 records made in the 1958-1959 dairying year shows that the average production of 2-yr-olds is 80% of the mature cow production. This is in keeping

with the averages shown in Table 1, the 2-yr-old average there, 306, being 80% of the mature cow average, 383. The multiplicative age-correction factor based on this result is 1.25 and the corresponding herd-level factor is 20%. The effects of these changes in the factors for 2-yr-olds were investigated by repeating the Method I analysis on the first group of herds (8,487 cows in 144 herds), using the changed factors. Results are shown in Table 3. There

TABLE 3

Variance components using age-correction factors adjusted for earlier calving of 2-yr-olds

(8,487 cows in 144 herds)

Variance due to:	Actual records	Corrected records	
		Multi-plicative	Herd-level
Error	4,821	5,601	4,821
Age	1,400	−9	−7
Herd	1,913	2,250	2,274
Age × herd	223	307	225

is, of course, no change in the components for the actual records, nor in the error component for records corrected by the herd-level factors. The error component when using multiplicative factors is slightly reduced (from 5,706 to 5,601), as would be expected if the new factors correct for age more satisfactorily than the old ones. Similarly, the age components in Table 3 are less than their counterparts in Table 2, the herd components are also slightly lower (by only a small amount), and the interaction component is changed from 322 to 307 with the multiplicative factors, and from 226 to 225 with the herd-level factors. In general, then, the new age-correction factors for 2-yr-olds lead to small changes in the variance components which are insignificant in magnitude, but they are all reductions, indicating that the new factors probably correct for age more satisfactorily than the old.

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HERITABILITY AND REPEATABILITY OF CONCEPTION RATE OF BULLS IN ARTIFICIAL BREEDING

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SUMMARY

The conception rate for bulls used in artificial breeding is defined as the proportion of cows not returning to first service within 49 days of insemination. Heritability of conception rate is estimated as 0.55 ± 0.26 from 75 sire-son pairs in 3 yr of breeding, the sires averaging 1,792 inseminations and the sons 376. Repeatability is estimated from 314 sires used in 2 yr as 0.69 ± 0.05, based on an average of 1,344 inseminations per sire per year. These estimates are obtained after eliminating from the mean squares the inherent sampling variation that arises from the binomial distribution in estimating the conception rate of a bull. The mathematical method of doing this is presented.

Efficiency of reproduction in dairy cattle is particularly important in New Zealand, where grass is the main (almost sole) item of feed and calvings are largely restricted to the spring months. Thus, the period of maximum milk production coincides with the period of greatest pasture growth and, in consequence, late-calving cows have shorter lactations and lower production, as shown in (5), and are less valuable to the farmer. Fertility of the male is also important, especially in artificial breeding organizations, where a minimum number of inseminations per cow in calf is highly desirable. The productivity of a sire's daughters is the most important factor affecting his value but, other things being equal, the sire with a high conception rate is more desirable than one with a low conception rate. Knowledge of the heritability of conception rate is, therefore, useful as an indication of the effectiveness of selecting for this character when procuring new bulls for use in artificial breeding, that are sons of sires already in the stud.

Reported estimates of the heritability of conception rate appear to be few in number. Dunbar and Henderson (2) estimated the heritability of the proportion of nonreturns to first service as 0.004, using the value 0 for a return and 1 for a nonreturn. Robertson (4) quotes an estimate of 0.01, but gives no definition of conception rate, and Bayley (1) reports estimates obtained by Cornell and North Carolina workers of 0.07 and 0.03 for the heritability of number of services per conception. These reports relate to fertility of the cow and there appear to be none relative to the bull. Therefore it seems of interest to record estimates

obtained from insemination data collected from sires and sons used at an artificial breeding stud.

DATA AND METHODS

Records were available of the percentage of nonreturns to first service within a period of 49 days, for each sire used at the New Zealand Dairy Board Artificial Breeding Centre in 1958, 1959, and 1960. Some of the sires had sons in service, thus providing data suitable for estimating the sire-son regression of nonreturns to service (hereafter referred to as conception rate) and, hence, the heritability of conception rate. Data were available in all 3 yr for inseminations made on the day the semen was collected and on the day after collection. These data were kept separate in the analyses, since bulls in New Zealand are classified on the keeping quality of their semen, as discussed by Probine et al. (3), and those with better keeping quality are used more widely in the regions far distant from the Centre, where transport delays result in semen being used mostly on the day after collection. Several bulls had more than one son in service in the same year, and since repeating such bulls with each son would give an untrue picture of the bull variance (there being only a small sample of bulls—12 in most instances), the regression analysis was made of the average conception rate of a sire's sons on the sire's own conception rate. The average for the sons was obtained as the proportion of nonreturns among all inseminations of all sons, so that all inseminations of the sons of a bull were analyzed together and the identity of the individual sons not retained.

Bulls having sons in service were only part of the data. These and many others had inseminations in 2 yr, thus allowing estimation of the repeatability of conception rate. Although some bulls had inseminations in all 3 yr, estimates were obtained from the regression of 1 yr's results on those of the preceding year, and not from a variance component analysis of the 3 yrs' records. Again, the data for the two types of semen usage (day of, and day after, collection) were kept separate. Pooled estimates were also obtained, by pooling the mean squares and mean products over years and times of usage.

Regression estimates of heritability and repeatability of milk production are free of the effects of the selection that has taken place on the production records. These selection effects do not arise in analyzing sire conception rates, because there is little or no selection practiced on this trait. There is certainly selection against bulls having daughters with low milk and fat production, but it is assumed these traits are independent of conception rate.

SAMPLING VARIATION

The observed conception rate of a sire is the number of nonreturns to first service within 49 days, divided by the total number of first inseminations. This value is an estimate of the parameter of a binomial distribution, assuming that the number of nonreturns (i.e., successful inseminations) is binomially distributed, the true conception rate being the probability that any insemination is successful. The observed conception rate, therefore, has a sampling variance arising from this binomial distribution and dependent on the total number of inseminations on which it is based. This sampling variance contributes to the mean square between the observed conception rates and must be removed before the mean square is used for estimating heritability and repeatability. This effect occurs only in the mean square and not in the mean product, because the samples of cows on which are based the conception rates used in the product are independent of each other. We, therefore, need only consider how to eliminate the sampling variance from the mean squares.

Let p_i be the true conception rate of the ith bull in the study and let $\hat{p}_i$ be the observed conception rate based on n_i inseminations. For a sample of B bulls chosen from the whole population of bulls, the variance we wish to estimate is

$$V = \frac{\Sigma p_i^2 - (\Sigma p_i)^2/B}{B - 1}.$$

The expected value of V over repeated sampling of B bulls from the bull population is σ_p^2, the true variance among bull conception rates. The mean square among the observed conception rates is

$$M = \frac{\Sigma \hat{p}_i^2 - (\Sigma p_i)^2/B}{B - 1}.$$

The expected value of this over repetitions of the B binomial populations, using the same numbers of inseminations n_i, is not V, as we want but, writing $q_i = 1 - p_i$, is

$$E(M) = V + \frac{1}{B} \Sigma_i \frac{p_i q_i}{n_i}.$$

That is, M is a biased estimate of V, biased upward by the mean value of the sampling variance of the individual observed conception rates, $p_i q_i / n_i$ being the sampling variance of $\hat{p}_i$.

The bias in M can be removed by noting that the expected value of $\hat{p}_i \hat{q}_i / (n_i - 1)$ over repeated sampling in the binomial population (keeping n_i fixed) is $p_i q_i / n_i$. Thus, if we write

$$M' = M - \frac{1}{B} \Sigma_i \frac{\hat{p}_i \hat{q}_i}{n_i - 1}$$

we find that the expected value of M' is

$$E(M') = V,$$

namely, that M' is an estimator of V unbiased by the effects of the binomial sampling. We shall refer to the term $\frac{1}{B} \Sigma_i \frac{p_i q_i}{n_i - 1}$ as the adjustment for sampling, which has to be subtracted from the mean square of the observed conception rates to obtain an unbiased estimate of the variance among the sire conception rates. This has been done when estimating the variance components used in the estimates which follow, of heritability and repeatability and their standard errors.

We may note at this stage that corrections of this nature have also been developed for a between-and-within analysis where, for example, we wish to analyze batch results for batches of semen within bulls. In both cases, it is also possible to find the variance of the estimates obtained from expressions like M' and to find unbiased estimators of these variances. This work is proceeding and will be reported in due course.

It might be suggested that a more appropriate mean square than M would be one where the observed conception rates were weighted

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TABLE 1
Summary of data for heritability study

| Data | | | Sires | | | Sons | | | |
| | | | | | | | Avg no. of insems. | | |
Anal-ysis	Time of inseminating	Year	No.	Avg no. of insems.	Avg c.r.[a]	No.	Per son	Per ½-sib group	Avg c.r.[a]
					(%)				(%)
1	Day of collection	1958	12	1,220	65	17	442	627	65
2		1959	12	1,633	62	23	343	657	61
3		1960	27	1,673	62	54	456	902	63
4	Day after collection	1959	12	1,917	55	27	364	818	52
5		1960	12	1,663	61	25	213	444	62
Pooled			75	1,792	61	146	376	732	61

[a] c.r. = Conception rate, the percentage of nonreturns to first service 49 days after insemination.

by the number of inseminations on which they are based. Such a mean square, however, has an expected value which incorporates V in no simple manner. If we were interested in a weighted variance among the true conception rates, then the weighted mean square would be suitable, but since we are interested in the unweighted variance among the p_i's V is appropriate and M, adjusted to M', more suitable.

RESULTS AND DISCUSSION

Table 1 summarizes the data used for the heritability study. The most noticeable difference between the sires and the sons is the greater number of inseminations per sire, more than four times as many as for the sons. This is because the majority of the sons are unproven bulls used in service just sufficiently to obtain enough daughters for a reliable progeny-test. The conception rate for semen used the day after collection is higher in 1960 than 1959, the increase in 1960 coinciding with a less widespread use of day-after-collection semen

and correspondingly a more intense selection of bulls according to semen-keeping qualities, for use in areas having to use the older semen. Improvements in the semen extender in 1960 also contribute to this increase in conception rate. The average number of inseminations are shown for the sons and also for the half-sib groups of each sire, the latter being the average number of sons' inseminations per sire.

The data in the repeatability study are summarized in Table 2. The number of inseminations per bull are similar from year to year, there being a mixture of proven and unproven bulls. The conception rate for inseminations made the day after collection shows the same low tendency in 1959 as in the heritability data, although the figure 1 yr earlier, for the same bulls, is higher, namely 61%. This is accounted for by regional differences in conception rates that have occurred regularly throughout the country. One particular region has consistently had rates lower than elsewhere and in 1959 this region was forced to use almost predom-

TABLE 2
Summary of data for repeatability study

| Anal-ysis | Time of inseminating | Years | No. of sires | First year | | Second year | |
				Avg no. insems.	Avg c.r.[a]	Avg no. insems.	Avg c.r.[a]
					(%)		(%)
1	Day of collection	1958 and 1959	77	1,516	65	1,194	62
2		1959 and 1960	99	1,223	63	1,413	62
3	Day after collection	1958 and 1959	82	1,038	61	1,286	57
4		1959 and 1960	56	1,781	57	1,499	61
Pooled			314	1,346	62	1,341	61

[a] c.r. = Conception rate, the percentage of nonreturns to first service 49 days after insemination.

TABLE 3

Estimating heritability of conception rate (measured as a percentage)

Anal- ysis	No. of sires	Observed mean squares		Adjusted mean squares		Mean prod- uct	Heritability	
							Esti- mate	Stand- ard error
		Sires	Sons	Sires	Sons			
1	12	13.9	13.0	11.2	4.8	3.1	.56	.37
2	12	21.4	72.4	18.3	61.9	10.3	1.13	1.11
3	27	9.2	12.7	5.0	6.5	1.2	.47	.45
4	12	86.8	120.8	82.4	114.6	26.7	.64	.72
5	12	52.2	34.4	48.9	22.6	4.0	.16	.43
Pooled	75	31.2	43.2	27.5	34.9	7.5	.55	.26

inantly day-after-collection semen. In consequence, the conception rate for that semen is lower than in other years. This factor is additional to those given above in discussing the heritability data.

Results of the two studies are given in Tables 3 and 4, showing the observed mean squares and the adjusted values after allowing for binomial sampling. Pooled estimates, pooled within years and ages of semen, also are shown. The heritability and repeatability estimates are based on the adjusted values, using observed covariances calculated without adjustment. Values in the tables are in terms of percentage units, i.e., 11.2 is the variance (after adjustment) of the sire conception rate in the first analysis of the heritability study (from Table 1, a mean value of 61%), the standard error of the individual rate being $\sqrt{11.2} = 3.4\%$.

The pooled estimate of the heritability of conception rate is 0.55, with a standard error of 0.26. This is significantly greater than zero at the 5% level, based on 74 degrees of freedom. But with only 75 sire-son pairs and a resultant standard error of 0.26 a satisfactory estimate of the true value can scarcely be given. More data are needed and were some three to four hundred sire-son records available the

standard error of the estimate of heritability would be in the order of 0.10, a far more satisfactory situation.

It is of interest to note that the difference between the observed and adjusted mean squares in the heritability study (Table 3) is more than twice as large for the sons as for the sires, corresponding to the lesser number of sons' inseminations as shown in Table 1. On the other hand, in the repeatability study the difference between observed and adjusted mean squares is similar for both first and second years, corresponding to the similar number of inseminations per sire each year (Table 2). These results are, of course, as one would expect, from the nature of the adjustment that is made to the mean squares. They emphasize the need for caution when interpreting estimates of heritability and repeatability of conception rate based on records obtained from only small numbers of inseminations. The estimated (i.e., observed) conception rates will have high sampling errors about their true values and, although the adjustments to the mean squares will be relatively large, the regression estimates will still have large standard errors. This must also apply to heritability estimates obtained from daughter-dam pairs, using the (0, 1) variate for returns and nonre-

TABLE 4

Estimating repeatability of conception rate (measured as a percentage)

Anal- ysis	No. of sires	Observed mean squares		Adjusted mean squares		Mean prod- uct	Repeatability	
							Esti- mate	Stand- ard error
		1st year	2nd year	1st year	2nd year			
1	71	17.9	34.9	12.3	28.1	10.5	.85	.15
2	99	21.4	15.6	13.3	10.0	9.5	.71	.05
3	82	44.5	56.9	35.7	49.0	33.6	.94	.08
4	56	50.8	36.3	46.4	29.7	15.8	.34	.10
Pooled	314	31.8	34.8	24.8	28.1	17.2	.69	.05

turns to service. Such a variate, used as an estimate of the female's conception rate, has a high standard error, as it is based, in effect, on one insemination only. It is, of course, impossible to estimate a cow's conception rate from a large number of inseminations, because she receives no further services once she is in calf and, in many instances, only one insemination is made. If this restriction on the binomial situation did not exist, estimates of the heritability of female conception rate might be larger than the values reported in the literature.

The heritability estimate obtained here is considerably greater than previously reported estimates (for female conception rate) and although it has a high standard error the indication is that the true value is probably greater than zero. This suggests that it may be worth while discriminating against sons of a sire known to have a low conception rate, when procuring new bulls for an artificial breeding stud. Selection of this nature could be practiced quite easily, because the bulls with higher conception rates will usually have more sons available than those with low conception rates. Too much selection in this direction, however, could lead to an over-concentration of the sons of only a few sires in the stud, those with high conception rates and high-producing daughters.

The estimates of repeatability are shown in Table 4, the pooled estimate being 0.69, with a standard error of 0.05. This indicates that the repeatability of sire conception rates from year to year is undoubtedly high, and very likely something exceeding 0.60. The standard errors of the various estimates are noticeably smaller than in the heritability study, due to there being considerably more bulls in the analyses. The estimate 0.34 for Analysis 4 (semen used the day after collection, 1959 and 1960) is considerably lower than the three other values. This arises from the large mean square in 1959, which is brought about by the same factors that led to low conception rates in this year for day-after-collection semen (Table 2). One region of the country which has had consistently lower conception rates than elsewhere used this semen extensively in 1959, thus increasing the between-bull variation (49.0 in Analysis 3 and 46.4 in Analysis 4, compared to 35.7 for 1958 in Analysis 3). The reduction in 1960 to 29.7 is probably due to the new diluents, which increased the conception rates of bulls with otherwise low values more than it did for the bulls with high values.

A high repeatability value of conception rate has two useful implications in the managing of an artificial breeding stud. The first concerns estimating the conception rate of unproven bulls being used to obtain sufficient daughters for a progeny-test. These bulls will have relatively few inseminations initially, perhaps only two or three hundred, depending in large measure on the proportion of farmers using artificial breeding who also herd-test. Apart from the sampling variation, this first estimate of an unproven bull's conception rate will be a relatively reliable estimate of his future conception rate assuming, as this study suggests, that the repeatability of sire conception rate is high. The second implication of a high repeatability concerns arranging the daily roster of bulls, which is especially important in New Zealand, where the breeding season is short and the daily output of processed semen over the main two-month season is high, rising to over 15,000 cc per day. If prior estimates of conception rates are a reliable guide to future values, the roster can be designed so that the use of bulls with low conception rates is minimized. The high estimate of repeatability might also suggest, perhaps, that the heritability of conception rate is greater than hitherto thought from daughter-dam studies. The sire study reported here yields an estimate of 0.55 ± 0.26, and with the repeatability estimate being 0.69 ± 0.05 it seems reasonable to think that the true heritability value might be in the neighborhood of 0.30. This conjecture will be substantiated or rejected as further sire-son data become available.

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EFFECT OF LEVEL OF RATION INTAKE AND DURATION OF VITAMIN A DEFICIENCY UPON SOME BIOCHEMICAL CONSTITUENTS IN SERUM, CEREBROSPINAL FLUID, AND AQUEOUS HUMOR OF HOLSTEIN CALVES FED FIXED CAROTENE INTAKES [1]

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SUMMARY

Forty-eight male Holstein calves, averaging 79 ± 6 days of age and $7.2 \pm 1.8 \gamma$ of vitamin A per 100 ml of plasma, were fed one of two intakes of a vitamin A depletion ration, 100%, estimated to provide an anticipated weekly gain of approximately 10 lb, or 60%, which was six-tenths of the ration allowance of the 100% intake group, for one of two durations, 12-wk or 24-wk, and one of six carotene intakes from artificially dehydrated alfalfa, 10-, 20-, 30-, 40-, 50-, or 60-γ per pound of live weight per day. Marked differences between the 60% and 100% intake groups were observed in daily rate of live weight gain, 1.36 vs. 2.08 lb, in heart rate, 74 vs. 88 beats per minute, in the QT electrocardiographic interval, 0.364 vs. 0.314 sec, and in the systolic index, 0.400 vs. 0.378. These differences in the calves fed the 60% level of ration intake were accompanied by higher concentrations of plasma and liver vitamin A, by slightly lower cerebrospinal fluid pressures, and by an alteration in the protein distribution of serum and cerebrospinal fluid. The latter changes were primarily attributable to decreases in the beta-globulin fraction. Also, slightly lower concentrations of chloride in serum and of sodium and potassium in cerebrospinal fluid occurred in the 60% level of ration intake calves. To maintain equivalent cerebrospinal fluid pressures, calves fed the 60% level of ration intake required 0.74 as much carotene as calves fed the 100% level of ration intake, and to maintain equivalent liver vitamin A stores per unit of live weight, 0.86 as much carotene.

Duration of deficiency, either 12-wk or 24-wk, had an inappreciable effect on most of the criteria studied. There was some indication of greater cerebrospinal fluid pressures in the calves fed the three lowest carotene intakes for the 24-wk duration than in comparable calves of the 12-wk grouping, as well as higher serum magnesium and cerebrospinal fluid sodium concentrations.

Calves fed the three lowest carotene intakes, deficient as evidenced by elevated cerebrospinal fluid pressures, exhibited greater protein concentrations in serum and cerebrospinal fluid (also slightly greater in aqueous humor but not statistically significant) than did calves fed the highest three carotene intakes, adequate as evidenced by no elevation in cerebrospinal fluid pressures. These increased total protein concentrations were attributable, primarily, to increases in the beta-globulin fraction and to a lesser degree in the gamma-globulin fraction.

Reduced feed intake of cattle occurs in acute (9) and chronic (25) hypovitaminosis A, especially in the terminal stages of the deficiency. In addition, the higher the level of ration intake, the more rapid has been the rate of vitamin A depletion from the liver (6, 14). A greater occurrence of squamous metaplasia of the interlobular ducts of the parotid gland was observed in calves fed the higher of two intakes of a vitamin A depletion ration (27). Similar findings have been observed in other species, as reviewed by Moore (24).

Cattle can tolerate vitamin A deficiency of short duration without apparent ill effect, as evidenced by the lack of outward signs characteristic of this deficiency in either cows or their calves (29) and in calves by no elevation of cerebrospinal fluid pressure or decrease in subsequent ability to convert carotene to vitamin A (13). In contrast, relatively long periods of vitamin A deficiency result in a variety of anatomical (3), clinical (5, 18), histological (17), and physiological changes. Among the latter, night blindness (15), papillary edema (30), and increased cerebrospinal fluid pressure (22, 23) are well-defined changes characteristic of the deficiency. Alterations in the concentration of several biochemical constituents of serum (11, 12, 20), as well as decreased ability to convert carotene to vitamin A (13), also have been reported. While increased cerebrospinal fluid pressure appears to be the first discernible change to occur in vitamin A deficiency of cattle (8), the number of criteria exhibiting change, as well as each criterion's incidence, increases not only as the level of carotene intake is decreased but also with longer durations of deficiency (5, 18).

In possible contrast to the many changes observed in prolonged vitamin A deficiency, as just cited, calves fed graded levels of carotene (16-, 24-, 32-, and 40-γ per pound of live

Received for publication August 19, 1961.

weight per day), such that marked, slight, or no increases in cerebrospinal fluid pressures were produced at the termination of 16 wk of supplementation, exhibited no outward signs indicative of vitamin A deficiency (8). Also, except possibly at the lowest carotene intake which resulted in marked elevation in cerebrospinal fluid pressures, small differences were observed with respect to protein and mineral concentrations of serum and cerebrospinal fluid. However, slight differences existed in some of the mineral constituents of aqueous humor between calves fed the 40-γ intake, and calves fed the 16-, 24-, and 32-γ intakes.

Because of the apparent importance of level of feed intake upon the vitamin A economy of cattle, and of the numerous changes occurring in vitamin A deficiency of prolonged duration, as contrasted to the few of early deficiency, the present investigation was undertaken to ascertain the effect of these two variables, level of ration intake and duration of deficiency, upon some of the biochemical constituents in serum, cerebrospinal fluid, and aqueous humor of calves fed a range of carotene intakes sufficient to produce marked to no elevation in cerebrospinal fluid pressures.

EXPERIMENTAL PROCEDURE

Animals and feeding. Forty-eight one-day-old Holstein male calves purchased from various State institution herds, during the period November, 1959—January, 1960, were transported to the Animal Nutrition research barn and placed in individual, sawdust-bedded tie stalls in a portion of the barn in which the ambient temperature was maintained at a minimum of 50 F. Thereafter, each calf was raised to approximately the 63rd day of age on a ration consisting of limited whole milk, limited calf starter, and ad libitum chopped alfalfa hay, essentially as previously cited (8).

On approximately the 64th day of age, each calf was fed a vitamin A depletion ration (8), such that its anticipated increase in live weight for each seven-day period was 10 lb. When the plasma vitamin A concentration, based on analyses of blood samples obtained at successive seven-day periods, for each calf had decreased to or less than 12.0 γ per 100 ml, it was placed, after the next seven-day period, on one of 24 treatments for a 24-wk comparison period. The treatments, consisting of a $2 \times 2 \times 6$ factorial design were: two levels of ration intake, 100%, which was the vitamin A depletion ration allowance as cited above and provided an estimated seven-day increase in live weight of 10 lb and, 60%, six-tenths of the ration allow-

ance of the 100% intake group; two durations, 12- and 24-wk; and six carotene intakes, 10-, 20-, 30-, 40-, 50-, and 60-γ of carotene from artificially dehydrated alfalfa meal per pound of live weight per day. In order that measurements would be obtained at an essentially equivalent age, the calves allotted to the 12-wk duration groupings received the 100% depletion ration allowance plus 36 γ of carotene per pound of live weight per day for the first twelve seven-day periods and, thereafter to the completion of the comparison period, their respective levels of ration intake and carotene intake.

The 100% level of ration intake was expected (8, 26) to be almost completely consumed by the calves. The 60% level of ration intake was anticipated (27) to result in live weight increases of slightly greater than 50% of those for the animals receiving the 100% level of ration intake. While insufficient data existed to make a firm decision as to the minimum duration of vitamin A deficiency of calves in which to expect elevated cerebrospinal fluid pressure, a period of as few as 4 wk was definitely too short (13) in calves depleted to equal to or less than 12 γ of vitamin A per 100 ml of plasma and subsequently fed 12 γ of carotene intake per pound of live weight per day. A period of 8 wk appeared to be sufficient after essentially similar vitamin A depletion and subsequent feeding of carotene intakes up to approximately 20 γ, whereas a period of 12 wk of carotene supplementation at a 30 γ intake resulted in increases in pressure (unpublished data). Therefore, the latter period, 12-wk, was chosen for the shortest duration. The longest duration, 24-wk, was based on the observations of others (12, 20), in which outward changes characteristic of the latter stages of vitamin A deficiency were produced in cattle. The 10-, 20-, and 30-γ carotene intakes, deficient, were expected (8, 26) to result in marked to slight elevation in cerebrospinal fluid pressures, whereas the 40-, 50-, and 60-γ intakes, adequate, were expected to result in inappreciable change. The 36-γ carotene intake, chosen to feed the calves of the 12-wk duration groupings during the initial 12 wk of the comparison period, was anticipated to result in no increases in pressure.

The first 24 calves, first replicate, to arrive at the research barn were assigned to one of the 24 treatments according to a previous random allotment. This procedure was repeated for an additional 24 calves, second replicate. Average age at the start of the comparison period and its standard deviation were 79 and

six days, respectively. Comparable values for live weight were 195 and 21 lb, and for plasma vitamin A concentration, 7.2 and 1.8 γ per 100 ml. Calves were slaughtered either one or three days following the termination of the comparison period, during which time each calf continued to receive its designated levels of ration and carotene intake.

Calculation of the amount of artificially dehydrated alfalfa to supply the fixed carotene intakes, and the treatment for scours, were essentially identical to those reported for previous studies from this Station (26). However, the incorporation of the alfalfa into the vitamin A depletion ration was slightly modified (13), to ensure complete consumption of the carotene supplement.

Observations and analyses. With a few exceptions as listed below, these were identical to a previous study (8). In addition to terminal cerebrospinal fluid pressures, this measurement was taken the 12th wk of the comparison period also, for the purpose of ascertaining whether those calves in the 12-wk duration grouping had elevated pressures. No intraocular pressure measurements were made. Numerous other tissues in addition to the eye were taken for histological examination, results of which will be published subsequently.

Total protein concentration of the aqueous humor was determined by the Lowry procedure (19), and the chloride content of all fluids, instead of being analyzed colorimetrically as previously, was determined by the Cotlove procedure (7), employing an automatic titrator (Aminco-Cotlove, American Instrument Company catalog No. 4-4420).

In addition, glyco-protein distribution was determined with a Spinco (Model R, Series D) paper electrophoresis system (1). Serum magnesium was calculated from the combined calcium-magnesium concentrations determined by complexometric titration, using a Sargent (Model SE) automatic spectrophotometric titrator (21) and subtracting the concentration of calcium as determined by flame photometry (8). Also, serum alkaline phosphatase (16) and glutamic-oxalacetic transaminase (2) activities were determined; the former, because of its reported reduction in hypovitaminosis A of cattle (20) and the latter because of its ability to detect the possible effects of treatment on tissue integrity.

The average minimum and maximum daily barn temperatures during the comparison periods for all calves, January through September, 1960, with their standard errors, were 60.8 ± 0.2 F and 71.7 ± 0.1 F, respectively.

Average artificial light intensity to which the calves were exposed daily from 6 AM to 6 PM, measured at 28-day periods (26), during the comparison period, was 6.2 ± 0.4 ft-c. Proximate analysis and carotene concentrations of the feeds fed are contained in Table 1.

24-wk duration plus the 50-γ carotene intake, developed a prolapsed rectum during the terminal weeks of the comparison period. While most criteria for this calf were within the expected range of values, cerebrospinal fluid pressure was extremely elevated, particularly

TABLE 1

Average chemical composition of feeds

| Feed | Per cent dry matter | Per cent of dry matter | | | | | Caro-tene |
		Crude protein	Ether extract	Crude fiber	N.F.E.	Ash	
							(mg/lb)
Milk replacer [a]	92.9 ± 0.7 [d]	28.1 ± 0.2	2.0 ±0.2	0.6 ± 0.1	60.6 ± 0.2	8.7 ± 0.2	0.21 ± 0.03
Calf starter [a]	89.7 ± 0.7	22.6 ± 1.1	2.9 ±0.2	6.4 ± 0.2	60.4 ± 1.0	7.7 ± 0.4	2.52 ± 0.21
Chopped alfalfa hay [a]	91.0 ± 0.5	17.3 ± 0.9	2.3 ±0.4	29.8 ± 0.9	43.2 ± 1.1	7.5 ± 0.3	8.47 ± 0.56
Vitamin A-depletion ration [b]	88.8 ± 0.5	16.8 ± 0.2	2.0 ±0.1	11.0 ± 0.2	62.5 ± 0.5	7.6 ± 0.3	0.12 ± 0.02
Artificially dehydrated alfalfa meal [c]	90.9 ± 0.6	23.4 ± 0.2	4.3 ±0.2	20.4 ± 0.2	39.5 ± 0.5	12.4 ± 0.2	69.72 ± 2.26

[a] Average of six samples.
[b] Average of nine samples.
[c] Average of 14 samples.

The analysis of variance consisted of isolating variability attributed to replicates of calves, levels of ration intake, durations of vitamin A deficiency, and carotene intakes, as well as first- and second-order interactions of the last three named sources of variation. As decided before the experiment, the carotene intakes variability was separated into linear and quadratic trends (plus residual) across all carotene intakes, between the 10- plus 20- plus 30-γ intakes vs. the 40- plus 50- plus 60-γ intakes, the linear trend (plus residual) across the three lowest intakes, and the interactions of level of ration intake and duration with the above-mentioned sources of variability. The biological reasons for these comparisons were that a trend might exist in the various criteria across all carotene intakes but, more possibly, such a trend might be expected to exist only at the three lowest carotene intakes, 10- through 30-γ, in which marked to slight elevations in cerebrospinal fluid pressures were anticipated (8, 26).

One calf in the first replicate fed the 100% level of ration intake for the 24-wk duration plus the 10-γ carotene intake, died from strangulation during a convulsion the 16th wk of the comparison period. Missing values for all criteria for this calf were calculated. Another

calf fed the 60% level of ration intake for the 24-wk duration plus the 50-γ carotene intake, developed a prolapsed rectum during the terminal weeks of the comparison period. While most criteria for this calf were within the expected range of values, cerebrospinal fluid pressure was extremely elevated, particularly during defecation. In addition, there was a relatively marked increase in the gamma-globulin fraction of serum. Therefore, the terminal cerebrospinal fluid pressure value, protein distributions, and the concentrations of the four serum protein fractions were calculated. In addition, a few calves were extremely excited during cerebrospinal fluid pressure measurements, a condition known to temporarily cause elevated pressures (28). Repeat punctures on some of these calves were attempted within a three- to seven-day period, but were rejected because of the continued excitability. Missing values were calculated for these calves, as indicated in Table 2.

RESULTS

Feed consumption, growth, and health (Table 2). The average percentage of the total days during the comparison period in which each calf consumed its ration allowance was greater ($P < 0.05$ for the arcsin $\sqrt{\%}$) in those calves

TABLE 2

Effects of level of ration intake and duration of vitamin A deficiency upon feed consumption, liveweight, and cerebrospinal fluid pressure of Holstein calves fed fixed carotene intakes [a]

Treatments			Avg days consuming ration allow-ance	Live weight			Cerebrospinal fluid pressure			
				At 12-wk	Increase to the termination of 24-wk		At 12-wk		At 23-wk	
Ration level	Dura-tion	Caro-tene intake			Ac-tual	Ad-justed [d]	Ac-tual	Com-mon loga-rithm	Ac-tual	Com-mon loga-rithm
(%)	(wk)	(γ) [b]	(%)	(lb)	(lb/day)		(mm saline)			
60	12	10	100.0(90) [c]	396	1.54	1.46	98	1.99	171	2.22
60	12	20	100.0(90)	346	1.42	1.45	92	1.96	108	2.01
60	12	30	100.0(90)	384	1.54	1.45	88	1.94	107	2.02
60	12	40	97.9(82)	356	1.46	1.45	122	2.08	140	2.13
60	12	50	100.0(90)	369	1.54	1.52	89	1.94	98	1.98
60	12	60	99.1(86)	381	1.60	1.56	106	2.00	88	1.95
100	12	10	99.1(86)	356	2.24	2.23	116	2.06	344	2.51
100	12	20	99.7(87)	348	2.08	2.09	92	1.95	172	2.10
100	12	30	99.7(87)	351	1.94	1.94	116	2.06	100	2.00
100	12	40	97.9(84)	340	2.04	2.08	80 [e]	1.85 [f]	109	2.03
100	12	50	99.4(87)	414	2.40	2.29	78	1.89	51	1.71
100	12	60	97.9(84)	335	1.97	2.00	63	1.78	94	1.97
60	24	10	99.1(84)	253	1.19	1.27	181	2.26	346	2.54
60	24	20	99.1(84)	264	1.25	1.30	86	1.93	150	2.17
60	24	30	98.8(84)	264	1.26	1.31	78	1.89	106	2.02
60	24	40	99.7(87)	272	1.19	1.19	95	1.95	98	1.99
60	24	50	100.0(90)	296	1.15	1.12	80	1.90	102 [e]	1.96 [f]
60	24	60	100.0(90)	264	1.26	1.28	78	1.89	95	1.98
100	24	10	97.6 [e](80) [f]	311 [f]	1.88 [f]	1.97 [f]	335 [e]	2.48 [f]	320 [e]	2.46 [f]
100	24	20	98.2 (82)	331	1.93	1.98	154	2.13	160	2.18
100	24	30	100.0 (90)	340	1.93	1.96	160	2.20	207	2.32
100	24	40	95.5 (78)	371	2.05	2.02	120	2.08	68 [e]	1.79 [f]
100	24	50	96.7 (80)	385	2.21	2.15	94	1.97	118	2.06
100	24	60	90.2 (77)	355	2.20	2.19	108 [e]	2.08 [f]	75 [e]	1.83 [f]
Standard deviation per calf			...... (5)	29	0.13	0.12	......	0.11	......	0.15

[a] Values represent averages based on two calves per treatment group, except as otherwise noted.
[b] γ/lb live weight/day.
[c] Are sin $\sqrt{\%}$.
[d] Value adjusted for initial live weights by covariance.
[e] Value represents data from one calf per treatment group.
[f] Value represents data from one calf plus a calculated missing value.

fed the 60% level of vitamin A depletion ration intake than in those fed the 100% level. The 12-wk duration resulted in a slightly higher average percentage of days consuming the ration allowance, 99.2%, than the 24-wk duration, 97.9%.

Growth, while not significantly influenced by carotene intake was, as expected, altered by level of ration intake and duration of vitamin A deficiency. Average daily increases in pounds of live weight adjusted by covariance for the initial value the day before the start of the comparison period were for those calves receiving the 60% ration allowance for the 12-wk duration, 1.48, for the 100% ration-12-wk duration, 2.10, for the 60% ration-24-wk duration, 1.24, and for the 100% ration-24-wk duration, 2.04. Increases in height at withers for the comparison period (unadjusted) were, respectively, 8.5, 9.9, 8.4, and 9.8 inches; in heart girth, 13.8, 17.0, 12.2, and 16.5 inches; and in girth of paunch, 14.7, 20.3, 12.4, and 18.2 inches. The respective standard deviations per calf of these three linear growth increases were 0.5, 0.8, and 2.5 inches.

Scours was not a problem in this experiment, since the average percentage of comparison-period days free of scours per calf was 99.7% (these percentages transformed to the arcsin $\sqrt{\%}$, averaged 88.8 with a standard deviation of 2.5) and only two calves of the 48 exhibited

scours, accompanied by an elevated rectal temperature of 103 F or greater.

Outward changes characteristic of hypovitaminosis A of cattle (5, 18), with the exception of convulsions, were absent in the calves of this experiment. Convulsions occurred during the 24th wk of the comparison period in one calf fed the 100% level of ration intake for the 12-wk duration and the 20-γ carotene intake. After the 20th wk of the comparison period, convulsions occurred in another calf fed the 100% ration-12-wk duration-10-γ carotene intake, and after the 14th wk of the comparison period, in both calves fed the 100%

ration-24-wk duration-10-γ carotene intake. One of these two calves died in convulsion during the 16th wk of the comparison period.

Cerebrospinal fluid pressures, electrocardiograms, and carotenoids and vitamin A concentrations (Tables 2 and 3). At the mid-point of the comparison period, those calves in the 12-wk duration grouping, previously fed the 100% level of ration intake and 36-γ carotene intake before subjecting them to their respective levels of ration (either 60% or 100%) and carotene intake (one of the six carotene intakes from 10- to 60-γ), had an arithmetic average cerebrospinal fluid pressure of 96 mm of saline

TABLE 3

Effects of level of ration intake and duration of vitamin A deficiency upon carotenoids and vitamin A concentrations in plasma and liver of Holstein calves fed fixed carotene intakes [a]

Treatments			Plasma				Liver at termination of 24-wk					
								Carotenoids		Vitamin A		
			Carotenoids		Vitamin A				Common logarithm		Common logarithm	Common logarithm per unit live weight
Ration level	Duration	Carotene intake	At 12-wk	At 24-wk	At 12-wk	At 24-wk	Weight	Actual		Actual		
(%)	(wk)	(γ)[b]	—(γ/100 ml)—				(kg)	—(γ/100 g)—				(γ/kg live weight)
60	12	10	37	18	13.8	5.5	2.7	28	1.44	10	1.00	0.09
60	12	20	34	43	14.3	8.5	2.4	58	1.76	131	2.11	1.21
60	12	30	30	43	13.4	11.0	2.8	72	1.86	120	2.02	1.12
60	12	40	30	68	13.7	15.4	2.7	88	1.94	427	2.63	1.75
60	12	50	38	82	14.4	16.2	2.4	140	2.15	547	2.72	1.78
60	12	60	26	76	15.9	20.1	2.7	115	2.06	497	2.70	1.80
100	12	10	35	16	11.7	5.3	3.8	24	1.38	8	0.77	−0.07
100	12	20	32	25	13.2	9.2	3.8	30	1.47	12	1.07	0.26
100	12	30	32	36	11.2	10.8	3.8	54	1.73	53	1.71	0.92
100	12	40	24	38	12.3	12.5	3.6	50	1.69	166	2.20	1.38
100	12	50	42	53	14.6	17.4	4.4	98	1.98	473	2.67	1.86
100	12	60	22	48	11.4	15.2	3.6	84	1.92	347	2.53	1.72
60	24	10	12	16	5.2	5.1	2.2	21	1.32	6	0.79	−0.10
60	24	20	18	27	10.6	9.6	2.4	41	1.61	31	1.49	0.62
60	24	30	40	46	11.1	15.6	2.4	56	1.74	108	2.01	1.14
60	24	40	56	64	12.8	11.8	2.3	109	2.04	264	2.42	1.53
60	24	50	57	51	15.4	13.6	2.4	106	2.03	273	2.34	1.49
60	24	60	61	70	14.2	14.6	2.3	106	2.02	738	2.86	1.97
100	24	10	10	13	5.8[d]	4.1[d]	4.0[d]	16[c]	1.19[d]	3[c]	0.60[d]	−0.14[d]
100	24	20	16	27	8.6	10.3	3.6	46	1.66	56	1.71	0.90
100	24	30	32	38	10.2	8.2	3.9	54	1.71	103	1.76	0.99
100	24	40	46	48	16.0	16.1	3.2	68	1.83	240	2.32	1.43
100	24	50	41	42	16.0	16.2	4.0	70	1.84	265	2.39	1.57
100	24	60	46	54	20.6	17.7	3.9	86	1.94	354	2.55	1.72
Standard deviation per calf			6	12	2.2	3.0	0.3	....	0.07	......	0.21	0.20

[a] Values represent averages based on two calves per treatment group, except as otherwise noted.

[b] γ/lb live weight/day.

[c] Value represents data from one calf per treatment group.

[d] Value represents data from one calf plus a calculated missing value.

(logarithmic average of 1.96 log units of milli-meters of saline equivalent to a geometric mean pressure of 91 mm of saline). Thus, these calves exhibited normal to slightly elevated pressures (10, 28). At the same time, the 24-wk duration calves fed the lowest three caro-tene intakes, 10-, 20-, and 30-γ, for the pre-vious part of the comparison period had ele-vated pressures, which decreased when expressed as the common logarithm of millimeters of saline, 0.72 $\pm$ 0.16 unit per unit log microgram increase in carotene intake. Slight, but sta-tistically insignificant, decreases in pressure occurred in the 60% level of ration intake grouping as carotene intake was increased from the 40-γ to the 60-γ level. No statistically sig-nificant difference in the rates of decrease be-tween the 60 and 100% level of ration intake groupings was found for the data at the three lowest carotene intakes (-0.80 ± 0.22 vs. -0.64 ± 0.22); however, the 60% level of

ration intake calves maintained lower pressures on the average than did the 100% calves, 2.02 vs. 2.27 for the three lowest carotene intakes ($P < 0.01$) and 1.97 vs. 2.16 for all carotene intakes ($P < 0.001$).

Terminal cerebrospinal fluid pressures, meas-ured during the 23rd wk of the comparison period, decreased 0.75 ± 0.15 log unit of milli-meters of saline per log unit increase in micro-grams of carotene intake for those calves re-ceiving the three lowest carotene intakes, but were not appreciably influenced in those calves fed the three highest carotene intakes. At the three lowest intakes, the 60% level calves had lower average pressures than the 100% level calves, 2.16 vs. 2.26, and the 12-wk duration calves lower values than the 24-wk duration calves, 2.14 vs. 2.28.

The average heart rate in beats per minute, for all calves in the experiment during the 24th wk of the comparison period, was 81 with a

TABLE 4

Effects of level of ration intake and duration of vitamin A deficiency upon protein distribution and concentration in serum of Holstein calves fed fixed carotene intakes [a]

Treatments			Protein distribution				Protein concentration					Ratio of albu-min to globu-lin
Ra-tion level	Dura-tion	Caro-tene in-take	Albu-min	Alpha-globu-lin	Beta-globu-lin	Gamma-globu-lin	Total	Albu-min	Alpha-globu-lin	Beta-globu-lin	Gamma-globu-lin	
(%)	(wk)	(γ)[b]	—(%)—				—(g/100 ml)—					
60	12	10	68.2	8.2	11.6	12.0	7.0	4.74	0.57	0.80	0.83	2.14
60	12	20	67.8	8.5	9.4	14.3	7.2	4.88	0.61	0.68	1.03	2.10
60	12	30	69.0	6.5	10.2	14.4	7.0	4.81	0.45	0.71	1.00	2.22
60	12	40	69.4	8.3	10.9	11.3	6.5	4.53	0.54	0.71	0.74	2.28
60	12	50	66.6	8.3	10.0	15.1	7.0	4.70	0.58	0.71	1.06	2.00
60	12	60	68.8	6.8	8.8	15.6	7.2	4.93	0.48	0.63	1.12	2.22
100	12	10	63.0	8.1	12.1	16.8	7.4	4.65	0.60	0.89	1.24	1.70
100	12	20	68.6	7.4	11.0	12.9	7.4	5.08	0.55	0.81	0.96	2.19
100	12	30	67.4	7.7	12.1	12.7	6.8	4.58	0.52	0.82	0.86	2.07
100	12	40	68.0	7.6	13.5	10.8	7.0	4.77	0.54	0.95	0.75	2.13
100	12	50	70.2	7.5	9.6	12.6	6.9	4.82	0.52	0.66	0.87	2.40
100	12	60	64.8	7.5	12.1	15.7	7.2	4.64	0.53	0.87	1.13	1.85
60	24	10	67.7	8.7	10.1	13.5	6.8	4.61	0.60	0.69	0.93	2.13
60	24	20	64.9	7.9	10.5	16.6	7.6	4.91	0.60	0.80	1.26	1.86
60	24	30	65.9	7.0	11.3	15.8	7.6	5.00	0.53	0.86	1.20	1.93
60	24	40	67.4	7.2	10.9	14.5	7.2	4.86	0.52	0.79	1.05	2.07
60	24	50	67.4[c]	7.7[c]	11.5[c]	13.3[c]	6.7	4.23[c]	0.48[c]	0.72[c]	0.83[c]	2.08[c]
60	24	60	70.2	8.1	8.8	12.8	7.1	5.02	0.58	0.63	0.92	2.39
100	24	10	64.8[c]	7.1[c]	12.2[c]	15.9[c]	7.4[c]	4.80[c]	0.53[c]	0.91[c]	1.17[c]	1.83[c]
100	24	20	66.2	7.6	12.0	14.2	7.2	4.74	0.54	0.86	1.02	1.96
100	24	30	63.9	8.6	10.5	16.9	7.4	4.71	0.64	0.77	1.26	1.78
100	24	40	69.4	8.3	10.8	11.5	7.0	4.89	0.58	0.76	0.81	2.27
100	24	50	66.0	8.2	11.9	13.9	6.9	4.54	0.56	0.82	0.96	1.94
100	24	60	67.3	7.6	10.4	14.7	7.4	4.94	0.56	0.77	1.08	2.06
Standard devia-tion per calf			2.2	0.9	1.1	2.2	0.3	0.20	0.07	0.08	0.18	0.22

[a] Values represent averages determined on serum obtained the 24th wk of the comparison period and from two calves per treatment group, except as otherwise noted.

[b] γ/lb live weight/day.

[c] Value represents data from one calf plus a calculated missing value.

TABLE 5

Effects of level of ration intake and duration of vitamin A deficiency upon some inorganic constituents, osmotic pressure (osmolality), and enzymatic activity in serum of Holstein calves fed fixed carotene intakes [a]

Ration level	Duration	Carotene intake	Calcium	Inorganic phosphorus	Sodium	Potassium	Chloride	Osmolality	Alkaline phosphatase activity [c]	Glutamic-oxalacetic transaminase activity [d]
(%)	(wk)	(γ)[b]		—————(mg/100 ml)—————				(milliosmols/ kg H₂O)	(units/ 100 ml)	(units/ ml)
60	12	10	10.2	9.5	346	17.2	359	286	8.2	34
60	12	20	11.1	9.6	345	18.5	359	290	6.2	29
60	12	30	9.8	9.5	345	17.8	358	289	4.6	35
60	12	40	10.0	9.9	355	17.6	362	293	6.6	33
60	12	50	10.0	9.7	346	17.7	364	290	5.2	41
60	12	60	10.0	9.2	349	16.8	363	286	3.6	33
100	12	10	10.0	10.2	354	19.4	376	297	5.7	41
100	12	20	10.4	9.0	349	16.6	368	286	7.0	42
100	12	30	10.5	9.5	349	17.1	371	296	5.6	40
100	12	40	10.0	9.8	352	18.3	369	290	7.6	35
100	12	50	10.2	10.1	354	17.6	368	292	7.4	36
100	12	60	10.2	9.8	344	17.0	364	285	5.6	36
60	24	10	10.6	9.5	356	18.3	363	287	6.4	51
60	24	20	10.0	10.5	346	18.5	365	290	5.9	42
60	24	30	10.2	10.3	352	18.2	369	296	6.6	34
60	24	40	10.0	9.5	342	17.8	361	288	7.5	36
60	24	50	9.8	9.3	352	17.8	364	292	5.0	39
60	24	60	9.8	9.4	355	17.6	358	290	7.6	36
100	24	10	10.6[e]	9.6[e]	342[e]	19.5[e]	363[e]	294[e]	8.8[e]	43[e]
100	24	20	10.4	9.2	353	17.0	360	292	6.1	32
100	24	30	9.6	8.9	354	17.3	363	288	6.8	41
100	24	40	10.0	8.9	349	16.8	368	287	7.0	39
100	24	50	10.4	9.1	350	17.7	359	286	5.6	39
100	24	60	10.1	9.7	344	17.9	367	295	6.1	45
Standard deviation per calf			0.6	0.9	6	1.2	7	4	1.9	9

[a] Values represent averages determined on serum obtained the 24th wk of the comparison period from two calves per treatment group, except as otherwise noted.

[b] γ/lb live weight/day.

[c] Bodansky units.

[d] Sigma-Frankel units.

[e] Value represents data from one calf plus a calculated missing value.

standard deviation of 12. The average electrocardiographic intervals in seconds obtained from the tracings of Lead II, with their standard deviations in parentheses were, for the PR interval 0.17 (0.02), for the QRS interval 0.06 (0.01), and for the QT interval 0.34 (0.03); whereas, the systolic index averaged 0.389 (0.021). The 60% level of ration intake resulted in significantly slower (P < 0.001) heart rates, 73.9, than did the 100% level, 88.3. These slower heart rates were accompanied by longer average QT intervals, 0.364 vs. 0.314 (P < 0.001), and greater average systolic indexes, 0.400 vs. 0.378 (P < 0.01). Body temperature obtained by inserting a clinical thermometer four inches into the rectum for 4 min immediately before recording electrocardiographic intervals, averaged for all calves 101.5 F, with a standard deviation of 0.6. The 60% level calves had a lower average value of 101.3 F, vs. 101.7 F for the 100% level calves (P < 0.10).

Calves fed the 60% level of ration intake had greater concentrations of carotenoids and vitamin A in their plasma and liver, at the termination of the comparison period, than did calves fed the 100% level ration intake. The respective averages of these two treatment categories were, for plasma carotenoids, 50 vs. 36 γ per 100 ml, for plasma vitamin A, 12.2

vs. 11.9 γ per 100 ml, for liver carotenoids, 1.83 vs. 1.70 common logarithm of micrograms of carotene per 100 g of liver, and for liver vitamin A, 2.09 vs. 1.86 common logarithm of micrograms of vitamin A per 100 g of liver. The difference still existed if the liver vitamin A was expressed as per unit of live weight, 1.20 vs. 1.04 common logarithm of total liver vitamin A in micrograms per kilogram of live weight. The latter calculation was necessary because the livers of the 60%-level calves averaged 12.9 g per kilogram of live weight vs. 15.5 g per kilogram of live weight for the 100% level calves. Duration of vitamin A deficiency did not significantly affect these concentrations; however, the differences between the 60 and 100% calves were more pronounced for the 12-wk duration calves than for the 24-wk duration calves, resulting in significant interactions between level of ration intake and duration for log liver vitamin A concentration

(per 100 g of liver) and log liver vitamin A concentration per unit of live weight (per kilogram). Plasma carotenoids concentration increased 62 ± 7 γ per 100 ml for each log microgram increase in carotene intake, plasma vitamin A concentration, 15.4 ± 1.7 γ per 100 ml, liver carotenoids concentration 0.88 ± 0.04 log micrograms per 100 g of liver, liver vitamin A concentration 2.45 ± 0.12 log micrograms per 100 g of liver, and liver vitamin A concentration per unit of live weight, 2.42 ± 0.11 log micrograms per kilogram of live weight. These linear regression coefficients were not significantly (at P ⩽ 0.05) affected by either level of ration intake or duration.

Serum constituents (Tables 4 and 5). Calves fed the 60% level of ration intake exhibited a higher average percentage of serum albumin (P < 0.10), and a lower percentage and concentration of beta-globulin (P < 0.01), than calves fed the 100% ration intake. As a result

TABLE 6

Effects of level of ration intake and duration of vitamin A deficiency upon protein distribution and concentration in cerebrospinal fluid of Holstein calves fed fixed carotene intakes [a]

Treatments			Protein distribution					Protein concentration				Ratio of albu- min to globu- lin
Ra- tion level	Dura- tion	Caro- tene in- take	Albu- min	Al- pha glob- ulin	Beta- glob- ulin	Gam- ma glob- ulin	Total	Albu- min	Alpha- globu- lin	Beta- globu- lin	Gam- ma globu- lin	
(%)	(wk)	(γ)[b]		(%)					(mg/100 ml)			
60	12	10	55	17	16	12	37	20	6.0	6.1	4.5	1.24
60	12	20	57	16	17	10	35	20	5.4	5.9	3.4	1.38
60	12	30	52	17	20	11	34	18	5.9	6.6	3.8	1.12
60	12	40	56	17	15	11	40	22	6.6	6.2	4.5	1.32
60	12	50	56	18	16	10	37	21	6.6	5.9	3.7	1.28
60	12	60	40	22	21	16	30	12	6.8	6.3	4.9	0.70
100	12	10	62	13	14	11	55	35	6.8	8.0	5.8	1.67
100	12	20	51	19	19	11	53	26	10.3	10.2	5.8	1.04
100	12	30	52	17	20	10	37	19	6.4	7.4	3.8	1.10
100	12	40	61	14	16	9	43	26	5.8	7.0	3.9	1.57
100	12	50	52	18	17	13	41	22	7.1	7.2	4.8	1.12
100	12	60	50	16	22	12	42	21	6.8	8.8	5.0	1.02
60	24	10	69	11	12	7	61	43	6.7	7.0	4.3	2.40
60	24	20	52	20	16	12	38	19	7.4	6.1	4.7	1.07
60	24	30	48	21	19	11	42	20	9.0	8.1	4.8	1.00
60	24	40	54	17	18	10	32	18	5.4	5.8	3.5	1.18
60	24	50	52	18	18	13	32	16	5.6	5.6	4.1	1.16
60	24	60	62	16	12	10	42	27	6.6	4.9	4.3	1.66
100	24	10	46[c]	16[c]	22[c]	16[c]	44[c]	20[c]	7.2[c]	9.9[c]	7.0[c]	0.83[c]
100	24	20	55	16	18	11	40	22	6.5	7.1	4.2	1.22
100	24	30	48	21	17	14	34	16	7.1	5.9	4.8	0.94
100	24	40	42	16	24	18	44	19	6.8	10.3	7.9	0.77
100	24	50	61	14	17	9	36	22	4.9	6.3	3.1	1.56
100	24	60	46	20	20	14	35	16	7.0	7.0	4.9	0.88
Standard devia- tion per calf			6	3	3	3	10	7	1.6	1.8	1.3	0.35

[a] Values represent averages determined on cerebrospinal fluid obtained the 24th wk of the comparison period and from two calves per treatment group, except as otherwise noted.
[b] γ/lb live weight/day.
[c] Value represents data from one calf plus a calculated missing value.

TABLE 7

Effects of level of ration intake and duration of vitamin A deficiency upon some inorganic constituents and osmotic pressure (osmolality) in cerebrospinal fluid of Holstein calves fed fixed carotene intakes [a]

Treatments				Inor-ganic phos-phorus	So-dium	Potas-sium	Chlo-ride	Osmol-ality
Ration level	Dura-tion	Caro-tene intake	Cal-cium					
(%)	(wk)	(γ)[b]	←————————(mg/100 ml)————————→					(milli-osmols/ kg H₂O)
60	12	10	4.8	1.8	339	11.8	433	284
60	12	20	4.7	2.0	340	12.0	431	285
60	12	30	4.6	2.1	334	12.2	422	284
60	12	40	4.7	2.0	337	11.9	432	288
60	12	50	4.7	1.8	350	12.4	438	290
60	12	60	4.6	1.6	342	12.3	430	288
100	12	10	4.6	1.8	346	12.0	434	286
100	12	20	4.7	2.0	342	12.5	429	284
100	12	30	4.4	1.6	340	12.8	427	282
100	12	40	4.8	1.8	350	12.6	437	274
100	12	50	4.4	1.6	352	12.3	426	286
100	12	60	4.6	1.9	348	12.1	427	288
60	24	10	4.9	1.7	343	12.1	432	284
60	24	20	4.8	1.9	346	12.1	427	288
60	24	30	4.8	1.8	344	12.0	430	285
60	24	40	4.5	1.8	349	11.6	427	285
60	24	50	4.7	1.7	341	11.8	433	286
60	24	60	4.5	2.0	347	11.8	423	287
100	24	10	4.9 [c]	1.8 [c]	344 [c]	12.2 [c]	435 [c]	290 [c]
100	24	20	5.6	1.9	348	12.2	422	290
100	24	30	4.4	1.6	350	12.4	428	284
100	24	40	4.8	1.9	351	11.9	434	286
100	24	50	4.9	1.8	352	12.4	432	288
100	24	60	5.0	1.6	350	12.4	430	286
Standard deviation per calf			0.3	0.2	6	0.4	9	4

[a] Values represent averages determined on cerebrospinal fluid obtained the 24th wk of the comparison period and from two calves per treatment group, except as otherwise noted.

[b] γ/lb live weight/day.

[c] Value represents data from one calf plus a calculated missing value.

of these differences, the albumin to globulin ratio was slightly higher in the calves fed the 60% ration. With increasing carotene intake, the percentage of serum albumin increased (P < 0.10), and the gamma-globulin percentage was higher at the three lowest carotene intakes, 10- plus 20- plus 30-γ, than at the three highest carotene intakes, 40- plus 50- plus 60-γ (P < 0.10). Concentrations of total protein, beta-globulin, and gamma-globulin were greater (P < 0.05, P < 0.10, and P < 0.05, respectively) in those calves fed the three lowest carotene intakes than in calves fed the highest carotene intakes. The increases of the two globulin fractions were reflected in lower albumin to globulin ratios (P < 0.05) for the calves fed the three lowest carotene intakes, as well as a linear increase in these values as carotene intake was increased (P < 0.10). Herdan's index (8) averaged 1.41 for all calves, with a standard deviation of 0.06. The index reflected

the influence of the 60% ration intake on the distribution of the four protein fractions, since the average value for these calves was 1.40 vs. 1.43 for the calves fed the 100% intake (P < 0.10). Also, the index decreased from a value of 1.45 for the lowest carotene intake, 10 γ, to 1.39 for the 60-γ carotene intake, P < 0.05 for the linear trend of the indexes on carotene intake.

Average percentages of the various glyco-protein fractions for all calves, with their respective standard deviations in parentheses, were 9.7(3.5) for the A-fraction, 43(5) for the alpha, 21(3) for the beta, and 26(4) for the gamma. Herdan's index, derived from the values of the four fractions for each calf, averaged 1.81(0.07). Possibly due to the relatively large variability in the glycoprotein fractions, the only treatment effect which exhibited statistical significance was a lower beta-fraction percentage for the 60% intake calves, 19.6,

TABLE 8

Effects of level of ration intake and duration of vitamin A deficiency upon protein concentration, some inorganic constituents, and osmotic pressure (osmolality) in aqueous humor of Holstein calves fed fixed carotene intakes [a]

Treatments								
Ration level	Dura- tion	Caro- tene intake	Pro- tein	Inor- ganic phos- phorus	So- dium	Potas- sium	Chlo- ride	Osmol- ality
(%)	(wk)	(γ) [b]	(mg/100 ml)		(mg/100 ml)			(milli- osmols/ kg H_2O)
60	12	10	65	3.5	337	17.0	416	293
60	12	20	66	3.9	334	18.6	412	290
60	12	30	61	3.7	340	16.8	411	293
60	12	40	63	4.0	345	17.4	420	290
60	12	50	63	3.8	337	17.4	421	293
60	12	60	72	3.7	335	17.2	410	289
100	12	10	80	3.5	335	17.3	407	290
100	12	20	58	4.2	339	16.9	419	292
100	12	30	55	4.1	348	16.8	416	292
100	12	40	63	4.3	337	17.0	416	288
100	12	50	79	3.8	335	17.5	411	285
100	12	60	68	3.4	339	17.4	421	288
60	24	10	71	4.1	340	16.7	419	290
60	24	20	74	4.0	340	17.2	417	292
60	24	30	67	3.8	341	16.2	415	290
60	24	40	68	3.8	336	16.2	408	286
60	24	50	70	4.0	342	17.4	430	294
60	24	60	73	3.8	338	16.9	410	286
100	24	10	79 [c]	4.2 [c]	337 [c]	17.1 [c]	408 [c]	290 [c]
100	24	20	71 [c]	4.2	340	16.8	410	292
100	24	30	54	3.7	334	16.1	415	290
100	24	40	56	3.9	338	17.5	414	290
100	24	50	61	3.8	341	17.1	420	288
100	24	60	58	4.0	331	17.1	410	292
Standard deviation per calf			11	0.3	5	0.9	8	4

[a] Values represent averages determined on aqueous humor obtained at slaughter upon the completion of the 24th wk of the comparison period from two calves per treatment group, except as otherwise noted.

[b] γ/lb live weight/day.

[c] Value represents data from one calf plus a calculated missing value.

as contrasted to 22.5 for the 100% calves (P < 0.01).

Of the inorganic constituents of serum, potassium concentrations decreased linearly with increasing carotene intake (P < 0.10) and chloride content of those calves fed the 60% level of ration intake was slightly less than that for the 100% calves (P < 0.10). The latter effect was more apparent in the 12-wk duration calves than in the 24-wk duration calves (P < 0.10 for the interaction of level of ration intake times duration). Magnesium concentration averaged 3.51 mg per 100 ml of serum for all calves, with a standard deviation per calf of 0.37. The calves in the 24-wk duration grouping had a higher (P < 0.01) average serum magnesium concentration, 3.69, than did the 12-wk duration calves, 3.34. Neither osmolality of serum nor alkaline phosphatase or glutamic-oxalacetic transaminase enzymatic ac-

tivities were materially influenced by level of ration intake, duration, or carotene intake.

Cerebrospinal fluid constituents (Tables 6 and 7). The average percentage of beta-globulin, as well as the average concentrations of beta- and gamma-globulin in cerebrospinal fluid of those calves fed the 60% ration intake, were lower than comparable values for the 100% ration calves (P < 0.05, P < 0.01, and P < 0.05, respectively). While comparisons of the 12- and 24-wk duration average percentages or concentrations indicated little effect between the two, the effects of level of ration intake 60% vs. 100% were not consistent at the two durations, as indicated by the presence of significant interactions between these two treatment categories (P < 0.05 for albumin and gamma-globulin percentages and for albumin concentration). As carotene intake was increased, there were linear decreases for the

albumin percentages $(P < 0.10)$ and concentrations $(P < 0.05)$, as well as for the total protein concentrations $(P < 0.05)$. The changes in the various protein fractions were largely reflected in the albumin to globulin ratios, which tended to decrease as carotene intake was increased $(P < 0.10)$. Also, an interaction between level of ration intake and duration $(P < 0.05)$ was present. This was due to smaller ratios for the 60% grouping at the 12-wk duration than for the 100% grouping; whereas, the opposite held at the 24-wk duration. Herdan's index, calculated from the four separate protein fraction percentages for each calf, averaged 1.70, with a standard deviation of 0.11. As carotene intake increased from 10 to 60 γ, the indexes increased from 1.61 to 1.75 $(P < 0.10$ for the positive linear trend). Also, an interaction between level of ration intake and duration existed $(P < 0.05)$ since, in the case of the 12-wk duration grouping, the 60% calves had a greater average index whereas, in contrast, this was reversed for the 24-wk duration.

Sodium and potassium were found in slightly lower concentrations in the cerebrospinal fluid of calves fed the 60% level of ration intake than of calves fed the 100% level intake $(P < 0.01$ and $P < 0.05$, respectively). Calves subjected to the 12-wk duration had lower cerebrospinal fluid calcium than the 24-wk duration calves $(P < 0.10)$. The same effect of duration was present for sodium $(P < 0.05)$, as well as lower concentrations for calves fed the three lowest carotene intakes $(P < 0.01)$. Osmolality appeared to be unaffected by treatment.

Aqueous humor constituents (Table 8). For all calves, the average volume of aqueous humor obtained from both eyes at slaughter and pooled was 2.9 ml, with a standard deviation of 0.4. While total protein and osmotic pressure appeared to be greater and potassium concentration less in those calves on the three lowest carotene intakes, in comparison to comparable values for the calves on the three highest carotene intakes, none of these differences approached statistical significance at $P < 0.10$.

DISCUSSION

This study confirms previous observations (6, 14) with respect to the inverse relationship between feed intake and vitamin A economy of the animal and allows quantitative estimates of the effect of feed intake on the carotene requirement of calves. For example, linear regressions of liver vitamin A stores per unit of live weight across all carotene intakes

and cerebrospinal fluid pressures across the three lowest carotene intakes, respectively, for the 60 and 100% levels of feed intake are graphically presented in Figure 1. From these functions, the amount of carotene to maintain equivalent responses for either criterion can be determined by obtaining the antilogarithm of the horizontal distance between the regression lines representing the two levels of feed intake (4). Based upon liver storage of vitamin A, the 60% calves required 0.86 as much carotene as the 100% level calves. A similar value based upon cerebrospinal fluid pressure was 0.74.

Duration of supplementation, either 12- or 24-wk, had inappreciable effect on most of the criteria studied. However, the effects of level of feed intake, 60% vs. 100%, were greater with the longer duration, as evidenced by the presence in several of the criteria of significant interactions between duration and level of feed intake. This was not completely unexpected, due to the conduct of the experiment with the 12-wk duration calves fed the 100% level of ration intake for the first half of the comparison period.

The findings with regard to alteration of the serum proteins, with a decrease in carotene intake from an adequate to a deficient level, confirm the work of Madsen and Earle (20), as well as more recent studies (11, 12), and ex-
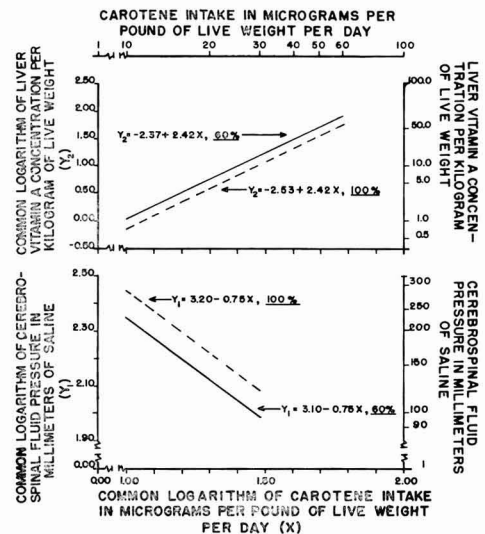
FIG. 1. Regression of cerebrospinal fluid pressure and liver vitamin A concentration per unit live weight on carotene intake for calves fed the 60% (————) and 100% (—— —— ——) levels of ration intake.

tend these observations to cerebrospinal fluid and possibly aqueous humor. It is of interest that the changes in protein due to involuntarily reduced feed intake, that is, the effects of the 60% versus the 100% level of ration intake, were in most cases opposite to those observed due to deficient carotene intake.

In this study, inappreciable change was observed in the osmolality of serum due to carotene intake. Therefore, the previous conclusions of Moore and Sykes (23), Madsen and Earle (20), and Dehority et al. (8), that the changes in the concentrations of the biochemical constituents in serum due to deficient carotene intake are apparently not related to either the elevated cerebrospinal fluid pressure or edema (anasarca) characteristic of bovine hypovitaminosis A, are supported.

Why there were no statistically significant alterations in the constituents of aqueous humor in the present study, as contrasted to those of previous ones, (8) is, at this time, inexplicable.

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EFFECT OF DIET pH ON FECAL CONSISTENCY OF YOUNG CALVES[1,2]

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SUMMARY

Fifteen young male dairy calves of different breeds were fed, in various sequences, normal milk pH (6.8) and milk with pH's adjusted to 5.0 with 1 N HCl, and 8.0 with 1 N NaOH. The feeding of acidified and alkalized milk did not significantly affect dry matter content of the feces, daily fecal excretion, or incidence of scours.

Incidence of scours is often high in young dairy calves. Withers (19) found that 33% of the deaths in calves could be attributed to infantile diarrhea, either due to coliform infection or associated with improper feeding. Although it is difficult to establish the exact cause for a particular case of diarrhea, there is a general agreement among workers that the kind of ration, its manner of feeding, and management may be factors contributing to attacks of diarrhea in calves. Such dietary factors as abnormally high levels of lactose, minerals, or fat have been postulated as important factors in the etiology of calf scours (1-7, 9-15). Although fecal consistency has often been mentioned in experimental observations, very few studies have been planned specifically for studying the incidence of calf diarrhea as associated with diet pH. Wiese et al. (18), in synthetic milk feeding studies with calves, emphasized the importance of a diet between 6.5 and 6.8. Morrison (8) recommends that sour milk should not be fed to young calves. Little evidence has been reported concerning the effect of pH of the diet on the consistency of feces of the young calves, except for the report by Blaxter and Wood (2) and as suggested by Velu et al. (17).

Since the advent of whole milk replacers for calf feeding, the influence of various constituents, as well as certain properties of the milk replacer such as consistency and pH, as related to diarrhea incidence, has become of funda-

mental as well as of greater practical importance. The present experiment, therefore, was planned to study the effects of the pH of a diet per se on the consistency of the feces in young calves.

EXPERIMENTAL PROCEDURE

Fifteen male dairy calves, four to eight days of age, housed in individual pens, and randomly assigned as shown in Table 1, were fed freshly drawn milk with a pH of 6.8 or milk adjusted to a pH of 5.0 with 1 N HCl and a pH of 8.0 with 1 N NaOH. The calves were fed the milk diets twice daily from nipple pails. The rate of feeding per day was equal to 10% of the body weight of the calves.

Fecal consistency was measured by determining the moisture content of collected feces. For collecting the feces, a harness as designed by Grimes and Gardner (5) was used. A clean harness was attached to the animal the same day a different diet was fed. The feces collected during the first 24 hr were discarded, after which collections were made and weighed daily for each of the four-day diet feeding periods. After weighing, the feces were well mixed and aliquoted and placed in stoppered bottles. The samples were refrigerated at 0 F until analyses could be made.

In instances where calves scoured severely, the collection period was reduced to three days. The next diet in the sequence was not given in such cases, until 24 hr after the feces had returned to normal appearance. In cases of severe diarrhea, Terramix (mixture of terramycin and vitamins A and D) was added to the whole milk for two or three feedings to prevent secondary infections.

The dry matter content of each fecal sample was determined individually, under nitrogen, in a vacuum oven at a temperature of 65 C. Eight to ten days were required for drying samples to reach constant weight.

TABLE 1

Feeding sequences of normal, alkalized, and acidified whole milk

Sequences	No. of calves	First period (5 days)	Intermediate period (3 days)	Second period (5 days)	Intermediate period (3 days)	Third period (5 days)
I	5	Acidified	Normal	Alkalized	Normal	Normal
II	3	Normal	....	Alkalized	Normal	Acidified
III	2	Normal	....	Acidified	Normal	Alkalized
IV	5	Alkalized	Normal	Acidified	Normal	Normal

RESULTS AND DISCUSSION

Results of the experiment are summarized in Table 2, which shows that some calves on each treatment suffered to varying degrees from scours. The incidence of diarrhea observed could not be attributed to any specific dietary factor or combination of factors.

The ranges in per cent of dry matter content of the feces as associated with diet fed were as follows: 10.6 to 27.6% for milk, 7.6 to 37.2% for acidified milk, and 5.0 to 27.9% for alkalized milk. The average fecal dry matter levels for all calves fed normal, acidified, and alkalized milk were 21.4, 21.5, and 20.8%, respectively; whereas, the dry matter values for apparently normal feces averaged 23.6, 24.1, and 24.0%, respectively.

The daily fecal output ranged from 61 to 524 g for normal milk, 52 to 588 g for acidified milk, and 79 to 1,123 g for alkalized milk. The average daily fecal excretion per calf for these treatments was 161, 192, and 251 g, respectively. The daily fecal excretion for the calves without diarrhea ranged from 61 to 182 g, 52 to 214 g, and 79 to 310 g when fed normal, acidified, and alkalized milk with average outputs of 104, 120, and 131 g, respectively.

The average daily fecal output in the scouring calves varied from 302 to 1,123 g, whereas the dry matter content of these feces ranged from 5.0 to 15.1%. The dry matter excretion per pound of milk ingested ranged from 2.7 to 18 g. These findings, suggesting that the dry matter digestibility is variously affected in

TABLE 2

Dry matter percentages and daily fecal outputs of all the calves when fed milk at different pH levels

Calf no.	Normal		Acidified milk		Alkalized milk	
	Per cent dry matter	Daily fecal output	Per cent dry matter	Daily fecal output	Per cent dry matter	Daily fecal output
		(g)		(g)		(g)
B1S	25.7	74	28.1	195	5.0[a]	711[a]
243	14.0[a]	302[a]	21.1	79	25.4	90
197	21.1	95	10.4[a]	549[a]	25.0	132
203	21.5	61	7.6[a]	588[a]	24.8	79
1662	27.6	73	28.3	214	11.1[a]	1123[a]
M5	10.6[a]	524[a]	18.4	134	20.9	148
G3	25.7	88	15.1[a]	305[a]	19.0	198
B4	13.2[a]	336[a]	37.2	84	21.1	150
M6	19.3	151	20.1	153	7.7[a]	354[a]
290	24.0	75	28.6	80	25.2	80
289	25.6	148	20.1	107	27.9	109
240	23.5	182	17.0	160	25.0	95
1553	26.4	146	29.6	52	24.5	88
1624	23.2	88	21.3	52	22.4	310
1608	20.0	73	19.7	128	26.9	97
Average[b]	21.4	161	21.5	192	20.8	251
Average[c]	23.6	104	24.1	120	24.0	131

[a] Feces condition, loose.
[b] Average for all calves.
[c] Average for nonscouring calves.

TABLE 3

Analysis of variance in the daily fecal outputs as related to treatment

Source of variations	Degrees of freedom	Sum of squares	Mean square	F
1. Total groups	44	1,895,526.00		
2. Between means of individuals	14	446,277.33		
3. Between means of treatments	2	62,530.13	31,265.06	2.26
4. Error	28	386,718.57		

scours, are in agreement with those of Nezvesky et al. (9), Blaxter and Wood (2), and Grimes and Gardner (5).

The average daily intake per callf was 10.0, 8.9, and 9.0 lb, respectively, for the normal, acidified, and alkalized milk diets. It would seem, therefore, that differences in the average fecal excretion values could not be attributed to the difference in levels of diet intake. These values suggest that when the pH of the milk is shifted towards either acidity or alkalinity, the level of fecal excretion may be affected. The analysis of variance (16), however, shows that these variations were not significant at $P = .05$, as shown in Table 3.

It was further observed that the calves did not pass feces regularly each day, which supports the earlier findings of Blaxter and Wood (2) and Grimes and Gardner (5). Also, the scouring calves lost hair from the rear legs, with the extent of loss depending upon severity of diarrhea. Similar observations have been reported by Blaxter and Wood (2).

Variations in the consistency of the feces are a continuous change; therefore, it was difficult to establish the exact time at which feces may have become abnormal or the condition considered diarrhea. Blaxter and Wood (2) have indicated the mean dry matter content of normal, loose, and watery feces to be 19.6, 13, and 9.1%. If these values are applied to the present studies, three calves out of 15 scoured in each of the three treatments.

The findings reported here are not in agreement with those of Blaxter and Wood (2), who reported that addition of an acid to the milk produces a curd of low tension and that feeding of such milk tended to cause calves to scour. These results, however, are in agreement with those of Owen et al. (11), who found that reducing curd tension by adding sodium citrate did not induce scouring. Blaxter and Wood (2) indicated that the addition of salts of the alkali metals to the milk interferes with the rennin action upon curd formation in milk and thus produces scours.

During the entire 5-wk study, nine of the 15 calves (60%) scoured at one time or another. This incidence is in agreement with the incidence of 56% reported by Pounden et al. (14) and of 64% reported by Norton et al. (10).

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FATE OF LACTIC ACID IN RUMEN INGESTA

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SUMMARY

The production of lactic acid in ruminal ingesta in vitro was stimulated by commercial alfalfa meal, heated and unheated alfalfa, glucose, and heated starch. The heating of starch and alfalfa increased production although no substrate, at levels comparable to the amounts of similar material in commercial alfalfa meal, caused as much lactic acid to be produced as did alfalfa meal. Propionate production was stimulated by mixtures of peptone and glucose in the absence of lactic acid accumulation or by alfalfa meal in the presence of lactic acid. Product composition varied with fermentation pH. At pH 6.3, 35% of added uniformly labeled C-14 lactic acid was converted to acetic acid, whereas 33% was recovered in the ether-extracted aqueous residues and 9% in the propionate. The remainder was distributed in other fatty acids and fermentation gases.

Bauman and Foster (3), Briggs et al. (4), Elsden (8), Waldo and Schultz (20), and Reid et al. (16), among others, have demonstrated that detectable quantities of lactic acid accumulate in the rumen under special conditions, and many authors have isolated cultures of bacteria from the rumen which produce lactic acid (5). The low levels of lactate usually present in the rumen have led investigators to study the pathways by which lactate may disappear. The work of Waldo and Schultz (20), Elsden (8), and Hueter et al. (11) suggests that lactate may be converted to other fatty acids, especially propionate. Johns (13) demonstrated this conversion with *Veillonella gazogenes* and *Propionibacterium*, and Ladd and Walker (15) investigated it with *Peptostreptococcus elsdenii*.

With special rations, usually including cooked starch, heat-processed ground roughage, or high starch, an increase in propionate has been obtained in the ruminal fermentation (1, 8-10, 17, 18). It has been suggested that the production of propionate with special rations is related to the rapid conversion of lactate produced when glucose or other readily available carbohydrates are fed.

The work of Jayasuriya and Hungate (12) demonstrated that under their conditions an average of 81% of the C-14 from that portion of the two-labeled lactate converted to steam-distillable material during in vitro fermenta-

tion was recovered as acetate. Only 14% of the activity was found in propionate and 5% in butyrate. Differences between manometric and isotopic results suggested that the use of uniformly labeled substrate would be desirable. The present studies were designed to extend the above observations and follow the formation of lactic acid and the conversion of uniformly labeled lactate in ruminal ingesta.

MATERIALS AND METHODS

To determine the optimum conditions for lactic acid production in ruminal ingesta, samples of ingesta were taken from 2-yr-old fistulated steers fed poor-quality mixed hay ad libitum and 4 lb of 12% protein concentrate per day. The material was taken from the center of the rumen about 20 min after feeding. Two hundred and twenty-five milliliters of ruminal ingesta was strained through two layers of cheese cloth into 250-ml Erlenmeyer flasks. Each flask contained 15 g of commercial alfalfa meal or ground alfalfa, or 5.73 g of heat-treated or untreated carbohydrate (calculated to be equivalent to a maximum of 38.2% N.F.E.), or 2.42 g of each protein source (equivalent to a maximum value of 16.1% protein) or both. Control flasks contained no added substrate. In the experiments concerning the effect of heat (autoclaved at 121 C for 30 min) upon the substrate, the unheated samples served as controls. To determine the effects of pH upon lactic acid production, 200 ml of rumen fluid was added to flasks contain-

ing buffer solutions of KH_2PO_4 and K_2HPO_4, which were calculated to give a final concentration of 0.2 M phosphate ions in each case. The measured pH values of the resulting buffered rumen fluid samples were 6.9, 6.5, 6.05, and 5.5. Ten grams of commercial alfalfa meal served as substrate in each flask. Flasks in all experiments were incubated in water baths at 39 C under a nitrogen atmosphere.

Fermentation acids were determined by a modification of the silicic acid column chromatography method of Smith et al. (19). One hundred–mesh silicic acid (Mallinckrodt) was sized by repeated suspension in water, the suspended fine particles decanted from the settled material and discarded. The acid was then dried at 55 C. Four grams of the dry acid were suspended in excess chloroform. The acid was then slurried to a fine suspension by stirring and grinding in 3 ml of 0.1 N H_2SO_4. The slurry was poured into a one-half inch i.d. column approximately 16 inches long, which had been drawn to a tip at the lower end. The tip contained glass wool covered by a filter paper disk. Additional thin layers of asbestos fiber and Celite prevented leaching of the column and erratic results during later use of the column. The silicic acid slurry was pressed into the column with an additional filter paper disk, using a glass tube. One gram of dry silicic acid was then added to the top of the column and mixed with a small amount of chloroform to remove the trapped air. This top layer was compressed with a final filter paper disk. A 1-ml acidified aqueous sample (pH 2.0 or less with 50% conc H_2SO_4/50% H_2O) was forced into the dry silicic acid cap under air or nitrogen pressure. The column was then developed with approximately 75 ml of benzene integrated with 75 ml of ether or with 25% butanol in benzene as the second solvent. The solvents were saturated with 0.1 N H_2SO_4. The air or nitrogen pressure was regulated to give a solvent flow rate of 30 to 60 drops per minute. The integrator reservoirs for the solvents were made from two 50-ml Erlenmeyer flasks placed side by side and connected by a stopcock. The first flask was provided with an opening in the bottom and placed upside down, as shown in Figure 1.

This arrangement allowed minimal addition of the second solvent during the initial stages of development and minimal addition of the first solvent later. Forty-drop fractions were eluted and titrated to the phenolphthalein or brom-cresol-purple end point with 0.01 N C_2H_5O Na or 0.01 N NaOH in absolute ethanol. The top 1 g of the column was removed with
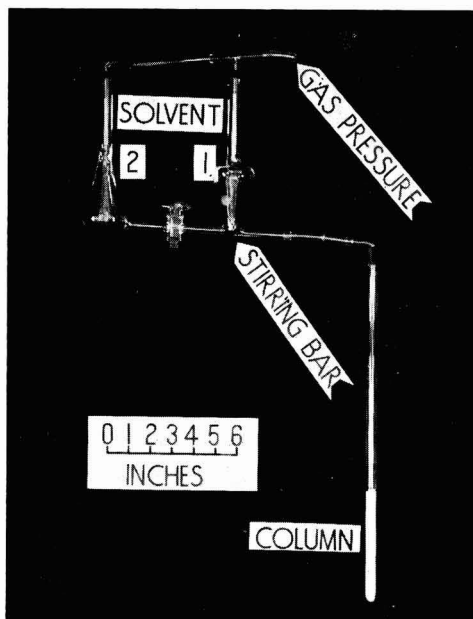
FIG. 1. Equipment for chromatographic separation of fermentation acids.

a glass tube for reuse of the column, which does not begin to leach for nine or ten uses.
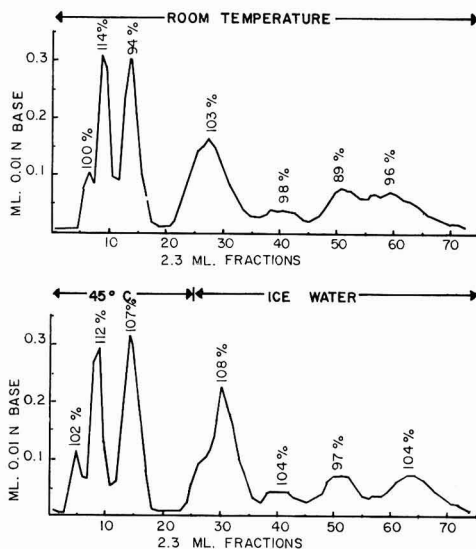
FIG. 2. Chromatograms of a standard mixture of fermentation acids run under different temperatures. Peaks, from left to right are: valeric, butyric, propionic, acetic, formic, succinic, and lactic acids. Recovery based on calculated amount in the sample is shown for each peak.

Known samples of valeric, butyric, propionic, acetic, formic, succinic, and lactic acids were recovered satisfactorily, as illustrated in Figure 2. Isobutyric and butyric acids were separated if the solvent flow was less than 30 drops per minute and if 20-drop fractions were collected. Five- and six-carbon acids eluted within the first 10 ml were overlooked unless titration continued beyond the first false end point. This precaution was not necessary with the other acids. A column thinner than one-half inch i.d. allowed more rapid development with equal separation. Resolution of five-carbon acids could be increased even further by enclosing the column in a jacket at approximately 45 C. Resolution of succinic and lactic acids is improved at lower temperatures. The entire packing and development of the column can be accomplished in 30 to 50 min.

For the biosynthesis of uniformly C-14 labeled lactic acid, 0.4 g of glucose was added to 25 ml of rumen fluid, together with a trace of uniformly labeled glucose. The mixture was incubated for 18 min at 39 C, after which the fermentation was stopped by the addition of 0.5 ml of 50% H_2SO_4. The CO_2 evolved was trapped in aqueous NaOH. Several such fermentations were carried out, to obtain required amounts of labeled lactic acid. Lactic acid was separated from the aqueous centrifugal supernatant by the anion exchange method of Busch et al. (6). The lactic acid was identified by the method of Eegriwe (7) and purity was determined with paper chromatography, using the method of Jones (14). The chromatograms were counted in a chromatogram strip counter.

For the in vitro fermentation of purified uniformly labeled lactic acid, the substrate was incubated at 39 C with 25 ml of fresh rumen fluid under a mixture of 65% CO_2, 34% CH_4, and 1% H_2 in a closed system connected to a burette for the collection of gas over mercury. The incubation flask was agitated continuously for 1.5 hr, when the reaction was stopped with H_2SO_4. The gas was transferred quantitatively to the collection burette for analysis, in a precision mine air apparatus. The CO_2 was trapped as carbonate for direct plating and the CH_4 was converted to CO_2 and trapped separately. An unfermented control sample of labeled lactate was added to preacidified rumen fluid. Both acidified samples (fermented and unfermented control) were divided in half for plating and for duplicate fractionation by continuous liquid-liquid ether extraction and column chromatography. Subsamples were adjusted to the appropriate pH and direct-plated in triplicate on stainless steel planchets for

counting in a gas-flow Geiger system. The outer edge of each planchet was ringed with a thin layer of vaseline, and a drop of 5% Sparkleen solution added to each sample to facilitate plating. Triplicate subsamples containing an internal C-14 standard were also plated, to allow direct corrections for self-absorption, geometry, and efficiency.

RESULTS AND DISCUSSION

The production of lactic acid and its subsequent disappearance during in vitro incubation of commercial alfalfa meal with rumen fluids is illustrated in Figure 3. During the
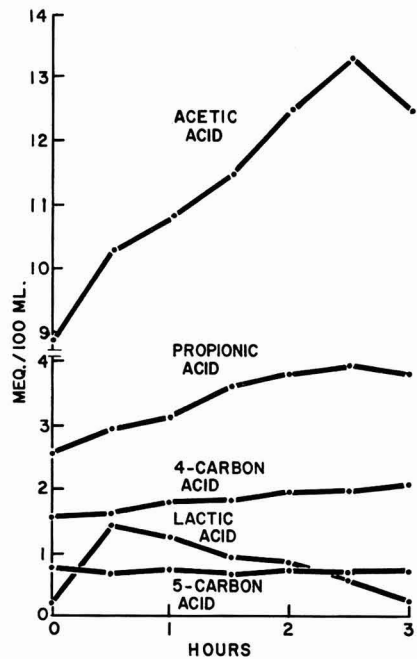
FIG. 3. Concentration of fermentation acids during in vitro incubation of commercial alfalfa meal.

incubation, lactic acid reached a peak value of 8.2% of the total acids within the first hour. At the end of 3 hr the total concentration of fermentation acids increased from 14.0 to 19.3 meq/100 ml. The concentration of butyric increased 38%, propionic 50%, and acetic 38% of their original concentrations.

It is evident from the data in Figure 4 that fermentation rates were greatest during the first hour of incubation in vitro. Substrates producing the greatest increase in lactic acid also caused the largest increases in acetic and
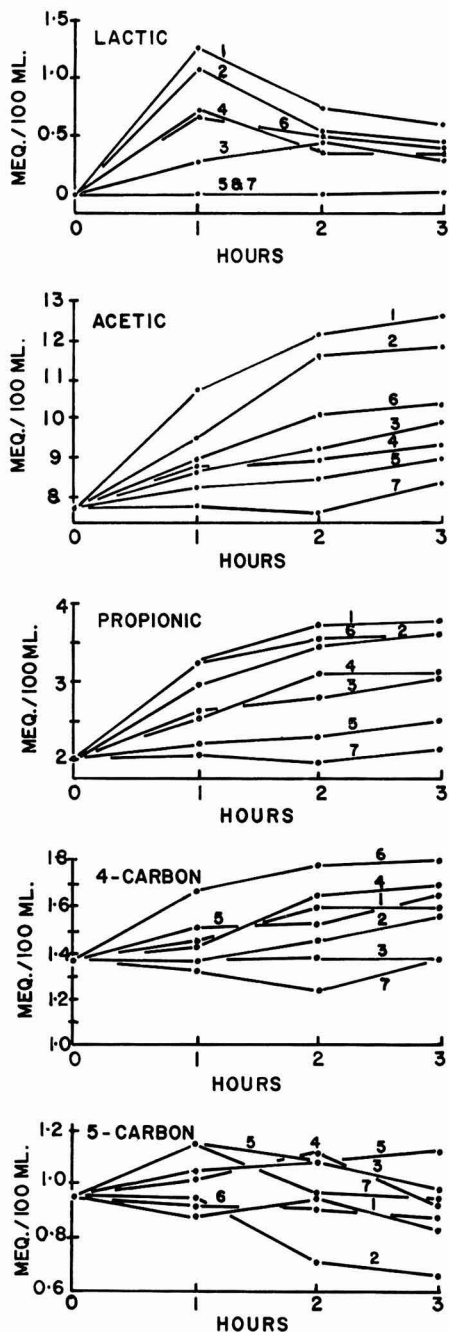
FIG. 4. Comparison of the concentrations of fermentation acids during the in vitro incubation of commercial alfalfa meal (1), heat-treated ground alfalfa hay (2), unheated ground alfalfa hay (3), glucose (4), peptone (5), glucose and peptone (6), and rumen fluid alone (7).

propionic acid. A notable exception was the production of proportionately larger quantities of propionate with the glucose-peptone mixture. This exception may result from the operation of a different pathway for the conversion of carbohydrate to fatty acids, possibly in response to a different pH as the result of peptone deamination, or in response to the stimulation of a different segment of the microbial population.

The production of butyrate was not well-correlated with the production of any other acid. Valeric acid production was stimulated only by peptone alone. None of the substrates was equal to alfalfa meal as a precursor of lactic acid. The comparison of heated and unheated ground alfalfa supports the observation of Shaw et al. (17), that heat treatment of substrate is important in the production of ruminal propionate. Heat-treated glucose, sucrose, and unheated sucrose gave responses similar to glucose alone. No lactic acid was produced with heat-treated or unheated casein or with unheated starch, whereas heated starch produced approximately one-half as much lactic acid as did glucose. Microscopic observation of the heated starch granules revealed a change in the physical structure of the granules, which may account for the change in fermentation activity. After heating, much of the granular material was reduced to an amorphous form, which undoubtedly provided more surface area and perhaps made the starch more readily available to microorganisms.

The transitory accumulation of lactic acid which occurs with glucose, heated starch, or heat-treated alfalfa supports the observation that large amounts of readily available carbohydrate are necessary for the accumulation of lactate in vivo. This might only reflect the resistance of the lactic fermentation pathways to low pH values. Such a mechanism is suggested by the data of Figure 5. Here, the response in lactic, acetic, and propionic acid production to changes in pH was immediate, indicating that a major shift in microbial population is not essential. In this experiment lactic acid reached its maximum concentration at lower pH values after 2 hr, whereas at the higher pH values it had decreased slightly after 2 hr. Essentially the same results were obtained with heat-treated finely ground alfalfa hay.

Uniformly C-14 labeled lactic acid was prepared by fermentation of uniformly C-14 labeled glucose in ruminal ingesta. Table 1 contains the data obtained from the fermentation of approximately 0.85 meq of purified labeled lactic acid with 25 ml of ruminal ingesta for

4

3

MEQ./100 ML.

1

0

ACETIC

PROPIONIC

LACTIC

4-CARBON

5-CARBON

5   6   7
          pH
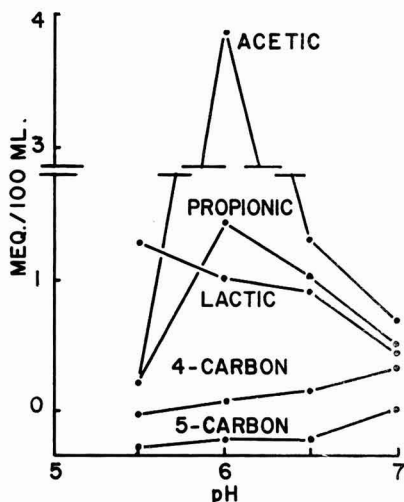
FIG. 5. Effect of buffered pH upon the in vitro production of fermentation acids by ruminal ingesta during the fermentation of commercial alfalfa meal. Each point represents the increase in concentration of each of the fatty acids after 2 hr of incubation.

1.5 hr, as described previously. Results of analysis of the preacidified unfermented control sample are shown in Table 2. The initial pH

of the unbuffered rumen fluid in which the uniformly labeled lactic acid was incubated was 6.3. At that pH value, lactic acid does not normally accumulate in the rumen. The experiment demonstrates that lactic acid is rapidly metabolized at pH 6.3. However, the experiment cannot indicate the importance of lactic acid as an intermediate in rumen fermentation, since its normal absence at pH 6.3 may reflect either the rapid metabolism or a decreased rate of production. At lower pH values, where lactate often accumulates, it might be metabolized much more slowly and may be converted to much different products. The results support the observation of Jayasuriya and Hungate (12). The carbohydrate converted to lactic acid as an intermediate at the pH of this experiment is ultimately converted primarily to acetic acid. The presence of activity in the four- and five-carbon acids suggests either carboxyl exchange, or the condensation of two- and three-carbon acids, as reported by Barker (2).

The presence of more than one important pathway for the production of propionate in ruminal ingesta might be inferred from some of the data. With commercial alfalfa meal, where lactic acid accumulated, propionate increased proportionately more than acetate and

TABLE 1

Distribution of C-14 following the fermentation of uniformly C-14 labeled lactic acid by ruminal ingesta in vitro

		d/m [a]	Per cent of total in sub- sample	Average per cent of total	Per cent of ether extract	Avg per cent of ether extract
		(in thousands)				
Radioactivity added		3,983 ($\div$2)	100			
Ether extracted	(a) [b]	636	31.9	32.6		
aqueous residue	(b)	666	33.4			
Ether extract	(a)	1,150	57.7	55.8		
	(b)	1,072	53.8			
CO_2		94 ($\div$2)	2.4	2.4		
CH_4		104 ($\div$2)	2.6	2.6		
Total recovery	(a)	1,885	94.6	93.4		
	(b)	1,837	92.2			
Ether extract						
5-Carbon Acid	(a)	48	2.4	2.2	4.2	4.0
	(b)	41	2.1		3.8	
4-Carbon Acid	(a)	62	3.1	3.3	5.4	6.0
	(b)	70	3.5		6.5	
Propionic	(a)	177	8.9	9.2	15.4	16.4
	(b)	188	9.4		17.5	
Acetic	(a)	683	34.3	34.9	59.4	62.7
	(b)	707	35.5		66.0	
Lactic	(a)	133	6.7	6.0	11.6	10.6
	(b)	103	5.2		9.6	
Recovery from column	(a)	1,102	55.3	55.5	95.8	99.6
	(b)	1,110	55.7		103.5	

[a] d/m—disintegrations per minute.
[b] (a) and (b) are duplicate subsamples.

TABLE 2

Distribution of C-14 labeled lactic acid in an unfermented control sample of ruminal ingesta

		d/m [a]	Per cent of total in sub-sample	Average per cent of total	Per cent of ether extract	Avg per cent of ether extract
		(in thousands)				
Radioactivity added		4,275($\div$2)	100			
Ether extracted	(a) [b]	487	22.8	16.2		
aqueous residue	(b)	206	9.6			
Ether extract	(a)	1,390	65.0	73.4		
	(b)	1,748	81.8			
Total recovery	(a)	1,877	87.8	89.6		
	(b)	1,954	91.4			
Ether extract						
5-Carbon Acid	(a)	6	0.3	0.3	0.4	0.4
	(b)	7	0.3		0.4	
4-Carbon Acid	(a)	5	0.2	0.4	0.4	0.6
	(b)	14	0.7		0.8	
Propionic	(a)	6	0.3	0.4	0.4	0.5
	(b)	10	0.5		0.6	
Acetic	(a)	9	0.4	0.4	0.6	0.5
	(b)	8	0.4		0.5	
Lactic	(a)	1,188	55.6	61.9	85.5	84.4
	(b)	1,458	68.2		83.4	
Recovery from column	(a)	1,214	56.8	63.4	87.3	86.4
	(b)	1,497	70.0		85.6	

[a] d/m—disintegrations per minute.
[b] (a) and (b) are duplicate subsamples.

butyrate. That some of this propionate was derived from lactate is demonstrated by the results with tracers. However, propionate increased significantly in the absence of accumulated lactate when glucose and peptone were used as substrate. Either lactate was converted to propionate so rapidly at this pH that it could not be detected, or the increased production of propionate in the absence of accumulated lactate resulted from an independent pathway for propionate formation which is quantitatively more significant at higher pH values.

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TECHNICAL NOTE

SOME FACTORS INFLUENCING INTAKE OF DIRECT-CUT SILAGE BY DAIRY COWS [1]

The current interest in forage-testing programs as a means of improving dairy-feeding programs has resulted in a search for suitable methods for predicting forage intake by dairy cows. Due to animal variation, there is little current information to indicate that the individual cow consumption can be accurately estimated. This is not necessarily a major problem, since predictions of intake on a herd basis would be a useful tool in providing improved feeding information to dairy farmers.

Data from feeding trials with 34 silages, fed as the only roughage, were available for analysis. The silages represented a wide variety of forages including summer grasses, alfalfa, small grains and corn, and silages made with and without additives. The silages were all fed free-choice and supplemented with grain according to milk production. The data were analyzed by least-squares multiple regression. Factors included in the study as independent variables were: body weight of cows, level of milk production, dry matter digestibility, silage dry matter, silage pH, silage crude protein, silage crude fiber, and calculated TDN required by the cows. Of the variables studied, only dry matter digestibility, crude protein, crude fiber, and calculated TDN required yielded significant influences on dry matter intake. The regression coefficients are shown in Table 1. The four factors accounted for 93% of the variation in average silage dry matter intake ($R^2 = .928$). The standard error of estimate was ± 1.28 lb dry matter. The resulting equation for predicting silage dry matter intake was $Y = 17.60 + .209\ X_1 - .477\ X_2 - .239\ X_3 + .346\ X_4$, where Y is dry matter intake, X_1 is dry matter digestibility, X_2 is crude protein, X_3 is crude fiber, and X_4 is calculated TDN required. Of the variables, only the negative value for crude protein would appear to require explanation. This value probably reflects the intake problem frequently encountered on high-protein silages due to compounds formed during fermentation. As yet, this factor is not understood, although frequently encountered (1). In terms of a 1,200-lb cow producing 50 lb of 4% milk, the formula estimates a daily intake of 17 lb TDN on an early-cut forage and 13 lb on a mature silage. This mean value and the resulting grain requirement fit well with data reported from this and other experiment stations (3, 4). Despite the excellent success of the formula in accounting for variations in intake, the formula is suggested only as a basis for developing similar information in specific

TABLE 1

Influence of variables on silage dry matter intake (Y_1) and nutritive value index (Y_2)

Variable	Dry matter intake (Y_1)	N.V.I. of silage (Y_2)
Constants		
No preservative	......	−11.110*
100 lb per ton	......	4.433*
200 lb per ton	......	6.677*
Net Partial Regressions		
Crude fiber of silage (%)	......	.784
Silage dry matter (%)	......	2.545**
Dry matter loss in silo (%)	......	.724*
Dry matter digestibility (%)	.209**	......
Crude protein of silage (%)	−.477**	......
Crude fiber of silage (%)	−.239**	......
Calculated TDN required (%)	.346**	......
R^2	.928	.731
N	34	29

* Significant at the 5% level.
** Significant at the 1% level.

feeding situations and not as a universal method for calculating intake.

In addition to the analysis of factors influencing intake, data from 29 direct-cut silages were studied for the factors influencing nutritive value index (NVI) (2). The results shown in Table 1 are of interest in evaluating the relative influence of several items on the feeding value of silage. The influence of adding 100 lb of a feed such as ground snap corn or citrus pulp per ton of silage is of particular interest. The use of this rate of additive increased the NVI by 15.5 points, an equivalent influence to increasing silage dry matter by 6.2 percentage points. The fact that adding a second 100 lb resulted in only a 6.7-point increase in NVI indicates that the effect of feed additives is nutritional as well as merely adding dry matter to the silage. The significant effect of dry matter loss in the silo is of interest and indicates the importance of keeping this factor at a minimum. The relative influence of adding 100 lb of grain, vs. the loss of dry matter in the silo, indicates that many of the effects of loss of dry matter can be overcome by additives.

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SYMPOSIUM: Changes Which May Be Forced Upon Us
by Trends in College Enrollment[1]

PROBLEMS FACING COLLEGE ADMINISTRATIONS

A. E. Darlow

Vice-President in Charge of Agricultural Sciences
Oklahoma State University, Stillwater

I have been asked to discuss college administration problems, problems brought about by increased enrollments and relatively decreased appropriations.

I know that people in dairying are concerned with these problems, also, but it has been my impression that we in departments have sometimes resisted change, at least changes of major proportion. Too, college and university administrations at times may not have moved rapidly enough to meet changing conditions. So, in some instances, changes in individual institutions have been originated by boards of regents, or by state legislatures.

I believe that if we're going to continue to be the leaders in agricultural education, and in educational policy in agriculture, we must lead. At Oklahoma State University, many of the changes we in agriculture have made were as a result of pressure from the administration, changes that should have been made within the structure of individual departments. It is encouraging indeed that you in dairying are taking a close look at the situation as it applies to your field. If each department, in each college of agriculture, will do likewise, perhaps we can lead and stay ahead of the layman in the changes that are made.

General figures of enrollment prospects are useful to state governments, to university administrations and, up to a point, to colleges of agriculture. But always, regardless of the size of the general increase, there will be areas within universities that will have few students and will not share in the increased enrollment.

The consensus is that state-supported college and university enrollments will very likely increase more rapidly than will total enrollments in all institutions of higher learning. Many private institutions restrict enrollment, but should this policy be changed sufficient resources are not available to expand services at the rate the student body in the entire United States is expanding.

As a result, state-supported institutions will have an extra-heavy load. Do the states have sufficient resources for this tremendous, gigantic educational responsibility and, if so, how will these resources be used?

At Oklahoma State University, shortages in faculty and in physical facilities, particularly

laboratories, have been our main problems. If there is any shortage of classrooms, it is because our classroom space is distributed among rooms of sizes that do not quite fit our present enrollment and scheduling practices.

There is some discussion these days of a trimester plan of study which we believe would result in no more efficient use of our facilities than at present. Many institutions, including our own, have Saturday morning classes, but we believe students, faculty, and parents of students would look with disfavor on full-day Saturday classes.

It is disconcerting that among the departments which represent the least important areas of agriculture we find departments whose instruction is by far the most expensive.

It is important that we take a close look at the number and kinds of courses we offer, with the aim of bringing our curriculum more nearly in line with training needed by our graduates and of lowering costs if possible.

Pressure to offer undergraduate courses with less than ten students will continue and will increase. If we persist in offering such high-cost courses we are, in a very real sense, asking the entire staff to subsidize the low-enrollment, high-cost areas.

Low-enrollment departments on the Oklahoma State University campus have made two adjustments to lower costs. All departments have revised their curricula, and in many of the upper-division classes a sequence of upper-division courses is offered in alternate years. Fortunately for us in agriculture, qualified men can be transferred from teaching into needed areas of research.

We've also increased the size of freshman and sophomore sections in those departments with large enrollments.

It is disconcerting that these changes in individual departments have resulted from pressure by the Dean's office, and by the university administration itself. These changes did not originate in the departments.

If we in colleges of agriculture can consolidate courses, offer courses in alternate years, and make other adjustments, we will strengthen some of our weak spots. Then, when the pressure comes and the question of interuniversity cooperation to lower teaching costs is raised, we shall be better able to answer that question realistically.

Such arrangements are now in effect. Maine and Vermont have an exchange program in

forestry and dairying. Dairy technology can be studied at Vermont or Massachusetts by students from Maine or New Hampshire or Rhode Island by the payment of in-state tuition. I understand that Massachusetts is the accepted school of landscape architecture for all of New England. Massachusetts and Maine have an exchange plan that involves food technology and agricultural engineering students. And, among Southern states, there is a working arrangement for training of students in dentistry and in veterinary medicine.

The details are not important. What I am trying to call to your attention is that there are interstate and regional arrangements whereby students may transfer to another state without financial penalty and with the hope of increasing their educational responsibilities.

I rather feel that college and university administrations should explore these possibilities, particularly in those areas where cost of instruction is very high.

Let us raise a few questions about dairying.

Just what is required in a dairy herd to teach dairy judging and dairy production? This is, of course, aside from research work. Four breeds of dairy cattle, in sufficient numbers to justify a breeding program, can hardly be justified for the purpose of teaching cattle judging to freshmen or sophomores, particularly if the course has only 20 to 30 students, and for the purpose of teaching five to eight dairy majors.

I raise serious questions as to what is required for teaching dairy processing. Must we have a costly dairy manufacturing plant, particularly since it is difficult, if not impossible, for the average university to keep its processing machinery current with that being used in commercial plants?

One possible alternative is an arrangement for apprenticing dairy students to one of the university's graduates who is a successful creamery operator. If this can not be done during the regular school year, perhaps the students could arrange to get summer employment at one of the very successful, up-to-date creameries. Perhaps these majors could be thoroughly trained in the academic areas of dairy processing, and after graduation get their practical experience in a training program where they are employed.

My approach to teaching is not entirely on a "what does it cost?" basis, but I believe we should never forget that we do not operate in a vacuum. Agriculture is not isolated from other segments of the university, nor is the university isolated from the general public. Whether we will it or not, we must make certain adjustments and justifications from time to time. This we must do in order to continue an on-going program.

I realize, as you do, that we must insist on a good general education. May I quote from an article by Dr. Stanley Musgrave, head of the dairy department at Oklahoma State: "It could be stated that research funds, both public and private, have not been pumped into specialized areas on an equal basis. State, federal, and private grants have been employed in the areas of expected greatest returns. As these interests increase, we should take special care to see that these special interests do not cause us to let our basic responsibility, that of good teaching at all levels, in all areas, be sidetracked."

We are preparing to celebrate the 100th anniversary of the establishment of the land-grant college system in the United States. As we look back over the past century, we see what innovations have been accomplished. My plea to you is not that we go home and say that no course that costs more than a certain number of dollars per credit hour will be offered, but that we take a new look, a fresh look, at what we are doing. Let us keep this entire land-grant concept, particularly the agricultural part of it, alive and moving. If we are content to stay where we are, if we resist change, then whatever is moving will prevail and it will not be agriculture. If all of us together keep working at this job, agriculture will continue to be in the forefront of our economy and, I may even say, of our educational system.

CHANGES IN DAIRY MANUFACTURING EDUCATION

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The Education Committee is responsible for watching developments in the field of education, and for calling the attention of the Association to changes of major importance. Your Committee has been concerned by predictions of an enormous increase in college enrollment in the next few years, and by the possible effect that such an increase may have upon our own teaching programs. In my own state, for example, the state university is preparing to expand from 42,000 students to 145,000 within a decade. In many states, college enrollment

is expected to double. It seemed to your Committee that this flood of students will strain the financial resources of all our colleges. Much more money will be needed for teaching staff, for equipment, and for floor space. We must soon expect very tight budgets and intense competition between departments for financial support. There seems to be stormy weather ahead.

Under these conditions, your Committee thought it wise to seek a long-range weather forecast for the educational world. Dean Darlow was asked to give us that. Then, it seemed wise for us to examine our own houses, to see how well we are prepared for stormy weather. If we are vulnerable, if we have loose shingles on our roof, we should take steps at once to fasten them down. If we may be flooded by high water, we should look now for safer ground, and make plans to move in an orderly fashion whenever a move becomes necessary.

It is not the responsibility of the Education Committee to provide answers, but it is our responsibility to bring problems to the attention of the Association. Professor Hyatt of North Carolina will discuss the problem from the viewpoint of Dairy Production. I agreed to talk about the possible effects of this flood of students upon education in Dairy Manufacturing.

The preparation of this talk was very difficult. What I have to say represents only one man's opinion. Should that fact be revealed by the use of the singular pronoun, I, or concealed by the use of the editorial we? The question arises because these spoken words will probably appear in print.

We argued this question among myself. I I told us that if we were singular, the Editor would blue-pencil me and write us. On the other hand, we told me that our voice was singular; there can be no double-talking before this assembly. All can see that we are not plural. I reminded ourselves that on a former occasion the Editor had multiplied us by some factor to make me seem more important than we are. We argued among myself for a long time. Finally, I persuaded us to let the Editor do his darndest. If I look like we, you have had more than milk to drink.

Dean Darlow has given us a general forecast of weather conditions in the educational world during the next few years. We must all interpret it in terms of our own local situation. A forecast of a cold wave does not have quite the same meaning in Wisconsin that it does in Florida, but it should be taken seriously in both places. Likewise, you must interpret what I have to say in terms of your own local circumstances, remembering, also, that I give you only one man's opinion.

As I see it, Departments of Dairy Manufacturing live in three houses: Teaching, Extension, and Research. All three are parts of one homestead, but it is quite possible that one house might be blown away without disturbing the others.

We must consider the danger of each house separately. Research and Extension should be in little danger from the flood of students. They are organized to serve the needs of the dairy industry and the general public, not the needs of a changing student body. The importance of Research and Extension is increasing rather than diminishing. As processing operations and machinery become more complex, there is an increasing demand for technical aid and advice from the colleges. The cost of these services is not a part of the teaching budget and they should escape most of the economic pressure which will fall on the teaching program.

We should be much more concerned about the future of our teaching program. The flood of students will greatly increase the total cost of teaching. Each item of that cost will be scrutinized, to see where cuts can be made. We should begin now to examine the efficiency of our own teaching operation, and to improve it wherever we can.

Are we efficient? If we are not, what can we do to increase our efficiency? These are the questions I bring to you.

To guide our thinking, I sent a questionnaire to the agricultural colleges of 50 states. I received 44 replies which deserve careful attention. I asked for an opinion: How many graduates are needed per year to justify the cost of teaching specialized programs in Dairy Manufacturing? There was a very wide difference of opinion. The estimates ranged from four per year to 25. The average estimate was 11.5. However, the average number who did complete these specialized programs during the past three years was only 5.4; less than half the number considered necessary to justify the existence of these courses. By their own estimates, only seven of these who replied had enough students to justify specialized courses in Dairy Manufacturing.

A few days ago, I received the results of a questionnaire sent to dairy departments by Professor E. O. Anderson. He asked how many students would receive their Bachelor's degree in Dairy Manufacturing this June. Thirty-three colleges expected to give such degrees, but 19 will graduate three or less. Clearly, the teaching of Dairy Manufacturing is inefficient if classes are so very small.

If instruction in Dairy Manufacturing were a small operation, it might escape the notice of college administrators, but it is not a small operation. I asked, how many hours of instruction were offered in courses designed specifically for students majoring in Dairy Manufacturing? Some states, such as Hawaii, do not offer such training. Those that do reported from 16 semester hours (or their equivalent in

quarter hours) up to 50. One state reported 87 semester hours of specialized courses, but that seems incredible; I think that there is some misunderstanding. The average number of semester credit hours in courses designed specifically for Dairy Manufacturing majors was 29. It seems clear that our teaching programs are not only inefficient; they are inefficient on a large scale.

That is the situation today. Is it likely to grow better or worse? To answer that question, we need a crystal ball of the very largest size, and of the highest quality. Such balls are very scarce, and I found a great difference of opinion among those who answered my questionnaire. Seventeen of those who replied thought that our classes would grow larger; 14 thought they would grow smaller. Seventeen thought that it would become easier to get money for Dairy Manufacturing when our colleges are flooded with students; but 12 believed it would be more difficult.

I do not believe that we can expect a general increase in the size of our Dairy Manufacturing classes. They have been growing smaller for some time. Years ago, many students chose manufacturing courses as electives; sometimes because there were few other courses available, sometimes because they had a real interest in milk. But times have changed, and I do not believe that time will turn backward. Now, fewer students from other departments choose manufacturing courses as electives. The number of alternative courses has increased. Furthermore, as our courses have become more and more technical, and the number of prerequisites has increased, it has become more difficult for nondairy majors to take these specialized courses as electives.

We should note, also, that the character of our student body is changing. The farm boy of yesteryear was familiar with milk and appreciated its importance. It was natural that some of them should elect courses in milk processing, even though their major interest was elsewhere. But students of today come increasingly from urban areas; and they have little knowledge of, or interest in milk. This is shown by our experience at Cornell. During the past 25 years, the number of students in the general introductory course has dropped from more than 200 per year to about 50. Our college now offers training in 55 fields of specialization. As the number of competing programs increases, we must expect our own classes to become smaller.

I see no reason to believe that these trends will reverse themselves, and that enrollment in manufacturing will increase appreciably. It can only increase if the number of manufacturing majors increases, and I consider that unlikely. The number of dairy plants is decreasing. The number of employees in dairy plants is decreasing as operations are mech-

anized. It is true that these larger plants need better-trained men, but they are hiring chemists, engineers, bacteriologists, and accountants, as well as dairymen. Some dairy departments are seeking to train these other specialists, also; but I do not believe that we can improve our teaching efficiency by greater diversification of our product. The reverse is likely to be true.

Some departments have tried to strengthen themselves by becoming departments of dairy and food science. Replies to my questionnaire show that eight institutions have made such a change, and ten others expect a change in the next decade.

This consolidation will bring some economies. You can get rid of one department head, for example, and that may be very desirable. However, the consolidation of departments does not increase teaching efficiency unless the total number of class hours is reduced by combining two courses into one. In general, that has not happened. A large majority of those who answered my question said that instruction adequate for the needs of the dairy industry could not be given if courses in milk processing were combined with courses in other foods. We must seek other ways to increase the average size of our classes.

It is possible to increase the size of classes by offering courses only in alternate years. Some departments are doing that now. I fear, however, that those who teach only in alternate years will find it difficult to maintain the intense interest in their course that is needed to teach it well. If our courses are not well-taught, the enrollment may decline still more.

I have painted a dreary picture, but I think we should face it. Our teaching operations in Dairy Manufacturing are inefficient because we have far more teaching capacity in this country than we need, or are likely to need. Nearly every state has thought it necessary to offer instruction in the subject. That is nonsense. It is an extravagance than many states can not afford much longer.

We have too many men teaching very small classes. What can we do about it? The New England states are trying one solution. By interstate agreement, they have decided that each state should not teach every subject. Students in Maine, New Hampshire, and Rhode Island who wish to major in Dairy Manufacturing are sent to Vermont or Massachusetts. Students from Vermont are sent to Maine to study forestry, etc. Under their cooperative plan, these students do not pay out of state tuition.

If adopted generally, such interstate cooperation to concentrate the teaching of Dairy Manufacturing in a few states might increase our teaching efficiency a great deal, and release many men now teaching very small classes for more profitable work in extension and in research.

In my questionnaire, I asked if they thought a single dairy department, supported cooperatively by several states, could do a better job of teaching, at substantially less cost, than was being done by the separate states under our present system. Thirty-three said yes. Only seven said no. The opinion was nearly five to one that a single department supported by the joint effort of several states could do a better job, at less cost.

I asked if they thought such a program would impose much hardship upon those students who must go to a neighboring state for training. Twenty-seven said no; only nine said yes. I asked if any cooperative plan had been considered at their institution. Eight states, outside of New England, said that it had been discussed. How seriously it had been discussed, I do not know.

I believe that many states will have to consider such a plan in the years ahead. I do not believe we can afford the luxury of very small classes much longer. It makes economic sense to combine very small classes into larger ones, if we can. We must not curtail the subject matter taught; we must not deprive the student of an opportunity to major in Dairy Manufacturing; but I believe we can reduce the number of men required to teach, with no loss of effectiveness.

There will be many difficult problems to solve, but economic necessity is a very powerful solvent. We should plan now for adjustments, and not wait until they are forced upon us.

The cooperative plan has not worked as well in New England as some had expected, and we should profit by their experience. The students are supposed to spend the first two years in their home states in basic studies before going elsewhere for specialized training. However, after spending two years in one school, making many friends, perhaps joining a fraternity, the students are reluctant to leave. Many change their major rather than move to another school. That is human nature, and we should take it into account in any plans we make. Students who must go to another state should go as freshmen, or else wait until their four years of undergraduate work is finished. Perhaps Dairy Manufacturing should be taught only at the graduate level. Alternately, the Dairy Manufacturing curriculum might become a five-year program leading to a special baccalaureate degree. Five-year programs are not uncommon in engineering schools.

It is not my purpose to answer these questions, but to provoke discussion. We who are responsible for teaching have the added responsibility of seeing that it is done efficiently and well. Most of the decisions must be made by administration, but we who teach should be prepared with plans which are sound economically and educationally. Unless we plan carefully, it is possible that both extension and research may be swept away along with some of our inefficient teaching programs. We should prepare now, before the storm breaks, or we may all be swept away at a time when we can do little about it.

CHANGES IN DAIRY PRODUCTION EDUCATION

GEORGE HYATT, JR.

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It has been readily recognized by the public that science per se has made great strides in recent years; however, agriculture has not always been generally closely identified with other advances. An analysis has revealed the view that a new concept of agriculture is emerging. In this new concept, agriculture is defined to include three important segments of our economy. The first segment includes the farmers themselves, engaged in the production of crops and livestock. The second segment includes those industries which furnish supplies and service to agriculture. The third segment includes those industries which process, store, handle, and merchandise the products of agriculture. Taken together, these three groups employ approximately 37% of the total employed people in the United States. These changes in what we know as modern agriculture, coupled with the skyrocketing enrollments within our land-grant institutions, are giving rise to complex and new problems and opportunities. From this general background emerges several basic questions relating to dairy production education.

(1) Where does dairy production training, as currently presented, fit into the picture?

(2) Is there going to be a need for dairy production education in the future?

(3) If training is continued, what changes in emphasis are indicated?

In suggesting answers to these and other questions, it is well to remember that conditions in this great empire of ours vary greatly; therefore, what may be the answer to the problem in Maine may not be the solution in New Mexico. In some states there are six to eight institutions offering the full four-year course in agricultural training. This places the land-grant institution in a much different position than in states where only one institution is involved. Moreover, the industry is much more developed in some states than it is in others, making wide differences in training needs. At such a gathering as this, it is possible to talk only in terms of generalities.

Let us begin our discussion with Question No. (1). Certainly developments in industry and on the farm have revealed the obsolescence of conventional dairy production training. Science stimulation and training in secondary schools have raised the challenge threshold; hence, conventional dairy production offerings present little appeal.

One of the most encouraging developments of recent years has been the willingness of distinguished scholars and eminent scientists to give up for a time their research investigations and devote their best efforts to the problems of education. We see, for example, some of the ablest minds in physics in America (Noble Prize winners among them), members of the faculties of our leading universities, giving their attention to the study of what should be taught in the field of physics in the secondary schools. Working cooperatively with experienced high school teachers for several years, they have produced a totally new and imaginative approach to this discipline. New textbooks, new laboratory experiments and manuals, new guides for teachers, 50 or more motion pictures prepared to demonstrate critical experiments, and more than a hundred paperbacked monographs on specific subjects written by world authorities for the high school student have resulted. Similarly, the mathematicians, chemists, biologists, geologists, and others have developed new and effective treatments of their disciplines for the secondary schools. Never before in the history of education has so much high competence been focused upon the quality of instruction in the sciences.

It is true that much of this effffort has been directed to the secondary schools, but its impact upon college education will be considerable and prompt. We who are responsible for what is taught in colleges and universities must also take bold and imaginative steps to improve the quality of instructional programs. We can no longer afford the luxury of complacency. The cooperation which has had such dramatic results in improving the quality of science instruction in secondary schools can have equally beneficial results at the college and university levels.

On a number of occasions the Provost at the University of North Carolina suggested, facetiously, an exercise that might contribute to the significance of the Commencement activities. The idea was that it might be appropriate to follow the awarding of degrees by a celebration in which all who taught would burn their lecture notes. We all find the preparation of lectures a time-consuming task and, once we have done it well, it is hard to resist the temptation to use the results of our labors a second time.

Colleges and universities have been slow to diagnose the difficulty and more reluctant to use therapy. It seems that many of our institutions lament the continued decreasing enrollments in dairy production departments, but have failed to materially change the attractiveness of the goods offered to those they wish to interest. Professors are usually the first to observe that students and dairymen must change their ways to keep up with this dynamic age, but when it is suggested that one of their courses is obsolete and their teaching methods do not attract and hold student interest, the intelligence of those posing such possibilities is questioned, and there are grave doubts that the holy sanctuary of academic freedom may longer exist. We have had some recent experiences at North Carolina State relative to student enrollment and changes in the curriculum which bear on our subject.

In 1958, I became Head of the Department of Animal Industry at North Carolina State College. Before I was permitted to leave the main administrative office, I was instructed by the Director of Instruction that our costs per student were at such a high level in our department that any vacancies which might occur on the staff involving College funds could not be filled by use of these funds. In other words, we must give up some of our College budget as rapidly as possible. The freshman enrollment in Agriculture, which prior to 1955 had been constantly above 200, had dropped to 89 in 1958. Our whole program faced a most serious crisis. The consequences were, of course, few and mediocre students.

Now, let us examine Question No. (2). Do present trends in the dairy industry and in education indicate a need for dairy production training in the future? The answer to this question must be based on several assumptions. If we assume that dairy production departments exist only to train dairymen returning to farms, the need for such departments will be limited and numbers of students small. If we accept the broader concept of agribusiness, then dairy production training, at least in a modified form, has a much wider usage. Within this broader concept, students will be trained for positions in sales, related services, research, foreign service, and the

numerous other opportunities in the dairy production orbit. Within the broader concept, the future demands a redirected and up-to-date curriculum in dairy production.

The indicated need for talent that can be developed in dairy production education is for basic understanding and appreciation of science, literature, humanities, and so forth, linked to a strong and sustained interest in the "foster mother of the human race."

Our third question, No. (3), should then seek answers to changes in emphasis that are indicated if dairy production training is to be continued. Experience at North Carolina State College and at a few other institutions demonstrates that imaginative, carefully planned, and executed changes do bring about desirable results. It was previously pointed out that the freshman enrollment in Agriculture at North Carolina State, which prior to 1955 had been constantly above 200, had dropped to 89 in 1958. Fortunately, much work and thought had been given the problem. Vast changes in the curriculum and course content had been accomplished and a complete new approach to the recruitment of students had been developed. The school was now able to offer three curricula: agricultural science, agricultural business, and agricultural technology. Students now enrolled in the dairy production department could select either the science, business, or tehnology option for major emphasis.

1. The curriculum in Agricultural Science places much greater emphasis upon science and its applications to agriculture. It provides excellent training for employment opportunities, including research for public institutions and industry. It was developed to help meet the shortage of well-trained scientists.

2. Agricultural Business trains young men in business and agriculture. This program brings into existence a new combination of business, science, and technical agriculture. Men in this curriculum are trained to work in agricultural industries closely related to dairying. There is a great demand for men with the unique combination of training in science, business, and technical agriculture.

3. Agricultural Technology emphasizes applied science and technology. In this curriculum men are trained in dairy production and in the technical processes involved in the industry. There is a shortage of well-trained technologists.

This reorganized approach, emphasizing these three new curricula, is offering many new opportunities to the urban as well as the rural boys of our state. Our freshman enrollment jumped to 111 new students in 1959,

174 in 1960, and the present estimate for fall, 1961, is more than 200. The dairy production department will share in this increase. This new package of goods, combined with a vigorous recruitment program, is interesting many urban and rural youths in this area of training. We can not sit idly by and expect the students to come to us any more. They must be recruited.

I am firmly persuaded that if there is a place for dairy production curriculum within the future educational structure of our siciety, presumably it must be found somewhere between two alternatives. If we continually press fully in the direction of the fundamental principles, we could easily define ourselves out of business, as far as a dairy production or an agricultural curriculum is concerned. On the other hand, if the curriculum does not change with advances in knowledge and with changes in our society, the declining enrollment in the past decade would suggest that we could find ourselves without students. What should be the characteristics of such a curriculum? This is a question I can not answer; however, I do believe the steps taken at North Carolina State College have brought a better blending of the basic sciences, technology, and humanities than was to be found in the dairy curriculum of the past.

In making changes in the curriculum, I hope we won't forget that our enormous industry hinges on the dairy cow. The undergraduate student in dairy production must still learn some husbandry. This word, husbandry, has been abused and maligned in recent years to such an extent that it is often difficult to find a prospective staff member willing to teach dairy husbandry or to conduct research with dairy cows. The training in basic sciences is highly important, but there is still the great need to provide the dairy production student with the necessary professional training to successfully make a beginning in his chosen profession.

The objectives for professional curricula, in general, were well stated by a special committee of the University of California [2] appointed to study how to appraise professional and vocational educations:

(1) To provide the student with the necessary professional training, to successfully make a beginning in his chosen profession.

(2) To provide him with a basic understanding of the place of his profession in, and its relation to, society.

(3) To prepare the student for intellectual, political, and cultural citizenship and leadership.

[2] Proceedings University of California, pp. 28-29. Ninth All-University Faculty Conference. 1952.

(4) To instill in the student a respect and desire for knowledge and truth, which shall continue throughout his lifetime.

In preparing a curriculum to reach these stated objectives, Dr. H. H. Cole, Department of Animal Husbandry, University of California,[3] suggests it would seem essential to do the following:

(1) Give sufficient sciences, in order that students can comprehend biological processes.
(2) Give detailed information on the management of dairy cattle, using management in a broad sense, to include not only knowledge of genetics, physiology, and nutrition of the animal but also the producing of feeds for the animal, and marketing.
(3) Provide sufficient cultural courses to prepare the students for intellectual, political, and cultural citizenship and leadership.

In preparation for my remarks today, I did not plan to spend as much time as I have on curriculum content; however, curriculum content represents the goods offered for sale. It must carry a guarantee of excellence, be modern and attractive, and come alive in the hands of the teacher. The competition is enormous for the small pool of students interested in the various scientific areas. The group that does nothing will degenerate. The proper curriculum put in the hands of good teachers, undergirded by a hard-hitting recruitment program, aimed at high-school principals and counselors will, in many instances, keep dairy production departments open and maintained with adequate budgets.

Now, what about regional schools in the future? I am sure I do not have an answer to this question; however, let us ponder a few possibilities. Would it make economic sense to organize regional associations of state colleges which would consolidate their smaller departments on a regional basis? All students majoring in dairy production might be sent to State X for specialized training; all majoring in another subject might be sent to State Y. Under such a cooperative agreement, students in these special programs should be exempt from out-of-state tuition fees if they were residents of one of the cooperating states.

This arrangement is working exceedingly well in Veterinary Science at North Carolina State College. The College has an arrangement with the University of Georgia and Oklahoma State University to take a certain quota of preveterinary students from North Carolina State each year for their final years

of training. The out-of-state tuition fees for each student are paid by North Carolina State College. Personally, I have been extremely pleased with the arrangement. A Veterinary College is costly to set up and operate. We can, through such a cooperative agreement, mobilize our resources for a better program of preveterinary training and research much more effectively than if our resources were more outspread. Most of the young men trained in this area are returning to the state upon their graduation from the cooperating institutions.

It would appear that it might be difficult to have a strong production department without strong supporting disciplines in other areas. This may mean that proper training in the future can be offered only in the larger colleges.

Such mutual cooperation can be realized; however, it is much easier to initiate in such a manner than to change presently active departments which have served a given state for many years into regional working relationships. Nevertheless, I am convinced that such arrangements must be developed in certain areas of the country or the result will be slow death through attrition for some dairy production departments. Wouldn't it be a whole lot better for those of us interested in perpetuating competent training in this field, to come up with some specific suggestions for cooperative, regional organizations, rather than to slowly disintegrate through lack of budgetary support? This has been talked about for several years in certain areas of the country, but talking is not enough. I believe the only way such moves will become reality is for those most vitally interested in the field of dairy production to develop some plans that can be considered by administrators. The administrators will, no doubt, be forced by public pressures to make changes. Perhaps such changes, when they come, may fit the needs better for dairy production training if we take some initiative to solve our problems.

I would like to make one other observation. You will note from my remarks that I have said nothing about graduate training in the area of dairy production. This omission is not through oversight, but rather so that I might emphasize and re-emphasize one point. The point is, simply, if we don't make changes in the dairy production education at the undergraduate level now, we will not have any graduate students in this area. There is now a great shortage of graduate timber. The situation is indeed critical. We are failing to replace the supply of well-trained dairy scientists and to have a backlog of these scientists for the ever-expanding needs of educational institutions, governmental agencies, foreign service, and the food industries. Since nearly all graduate training has become highly

[3] Journal of Dairy Science, Vol. 40, p. 1370. 1957.

specialized in a functional area, seemingly the commodity element must be planted at the undergraduate level, to establish a real and continuing anchorage to the dairy cow.

CONCLUSIONS

There are two primary goals to which change must be directed:

(1) Quality training harmonious with changes in the field of science.

(2) Effective selling of opportunities.

If we fail to vigorously recruit bright young men, and condition and develop their minds through contact with outstanding teachers, who draw on subject matter contained in a modern, dynamic, purposeful dairy production curriculum, designed to prepare the student for occupation and improvement of the world in which we live, we will soon be out of business.

SYMPOSIUM: Problems in Dairy Cattle Identification[1]

IMPORTANCE OF PROPER IDENTIFICATION TO THE DHIA SUPERVISORS

KEITH FINCH
D.H.I. Supervisor
Winnebago, Wisconsin

1. The first question a supervisor should ask is, "How can I best serve my DHI member?"

   One of the best ways a supervisor can serve his DHIA members is in the area of animal identification. Complete information (especially identification) helps the supervisor in providing the DHIA member with a useful book at the start of his testing program, and is used as basic information for selecting breeding stock and culling poor cow families. Consequently, it is important to teach the principle that each cow is different and that, therefore, correct appraisal of each cow depends upon individual identity. In some cases, an entirely new herd identification system will need to be encouraged, so that the dairyman knows his individual cows. (Systems which label daughters with same number as dam often lead to confusion.)

2. What are some of the problems of early identification for the supervisor?

   The first problem is to get the member to identify his calves by some method soon after they are born. A good method of individual stalls is to put information on each calf on a card on the stall, so that the supervisor can easily identify the calf. Some use small neck chains with a number code referring to the dam. These chains can be used over and over again as soon as calves are permanently identified.

3. What method of early identification is

most widely used by our DHI Standard and Owner-Sampler members in Winnebago County?

   Supervisors attempt to ear-tag all calves raised in both Standard and Owner-Sampler herds. New calves are tagged and identification records are kept every month as the supervisor calls at the farm.

4. Are there other methods of early identification used in our county?

   Where ear-tagging becomes a problem, sketching is used, and is practiced extensively in several grade herds in addition to the registered herds. A few of our members have taken snapshots or slides of the calves for future records. Tattooing is done on offspring in certain breeds of cattle where it is necessary for registration. Some small neck chains are used on calves until ear-tagging or sketching can be done.

5. Why should a supervisor have positive identification for all cows in the milking herd?

   It is necessary to have positive identification, so each cow can receive her complete credits and not the production of another cow that may look like her, even if she is a twin. This gives additional reliability to standard and official records. A system needs to be worked out that is fast and relatively easy. A good system greatly aids the supervisor in his work.

6. How are neck chains used as identification?

   A neck chain (sealed for Standard DHIA and official herds) is used in large herds, or herds in a milking parlor or pen-type barn. The numbers should be easy to read and on both sides of

the tag, and of a material that won't break or crack in cold weather. This neck chain and number can be permanently assigned to this animal as long as it is in the herd. It will also aid hired help that may not know the cows, to record information necessary for the owner or herdsman.

7. What method of identification is used in herds on official test?

Herds on official test have registration papers with sketches or tattoo numbers. The papers should be in order and in a handy binder, to facilitate rapid and accurate use. If a permanent neck chain is used, the number can be written on the border of the registration paper, to facilitate rapid location. Numbering the cow with marking-crayon can also help to locate registration papers more rapidly. One difficulty I encounter in my largest herd on DHIA is having an individual envelope with a folded registration paper in each one. It takes a lot of time to properly identify this herd.

8. How can supplementary identification be of value to the members?

Some supplementary identifications that are of value and greatly add to knowledge of the herd by the owner and others, is the use of some type of name plate and production information. This is convenient for the owner, as well as for buyers and visitors, if it is current. The use of cow names is better than numbers, unless the numbers are a permanent

number for that cow. The use of stall numbers is confusing and is not reliable for identification use.

9. What is the need and value of tagging and to what extent is it used in our county?

An Owner-Sampler member, as well as the Standard member, needs complete information to improve his herd, and will be a more permanent member than those who have records inadequately identified. It is through positive identification that a member becomes interested and progresses in building his herd. We attempt to tag all calves, if for no other reason than to provide the member with records.

10. How does positive identification promote sales?

Good records with positive identification information nearly always add to the sale of animals. The buyer has more confidence in the breeder and his animals when this information is in order. A new Blue Tag calf law in Wisconsin is designed to further improve the sales value of animals identified in tested herds.

11. What are some problems of adequate identification by the supervisor?

The problem of adequate identification by the supervisor is helping dairymen understand the real value of proper identification and time involved, if a convenient system is not used. Above all is the loss of valuable information which a dairyman needs to build a high-producing herd of cattle.

IMPORTANCE OF PROPER IDENTIFICATION TO THE BREED ASSOCIATIONS

CHARLES H. BOHL

American Jersey Cattle Club, Columbus, Ohio

Positive identification is the basis of all Purebred Societies today. All breed programs such as registration, testing, showing, sales, and classification are founded on positive identification. We have always thought of positive identification as being able to identify the animal by means of tattoos or color descriptions with a Registration Certificate. If the animal checked with the certificate, this was positive identification, because the Breed Association had a complete record of the sire, dam, correct registration number, and date of birth. The full registration names were reported, so that cross-checks could be complete.

Today we find ourselves in a new world, a world of electronics where reports and sta-

tistical information can be completed in days or hours, that formerly required months. With progress there are some drawbacks, as only registration numbers can be reported because of limited space. A mistake in copying a number can change the entire identity of an animal.

In this new era we find that positive identification not only means checking the animal against the Registration Certificate; it necessitates accuracy in reporting the registration number, the sire, the dam, and the date of birth.

Studies completed by all breeds show that 11.5% of all DHIR records received are incorrect, either in registration number, sire

number, dam number, or date of birth. This means that if there was no checking, cows could be reported wrong, sires' averages would include wrong daughters, daughter-dam comparisons would be inaccurate, and 11.5% of the information reported from the Breed Associations and Washington could be incorrect. Any program that has 11.5% error would be difficult to sell to breeders and research men.

The Breed Associations are greatly concerned over the large per cent of error being encountered. It is imperative that this situation be corrected immediately. Those persons assuming the responsibility for the DHIA, DHIR, and HIR programs in each state must have the responsibility to see that cows are correctly identified and the information correctly reported.

PROCEDURES AND METHODS USED IN IDENTIFYING
COWS IN LARGE HERDS

C. L. PELISSIER
Agricultural Extension, University of California, Davis

Identification in large herds is difficult, but not a serious problem in DHIA. It is less of a problem with large herds than with medium-sized or small herds. Why? Because in large herds reliable identification is recognized as a necessity for proper management. Therefore, the major responsibility for identification is assumed by the herd manager. In general, the DHIA supervisor receives excellent cooperation and this is a major factor influencing accuracy. Cooperation is excellent because reliable identification is necessary for proper feeding, culling, breeding and, perhaps even more important, for breeding efficiency control and business management.

IDENTIFICATION SYSTEMS

Eartags are still the most common means of identifying cows in our dairy herds, but they are not foolproof. For reliable identification the tag should be as permanent as possible. This requirement governs the choice of tags in most cases. The numbers should be large, so that they can be easily and accurately read. Many dairymen double-tag their cows, so that if the tag in one ear is lost a new one can be stamped and inserted before the second tag is lost. These requirements of a reliable tagging program eliminate the possibility of using DHIA uniform series tags for herd management purposes.

Number brands provide commercial dairymen with a positive and reliable identification system. If the branding is done properly, and the hair around the brand is clipped occasionally, this numbering system is satisfactory and quite accurate.

Neck chains are being used more and more. Though they are costly, the dairymen like them because the number tags can be read easily without confining the cow. Immediate reassignment of neck-chain numbers from culled cows can confuse records, but a six-month reserve of number tags helps to eliminate this problem.

Marking crayons are widely used by DHIA supervisors for supplementary identification on test day, particularly in the conventional stanchion barns used in California. Bold numbers on the rump are easily read and reduce identification errors, particularly when eartags are used for permanent identification in the herd. Variations in order of milking are not a problem when these temporary numbers are used. Crayon numbers must be cross-indexed on the supervisor's barn sheet with the eartag number. To reduce disturbances, this can be done after a string of cows has been milked.

IDENTIFICATION RECORDS

A good, reliable, and permanent individual cow record is a valuable aid to identification. Such records are more prevalent in large herds because they are a management necessity. These records are more likely to be kept up to date in large herds and this increases their reliability. Index cards are extensively used for this purpose in large herds and are readily available from several sources. A few dairymen prefer to have record cards printed to fit their specific needs.

The use of these cards for herd management is facilitated by colored tabs that indicate the cow's status. Cows to be bred, turned dry, due to calf, etc., can be flagged with these colored tabs, so that they will not be overlooked.

CALVES

Some of our dairymen do not attempt to identify the calf with the dam and sire for proving sires. They leave the responsibility of providing good sires to the stud or purebred breeder of their choice. Herd size is not an

important factor in this matter. It is difficult to criticize this attitude when the excellent results in so many large herds are considered. Accurate calf identification requires prompt action when a calf is born. This is no more difficult in large herds than in small herds, and negligence is not as likely. We have no unique procedures in this regard; our methods are rather standard.

SUMMARY

It is not my intent to leave you with the impression that we are doing a perfect job of identification. Nevertheless, we are less concerned with the accuracy of identification in large herds than in others. Effective identification, like public relations, is not something that is accomplished once and for all; it must be worked at constantly.

PROCEDURES AND METHODS USED TO IDENTIFY COWS IN FARM HERDS

J. D. BURKE

Department of Animal Husbandry, Cornell University, Ithaca, New York

Positive and complete identification of all animals on test is essential if the National Cooperative Dairy Herd Improvement Program is to fulfill its purpose. Let's look at the problems from the standpoint of the dairymen, the DHIA supervisor, the Dairy Records Processing Laboratories, and the Breed Associations.

First, take the dairymen's problem. A calf is born. Chances are that it is placed in a pen with several other calves. The question now is, which calf is which? When was each calf born? Who were the sire and dam? The only logical answer is to register the calf immediately after birth if purebred, or to attach a number to the calf and record the essential information in a herd record book. This is simply good herd management, whether or not the herd is on test. These numbers can be tattoos, neck chains, or straps, ear tags, or registration numbers. Recorded pictures or a sketch of color markings also can be used as temporary identification. The important point is that the animal be positively identified as quickly as possible after birth.

Next, let's look at the DHIA supervisor's problem. He must know without question which cows he is testing. Obviously, a number that is easily seen from behind the cow or from the milking parlor is best. Neck chains or straps, ankle straps, or hip brands are first choice. Ear tags and registration certificates work well in stanchions if they can be supplemented with stall cards or cattle-marking crayons.

The Dairy Records Processing Laboratory must have positive identification numbers that can be handled on punch cards or tape. Registration numbers or the uniform series DHIA, AB, or Disease Control tags (two-digit state code, three-letter prefix, four-digit, i.e., 21-WAB-1234) are the only permanent identifications that will work. In addition, a cow index number (four digits) is assigned to each cow in the herd to keep cows and records in

proper sequence. This number is usually assigned by the Dairy Records Processing Laboratories.

The respective Breed Associations, likewise, need and demand positive identification, date of birth, sire and dam. Disease control officials also are interested and need positive and permanent animal identification.

There will be no problems if we understand the requirements of each program and work toward a coordinated identification system that will meet the needs of all concerned. This will include the following steps:

1. Ear-tag calves within 30 days (first test after birth) with uniform series tags.
2. Tattoo Jerseys and Brown Swiss immediately after birth.
3. Register purebreds as soon as possible, preferably before 6 mo. of age.
4. Transfer sold cows on date of sale. Provide buyer with transcript of identification, breeding record, and lactation to date.
5. Record all identification data in herd book.
6. Provide neck chains or strap number, ankle strap, or stall cards for quick supplemental identification.

It is best if the last three digits of the ear tag, tattoo, neck chain, or ankle number are the same as the central processing cow index number. These numbers also can be used to identify sample jars. This coordination requires:

1. The assignment to a herd, a block of ear tags to be used in sequence.
2. That the neck chain or strap numbers must match the last three digits of the ear tag and tattoo number.
3. That the DHIA supervisor and herd owner assign cow index numbers to match chain or strap numbers without duplication.
4. That a complete herd register be maintained by the DHIA supervisor.

OUR INDUSTRY TODAY

ECONOMIC ANALYSIS AND OBSERVATIONS OF AUTOMATED CLEANING IN SMALL PLANTS

S. J. CAVALLARO

Department of Agricultural and Food Economics, University of Massachusetts, Amherst

Since hand cleaning and setup operations may require up to 50% of the work-day in many small plants, and are unproductive in terms of labor and equipment usage, management is interested in a means to make more efficient use of labor and equipment and to improve sanitation as well. Automated CIP appeared to offer some possibilities of achieving these features.

A portable automated CIP unit was developed by the Department of Dairy and Animal Science, University of Massachusetts. The cost was $2,500. This unit, equipped with time, temperature, liquid level, solution flow, and pump controls, was installed and operated in four Massachusetts dairy plants ranging in size from a volume of 6,000 qt. to 17,000 qt. of milk processed daily. A preliminary survey was made in each plant to determine the usual plant operation as to scheduling, cleaning labor, and potential CIP installation. The CIP equipment was then installed and cleaning circuits and an operating schedule were developed. Instruction was given on the operation of the equipment and, after a period of operation, a second survey made to determine changes in plant operation due to CIP equipment. In two of the plants, automated CIP appeared to be feasible. (See Table 1, Costs and Savings Bases in Plants B and D.)

Both of the indicated time savings can be considered as a minimum. In Plant B, where circumstances allowed two surveys to be conducted, one after 5 wk. and the second after 15 wk. of operation, the first survey indicated a savings of 13 min. per cleanup man per day and the second indicated an increase to a total of 24 min. per cleanup man per day. As the plant men become more familiar with operating the equipment and adjusting their work habits, and as plant volume increases over a period of time so that the men gradually become more productive, the full benefits of automated CIP will be realized. It is not reasonable to expect a new system such as automated CIP to be operated at the most efficient level immediately. Furthermore, it must be pointed out that time savings can not be capitalized unless overtime is reduced, a man eliminated from the work force, or the time used more productively.

In Plant B, where there was a manager and only four plant men, the only current savings could be in a reduction of overtime. It would be impossible to eliminate a man. At Plant D, where there usually were 12 full-time and two part-time men, no immediate labor savings appeared possible. However, since the analysis was made, one man has retired and will not be replaced and another man is now able to spend some time on maintenance. The general manager could not determine how much of this savings in plant labor was due to in-place cleaning, but he felt that the use of CIP equipment was a contributing factor. This additional savings in labor is only slightly represented in Table 1.

Added expenses. An increased cleaner expense was noted in all plants due to CIP. Since the wash tank was still used for some pipeline and equipment in each plant, the only decrease in hand-cleaner cost was in Plant D, which could eliminate one of two tanks of washing solution. This resulted in a savings of about $67 per year. However, CIP cleaner costs were increased in all plants, due to the larger amount of cleaners needed for the larger systems CIP cleaned. The capacity of the makeup tank and the volume of the additional pipeline must also be added to any system previously cleaned in place.

An increase of $10 per month was estimated to cover any increase in water, steam, and power costs. There was a definite increase in the consumption of water estimated at $1 a month. Increased power and steam costs were not measured, but it appears that a total of $10 a month is reasonable when the added stress on the steam-generating equipment is considered.

Investment and operating costs of CIP equipment. The demonstration unit used in this study was built at an estimated cost of $2,500. Each

TABLE 1
Costs and savings bases in Plants B and D

	Plant B	Plant D
Labor per hour	$1.45	$1.80
Hours saved daily	1.62	2.22
Equipment cost	$1,600	$1,700
Yearly depreciation (salvage value after 3 yr., $500)	$ 367	$ 400
Yearly repairs, taxes, insurance, etc. (use cost)	$ 80	$ 85
Yearly additional cleaner expense	$ 132	$ 183
Yearly power and water (estimate, $10 per month)	$ 120	$ 120
Summary		
Labor savings (52 six-day weeks)	$ 733	
Labor savings (52 five-day weeks)		$1,040
Total additional costs	$ 699	$ 788
Net annual savings (minimum)	$ 34	$ 252

plant has since installed equipment based on the demonstration unit, but at a lower cost. At Plant B, the cost was estimated at $1,200 and at Plant D, $1,600. An additional investment in pipeline was necessary to complete the cleaning circuits—about $400 in Plant B, which had to purchase most of the extra pipe and fittings needed, and $100 in Plant D, which had a large supply in stock. The cost will vary considerably with the individual dairy plant, depending on the length of pipeline circuits. Another cost to be considered is installation of CIP equipment. Utilities must be brought to the equipment and the time spent designing and laying out the circuits could be included. No attempt was made to determine these costs.

The total annual cost to make an investment in CIP equipment was determined, to help management decide whether to make the additional investment. This cost was based on depreciation and a use cost (see Cost Summary Table).

This analysis was made using a conservative estimate of the amount of time saved and the installation of a minimum of equipment. While automated CIP was feasible in Plants B and D, it is conceivable that many dairies will not be able to construct a unit at a similar cost, since outside labor is needed. However, some plants will be able to devise a less expensive cleaning unit if certain parts are available. The greatest expense is for an adequate-sized pump and, if one is available, the cost can be lowered substantially.
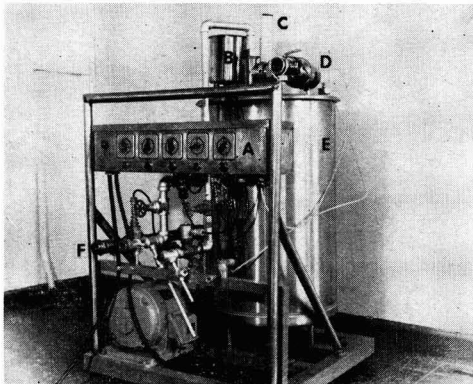

FIG. 1. Portable automated cleaning-in-place unit. A. Control panel. Thirty-minute adjustable timers control: (l. to r.) prerinse, wash, rinse, wash, rinse. B. Liquid acid dispenser. Opens on second wash. C. Thermometer and temperature control. D. Three-way air-operated valve. E. Makeup tank. F. Connections: (l. to r.) pump discharge, steam, water, air.

Operation: After a prerinse, the operator need only add alkali cleaner to the makeup tank and liquid acid to the dispenser. The unit will automatically heat the wash solutions and operate a pretimed wash, rinse, wash, rinse cycle that takes approximately 1 hr.

In Plant B, the $1,600 pipeline and equipment was estimated to have a salvage value of $500 at the end of 3 yr. A 3-yr. depreciation period was used, primarily due to obsolescence. This would indicate a yearly depreciation of $367. When a use cost of $80 for insurance, repairs, taxes, and interest is added, the total annual cost will be $447.[1] The estimated annual net savings potential available in this plant after additional cleaner, steam, and water expenses and the annual CIP equipment cost were deducted, ranged from $34, if wages could be reduced, to $1,653 if processing facilities could be used during the time saved.[2]

In Plant D, a total cost of $1,700 was needed for the pipeline and equipment, estimated to have a salvage value of $500. The total annual cost would be $485—$400 for depreciation and $85 for use cost. The estimated annual savings potential, less additional expenses, ranged from $252 to $1,848, depending on whether wage savings or the use of processing facilities are considered. In both instances, some means would have to be found to make productive use of the time saved.

In both plants, the greatest potential benefits appear to be in the form of increased plant capacity to process milk with the same size work force, providing storage facilities are adequate and sales can be increased. Automated CIP, by reducing nonproductive cleaning time, will enable a plant to be operated on the same fixed hour schedule but with an increased processing capacity. In Plant B an additional 640 qt. of milk could be processed daily, with no increase in labor or equipment costs. If a dairy plant has experienced a pattern of steadily increasing sales and volume, the increased plant capacity made available by automated CIP will be highly desirable.

SOME OBSERVATIONS OF PLANT ORGANIZATION AND MANAGEMENT

During the conduction of this project, distinct differences were found in organizations and in the ability of managers and employees within these organizations. These differences and their implications have had and will continue to have a more profound effect on these dairies than the use of automated CIP or any other innovation.

Management sets the tone of any organization and a plant manager's decisions over the years definitely affect the current status of that plant. The complexity of automated CIP equipment, cleaning circuits, and the scheduling

[1] French, C. E., The Dirty Five, American Milk Review. p. 44. May, 1957.

[2] To obtain this figure, the additional number of quarts that could be processed during the time saved was multiplied by $0.0117, the average net margin for 1954-59 as derived from the USDA, Agricultural Marketing Service publication, Milk Distributors Sales and Costs.

of cleaning operations to make the most desirable use of the equipment, requires a well-designed organization and capable employees.

The successful installations in this study were in the better organized and managed plants. The employees were fairly well chosen and trained and, in general, were conscientious and resourceful. In the other plants this was not as true. There appeared to be an over-all lack of planning that led to many plant and employee problems. The frequent operating delays in these plants confirmed this observation.

The managers of Plants B and D were of above-average caliber. One manager personally assumed responsibility for the operation of the equipment until an operator could be trained to continue this operation. He has continually directed all plant activities in such a manner that his employees are adequately trained and perform their work well at all times.

Management of the second plant was concerned with older and more experienced plant men. The men had originally been trained and organized so that the manager was relieved of most recurring activities and able to deal more with unusual circumstances. When the CIP unit was installed, the manager immediately delegated the responsibility for operating it to one of his employees, who was then instructed in its operation.

In the remaining plants numerous difficulties arose. Employees were less capable and less well trained and found it more difficult to adjust to the complexity of operating CIP systems. It was often difficult to schedule and coordinate cleaning activities successfully, if at all. Operating delays were frequent and many delays were not properly or thoroughly attended to and would often recur. Some of the difficulties were a result of the abilities of individual employees, but basically management had some involvement, especially since it was responsible for selecting and training the men and coordinating over-all plant operation.

The important factor in achieving successful results is to have employees capable of and willing to operate the equipment. Both of these factors originally stem from the nature of the organization, but in every CIP installation results are directly related to employees. If they want CIP to work, and are encouraged to make it work, then it will. Conversely, the employees can add many problems to an already complex installation, especially if management has built and sanctioned this type of an organization. Automation of in-place-cleaning procedures is not and will never be a substitute for good management and employees. In fact, automated CIP equipment requires more effective management and more capable employees.

Automated in-place cleaning offers many potential benefits, but will also cause some new problems, which will take a capable and alert dairy plant management to solve. It appears from this study that automated CIP will first appear in the more efficient dairy plants, and will be introduced less rapidly in the less efficient plants. This is due to the complexity of the new equipment and procedures and the ability of the better managers to incorporate CIP systems or any other new innovations into the plant routine. This management ability has had and will continue to have a great effect on the size of the dairy industry and, in particular, the future of the smaller plants. The survival of these plants depends on the capacity of their management and how well they can adjust their organizations to current and future economic conditions.

CONCLUSIONS

The following conclusions are based on information gathered from and observation of the four installations:

1. The use of automated CIP equipment was feasible in two of the four dairy plants studied.
2. A savings in time from the use of automated CIP equipment can not be capitalized on until that time can be employed to make more productive use of the plant and processing facilities or to reduce labor costs.
3. The major difficulties in obtaining desirable results from automated CIP equipment are directly related to the ability and interest of management and the plant men.
   a. Automated CIP will not work in a poorly managed plant or if employees are not capable of operating the equipment.
   b. Management and the employees must be interested in automated CIP, since the first results are often discouraging and time and patience are necessary to get desirable results.

FEATURES OF PORTABLE AUTOMATION CLEANING UNIT

1. Made of commercially available components at much lower cost than ready-assembled or custom-engineered installations.
2. Adjustable time periods of up to 30 min. each for prerinse, first wash, rinse, second wash, and final rinse.
3. Automatic water make-up. A level control switch opens and closes a solenoid valve in the water line to maintain level.
4. Pump can not start unless water level control is satisfied.
5. Automatic diversion to waste. A three-way valve opens to divert to waste or closes to permit recirculation, as called for by the timers.
6. Steam injector system will not function unless pump is operating. (This part of the equipment can be by-passed and the solutions heated in the press, if desired.)
7. Temperature of solutions is thermostatically controlled if steam injection system is used.
8. Unit capable of delivering circulating solutions at velocity of 5 ft. per second.

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UNIVERSITY OF MARYLAND
COLLEGE PARK

October 11, 1961

To the Members of the American Dairy Science Association:

The University of Maryland is honored to join with the United States Department of Agriculture as host to the 57th Annual Meeting of the American Dairy Science Association scheduled to be held on this campus June 18 to 21, 1962.

Both the production and processing aspects of dairying are important segments of the economy of Maryland. We shall look forward, with interest, to the scientific information and review of current problems which this meeting will bring to

W. H. Elkins

our campus. This year is of special significance to all those associated with scientific agriculture, since it marks the Centennial of establishment of the Land-Grant College System and the United States Department of Agriculture. We are pleased to note that your Association is giving special recognition to this historical event.

We hope you will find the facilities of our campus well adapted to your needs, and we urge you to join us for this important event. Our staff and campus will be at your disposal.

Sincerely yours,
WILSON H. ELKINS
President

DEPARTMENT OF AGRICULTURE
OFFICE OF THE SECRETARY
WASHINGTON

October 6, 1961

TO: Members of the American Dairy Science Association

The United States Department of Agriculture is honored to be joint hosts with the University of Maryland to the 57th annual meeting of the American Dairy Science Association, June 18-21, 1962.

It is significant that your Association is holding its annual meeting in the Washington, D. C. area on the beautiful campus of the University of Maryland in 1962 because it is the centennial year of the founding of the Land-Grant College System and the United

O. L. Freeman

States Department of Agriculture. I am pleased to note that you plan to recognize the centennial during your meeting.

The Department is pleased to join with the University of Maryland in welcoming you. You may be assured that you will receive a cordial reception at the Agricultural Research Center and at the several units of the Department concerned with the dairy industry. Our staff will be pleased to assist in any way possible in making your stay with us pleasant and profitable. We anticipate that these associations will be of mutual benefit.

Sincerely yours,
ORVILLE L. FREEMAN
Secretary

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BORDEN PRODUCTION AWARD

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Ray Albrechtsen, Chairman, Dairy Extension, Cornell University, Ithaca, New York

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Bruce Poulton, Chairman, Dairy Department, University of Maine, Orono

PAUL LEWIS AWARD FOR RESEARCH IN CHEESE

J. B. Stine, Chairman, Kraft Foods, 500 Peshtigo Court, Chicago, Ill.

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National Association of Artificial Breeders:
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U. S. Livestock Sanitary Association:
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RESOLUTIONS

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THE AMERICAN DAIRY SCIENCE ASSOCIATION
CONSTITUTION AND BY-LAWS
REVISED 1961

ARTICLE I—NAME

Section 1. The name of this organization shall be The American Dairy Science Association.

ARTICLE II—OBJECT

Section 1. The object of the Association is to promote the welfare of the dairy industry by stimulating scientific research, improving educational methods, encouraging worthy intra-industry and inter-industry cooperative endeavors, and by publishing the JOURNAL OF DAIRY SCIENCE and other official periodicals.

ARTICLE III—MEMBERSHIP

Section 1. Any person shall be eligible to

membership who has had college training in dairying or who is in a position of responsibility that requires a technical knowledge of dairy science.

Section 2. Any person shall be eligible to nonvoting membership as a student affiliate who is a regularly enrolled college student and who does not hold a rank of instructor or higher or the equivalent thereof.

Section 3. Any firm engaged in the dairy industry or in a business having an interest in the dairy industry shall be eligible for sustaining membership upon the payment of annual dues established by the Executive Board.

ARTICLE IV—OFFICERS

Section 1. The officers of the Association shall be President, Vice-President, Secretary-Treasurer, JOURNAL Editor, and seven Directors, one of whom shall be the immediate Past-President.

The Vice-President shall be elected by the vote of the membership for a term of one year beginning at the time of his installation during the first annual meeting of the Association following his election. If an annual meeting cannot be held, his term of office shall begin on July first. On the completion of his term as Vice-President, he shall automatically become President for one year beginning at the time of his installation during the annual meeting, or, in the absence of such a meeting, on July first.

The Secretary-Treasurer and the JOURNAL Editor shall be elected annually by the Executive Board.

Two directors shall be elected by the membership each year to hold office for a term of three years, beginning either at the time of the installation of officers at the first annual meeting following their election or on July first if an annual meeting is not held.

Section 2. The Executive Board shall consist of the President, Vice-President, seven Directors, and with the Secretary-Treasurer and the JOURNAL Editor as ex-officio members. The Board shall be responseible for the business of the Association.

ARTICLE V—MEETINGS

Section 1. Meetings of the Association shall be held at least once during each calendar year. The exact date and place of each meeting shall be fixed by the Executive Board. Notice of the time and place of meetings of the Association shall be given to all members not less than four weeks prior to the date of the meeting. In an emergency the annual meeting may be canceled by action of the Executive Board.

ARTICLE VI—AMENDMENTS

Section 1. The Constitution and By-Laws may be amended at any meeting of the Association by an affirmative vote of three-fourths of those members present, provided not less than five per cent (5%) of the voting membership is present at the meeting. All amendments must be submitted for approval only after they have been presented in writing to the membership at the previous regular business meeting or have been published in the JOURNAL OF DAIRY SCIENCE at least 30 days before the regular meeting at which the amendments are offered for approval. All amendments must have been acted upon by the Executive Board prior to final action by the Association.

Section 2. The Executive Board may submit proposed amendments, approved by the Board, to the members of the Association for vote by mail. In such a case, a minimum of twenty-five per cent (25%) of the membership must vote on the proposed amendment and an affirmative vote by two-thirds of all voting shall be necessary for its approval.

BY-LAWS

ARTICLE I—DUTIES OF OFFICERS

Section 1. The President of the Association shall preside at all meetings of the Association and the meetings of the Executive Board, and shall perform such other duties as pertain to that office. The President shall call meetings of the Executive Board, and notices of such meetings shall be sent to each member of the Board not less than ten days before the meetings. As chairman of the Executive Board, the President shall submit to the Executive Board for approval his nominations of members to fill vacancies that may occur among elective offices of the Association. The President shall appoint without the approval of the Executive Board the standing, nonelective committees of the Association.

Section 2. The Vice-President shall perform the duties of the President in the absence, illness, resignation, or death of the President.

Section 3.

(a) The Secretary-Treasurer shall manage the business of the Association in accordance with the policies established by the Executive Board.

(b) He shall have custody of the books and records of the Association, keep the minutes of all meetings of the Association and the Executive Board, maintain a list of all members and subscribers, keep the funds of the Association, maintain in current condition an official Procedural Handbook of the Association, submit an annual budget for consideration by the Executive Board, make disbursements as authorized in the budget approved by the Executive Board, and cause an annual audit of the books to be made by a certified public accountant.

(c) He shall receive applications for membership which are submitted in writing and endorsed by one member. He shall refer to the Executive Board applications of doubtful eligibility. He shall, upon receiving the annual dues of the successful applicant, enroll him as a member of the Association, and shall place his name upon the list of those to receive the JOURNAL OF DAIRY SCIENCE.

(d) He shall remove from the roll of members those individuals who have failed to pay the annual dues on or before January first. He shall restore these individuals to membership upon receiving their payment of current dues.

Section 4. The Editor of the JOURNAL OF DAIRY SCIENCE shall have direct charge of all editorial details of the Journal and of other official regular publications of the Association under the general supervision of the Journal Management Committee and shall assume such other management responsibilities as may be designated by the Executive Board upon the recommendation or with the approval of the Journal Management Committee.

Section 5.

(a) The Executive Board shall have full control of the business of the Association and shall report its official actions to the members of the Association at the annual business meeting, or, if such a meeting is not held, through the JOURNAL OF DAIRY SCIENCE.

(b) The Executive Board shall hold the title to all property and funds of the Association and shall have all the rights and powers vested in the Association by the laws of the District of Columbia under which it was incorporated.

(c) The Executive Board shall pass upon all applications for the establishment of divisions, sections, and student branches of the Association.

(d) The Executive Board shall fix the amount of annual dues to be paid by members and student affiliate members, and the amount to be paid by nonmember subscribers to the JOURNAL OF DAIRY SCIENCE.

(e) The Executive Board shall adopt the annual budget under which expenditures of Association funds will be authorized by the Board.

(f) The Executive Board annually shall elect the Secretary-Treasurer and the Editor of the JOURNAL OF DAIRY SCIENCE.

(g) The Executive Board shall elect three members of the Association, who, with the Secretary-Treasurer and Editor of the JOURNAL OF DAIRY SCIENCE as ex-officio members, shall constitute the Journal Management Committee. This Committee shall be responsible to the Executive Board and shall have general supervision of the JOURNAL OF DAIRY SCIENCE and other official periodicals of the Association. The Executive Board shall elect to the Journal

Management Committee one member each year for a term of three years. Members may be elected to succeed themselves for one term only. The elected member having seniority of service shall be the chairman of the Journal Management Committee. A member reelected for a second term shall become the junior member of the committee.

(h) The Executive Board may appoint or cause to be appointed such committees of the Association as it deems necessary.

(i) The Executive Board shall have the authority to approve or disapprove nominations made by the President to fill vacancies in unexpired terms of office that may occur among the elective officers of the Association.

(j) The Executive Board shall have the authority to present to the Association a resolution asking the expulsion of any member whose conduct has been shown to be damaging to the Association, or its reputation, or to the objects of the Association after: (1) the individual has appeared before the Board; (2) the individual has heard reasons for his expulsion presented by his accuser; and (3) the individual has had opportunity to present his witnesses and to plead his case, with or without the benefit of counsel, who must be a member of the Association.

(k) The Executive Board shall have the authority to define and establish awards and to grant life memberships according to conditions described in Article III of the By-Laws.

ARTICLE II—ELECTION OF OFFICERS

Section 1. Nominations for the offices to be filled by membership voting shall be by a Nominating Committee appointed by the President.

Section 2. The nominating committee shall consist of five members, including the immediate past-president, one representative each from the extension, manufacturing, and production sections, and one representing commercial interests. The JOURNAL OF DAIRY SCIENCE for October shall contain the names of the nominating committee and a statement from the chairman, inviting members to suggest names of candidates for office. These suggestions must be in the hands of the committee by January first.

Section 3. The Nominating Committee shall select two candidates for each office to be filled. The selections shall be made preferably to permit yearly alternation of the office of Vice-President between the two broad fields of interests of production and processing of milk. Selection of candidates for the offices of Directors shall be chosen to provide equal representation of the production, manufacturing, and extension sections.

Section 4. The chairman of the Nominating Committee shall send the names of the nomi-

nees for each office with their photographs and biographical sketches to the Editor by March 1st for publication in the JOURNAL OF DAIRY SCIENCE for April. Also, the chairman shall send duplicate copies of the biographies to the Secretary-Treasurer.

Section 5. The official ballot, containing the nominations of the Nominating Committee and pertinent biographical information regarding each candidate, shall be mailed by the Secretary-Treasurer to each member of the Association on or before May 1. Ballots shall be returned within 30 days of mailing to the office of the Secretary-Treasurer by the members voting.

Section 6. The ballots shall be opened and counted by a three-member Balloting Committee appointed by the President, one of whom may be the Secretary-Treasurer, and the results certified to the President. A tie vote shall be broken by the Executive Board.

Section 7. The elected officers shall be so informed by the President prior to the annual meeting at which the election results will be announced publicly. New officers will begin their term of office on July first if the annual meeting is not held.

ARTICLE III—AWARDS AND RECOGNITIONS

Section 1.

(a) An Award of Honor may be bestowed by the Association upon any person who has been a member of the Association for not less than 25 years. and who has made a distinguished contribution to the Association.

(b) Award of Honor members shall be entitled to all rights and privileges of membership and shall receive the JOURNAL OF DAIRY SCIENCE without the payment of annual dues.

(c) The citation and Award of Honor member shall be presented at a suitable function of the Association during the annual meeting.

(d) No more than one Award of Honor may be awarded during any one year.

Section 2. Upon application by, or on behalf of the individual concerned, the Association, through action of the Executive Board, may grant the title of Life Member to:

(a) Active members who retire after having belonged to the Association for 25 years or more. Retirement is understood to mean withdrawal from full time employment in dairy science or in the dairy industry.

(b) To any active member who has paid membership dues, to include those of a student affiliate, for 40 years. The status of Life Membership shall entitle the holder to all the rights and privileges of regular members without the payment of annual dues.

Section 3. The American Dairy Science Association may honor chosen individuals for distinguished services or achievements. The Executive Board shall be charged with the responsibility of defining and establishing, or causing to be defined and established, suitable awards or recognitions to accomplish this purpose. The Executive Board may accept the cooperation, financial or otherwise, of organizations or individuals who may wish to participate in honoring the individuals chosen to receive such awards or recognitions.

ARTICLE IV—ORGANIZATION OF DIVISIONS, SECTIONS, AND STUDENT BRANCHES

Section 1.

(a) Divisions of the Association, organized by members of the Association on the basis of geographical location, may be authorized by the Executive Board upon petition of not less than 25 members of the Association.

(b) Membership in Divisions is open only to those who are members of the Association.

(c) The Divisions shall select officers, one of whom will be the chairman, and shall govern themselves in a manner consistent with the Charter, the Constitution, and the By-Laws of the Association.

(d) Divisions may collect funds and/or dues, and in addition, the Association shall grant a subsidy of $25 per annum, unless this sum is modified by the Executive Board. These monies may be expended by the divisions for their own purposes.

Section 2.

(a) Professional groups organized by members within the Association on the basis of specialized interests and to be known as sections, may be authorized by the Executive Board upon petition of not less than twenty-five members.

(b) The Sections shall elect their own officers and make rules for their own guidance consistent with the Constitution and By-Laws of the Association.

(c) Sections shall conduct their official business during the annual meeting of the Association.

Section 3. Student Branches of the Association, organized by college and university groups with interests in the dairy industry, may be authorized by the Executive Board on petition from a majority of the local group's members and on recommendation by two faculty representatives who are regular members of the Association. Annually, not later than June 1st, the secretary of each student branch shall submit a brief report of its activities to the Secretary-Treasurer of the Association. Upon affirmative vote of at least two-thirds of the existing student branches a National Student Branch of the Association may be formed.

PROGRAM

ANNUAL MEETING SOUTHERN DIVISION, AMERICAN DAIRY SCIENCE ASSOCIATION JACKSONVILLE, FLORIDA

February 5-7, 1962

Monday AM
JOINT SESSION DAIRY PRODUCTION, DAIRY MANUFACTURING, AND DAIRY EXTENSION

Presiding: J. B. FRYE, JR.

8:30 AM

North Ballroom, Hotel Roosevelt

Historical review of Florida's contribution to dairying. R. B. Becker, Florida Agricultural Experiment Station.

Comparison of the methods for determining thyroid function in dairy cattle in Louisiana. A. J. Guidry and E. J. Stone, Louisiana Agricultural Experiment Station.

Within-cow variability of milk constituents in samples taken at daily intervals. C. J. Wilcox and W. A. Krienke, Florida Agricultural Experiment Station.

Palatability studies within dairy animals of volatile fatty acids usually found in grass silage. L. L. Rusoff and Paul Randel, Louisiana Agricultural Experiment Station.

Physiological characteristics of some staphylococci isolated from aseptically drawn cows' milk. K. L. Smith, Florida Agricultural Experiment Station.

Uptake of strontium and calcium by *Streptococcus lactis*. B. J. Demott and H. C. Holt, University of Tennessee.

11:00 AM

General Convention Session, ASAW
George Washington Hotel

Monday PM
DAIRY MANUFACTURING

Presiding: B. E. GOODALE

2:00 PM

Emerald Room, Hotel Roosevelt

Development of a test for predicting the shelf-life of Cottage cheese. R. J. MacDonald, J. J. Willingham, and M. L. Peeples, Texas Technological College.

Effect of preservatives on the shelf life of Cottage cheese. R. Y. Cannon, Auburn University.

Determination of the polypeptide content of Cottage cheese. David N. Naff and W. K. Stone, Virginia Polytechnic Institute.

Chloride content of fortified skimmilk. W. A. Krienke, Florida Agricultural Experiment Station.

Nutritional characteristics of *Pseudomonas fluorescens*. C. Vanderzant and T. J. Ousley, Texas Agricultural Experiment Station.

Changes in cation composition of milk caused by ion exchange treatment. B. J. Demott, H. C. Holt, and R. G. Cragle, University of Tennessee.

Monday PM
JOINT SESSION, DAIRY PRODUCTION, DAIRY EXTENSION, ANIMAL HUSBANDRY, AND AGRONOMY

Presiding: U. S. JONES

2:00 PM

Seminole Room, Seminole Hotel

Effects of fertility levels and stage of maturity on forage nutritive value. R. E. Blaser, Virginia.

Pilot or laboratory methods of estimating forage nutritive value. W. B. Anthony, Alabama.

Nutritive value of forage as affected by physical form and harvesting and preserving methods. L. A. Moore, D. W. Beardsley, USDA, and Georgia.

Example of inter-disciplinary pasture research—animal, agronomic, economic, and other aspects. T. J. Cunha, University of Florida.

Monday PM
SOUTHERN DAIRY STUDENTS' ASSOCIATION

2:00 PM

Attend Dairy Sessions of Your Choice

4:30 PM

Presiding: JAMES K. CAUGHMAN
Directors' Room, Hotel Roosevelt
Student Session

7:00 PM

Student Mixer

Tuesday AM

JOINT SESSION, DAIRY PRODUCTION, DAIRY MANUFACTURING, AND DAIRY EXTENSION

Presiding: J. B. Frye, Jr.

8:30 AM

North Ballroom, Hotel Roosevelt

Seasonal variations in the fertility of dairy cattle. H. C. Kellgren, T. E. Patrick, J. O. Shelwick, and J. D. Roussel, Louisiana Agricultural Experiment Station.

Value of feed additives in storing and feeding grass silage. Lee R. Sisk and M. E. McCullough, Georgia Experiment Station.

Effects of injecting testosterone into pregnant cows on the reproductive organs of their heifer calves. Victor Hurst, South Carolina Agricultural Experiment Station.

Effect of feeding low-level rates of phenothiazine and diethystilbestrol to young dairy heifers. O. T. Fosgate and K. S. Hegde, University of Georgia.

Dry lot feeding vs. supplemented pasture for lactating cows. W. R. Murley, North Carolina Agricultural Experiment Station, and J. R. Edwards, Mountain Research Station.

Results of selection for production in a Holstein herd. Robert E. Walton, University of Kentucky.

9:45 AM

Southern Division, ADSA, Business Meeting
North Ballroom, Hotel Roosevelt

11:00 AM

General Convention Session, ASAW
George Washington Hotel

Tuesday PM

DAIRY MANUFACTURING

Presiding: J. J. Willingham

2:00 PM

Emerald Room, Hotel Roosevelt

Combined effects of agitation and temperature treatments on the lipolytic activity in milk. P. E. Johnson and R. L. Von Gunten, Oklahoma Agricultural Experiment Station.

Quality study on retail market creams. W. W. Overcast and J. D. Skean, University of Tennessee.

Effect of certain steam infusion deodorizer treatments on some physical and chemical properties of chocolate milk. Joe T. Cardwell and T. J. Ousley, Mississippi State University.

Milk solids-not-fat values determined by evaporation and by computation using specific gravity measurements. R. W. Henningson, Clemson Agricultural College.

Simplified nusselt's-type equation of describing some of the heat transfer characteristics of several fluid dairy products. M. L. Peeples and J. Eastham, Texas Technological College.

Consumer preference for sugar levels in ice cream and frozen desserts. II. Vanilla levels in ice cream. J. J. Sheuring and Eugene Finnegan, University of Georgia.

6:30 PM

Annual Banquet and Honors Program
Speaker: P. H. Tracy, Past-President A.D.S.A.

Tuesday PM

DAIRY PRODUCTION

Presiding: W. E. Thomas

2:00 PM

North Ballroom, Hotel Roosevelt

Palatability and digestibility of corn and grass silages fed alone and in combination to young dairy heifers. J. T. Huber, G. C. Graf, and R. W. Engel, Virginia Polytechnic Institute.

Ad libitum consumption of ground corn or corn silage by lactating dairy cows on pasture. J. T. Huber, R. W. Engel, and G. C. Graf, Virginia Polytechnic Institute.

Performance of dairy cows on sart sorghum silage cut at different stages of maturity. George E. Hawkins, Aubrey Smith, Harold Grimes, and Joe Little, Auburn University.

Relationship between several laboratory evaluations of frozen semen and its fertility level. J. W. Kelly and Victor Hurst, South Carolina Agricultural Experiment Station.

Methylene blue reduction test for frozen semen. S. B. Hayes and Victor Hurst, South Carolina Agricultural Experiment Station.

Effects of nitrogen and argon gases on metabolism freezability and livability of bovine spermatozoa. T. E. Patrick, J. D. Roussel, H. C. Kellgren, and J. O. Shelwick, Louisiana Agricultural Experiment Station.

Comparison of cold shock and sperm longevity at 38 C as methods for evaluating frozen semen. B. C. Pass and Victor Hurst, South Carolina Agricultural Experiment Station.

Effect of method of semen collection and tranquilization on semen quality. M. E. Wells, S. D. Musgrave, W. N. Philpot, E. W. Brock, and E. W. Jones, Oklahoma Agricultural Experiment Station.

Coconut water as an extender in bovine semen at room temperature. Fernando Luis Oliver, Puerto Rico Agricultural Extension Service.

Effect of electroejaculation and tranquilization on other than semen characteristics. M. E. Wells, S. D. Musgrave, W. N. Philpot, W. E. Brock, and E. W. Jones, Oklahoma Agricultural Experiment Station, Stillwater.

6:30 PM

Anual Banquet and Honors Program
Speaker: P. H. TRACY, Past-President A.D.S.A.

Tuesday PM

DAIRY EXTENSION

Presiding: V. L. BALDWIN

2:00 PM

Directors' Room, Hotel Roosevelt

2:00-2:30 PM

Team approach for building effective dairy extension programs. R. E. Burleson, Federal Extension Dairyman, Washington, D. C.

2:30-3:10 PM

Impact milk marketing quotas might have on the dairy industry. W. H. Alexander, Louisiana State University.

3:10-3:20 PM

Milk Break

3:20-3:50 PM

Approach to improved milking machine maintenance, operation, and use. J. D. George, North Carolina State College.

3:50-4:20 PM

Using dairy farm business analyses to determine changes needed. H. W. Anderson, Louisiana State University.

4:20-5:00 PM

Business Session

6:30 PM

Annual Banquet and Honors Program
Speaker: P. H. TRACY, Past-President A.D.S.A.

Tuesday AM

SOUTHERN DAIRY STUDENTS'
ASSOCIATION

Presiding: RALPH L. HILL

8:00 AM

Tour of Dairy Plant or Farm

11:00 AM

General Convention Session, ASAW
George Washington Hotel

Tuesday PM

SOUTHERN DAIRY STUDENTS'
ASSOCIATION

Presiding: JAMES K. CAUGHMAN

2:00 PM

Roosevelt Hotel

Information on gradute programs offered in the dairy departments of the southern colleges and universities, Heads of Departments.

4:30 PM

Student Business Session
Roosevelt Hotel

6:30 PM

Annual Banquet and Honors Program
Speaker: P. H. TRACY, Past-President A.D.S.A.

Wednesday AM

JOINT SESSION, DAIRY PRODUCTION,
DAIRY MANUFACTURING, AND
DAIRY EXTENSION

Presiding: L. J. BOYD

8:30 AM

North Ballroom, Hotel Roosevelt

Effect of stilbestrol on the development and reproductive performance of dairy cattle. L. J. Bush and H. W. Reuber, Oklahoma Agricultural Experiment Station.

Sesame meal vs. soybean oil meal as a source of protein in calf starters. J. K. Miller, W. J. Miller, and C. M. Clifton, University of Georgia.

Effect of roughages on the calf's stomach development. S. P. Marshall and R. B. Becker, Florida Agricultural Experiment Station.

Digestibility of certain plant fractions from animals on bloat-producing and bloat-inhibiting diets. E. J. Stone, A. J. Guidry, and J. B. Frye, Jr., Louisiana Agricultural Experiment Station.

Relative value of corn and sorghum silages for dairy cattle. T. A. Taylor, B. F. Hollon, and R. D. Mochrie, North Carolina Agricultural Experiment Station and Coastal Plain Research Station.

Applicability of the chromogen method to determination of digestibility by forages when concentrate is fed. George E. Hawkins, Auburn University.

Comparison of irrigated Coastal Bermuda, Ladino clover, and a Coastal-Ladino mixture for summer grazing. C. B. Browning, Mississippi State University.

Effect of the physical state of hay on the rate of passage through the digestive tract of dairy heifers. G. D. O'Dell, W. A. King, and S. L. Moore, South Carolina Agricultural Experiment Station.

Wednesday AM

SOUTHERN DAIRY STUDENTS'
ASSOCIATION

New Officers in Charge

8:30 AM

Directors' Room, Hotel Roosevelt
Student Business Session

9:30 AM

Attend Dairy Sessions

Protein testing in the United States and the Netherlands. A. R. CORWIN, F. E. POTTER, AND S. N. GAUNT, University of Massachusetts, Amherst.

Reports from the Netherlands reveal a well-established protein-testing program. This is carried on under the supervision of the Central Milk Recording Organization. The program was initiated in 1958, with 153,000 cows tested. For 1960, the number of cows had increased to 310,000. All testing is done in ten central laboratories, with five doing 80% of the samples. Protein determinations have been made by different methods, but by the end of 1961 it is expected that all will be performed by the Amido Black technique. In the larger laboratories special mass production techniques have been employed so that 15,000 samples per day can be handled.

In the United States the Orange G dye method has been used with only a limited number of studies on the Amido Black method. At the present time there are approximately 56,000 cows being tested in 20 states. In some states, dye samples are supplied to the DHIA tester, who adds the milk and returns the sample to a central laboratory for testing. In Massachusetts, approximately 1,000 samples are tested per month. Milk samples are taken by the DHIA testers and are brought to the laboratory for analysis. Slides showing some of the techniques used in the Netherlands, New York, and Massachusetts will be presented.

Considerations in pricing milk on protein content. S. N. GAUNT, D. J. HANKINSON, A. R. CORWIN, AND J. H. BRAGG, University of Massachusetts, Amherst.

Criteria used to decide the component or components of milk on which milk should be priced ought to include: (1) relative nutritive importance to humans, (2) its appeal to consumers, (3) relative cost to consumers, and (4) ability to increase its percentage, (5) simplicity of the system, and (6) ease of shifting to it. Protein rates high on all six.

Pricing systems based on the price per pound of protein show that with high milk prices, producers of high protein milk would gain over the present system, whereas with low prices they would receive less. Data used were from 14 farms and average breed values. Since there is a smaller range percentagewise for SNF than for protein or fat, pricing milk on SNF on its pound value would underpay many producers, compared to the present system or to protein. Three systems were compared (1) pricing Class I on protein and Class II on fat, (2) assigning values $0.75 per pound of fat and $0.92 for protein compared to $0.40 and $1.34, respectively, and (3) giving weights, using differentials to fat and protein as 50-50, 33% fat to 67% protein, and 25% fat to 75% protein.

Some interrelationships between the percentages of SNF, protein, and fat in Holstein milk. N. H. SLACK, R. E. MATHER, AND K. O. PFAU, New Jersey Agricultural Experiment Station, Sussex.

The first ten monthly samples form 56 individual lactations were analyzed for fat, total protein (formol titration), and SNF (lactometer). Season (2-mo. periods) and stage of lactation effects were evaluated by the method of unweighted means. Means with standard deviations within and among lactations, respectively, were for protein, 3.48, 0.42, and 0.21; SNF, 8.72, 0.47, and 0.36; fat, 4.18, 0.57, and 0.35; and the ratio of protein to fat, 0.85, 0.10, and 0.14. Season, stage of lactation, their interaction, and cow effects were highly significant sources of variation for all measures. Average lactation curves for the three constituents were similar with high values in the first month, low values around the second, followed by gradual increases becoming more pronounced near the end of the lactation. Seasonal values were lowest in late winter and midsummer, and highest in early summer and fall. The animals were grouped by lactation months in which they conceived; after about the fifth month of gestation, sharp increases occurred in these constituents. Increases throughout lactation were less marked in animals conceiving late in lactation. Correlations within lactations between fat and protein were 0.63; fat and SNF, 0.62; and protein and SNF, 0.69.

The nature of the solids-not-fat curve during lactation. A. A. RIMM, R. E. MATHER, AND W. P. APGAR, New Jersey Agricultural Experiment Station, Sussex.

Each of 16 cows (12 Holsteins and four Guernseys) received one of four levels of grain (0, 7, 14, and 21 lb. maximum) for two lactations. Solids-not-fat (SNF) deter-

minations were made every 2 wk. There was a sharp decline in SNF immediately after calving, which leveled off about the sixth week. From 6 wk. after calving to 15 wk. after conception changes in SNF were small and linear. Changes for this period per week by ration groups were: 0.0084, 0.0132, −0.0096, and −0.0026% for zero, low, medium, and high levels, respectively. Differences among these values were significant (P < 0.01). Variables (total energy intake, change in weight, and weeks to lowest weight) representative of plane of nutrition were used to determine their effects on: (1) regression coefficients from the middle period of lactation and, (2) SNF level. There were no significant relationships. From the period from conception to 30 wk. after conception a second-degree polynomial curve fit the data better than an exponential curve. Correlation between fat and SNF percentages among lactations was 0.84.**

Fertility and motility of bovine spermatozoa frozen with liquid nitrogen. R. C. MARTIG, B. W. PICKETT, AND W. A. COWAN, University of Connecticut, Storrs.

A total of 43 ejaculates from 25 Holstein, Guernsey, Jersey, Ayrshire, and Angus bulls were extended in egg yolk–citrate, to a final concentration of 30 million progressively motile sperm per milliliter of extender, and frozen to −80° C. with a Linde (Model BF-1) liquid nitrogen (LN) semen freezer. Immediately after freezing, the ejaculates were divided equally and one-half transferred directly to dry ice–alcohol (DI) storage at −79° C., and the remaining one-half was transferred to LN storage at −196° C.

Motility estimations made after 3 wk. post-freeze averaged 40.4 and 25.7%, indicating a sperm survival of 19.2 and 12.2 million cells per milliliter of extender in LN and DI storage, respectively. The difference in survival between storage temperatures was statistically significant (P<0.001).

Semen samples from nine Holstein bulls, from the laboratory study, were used to breed unselected first-service cows. A total of 299 cows was bred with semen stored in LN, whereas 330 were bred with semen stored in DI, for a 60- to 90-day nonreturn rate of 73.24 and 67.58%, respectively. The 5.25% weighted difference in nonreturn rate in favor of LN was not significant (P = 0.05).

Comparison of a high-milk and a low-milk method of raising calves. W. S. GAUNYA, University of Connecticut, Storrs.

A 2 × 2 × 2 factorial experiment, utilizing the growth responses of 48 calves, was conducted. The treatment variables were Guernsey vs. Holstein heifer calves, timothy vs. alfalfa hay, and a high-milk system which provided milk at the rate of 10% of body weight to a maximum of 9 lb. for Guernseys

and 11 lb. for Holsteins to 63 days of age, vs. a low-milk system, which allowed up to 170 lb. of milk fed over 35 days. Limited starter and unlimited hay was fed to the 112th day, followed by a period of uniform pen feeding to 200 days of age. The mean gains of calves fed high-milk were 158.4 lb. to 112 days, 271.3 lb. to 200 days, and 7.09 in. in stature to 112 days. These values for low-milk calves were 128.5 lb., 240.2 lb., and 5.96 in., respectively. Mean gains for alfalfa-fed calves were 145.6 lb. to 112 days, 260.5 lb. to 200 days, and 6.87 in. in stature to 112 days. These values for timothy-fed calves were 141.3 lb., 251.0 lb., and 6.18 in., respectively.

Inherent appetite differences among milking cows. R. E. MATHER AND A. A. RIMM, New Jersey Agricultural Experiment Station, Sussex.

Appetite for forage was estimated in 2-wk. trials following 1-wk. adjustment in milking cows about 100 days after calving in three successive years. Excellent alfalfa hay was fed ad libitum twice daily, with limited alfalfa silage, and concentrates fed at about standard. A total of 120 trials on 79 daughters of 18 sires was conducted. Adjustments were made for lot of hay, amount of concentrates, days fresh, lactation number, condition score, and weight by least-squares methods. Condition score, as well as body weight, was an important source of variability in forage dry matter intake. Components of variance from the adjusted mean squares led to a within-cow correlation between records in different lactations (repeatability) of 0.19; an intraclass correlation between paternal sisters was also 0.10. Repeatability estimates from the same experiment in previous years were much higher.

Soilage vs. silage as a summer maintenance program for dairy cattle. B. W. HENDERSON, JR., AND W. M. ETGEN, Rhode Island Agricultural Experiment Station, Kingston.

For each of three summer feeding periods of 16, 10, and 15 wk., grass-legume soilage and grass-legume silage were compared as the sole forage for lactating dairy cows. Twelve cows were assigned to each feeding regime. Soilage was cut and fed twice daily. Direct-cut silage, with preservative, was fed twice daily. A 16% crude protein concentrate was fed at the rate of 1 lb. for each 5 lb. of FCM produced the previous week. Mean daily production of FCM on soilage was 35.9, 41.1, and 37.2 lb. for each of the three periods. Production on silage for the three respective years was 36.3, 36.0, and 39.7 lb. These yearly differences were not significant at the .05 level of probability. Daily dry matter intake for the three trials was 32.7, 30.4, and 34.7 for the soilage-fed cows, as contrasted to 23.3, 21.0, and 30.4 for the silage-fed cows. Although changes in body weights varied from one year

to the next, no significant changes occurred between groups in any given year.

Effect of feeding chlortetracycline to lactating dairy cattle—a summarization of field experiments. A. L. SHOR, J. J. DRAIN, AND R. A. LAMM, American Cyanamid Company, Princeton, New Jersey.

The effect of feeding chlortetracycline (0.1 mg/lb body weight) to lactating dairy cows was studied in 14 experiments conducted under practical conditions throughout the United States. Control and supplemented groups in an experiment were initially balanced according to accepted procedures. Duration of experiments ranged from 60 to 399 days. Due to the low incidence of clinical disease, it was not possible to evaluate chlortetracycline against infectious conditions.

Covariance analyses were performed on all herd data, adjusting production during the experimental period for differences in pretrial production. Nine out of 14 experiments showed a beneficial response in milk production attributable to feeding chlortetracycline. The over-all average increase for 14 experiments was 0.620 ± 0.600 lb. per head per day.

Data from 33 Michigan experiments (J. Dairy Sci., 43:890. 1960) were collected and processed in a similar manner as the 14 experiments reported above; therefore, all 47 experiments (582 control and 588 treated cows) may be pooled and summarized. Sixty-eight per cent of the herds responded positively to chlortetracycline supplementation. The average increase in milk production due to chlortetracycline, over all experiments, was 0.594 ± 0.356 lb. per cow per day (probability of no increase = 5%).

Effect of a plant growth regulator upon in vitro cellulose digestion. M. C. STILLIONS, J. L. EVANS, W. V. CHALUPA, AND J. L. CASON, Rutgers—The State University, New Jersey.

Kinetin I (6-furfurylaminopurine), a plant growth regulator, was employed in the artificial rumen on three different cellulose substrates — alfalfa, poor-quality timothy, and orchardgrass. Also, Kinetin analogs K_1 (6 benzyl adenine), K_2 (P-methoxy benzyl adenine), K_3 (B-diphenyl ethyl adenine), K_4 (naphthyl methyl adenine), and K_5 (B-naphthyl ethyl adenine) were studied, using alfalfa hay as the cellulose source. Preliminary studies showed that either 0.01 $\mu g/ml$ or 0.2 p.p.m. of hay dry matter was the optimum level. Cellulose digestion was increased more and with more consistent results for the poorer-quality hays than for the alfalfa hay. Orchardgrass cellulose digestion increase was 5.28% over the controls, P<.05. Timothy was 5.74% increase, P<.05. Alfalfa results showed cellulose digestion to be significant at either P<.10 or P<.05. Cellulose digestion was

significant at P<.01 for the analogs, with the range being $K_2 > K_3 > KI > C > K_4 > K_5 > K_1$.

Influence of the addition of a plant growth regulator upon in vivo ruminant digestion and rat growth. M. C. STILLIONS, J. L. EVANS, W. V. CHALUPA, AND J. L. CASON, Rutgers—The State University, New Jersey.

Four levels (20, 2.0, 0.2, 0 p.p.m.) of Kinetin I (6-furfurylaminopurine) were sprayed on second-cutting alfalfa hay to determine its influence upon in vivo ruminant digestion. A total collection digestion trial (4×4 Latin-square) was used. Digestion coefficients for protein and crude fiber were 74.7, 51.7—20 p.p.m.; 77.1, 55.3—2.0 p.p.m.; 75.1, 53.3—0.2 p.p.m.; 75.9, 53.1—0 p.p.m. TDN and coefficients for ether extract, cellulose, and NFE followed similar trends. Although, in some cases, a degree of biological significance may be implied, these slight increases did not exhibit statistical significance. Two trials were conducted using 21-day-old Hooded-Norway rats injected subcutaneously with Kinetin I (corn oil carrier). In Trial I, rats were injected daily with 0, 40, or 80 $\mu g/g$ body weight. Weight gains were 118, 116, 114 g. (females) and 131, 132, 127 g. (males), respectively. In Trial II, 32 males were injected with 0, 5, 20, or 200 $\mu g/g$ body weight. Gains were 136, 136, 128, 129 g., respectively. Fifteen parts per million dietary Kinetin for 6 wk. did not influence weight gains. No significant effect on growth rate of these rats was found, nor was any deteriorative effect noted.

Effect of varying regrowth intervals and initial harvest dates in the nutritive value of aftermath timothy hay. R. C. HAVEN, B. R. POULTON, AND M. J. ANDERSON, University of Maine, Orono.

Aftermath timothy forage was harvested at 4-, 6-, 8-, and 10-wk. intervals from June 3, June 10, June 24, and July 9 initial cutting dates. Analyses of the forage showed, on a dry matter basis, a decline in crude protein composition with each increase in length of regrowth interval. The apparent digestibility of protein declined significantly (P < .05) between the 6- or 8-wk. intervals and the 10-wk. regrowth interval; and between the June 10 aftermath cuttings and the July 9 aftermath cuttings.

The digestibility of the dry matter exceeded 65% in only one instance and never fell below 58%. A significant decrease in dry matter digestibility (P < .05) occurred between the 6- and the 10-wk. regrowth intervals. Dry matter digestibility of the aftermath forages from the plots initially harvested early (June 3 and June 10) was found to be significantly higher (P < .05) than those of the later harvested plots (June 24 and July 9). Digestible energy content was highly correlated ($r = +0.93$) with the digestible dry matter

content in these aftermath forages. Highly significant (P < .01) interactions for the digestibility of dry matter and energy occurred between 6- and 8-wk. regrowth intervals and the June 10 initial cutting date.

Comparison of the results of several in vitro forage evaluation techniques with in vivo values. B. R. POULTON, R. C. HAVEN, H. E. FOSS, AND T. E. MELLIN, University of Maine, Orono.

Three in vitro techniques for evaluating the nutritive worth of a forage were compared with in vivo values. Dry matter digestibility was used as the criteria of nutritive value. Climax timothy forage grown in pure stands under carefully controlled conditions was used in all studies. To make it possible to study the effects of forage maturity on the reliability of these in vitro techniques, the forages were harvested at 11 stages of their growth cycle.

The in vitro techniques used involved predicting dry matter digestibility from: (1) the cellulose digestion rate, (2) the acid-insoluble lignin content, and (3) the content of crude fiber and digestible protein. The in vivo data were obtained by feeding each forage to four wether sheep in standard total collection digestion trials.

The predicted dry matter digestibility values for each of the in vitro techniques was found to be highly correlated (P < .01) with the in vivo dry matter digestibility values. Correlation coefficients were 0.96, 0.94, and 0.88 for the cellulose digestibility, acid-insoluble lignin, and crude fiber-digestible protein techniques, respectively. A prediction equation has been developed for predicting dry matter digestibility from in vitro cellulose digestion: $Y = 23.43 + 0.671 X$ where Y = dry matter digestibility and X = in vitro cellulose digestibility in per cent.

Studies on the hay evaluation. I. Correlation coefficients between appearance and nutritive value of alfalfa hay. NOBUO TAKANO[1] AND MASATOSHI MITSUMATA, Hokkaido National Agricultural Experiment Station, Sapporo, Hokkaido, Japan.

Twenty-six pure hay samples were collected from dairy farms in Hokkaido to determine the correlation coefficients between appearance (green color, leafiness, and stem diameter) and nutritive value.

Almost all hay was harvested by field-cured method without using mechanical conditioners. Green color in hay was observed by a special color conversion table which has 11 grades from 100 to 0%. Percentage of leafiness indicated only leaf blade removed from stem by hand separation. Stem diameter was determined by means of a caliper measurement 2 in. from the cut end of the stem.

The correlation coefficients between leafiness and nutritive value were 0.72, −0.75, 0.69, and 0.80 for crude protein, crude fiber, calculated DCP and TDN, respectively, green color and nutritive value showed 0.67, −0.59, 0.67, and 0.70. Also, stem diameter and nutritive value were shown to be −0.57, 0.52, −0.59, and −0.63.

The multiple regression equation was calculated: Y (DCP) $= 9.90 + 0.09 X_1 + 0.02 X_2 − 0.78 X_3$; Y (TDN) $= 47.97 + 0.11 X_1 + 0.01 X_2 − 0.84 X_3$ (X_1 = percentage of leafiness, X_2 = percentage of green color, and X_3 = millimeter of stem diameter).

The correlation between appearance and nutritive value of red clover, timothy, and orchardgrass hay also was observed.

[1] Presently an Exchange Professor at the University of Massachusetts.

A critical analysis of New York DHIA feeding records. D. G. DAVENPORT, J. T. REID, AND J. D. BURKE, Cornell University, Ithaca, New York.

Lactation records of ENE intakes and FCM production for 6,020 cows of various breeds representing 205 herds in five New York Counties were analyzed. The precedural assumptions that levels of roughage consumption within herds vary linearly with the individual cows' body weights and are not affected by variation in the amounts of concentrates fed or milk produced were investigated. Gross efficiency [100 (0.340 FCM) ÷ (therms ENE intake)] and net efficiency [100 (0.340 FCM) ÷ (therms ENE intake − Morrison's maintenance and growth allowances)], were used as indices of the agreement between the calculated ENE intakes and the net energy requirements for individual cows. Gross and net efficiency both decreased with increasing weight; $b = −1.02\%/\text{cwt}$ and $−3.04\%/\text{cwt}$, respectively. When gross efficiency $= Y$, the ENE from roughage $= X_1$, the ENE from concentrates $= X_2$, and FCM production $= X_3$, by 1.23 $= −5.02\%/500$ therms, by 2.13 $= −5.02\%/500$ therms, and by 3.12 $= 5.64\%/1,000$ lb. When net efficiency $= Y$, by 1.23 $= −9.64\%/500$ therms, by 2.13 $= −11.13\%/500$ therms, and by 3.12 $= 8.26\%/1,000$ lb.

Transmission of some flavor components of silage to cow's milk. W. F. SHIPE AND R. W. DOUGHERTY, Cornell University, Ithaca, N. Y., and M. E. MORGAN, University of Connecticut, Storrs.

Two cows having ruminal and tracheal fistulas were used to determine what components could be transmitted to milk through the lungs or the rumen or both. Ethyl propionate, and the methyl, ethyl, propyl, and butyl acetates were transmitted by both routes; and they imparted a sweet, fruity-like flavor to milk.

Ethanol and *n*-propanol imparted a sweet, vanilla or alcohol-like flavor. Secondary butanol gave a more unpleasant flavor described as ester-like or chemical. Neither butyric nor propionic acid produced an off-flavor.

Attempts to study the effect of aldehydes had to be terminated because they had toxic effects. However, enough *n*-butanal was administered to impart a malty or chemical flavor. Acetone imparted a flavor described as feedy, silage, cowy, or sweet. A similar effect was noted with 2-butanone. Grassy, weedy, or musty were the terms used to describe the flavor imparted by *cis*-3-hexen-1-ol. A similar flavor was noted when the cows ate green grass. Dimethyl sulfide produced a strong flavor described as onion, weedy, feedy, or unclean.

Some problems in the determination of milk solids. B. L. HERRINGTON, J. M. A. PALMER, G. W. ROM, AND A. VALDAMBRINI, Cornell University, Ithaca, New York.

Payment for milk on a solids basis would necessitate adoption of a standard method for analysis, and the examination, licensing, and supervision of the testers. We are not prepared to do this. We can not get close agreement on solids tests made in different laboratories. Some of the causes are mechanical: differences in temperature of hot plates and ovens, in predrying, in ventilation of ovens, in desiccators used for cooling, in weight of sample per square centimeter of dish. Some are due to changes in the sample during drying. There is a weight loss due to protein-lactose interaction whose magnitude depends upon the temperature, and upon the time required to remove the water; hence, upon sample size. The physical state of the lactose is a variable. It may remain as noncrystalline hygroscopic glass. It may be present as the very hygroscopic desiccated α-lactose, or as the nonhygroscopic forms, α-hydrate, and β-anhydride, which differ in water content. Which of these is formed during drying is partly a matter of chance. In general, we do not know what we are weighing. There is not even agreement whether the lactose should be weighed as hydrate.

Comparison of Orange G dye and Buffalo Black methods for milk protein determination. F. R. ALLAIRE, A. R. CORWIN, F. E. POTTER, AND S. N. GAUNT, University of Massachusetts, Amherst.

The Orange G and Buffalo Black dye methods were compared with the Kjeldahl method for determinations of total milk protein in individual cow milk samples. All samples were done in duplicate.

Repeatability estimates between duplicates were .998 for Orange G, .990 for Buffalo Black (filtered), .987 for Buffalo Black (centrifuged), and .987 for Kjeldahl. The numbers of samples used for the above estimates were 144, 84, 96, and 144, respectively.

Correlations with the Kjeldahl were .966 for Orange G, .965 for Buffalo Black (filtered), and .989 for Buffalo Black (centrifuged). The standard error of estimate for per cent protein for the above correlations were .109, .099, and .062%, respectively. The correlations between dye binding methods Orange G and Buffalo Black (centrifuged) was .928. The correlation coefficients were more uniform by breeds for the Buffalo Black (centrifuged) and Kjeldahl.

The average protein percentages using all breeds for 144 samples were 3.51% by Kjeldahl and 3.50% by Orange G dye. On 84 samples, the average protein percentages by methods were 3.54, 3.53, 3.54, and 3.56% for Kjeldahl, Orange G, Buffalo Black (centrifuged), and Buffalo Black (filtered), respectively. The average protein per cent by breeds for the Ayrshire, Guernsey, Holstein, Jersey, and Brown Swiss were 3.33, 3.68, 3.26, 3.84, and 3.56%, respectively.

The fatty acid composition of pooled milk. R. G. JENSEN, G. W. GANDER, AND J. SAMPUGNA, University of Connecticut, Storrs.

One hundred samples of pooled raw milk were obtained at the rate of about eight per month during the period of June 10, 1960, to June 12, 1961. The lipids were extracted by the silica gel method and the fatty acid composition determined by gas-liquid chromatography. The fatty acids were converted to both methyl and butyl esters by refluxing with acidic methanol or butanol. The methyl esters were separated on a column of diethylene glycol succinate and the butyl esters were separated with a column of Apiezon L on glass beads and temperature programing. Distinct seasonal differences were noted; for example, oleic acid was present in greater concentration in the summer than in the winter, and the trend was reversed for palmitic acid.

Gas chromatographic analyses of cheese volatiles. W. W. NAWAR AND I. S. FAGERSON, University of Massachusetts, Amherst.

Methods for the recovery of cheese volatiles, in a form suitable for injection into the gas chromatograph, are described. Evidence is presented for the presence of the following compounds in Roquefort Cheese: 2-pentanone, 2-heptanone, 2-nonanone, acetaldehyde, propionaldehyde, acetone, isobutyraldehyde, 2-butanone, 2-propanol, diacetyl, 2-hexanone, 2-pentanol, 2-octanone, and 2-heptanol.

The significance of these components from the flavor standpoint is discussed.

Ultra high temperature pasteurization—high fat versus low fat milk. L. R. GLAZIER, University of Connecticut, Storrs.

Five replicate trials using raw milks of ap-

proximately 3.6 and 4.6% fat, with flavor scores as nearly identical as possible, were pasteurized in a plate-type pasteurizer at 193.5° F. for 1 sec. It was necessary at times to standardize the high-fat milk slightly with cream. The milks received 5° F. of treatment in a Vacutherm Chamber after leaving the raw regenerator and were then homogenized.

All samples were scored by the same two trained judges one day, seven days, and 14 days after processing. The flavor scores of the day-old high-fat milks averaged very slightly higher than the low-fat, whereas the reverse was true at the end of seven and 14 days. The flavor score of the low-fat milks improved approximately 0.9 point from the first to the 14th day, whereas that of the high-fat milk remained static. The HTST control milk scored slightly higher than the UHT milk on the first and seventh days, but slightly lower at the end of 14 days of storage.

Consumer complaints were not received from either milks when included randomly in home deliveries or in the University salesroom.

Effect of ice cream packaging material on ice cream quality. A. C. SMITH AND L. R. DOWD, Storrs Agricultural Experiment Station, Connecticut.

Ice cream in ten types of half-gallon packages was compared in three replicate trials with 6 mo. of storage at −20 and 0° F. Packaging materials studied included solid bleached board foil-lined inside, foil lined outside, plastic coated, waxed both sides, white paper laminated outside, protective coated, wax laminated to board, wax laminated to experimental board, and with gravure printing on the last two mentioned packaging materials. Package comparisons included hardening rate, insulating value, induced and normal storage shrinkage, surface dehydration and butteriness, adhesion to the package, flavor, and body and texture of the ice cream.

Statistical analysis failed to detect a difference among the packaging materials in induced and normal shrinkage, flavor, and body and texture of the ice cream during storage. However, the board foil lined outside had the least amount of induced ice cream shrinkage. Significant differences were noted between some of the cartons in all of the remaining comparisons, but ice cream quality was not affected or the differences were not considered of sufficient magnitude to cause customer complaints.

Flavor and physical characteristics of a hot-pack dairy product called sweet cultured cream. F. V. KOSIKOWSKI, AND H. F. GEERKEN, Cornell University, Ithaca, New York.

A satisfactory hot-pack product, called sweet cultured cream, was attained from regular, high-viscosity sour cream.

Spray-type dried skimmilk and dried sweet cream butermilk solids with a small concentration of a good quality food gum were stirred into chilled regular sour cream. The mixture was then heated to about 170° F. in a vat pasteurizer, pumped to an homogenizer double stage at 2,500—500 p.s.i., and packaged hot.

The addition of solids gave a sweeter flavor quality to the product by decreasing acid and increasing lactose and permitting diacetyl in the natural sour cream to predominate. A pH 4.6-4.7 appeared optimum.

Agitation during heating of the sour cream destroyed completely the body of the natural sour cream, but initial stiffness and smoothness were restored by proper homogenization. However, basic viscosity of sweet cultured cream, as distinguished from initial viscosity, never fully equalled that of the control regular sour cream.

In keeping-quality experiments sweet cultured cream shows no change in flavor at 4 wk. of 40° F. and no visible yeast and mold. Research is continuing on problems of holding whey pocketing to a minimum and attaining higher basic viscosity.

Diacetyl formation in creamed cottage cheese and butter by the use of citrated whey cultures of S. diacetilactis. E. LUNDSTEDT AND W. B. FOGG, H. P. Hood & Sons, Inc., Boston, Massachusetts.

A method has been developed whereby creamed cottage cheese, cream cheese, butter, and margarine can obtain any desired degree of aroma.

The addition to the finished creamed cottage cheese of from 0.25 to 1% of an 18-hr.-old citrated cottage cheese whey culture of *S. diacetilactis* will produce, in a few days, a medium to a very high aroma at temperatures between 32 and 45° F. without changing the pH of the cheese. Five per cent of such a culture calculated on the serum of the cream or margarine will impart within 3 wk. a high aroma, provided the serum has a pH of 5.0 and the serum is aerated before it is incorporated into the fat phase.

Such cultures can produce up to 1,000 p.p.m. of potential diacetyl in the form of alpha-aceto-lactic acid within 18 hr. at 72° F. at or near a pH of 6.0. No aroma can be detected in the cultures. However, the nonaromatic precursor materials will be converted into butter aroma (diacetyl, etc.) in a second effect or delayed aroma formation in cottage cheese or butter by an oxidative decarboxylation.

The process [1] is about 60 times more effective in aroma production than any other known method.

[1] Pat. pending.

Growth patterns of some aroma bacteria when cultivated in citrated rennet whey and citrated Cottage cheese whey. E. LUNDSTEDT, H. P. Hood & Sons, Inc., Boston, Mass.

A method has been proposed for the identification of aroma bacteria associated with dairy starters.

Isolated species and variants can be differentiated from each other by determining the daily pH values of the carbon dioxide–free cultures growing in Cottage cheese whey containing the addition of 5% sodium citrate, pentahydrate.

By plotting the pH values and corresponding time intervals in a coordinate system, a series of well defined growth patterns can be produced which differentiate and classify the various aroma bacteria found in starters.

S. diacetilactis bacteria initiate growth on citrate, and finish growth on lactose. All leuconostocs initiate growth on lactose. *L. citrovorum* finishes growth on citrate, whereas other leuconostocs finish growth on lactose. *S. lactis* var. *aromaticus* initiates growth on lactose, then ferments citrate and lactose simultaneously. The pH varies from 7.8 to 5.2. The method is valid only for aroma bacteria which have developed their citrate-metabolizing systems to an optimum by repeated transfer in citrated Cottage cheese whey.

Gassy diacetilactis starters and nongassy leuconostic-lactic starters can be classified in 18 hr. in citrated rennet whey and citrated Cottage cheese whey.

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: published in the JOURNAL; otherwise, the type will have been destroyed.

In case the original type has been destroyed, it is possible to supply reprints by a special photographic process, and their cost will be

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
600	37.50	51.25	93.75	137.50	177.50	221.25	266.25	313.75	346.25
700	41.25	56.25	105.00	153.75	197.50	246.25	297.50	350.00	387.50
800	45.00	62.50	115.00	168.75	218.75	272.50	328.75	386.25	427.50
900	48.75	67.50	126.25	185.00	238.75	298.75	358.75	422.50	467.50
1,000	57.25	73.75	136.25	200.00	258.75	323.75	390.00	458.75	507.50

If covers for reprints are desired, the cost of 50 covers will be $21.18, and for each additional 100 covers, the cost will be $8.75. Back copies of the JOURNAL will cost $2 each.

The reprints are made from standing type within 30 days after the papers appear in the JOURNAL. Requests for a few reprints of a paper should be sent to the authors, whose names and addresses appear with the title. The Secretary and the Editor's office do not keep supplies of the various reprints. Orders for large numbers of reprints should be sent to The Garrard Press, 510 North Hickory Street, Champaign, Illinois. These orders must be received within 30 days after the papers are 50% more than the regular ones. For example, 100 reprints of 32 pages will cost $217.50.

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.

CALL FOR PAPERS FOR THE 1962 ANNUAL MEETING OF THE AMERICAN DAIRY SCIENCE ASSOCIATION

M. E. SENGER, Chairman, Program Committee, A.D.S.A.
Department of Animal Industry, North Carolina State College, Raleigh

The 57th Annual Meeting of the American Dairy Science Association will be held June 18-21 at the University of Maryland, College Park. Members who wish to present papers must submit titles and abstracts not later than March 1. This deadline must be met to permit publication of titles with the complete program in the April, and abstracts in the June, JOURNAL.

All members of the Association, including graduate student affiliates, are entitled to present papers. Participation by members of the 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 appear as author on no more than two. This Committee, together with the Association membership, wishes to stimulate vigorous, sound research and realizes that these restrictions may penalize some members engaged in full-time research. Therefore, the Committee has liberalized this policy so that a member may exceed these limits, but only if he or his department rates the abstracts in order of preference for oral presentation.

Papers submitted for the annual meeting should be confined to research that has not been reported. Abstracts of papers accepted for publication by a scientific journal before the annual meeting are not eligible, as this constitutes duplication. If the total number of papers submitted by the membership is too great to include in the program, the Committee will assign some papers to be read by title only. In this event, consideration will be given to quality of research to be reported, number of abstracts per author and department, and importance of the research. Abstracts arriving late will be rejected.

Attention is called to the Dairy Manufacturing Extension Section program and members are urged to participate. This is a subsection of the Manufacturing Section.

Mimeographed copies of pertinent data are desirable for distribution when the paper is presented. At least 250 copies should be made available. This can be supplemented by slides for projection on screens, provided the author can adhere to the assigned time of 12 to 14 minutes for presentation of each paper.

PREPARING ABSTRACTS

The Program Committee encourages continued improvement in quality of papers and in oral presentation. Careful design of experiments and proper interpretation of results are necessary. Strict compliance with the instructions for preparation of abstracts will simplify the task of the Program Committee, and will improve the program. *Careful editing of abstracts before submission is essential.* Each year a number of abstracts must be returned due to incomplete details. Please follow instructions for preparing abstracts carefully.

1. All abstracts must be submitted on regular 8½- by 11-inch paper.
2. Abstracts must not exceed 200 words by actual count. Those exceeding 200 words will be returned to the author for revision.
3. An original (on bond paper) and three copies of each abstract should be typed double-spaced. The original and one copy should be mailed to the Chairman, another copy to the Vice-Chairman, and the fourth to the Secretary of the Section where the paper will be presented. The original copy will be used for publication in the JOURNAL.
4. The style and abbreviations of the JOURNAL OF DAIRY SCIENCE must be used. Please refer to abstracts in the June, 1961, 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 again in the text. The abstract should include, insofar as possible, the design and major results of the investigation. Only complete research should be reported. Brief, essential statistics will make the data 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 style used in the JOURNAL OF DAIRY SCIENCE. The author should send the first copy direct to the JOURNAL Editor, E. O. Herreid, before or immediately following the Annual Meeting.

GRADUATE STUDENT PRESENTATION CONTEST

This contest will be conducted in both the Production and Manufacturing Sections. Each institution is entitled to enter one participant in each contest. They must be student affiliate members. Complete rules for the contest are being sent to department heads. Those wishing to enter the contest must submit copies of their abstract to the Section Officers as outlined above. They should also include a letter indicating their desire to enter the contest. This letter should also be signed by the major Professor and the Department Head. A carbon copy of the letter and four additional copies of the abstract should be mailed to the contest representative.

For Production Section Award: Louis Boyd, Dairy Department, University of Tennessee, Knoxville

For Manufacturing Section Award: A. V. Moor e, Department of Dairy Science, Texas A&M College, College Station

Names and addresses of officers of sections to whom titles and abstracts should be sent are:

EXTENSION SECTION

Chairman: W. R. Van Sant, Department of Dairy Science, University of Arizona, Tucson.

Vice-Chairman: C. D. McGrew, Department of Dairy Science, Ohio State University, Columbus 10.

Secretary: D. E. Voelker, Department of Animal Husbandry, Iowa State University, Ames.

PRODUCTION SECTION

Chairman: J. C. Thompson, Ralston Purina Co., St. Louis, Missouri.

Vice-Chairman: L. H. Schultz, Department of Dairy Husbandry, University of Wisconsin, Madison.

Secretary: V. R. Smith, Department of Dairy Science, University of Arizona, Tucson.

MANUFACTURING SECTION

Chairman: M. L. Speck, Department of Food Science and Processing, North Carolina State College, Raleigh.

Vice-Chairman: D. M. Graham, Pet Milk Co., Research and Development Center, Greenville, Illinois.

Secretary: E. L. Thomas, Department of Dairy Industries, University of Minnesota, St. Paul.

(The Dairy Manufacturing Extension Section is a subsection of the Manufacturing Section. W. S. Arbuckle, Department of Dairy Husbandry, University of Maryland, College Park, is chairman, and A. L. Rippen, Department of Food Science, Michigan State University, East Lansing, is secretary. All abstracts for papers in this subsection should be submitted through the regular channels of the Manufacturing Section, but should be identified for presentation at the Dairy Manufacturing Extension Section meeting and a courtesy copy sent to W. S. Arbuckle.)