Journal of

DAIRY SCIENCE

Vol. 45 May, 1962 Number 5

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JOURNAL OF DAIRY SCIENCE

OFFICIAL PUBLICATION OF THE
AMERICAN DAIRY SCIENCE ASSOCIATION SINCE 1917

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INSTRUCTIONS TO CONTRIBUTORS

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

ORGANIZATION OF PAPERS

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

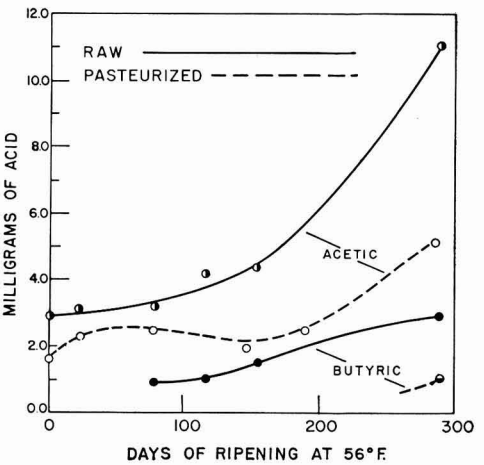

FIG. 1. Acetic and butyric acids in raw and pasteurized milk Cheddar cheese during ripening (milligrams in distillate obtained from 150 g of cheese oil).

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

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

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

Tables are costly. Use graphs whenever possible.

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

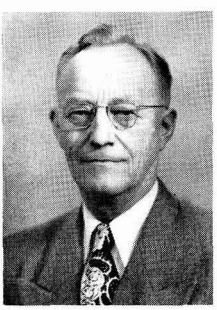
PEOPLE AND EVENTS

MEMORIALS

Thor Wilhelm Gullicksen

PROFESSOR T. W. GULLICKSEN of St. Paul, Minnesota, passed away on the afternoon of September 30, 1961. He was enjoying a football game when death came quickly and quietly.

Professor Gullicksen was born in Cushing, Wisconsin, August 26, 1887. He attended the rural and public schools in that community from 1894 to 1901 and later the academy at St. Olaf College, Northfield, Minnesota, from 1905 to 1908. His teaching career began in 1909 when he was selected principal of the grammar school in his home county; a position he held until 1912.



T. W. Gullicksen

He returned for further training to the State Normal School at River Falls, Wisconsin and, following graduation, he was appointed instructor in Agriculture at the Richland Center High School and served there from 1913 to 1916. Professor Gullicksen's quest for further training in Agriculture took him to the University of Minnesota where he earned the B.S. degree in 1916. For two years he was assistant dairy husbandman in the Bureau of Animal Industry of the United States Department of Agriculture. He became interested in an academic career and, in 1920, returned to the University of Minnesota as a research and teaching assistant. He earned the M.S. degree in 1922 and was appointed to the Dairy Husbandry Staff. The Ph.D. degree was conferred in 1934. He was promoted through the ranks of instructor, and assistant, associate, and professor of dairy husbandry. He retired in 1956 after 36 years of faithful and distinguished service to the state, the nation, and to dairy science, internationally. Professor Gullicksen was well known for his outstanding work in dairy cattle nutrition, involving mineral and vitamin metabolism in growing and adult cattle. His research was the basis of calcium and phosphorus requirements for present feeding standards. Other contributions were made on the energy and growth requirements for young cattle. He pioneered research on fat digestion and metabolism in calves. He was interested in the most efficient methods of storing, evaluating, and utilizing farm-grown roughages for milk production.

In 1951, the American Dairy Science Association honored Professor Gullicksen with the American Feed Manufacturers award for his outstanding contribution to animal nutrition. In 1959 this Association awarded him an honorary life membership. In addition, he was a member of the American Society for Animal Production, American Association for the Advancement of Science, Sigma Xi, Gamma Sigma Delta, Gamma Alpha, Alpha Zeta, Minnesota Academy of Science, and a life member of the General Alumni Association of the University of Minnesota.

Professor Gullicksen was a sturdy Scandinavian and proud of his Norwegian ancestry. He was quiet and unassuming and in his

characteristic way influenced the lives and careers of undergraduates and graduate students, many of whom sought his most respected advice and counsel. He exemplified the very highest ideals and ethics in his personal and professional life, but was tolerant of those who did not measure up to similar standards.

Thor W. Gullicksen and Gladys Martha Brown were married August 12, 1914, in Bay City, Wisconsin. They met at the State Normal School, River Falls, Wisconsin, where both graduated. There were twin children, Thomas William and G. Elizabeth.

The Gullicksens loved to travel. They attended, every year, the annual meeting of the American Dairy Science Association. This enabled them to visit every state in the United States. A foreign assignment, following his retirement, provided an opportunity to travel abroad. Professor Gullicksen was called to India by the International Cooperative Administration to serve as advisor to the government of India on problems of dairy cattle nutrition. He remained there two years.

Professor Gullicksen is survived by Mrs. Gullicksen of 1346 Raymond Avenue, St. Paul, the home where the Gullicksens lived for more than 30 yr; a daughter, Mrs. Darrel Vaughn of Minneapolis; a son, Thomas William Gullicksen of Milwaukee, Wisconsin, and two brothers, Harold of Minneapolis and Arthur who still lives on the ancestral farm in Cushing, Wisconsin.

Max Isaacs

MAX ISAACS died November 24, 1961, in Osteopathic Hospital, Chicago. Funeral services were held Monday, November 27, at Mooresville, Indiana. His parents, Mr. and Mrs. E. C. Isaacs of 207 S. 7th Street, New Castle, Indiana, one brother and two sisters survive him.

Max Isaacs was a most unusual person. He carried with him in every task of activity, an abundant capacity for work, radiant enthusiasm, and the rich humor which everyone enjoyed so much and will long remember. These fine traits were reflected in everything he did.

Max Isaacs

While Max's earlier years are obscured by his later accomplishments, he graduated from high school with an over-all average of 93. While this would have been work enough for most students, it was not for Max. He sang in the Glee Club,

played in the Band, served on the Student Council, worked on the yearbook . . . and, being the good farm boy he was, immersed himself in 4-H and FFA activities. Naturally, he was either President or Vice-President in most of the groups. But these were only high school activities.

He distinguished himself at Purdue University, receiving both his Bachelor of Science and Master of Science degrees with high honors. It was somewhat more difficult for Max at Purdue, because he worked his own way, paying most of his college expenses out of his own personal earnings. Because of restrictions imposed by these outside jobs, Max could participate only in the Purdue Reamer Club, an organization for outstanding students, Alpha Zeta fraternity, Ceres Club, Kappa Delta Pi, a student agricultural council, the Purdue Student Assembly, and the Student Co-Op Association.

Max Isaacs started his business career in 1954, when he joined the Hales and Hunter Company as associate biologist in research, and progressed rapidly through sales, then as livestock nutritionist and finally Manager of the Dairy Department. In this later position he made his finest and most personally satisfying contribution, because he was a dairyman and there was little else he loved quite so well. In his brief stay with the Hales and Hunter Company, Max added something special to the name Pioneer, for he turned all his boundless energy to enhancing the dairymen and the dairy industry through Pioneer Feed and its tradition.

Remarkable as it may seem, Max did not lose his zest even when faced with the knowledge that he had contracted the dread Hodgkin's Disease; and, even with the intense pain that was with him during the last weeks, Max could still smile and talk about his plans for next year and swap a joke or two.

All who knew Max will remember him as a unique and many-sided man. We shall remember him as an idealist, as an unreformed Indiana farm boy, as a dedicated dairyman, as a spellbinding talker, and as a humorist. But most of all, we shall remember him as a friend.

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Laesch Dairy Takes First Sustaining Membership

The Laesch Dairy of Bloomington, Illinois, was the first company in the state to subscribe for a sustaining membership in A.D.S.A. This independent company began selling milk in March, 1907. There were seven customers the first day but, by the end of the month, there were 70. The resources and equipment consisted of three cows, a few horses, some

machinery, and a used milk wagon. Milk sold for 7¢ a quart and was distributed to individual customers by pouring it out of 10-gal cans.

This dairy, started by MR. and MRS. FRANK LAESCH, has grown to a business of 6,200 customers, with 18 routemen delivering milk. In August, 1961, Laesch Dairy Company opened the first Cash and Carry Dairy Store in Bloomington. This year, a 50- by 75-foot annex will be added to the present building, for a Cottage cheese room, an unloading room for bulk milk, a cold storage room, a check-in room for route men, and office space.

DANIEL LAESCH, son of the founder of this company, states that his father and mother laid a solid foundation for the business by selling quality products and giving courteous service. He believes it is also essential to keep the plant looking well at all times and to take a personal interest in the welfare of the employees.

The Laesch Dairy Company has survived for 54 years and is still growing.

Earl Weaver Honored at Michigan State University

DR. EARL WEAVER recently was the recipient of the Distinguished Faculty Award at Michigan State University. This award of $1,000 was made at the Third Annual Distinguished Faculty Awards Convocation and Centennial Review Lecture at Anthony Hall.

Earl Weaver

He has been a member of the Dairy Department since 1937—this award coming at 25 years of service to the University.

Dr. Weaver has been recipient of two other outstanding honors during the past five years. In 1957, he received the American Dairy Science Association Award of Honor at its Annual Meeting, Stillwater, Oklahoma. In 1960 he received the American Dairy Science Association Master Teaching Award in Dairy Production, sponsored by the National Dairy Products Association, and symbolic of meritorious teaching accomplishments.

Professor Weaver became head of the Dairy Department, Michigan State University, in 1937, serving in that capacity 18 years. He relinquished his administrative duties in 1955 to head up the Colombia, South America, project where he served for the next four years, rendering outstanding guidance in Colombia, South American agriculture. While there he wrote several bulletins on agriculture which were printed in Spanish. Following his four-year work in Colombia he returned to the Dairy Department, not as an administrator but as a teacher within the department.

Not only is Dr. Weaver noted as an administrator and teacher but as a devoted member of the American Dairy Science Association. In addition to serving on many committees, he was a director of the Association in 1933, 1934, and 1937 and became its 23rd President in 1939. He received the B.S. degree in dairying at Oklahoma State University in 1913, the M.S. degree from Iowa State University, 1917, and the Ph.D. from the University of Minnesota, 1937. Before coming to Michigan State University he had served the faculties of the University of Minnesota, Iowa State University, and Oklahoma State University as teacher and administrator.

On Saturday evening, May 5, Dr. and Mrs. Weaver were guests of honor at a special dinner given by the Dairy Staff and their wives at the Forestry Cabin. It was a surprise affair and a most pleasant one. The children Jack, Harold, and Ruth were present. Marianna and her husband could not attend because of the expected arrival of Weaver grandchild no. 10.

Dr. Weaver was presented with an appropriately engraved automatic Omega watch. Mrs. Weaver was given an electric rotisserie. The children presented them with a dozen long-stemmed red roses.

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NEWS FROM THE UNIVERSITIES

Connecticut

Symposium on Fallout

A Symposium on Radioactive Fallout in Milk was held a part of Dairy Manufacturing Day, April 4, on the University of Connecticut campus. An audience of 200 heard various aspects of the topic discussed by: DR. D. W. MOELLER, PHS; DR. R. T. MOORE, PHS; DR. L. F. EDMONDSON, USDA; W. W. ULLMANN, Connecticut Department of Health; and NORMAN MYRICK of the Milk Industry Foundation. The day closed with a supper sponsored by the University of Connecticut Dairy Club.

Artificial Insemination Course Held

Nineteen cattlemen, representing ownership of 7,574 head of dairy and beef cattle, attended the annual five-day short course in Artificial Insemination at the University of Connecticut during April 16-20. Participants came from Connecticut, Maine, Massachusetts, New York, Vermont, and Rhode Island.

The course was presented by the staff of the Department of Animal Industries in cooperation with the Department of Animal Diseases.

New Jersey

Rutgers Schedules Food Products Seminar

A five-day seminar devoted to food products will be conducted by the Department of Food Science, Rutgers, The State University, at the Seaview Country Club, Absecon, New Jersey, July 9-13, inclusive, 1962. The Food Products seminar is the second in a series of short courses held at six-month intervals, devoted to advancement of the food sciences.

Like the highly successful first seminar held last January, which attracted a class of 60 members, enrollment in the Food Products seminar is open to persons holding responsible positions in the technical, sales, executive, and management areas of the food industry. The July seminar is expected to attract an even larger group, according to DR. C. OLIN BALL, Chairman of the Rutgers Food Science Department, and one of the most outstanding authorities in the food sciences in the United States.

Food Processing Conference at North Carolina State College

Over 200 of the State's top agricultural and business leaders were present April 17 for a conference on food processing and marketing. Leading the list of speakers were GOVERNOR TERRY SANFORD, Agriculture Commissioner L. Y. BALLENTINE, Conservation and Development Director HARGROVE BOWLES, JR., North Carolina University President WILLIAM C. FRIDAY, and Bank of Wachovia Board Chairman ARCHIE DAVIS.

Among industry leaders participating on the program were SENATOR RALPH SCOTT of Burlington, representing the dairy industry; PAUL MORGAN, Guilford College, broiler industry; JOHN HAMBY, Durham, egg industry; VINCE BODIE, Smithfield, meat packers; ARCHIBALD CRAIGE, Lexington, fruits and vegetables; ELMER WILLIS, Williston, seafood industry; DAN PAUL, Raleigh, representing food distributors; and A. G. BULLARD, Board of Farm Organizations and Agricultural Agencies.

Other participants on the program included JOHN T. CALDWELL, Chancellor, North Carolina State College; H. B. JAMES, Dean, College of Agriculture, North Carolina State College; L. L. RAY, Director of Foundations, North Carolina State College; and W. M. ROBERTS, Chairman, Food Science Department, North Carolina State College.

Governor Sanford said that food processing offers the greatest challenge and the greatest new opportunity to confront North Carolina agriculture in many years. He asserted, "The opportunities are there and if we are going to take advantage of them, we are going to have to throw ourselves into this program with great enthusiasm and complete confidence." Hargrove Bowles, Jr., told the conference that food processing in North Carolina already is big business, employing 36,000 persons and

having an annual payroll of $147 million and an output of $649 million.

Dr. W. M. Roberts said there are over 1,200 food processing plants in North Carolina and they add $350 million to the value of crops grown by farmers. However, he added, "we haven't reached the half-way mark yet." Problems to be overcome, Dr. Roberts asserted, include attitudes, lack of management, lack of technical skills and trained men and, generally, small acreages, small yields, or low volumes.

Some of the participants in the Food Processing and Marketing Conference held at North Carolina State College, April 17. Left to right: Dr. W. M. Roberts, Head, Dept. of Food Science, North Carolina State College; Hargrove Bowles, Jr., Director, Dept. of Conservation and Development; John T. Caldwell, Chancellor, North Carolina State College; Governor of North Carolina Terry Sanford; and William C. Friday, President, North Carolina Consolidated University.

STUDENT NEWS SECTION

W. W. SNYDER, Editor

A Section Devoted to News of Student Members

Illinois Dairy Technology Society Holds Banquet

The Dairy Technology Society had its annual banquet for students, faculty, and their wives at Holiday Inn in Champaign, April 12. Fifty persons attended.

President David Henning welcomed students and guests. Dr. L. W. Witter acted as Toastmaster.

The Society awarded three Certificates of Merit to three men who made outstanding contributions to the Dairy Industry. Carl Hansen, Vice President and District Manager of Beatrice Foods Co., Champaign; Nikolai King, Dairy Research Section, CSIRO, Melbourne, Australia, who is visiting lecturer at the University of Illinois in Dairy Technology; and John Wright, Sales Representative, Ambrosia Chocolate Company, Milwaukee, Wisconsin.

Illinois Dairy Production Club Holds Annual Banquet

The University of Illinois Dairy Production Club held their annual banquet April 3, in the University Y.M.C.A. building. Retiring club president, Dave MaComber introduced Dr. Albright, Dr. Brown, Dr. DeFries and Professor Cash as the advisors of the club for the coming year. He also presented the new club officers: president, Ray Ropp; vice-president, Jerry Miller; secretary, Dave Schingoethe; treasurer, Mike Campbell; reporter, Robert Henss; and Ag. Council representative, Don Pritchard.

The Dairy Cattle Judging Team for the past year was introduced by their coach, Dr. Albright. The team, consisting of Jerry Miller, Dave Gusse, Jim Dumphy, and alternate Marv Schlomer, placed 4th in the Intercollegiate Dairy Cattle Judging Contest at Waterloo, Iowa, with 30 teams competing. Jerry Miller was the 4th high individual and Dave Gusse 11th high among the 90 contestants competing in the contest. This same team placed 7th in the International Dairy Cattle Judging Contest in Chicago later in the fall. The team members expressed their appreciation to Dr. Albright by presenting him with a suitcase and a specially prepared fresh blueberry pie.

One of the club advisors, Dr. Brown, presented the Dairy Production Club Outstanding Sophomore Award to Ray Ropp and the Outstanding Senior Award to Dave MaComber.

Entertainment for the banquet was furnished by Peggy Pegler. The speaker was Dr. F. N. Andrews, Head of the Dairy Science Department at Purdue, who gave a very interesting and stimulating talk on the sub-

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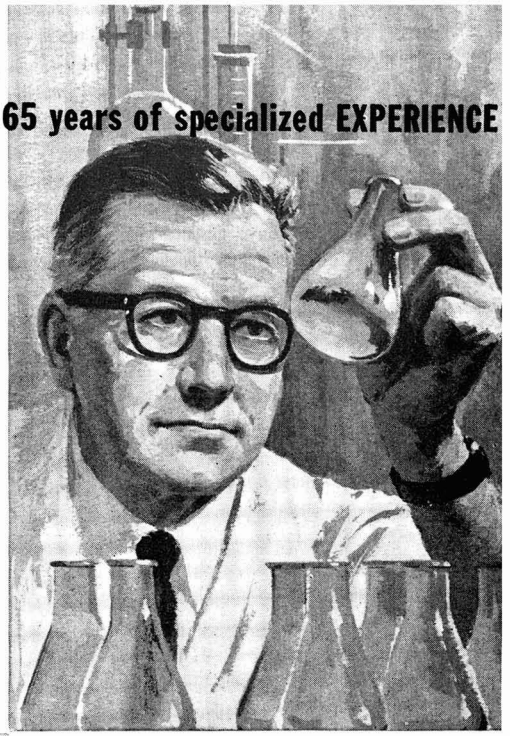

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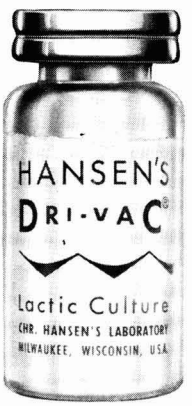

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ject, New Horizons in Food Production. The Master of Ceremonies for the entire program was Dave MaComber.

Southern Illinois University Students Take Field Trip

Students enrolled in Dairy Production, with their instructor, Dr. Howard Olson, visited dairy farms in the vicinity of Elgin, Lake Forest, Breese, Cary, and Hampshire, Illinois. Their tour included some of the outstanding breeding units in Illinois. Eight students made this tour April 26 to 28.

Scholarships Presented at Kentucky

Dairy students at the University of Kentucky were awarded a total of $850 in scholarships by the Kentucky Dairy Educational Committee. These scholarships were for the second semester of the 1961-62 school year.

The grants ranged from $100 to $150 each, depending on classification, with juniors and seniors receiving the largest amount and lower classmen the smaller sum. To receive an award a student must have completed at least one semester with no lower than a 2.25 average (A equals 4).

The scholarships were presented in the name of the Committee by Dr. D. M. Seath, Head of the Department of Dairy Science at the University. These awards represent the efforts of the entire dairy industry of the state. Serving on the Kentucky Educational Committee were representatives of both the production and manufacturing branches of the industry. This committee serves as aid in recruiting good students, as support to departmental student activities, and as advisers to the department in educational programs. All students were from Kentucky.

These young men proudly display their Dairy Scholarship checks awarded by the Kentucky Dairy Educational Committee. The awards are for $100 or $150 per man, depending on classification. Those receiving the second-semester grants are :(Seated from left to right): Jerry A. Brumagen, $100; John M. Peters, $150; Robert Allen Chiles, $100; (Standing): Neal Franklin Owen, $100; Brady James Deaton, $100; Dewane Bishop, $150; and Barney Lewis Hornback, $150.

Dairy Science Club's Annual

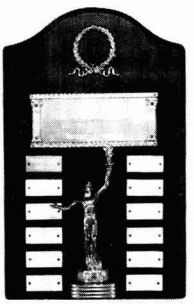
The U. K. Dairy Way was published by the Kentucky Dairy Science Club, April 12, 1962, at Lexington, Kentucky. The editor for the publication was James Davenport; Charles McKee served as business manager under the leadership of Barney Hornback, president of the club. Doctors R. E. Walton and T. R. Freeman served as faculty advisers for the publication.

The annual was mailed to approximately 750 dairy club alumni, county agents, vocational agriculture teachers, and other agricultural leaders in the state of Kentucky.

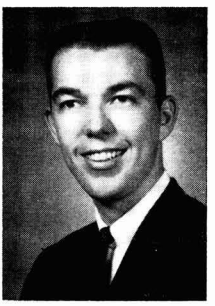
Missouri Dairy Club Receives Activities Award

On April 12, 1962, the Agricultural Council of the University of Missouri recognized the Missouri Dairy Club as the most outstanding club in the College of Agriculture. This was the first activities award presented by the Council and the presentation took place at the 34th Annual Awards Banquet, with 15 clubs competing for the award.

The awarding of the plaque was based on the per cent of students majoring in a department who are active in that department's club, the educational programs, fund-raising projects, club publications, public relations, social functions, and services rendered to the University of Missouri. The Missouri Student Chapter of the American Dairy Science Association is proud to have received the honor of being selected as the first recipient of the Agricultural Council Club Activities Award.



Plaque



Zane Akins

Mr. Zane Akins was the 1961 recipient of the annual Borden Agricultural Scholarship Award presented to a senior for achieving the highest grade-point average among students of agriculture, completing two or more dairy subjects. Mr. Akins is also president of the Dairy Club and has been very active in other campus activities. He was the third high individual in the 1960 Intercollegiate Dairy Cattle Judging Contest held at Waterloo, Iowa.

Recognition was also given Zane Akins for his being selected as the 1961 winner of the $300 American Guernsey Cattle Club Training scholarship. Mr. Akins worked three summers at the Cowan Guernsey Farms, Oshkosh, Wisconsin.

North Carolina Society Honors Secretary

The North Carolina Dairy Technology Society held its monthly meeting on April 11 in Raleigh.

In the evening, a piece of luggage was presented to Dr. R. B. Redfern, Dairy Products Extension Specialist at North Carolina State College, who has served as Secretary of the Dairy Technology Society. Dr. Redfern has accepted a position with Pet Dairy Products Company in Johnson City, Tennessee, and will be vacating his job in May as Secretary to the Society. He is assuming the position of General Production Manager and will serve a six-state area including North Carolina, South Carolina, Tennessee, Alabama, Georgia, and Virginia.

Dr. M. E. Gregory, Food Science Department, North Carolina State College, will assume the role as Secretary to the Society.

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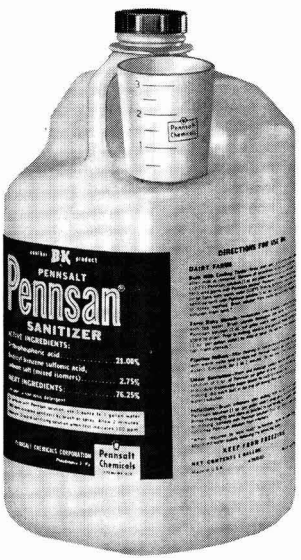

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INDUSTRY NEWS

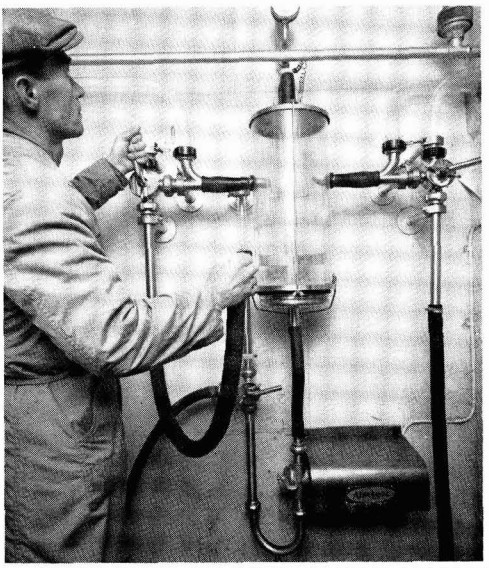
Swedish Milking Plant Saves 25% Time

One of the world's largest pipeline milking plants, including 1,300 feet of acrylic plastic tubing and electronically guided releasers, has recently been installed on the large country estate of Wambåsa, in Southern Sweden. Designed and built by the Swedish Alfa-Laval Company, the installation saves 25% in milking time and 50% in cleaning time.

Built to serve 240 cows, including 15 milking units, the plant was installed in a big cow barn of traditional type with two parallel feeding stalls. After the installation of the plant, it was possible to increase the number of milking cows at Wambåsa from 40 to 220 without adding to the staff of four men. With the new system it takes six monutes to milk one cow, compared to eight minutes with previous machinery.

The pipelines run in two separate coils around the feed racks and meet at a releaser installed in a milk collecting room at one end of the shed.

The Alfa-Matic electronically controlled releaser is made of stainless steel and toughened glass. It consists of an upper compartment, the air separating jar, which is constantly under vacuum during operation, and a lower compartment, the flow chamber, separated from the former by a stainless steel plate and communicating with it via a flap valve. A second flap valve forms the outlet.



In filling the releaser, the milk runs from the upper to the lower compartment, both being under vacuum. The air pressure holds the outlet flap shut. When the milk level has risen to the upper electrode, a circuit is completed, and air is admitted to the flow chamber, closing the intermediate flap and allowing milk to run out. When the level falls to the lower electrode, the circuit is broken.

The pump then passes the milk on to the coolers in the milk chamber, which is located some four feet above the barn level. The milk flows through two distributing conduits to the transport tanks.

The cleaning of the entire system is done by in-place flushing with warm water and circulating alkaline detergents and small foam-plastic sponges. The solution is discharged into a bowl from where it is sucked back into the pipeline. Since the discharge from the releaser is intermittent, the line sucks in detergent and air alternately, which results in a brush effect. This all-automatic cleaning takes 20 min, whereas it previously took 80 min in the morning and 40 min in the evening.

Beatrice Foods Promotes Kramer and Moore

KENNETH KRAMER has been promoted to manager of Beatrice Food Company's plant in Council Bluffs, Iowa, it has been announced by William G. Karnes, president. Karnes also announced that WILLIAM MOORE has been named to succeed Kramer as manager of the company's plant in Beatrice, Nebraska.

A native of Nebraska and veteran of World War II, Kramer joined Beatrice Foods as a wholesale route salesman for the Beatrice plant in 1954. He was advanced to sales manager, then named plant manager in 1958. Moore is also a native of Nebraska and a World War II veteran, and has been with the Beatrice branch four years as a salesman.

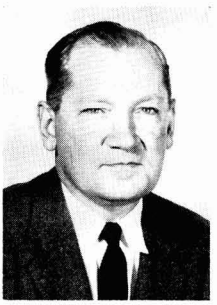
K. Kramer

W. Moore

Borden Names Research and Development Director

J. H. PETERSEN of La Grange, Illinois, has been named Director of Development and Research for the Central Division of The Borden Company. He was formerly Director of Quality Control.

Petersen started with Borden's in 1953 as a laboratory technician for the Chicago fluid milk operation. In 1954 he was placed in charge of the laboratory and, a year later, was made laboratory supervisor. In 1956 he was appointed Director of Quality Control for the Central Division, which covers Illinois, Indiana, Iowa, and Wisconsin.



J. H. Petersen

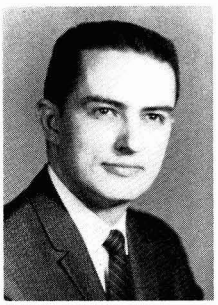
In his new position, Petersen will devote his time and talent to the development of new products and product improvement. The Borden Company, nationally, has placed much emphasis on research and development in recent years.

A graduate of Iowa State College with a Bachelor of Science degree, Petersen also earned the Master's Degree in Public Health from the University of Minnesota. He is a veteran of World War II and the Korean War. He is a member of the International Association of Milk and Food Sanitarians, American Dairy Science Association, American Public Health Association, and a Past President of the Chicago Dairy Technology

Society. His office will be at Borden's Central Division, 1821 S. Kilbourn Avenue, Chicago, Illinois.

Dr. Richardson Joins DFL as Manager of Research

DR. G. H. RICHARDSON has been appointed Manager of Research for Dairyland Food Laboratories, Inc. of Waukesha, Wisconsin. He was formerly associated with the Research Laboratories of Swift & Company in Chicago.



G. H. Richardson

Dr. Richardson will direct and supervise internal research on the firm's broad line of enzyme and enzyme-modified products for the food and dairy industries. The expanding research program on these products' use as flavor enhancers in foods will be Dr. Richardson's responsibility.

Dr. Richardson earned his B.S. degree at Utah State University in 1953. After a tour in the Armed Forces, he started graduate work at the University of Wisconsin. In 1960, he was awarded a Ph.D. degree from Wisconsin.

Dr. Richardson is an active member of the Institute of Food Technology, American Dairy Science Association, and Sigma Xi. With his family he now lives in Waukesha, Wisconsin.

Sluder Named Nestlé Director and Member of Executive Committee

Election of J. C. SLUDER, vice-president, manufacturing, as a director and member of the executive committee of The Nestlé Company, Inc., has been announced.

Sluder joined Nestlé in 1947 and was elected a vice-president in 1952. He is responsible for the manufacturing of all Nestlé products in the United States, as well as engineering and production planning.

Sluder served on the faculty of Massachusetts Institute of Technology from 1939 to 1946 and was named assistant professor in 1942, a year after receiving a doctoral degree from M.I.T. From 1950 to 1960, he served three 2-yr terms on the visiting committee of the M.I.T. Corporation.

Sluder is a member of Sigma Xi, The Institute of Food Technologists, Harvard Club, and M.I.T. Club. He lives in Armonk, New York, with his wife and three children.

Dr. C. C. Loo Granted Leave by Carnation's

DR. C. C. LOO has been granted a leave of absence from Carnation to assist in an FAO/UNICEF milk program in the Republic of Iraq. His responsibilities include: the installation, operation and maintenance of the equipment at the Abu Ghraib central plant, and in the collecting and chilling centers to be established as part of the scheme; the implementation of new department in the Abu Ghraib plant; the training of local personnel; and the administration of the plant with special reference to organization of work, control and recording of operations.

Dr. Loo will be stationed at Baghdad.

Dairy Technology Societies

Central Illinois—May 9 was Ladies' Night, with dinner at the Champaign Country Club and a tour of Beatrice Food's new milk plant included. Mrs. Rochelle Jaye, a special teacher in the Champaign schools, gave a talk on Problems in Speech Correction.

Central Michigan—The May meeting of this group was held jointly with the Western Michigan Society, in East Lansing, on the 16th. Judging contests for chocolate milk and chocolate ice cream were conducted. Feature speaker was Mort Neff, who conducts the Michigan Outdoors program.

Kansas—Featured speaker at the May 14 meeting was Dr. C. L. Norton, head, Dairy Science Department, Kansas State University. His topic: Our Mutual Responsibilities.

Metropolitan—Topic for the May 8 meeting was Sanitation Control Program of the Bureau of Foods and Drugs, New York City Department of Health, with Edwin Ludewig, director, Bureau of Food and Drugs, New York City Department of Health, as speaker. Locale was, as usual, Gasner's Restaurant.

Nebraska—Dr. D. H. Jacobsen, director of Product and Nutrition Research for the American Dairy Association, spoke at the May 9 meeting, held at Tower Motel and Restaurant, Omaha. His topic: Dairy Product Development Needs of the Day.

North Carolina—Topic of the month for this group was Ideas for June Dairy Month, Dr. George P. Gundlach of G. P. Gundlach & Company, Cincinnati, Ohio, speaking.

Oklahoma—May meeting took the form of an outing, with a steak fry, golf, bowling, and fishing contests included. Main objective: fraternization. Lake Carl Blackwell was the locale.

Philadelphia—Darold Taylor, Chief of the Milk & Food Sanitation Section, Milk & Food Branch, Division of Environmental Engineering and Food Protection, Department of Health, Education and Welfare, Washington, D. C., spoke at the May meeting on Cooperating Interstate Milk Shipment Program; also, on the present status of the National Milk Sanitation Act.

Tri-State—Changes and the Future in the Dairy Industry was discussed by guest speaker Dr. I. D. Porterfield, Chairman, Department of Dairy Science, West Virginia University, Morgantown, at the May 28 meeting.

Western Michigan—See Central Michigan entry above.

Theses Completed

M.S. Degree:

C. W. MOSS—Injury and death of Streptococcus lactis due to freezing, lyophilization, and frozen storage. North Carolina State College.

J. A. SINGLETON—A study of the mechanism of sunlight flavor development in milk. North Carolina State College.

Ph.D. Degree:

R. S. DAHIYA—Symbiosis among Lactic Streptococci. North Carolina State College.

W. T. WILLIAMSON—A study of the proteolytic activity of Lactic Streptococci. North Carolina State College.

A. E. WOODS—Studies on the volatile carbonyl compounds in Ladino clover and their influence on the flavor of milk. North Carolina State College.

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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. Seventy-five cents plus postage will be paid for each number.

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1928—January
1930—November
1931—January, March, May, November
1932—July, September, November
1934—October
1938—November, December
1943—January, February, March, April, August, December
1946—January, March, April
1947—January, February, March, April, May, June, December
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1960—October

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American Cyanamid Company
Agricultural Division
Post Box 400
Princeton, N. J.

Babcock Dairy Company
945 Berdan Avenue
Toledo 12, Ohio

Biltmore Dairy Farms
Biltmore, North Carolina

Brookshire Dairy Products Company
P. O. Box 289
Columbus, Mississippi

Carnation Company
5045 Wilshire Blvd.
Los Angeles 36, California

Chore-Boy Mfg. Co.
Cambridge City, Indiana

Challenge Cream & Butter Association
929 East Second Street
Los Angeles 12, California

Consolidated Dairies, Inc.
3415 Third Avenue, South
Birmingham 12, Alabama

Crowley's Milk Co. Inc.
145 Conklin Avenue
Binghamton, New York

Crystal Cream & Butter Co.
Box 1313
Sacramento 6, California

Dairymen's League Cooperative Association, Inc.
100 Park Avenue
New York 17, New York

Deltown Foods, Inc.
170 Saw Mill River Road
Yonkers, New York

Elgin Milk Products Company
3707 W. Harrison Street
Chicago 24, Illinois

Foremost Dairies, Inc.
425 Battery Street
San Francisco 11, California

Germantown Manufacturing Company
5100 Lancaster Avenue
Philadelphia 31, Pennsylvania

Irwin's Dairy, Inc.
512 S. 32nd Street
Camp Hill, Pennsylvania

Knudsen Creamery Company of California
P. O. Box 2335 Terminal Annex
Los Angeles 54, California

Laesch Dairy Company
P. O. Box 601
Bloomington, Illinois

Lily-Tulip Cup Corp.
122 East 42nd Street
New York 17, New York

Lilly Ice Cream Co.
P. O. Box 233
Bryan, Texas

Minnesota Valley Breeders' Association
New Prague, Minnesota

Moorman Mfg. Company
1000 North 30th Street
Quincy, Illinois

National Milk Producers Federation
30 F Street, N.W.
Washington 1, D. C.

Otto Milk Company
2400 Smallman Street
Pittsburgh 22, Pennsylvania

Ralphs Grocery Co., Creamery Division
2200 E. 89th Street
Los Angeles 2, California

Schepp's Dairy, Inc.
4935 Dolphin Road
Dallas 23, Texas

Sealtest Foods
260 Madison Avenue
New York 16, New York

Spreckels-Russell Dairy Co., Ltd.
1717 Mission Street
San Francisco 3, California

Turner Dairies, Inc.
P. O. 1161
Anniston, Alabama

Twin Pines Farm Dairy
8445 Lyndon
Detroit 38, Michigan

USI Solar Division of U. S. Industries
3315 West North Avenue
Melrose Park, Illinois

Vitex Laboratories
60 Park Place
Newark 1, New Jersey

FATTY ACID COMPOSITION OF THE PHOSPHOLIPIDS AND OTHER LIPIDS IN MILK

L. M. SMITH AND R. R. LOWRY
Department of Food Science and Technology, University of California, Davis

SUMMARY

Milk lipids were separated by silicic acid chromatography and identified by infrared spectrometry and paper chromatography. In agreement with our previous results, the major phospholipids were phosphatidyl cholines (PC), phosphatidyl ethanolamines (PE), and sphingomyelins, but cerebrosides, phosphatidyl serines (PS), and phosphatidyl inositols were also present. The various lipids showed marked differences in fatty acid composition as determined by gas-liquid chromatography. Mole percentages of saturated fatty acids were higher from the cerebrosides and sphingomyelins (86 and 87) than from the triglycerides (72), the PC (43), PS (32), and PE (24). Triglycerides contained less of the higher-molecular-weight even and odd C_{20} to C_{24} acids. No appreciable amounts of acids below C_{10} were found in the phospholipids, but traces were present in the cerebrosides. The principal saturated acid was stearic in PE and PS, and palmitic in the other lipids. Since the unsaturated fatty acids of PE, PS, and PC amounted to over 50% of the total acids, they must occur at both the α- and β-positions of the glycerol moiety of these phospholipids.

Various investigators have reported that phospholipids are involved in the synthesis of milk fat (12) and affect the quality of milk and milk products during processing and storage (6, 11, 12, 16). In addition, Billimoria et al. (2) found that butter has a greater in vitro thromboplastic activity in blood coagulation than do a number of other dietary fats, and they traced this activity to the phospholipid fraction. It is likely that fatty acid composition plays a part in determining the above properties of milk phospholipids.

Previous studies from this laboratory determined the composition of milk phospholipids (including sphingolipids). The respective mole percentages of cephalins, lecithins, sphingomyelins, and cerebrosides were 37, 31, 23, and 6, but small amounts of other lipids were also present (24). The amounts of the various types of unsaturated fatty acids in the total phospholipids were also reported (25). Earlier work on the component classes of milk phospholipids (1, 3, 14, 17, 19) and their fatty acid composition (3, 10, 15) was less detailed, but is in general agreement with our results. With regard to fatty acid composition, it has become apparent that the total phospholipids contain much larger amounts of polyunsaturated acids but negligible amounts of short-chain acids compared with milk triglycerides. However, the fatty acid composition of each individual phospholipid class and the distribution of specific fatty acids between the α^1-ester and β-ester positions of the glycerophospholipids are relatively unknown (11, 15, 19).

The work reported here is a continuation of our previous studies (24, 25) on improving the separation of the individual classes of phospholipids and sphingolipids in milk and determining the fatty acid compositions of fractions representative of the principal classes.

Received for publication January 22, 1962.

EXPERIMENTAL METHODS

Materials. Fresh buttermilk powder served as a convenient source of milk lipids (*cf.*, 19). The Holstein cream used in its preparation had been pasteurized for 30 min at 71 C, cooled, and churned. The resulting buttermilk was condensed in a double-effect (68 and 39 C) evaporator, and was spray-dried.

The beef brain and soybean lipid samples used as reference standards in paper chromatography were supplied by George Rouser.

Extraction of lipids. Extraction of the total lipids and separation of the triglycerides and phospholipids have been described (25). Briefly, the lipids were extracted from the buttermilk powder with ethanol, diethyl ether, and chloroform. Most of the phospholipids were separated from the triglycerides by acetone precipi-

tation, and the remainder were added following recovery on a silicic acid column. After the nonlipid contaminants were removed by washing twice with water, the phospholipid sample, which includes the sphingolipids, contained 3.53% phosphorus and 2.14% nitrogen.

Separation and isolation of lipid classes. Preparation of the silicic acid–Celite columns has been described in our previous paper (24). About 1.0 g of phospholipids was applied with 50 ml of chloroform to a column containing 75 g of silicic acid–Celite (2:1), and any residual triglycerides were eluted with 450 ml of chloroform. The phospholipids and sphingolipids were eluted, successively using 500 ml of acetone, 500 ml of 20% methanol in chloroform, 1,500 ml of 40% methanol in chloroform, and 600 ml of methanol. Fifty-milliliter fractions were collected at an average flow rate of 3.3 ml per minute. To follow the course of elution during the chromatographic run, 1.0 ml of each fraction was evaporated on a hot plate in an aluminum-foil dish (2 inches diameter by ½-inch deep). Elution curves (Figure 1) were plotted from phosphorus and sugar determinations (24) made on 1.0-ml portions of each fraction.

To identify the principal lipids, the infrared spectra of the peaks shown in Figure 1 were recorded with a Beckman Model IR-5 spectrophotometer from lipid films spread on potassium bromide disks as outlined in our earlier work (24). Films were more convenient to use for qualitative analysis than preparing disks by grinding the samples with potassium bromide.

To test the homogeneity of the peaks shown in Figure 1, fractions were examined by the paper chromatographic techniques of Rouser et al. (20).

Gas-liquid chromatographic (GLC) analysis of methyl esters of fatty acids. The methyl esters of the phospholipid and sphingolipid fractions were prepared by the method of Stoffel et al. (26), modified to eliminate the losses of esters of short-chain acids that would be incurred during the sublimation step in their procedure. Two milliliters each of pentane and an anhydrous methanol solution containing 5% hydrochloric acid were added to 25 to 50 mg of lipid in a 16- by 125-mm test tube fitted with a Teflon-lined screw cap. The tube was capped and heated 3 hr at 80 C, at first shaking occasionally to dissolve the lipid. After cooling, 2 ml each of water and pentane were added. The contents were shaken and the phases separated by centrifuging. The supernatant was removed and the lower phase extracted twice more in the same manner, using 2 ml of pentane each time. The combined extracts were chromatographed on a column 7 mm in diameter packed 40 mm deep with Mallinckrodt 100-mesh silicic acid. Above the silicic acid was placed a 10-mm layer of powdered dry potassium carbonate. The methyl esters were eluted into a 50-ml centrifuge tube with 20 ml of diethyl ether–pentane (1:1), and most of the solvent was evaporated at 20 C. Part of each ester sample was hydrogenated by the method of Farquhar et al. (4), except that hexane was used in place of absolute ethanol.

Methyl esters of the triglyceride fraction were prepared by alkali-catalyzed methanolysis. This procedure and details of the gas-liquid chromatographic analysis of the triglyceride fatty acids are described in detail by Smith (23). That was the procedure followed in determining the fatty acid composition of the phospholipids and sphingolipids, except that the correction

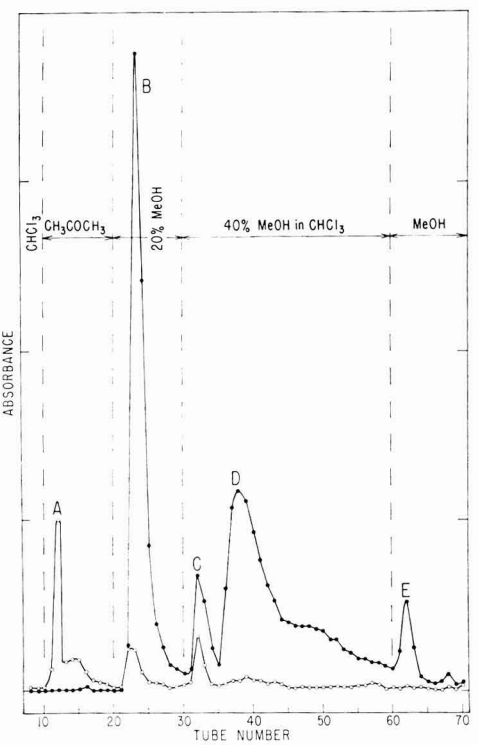

FIG. 1. Curve showing results of stepwise elution of 1.0 g of milk phospholipids from a 75-g silicic acid–Celite (2:1) column. Fractions, 50 ml, flow rate 3.3 ml/hr. Phosphorus (●) and sugar (○) concentrations in 1.0-ml portions expressed as relative absorbance.

factors for calculating the percentages of individual acids were obtained from mixtures of methyl esters of approximately the same composition as that of the lipid being analyzed.

Major ester peaks were identified by comparison of their retention volumes with those of pure esters run under the same conditions. Minor constituents, including saturated and unsaturated normal acids and saturated branched-chain acids, were tentatively identified on the basis of elution order, behavior on hydrogenation, and retention values compared with known values obtained under comparable conditions by other investigators (4, 9, 18).

RESULTS

Chromatographic separation of lipid classes. Figure 1 shows results obtained from a column chromatographic separation of milk lipids freed from triglycerides. The results thus obtained were reproducible when the separation was repeated on a second sample. The mixture was separated into five main peaks on the basis of relative concentrations of phosphorus and sugars as measured by anthrone reaction. The elution pattern suggests, however, that the fractions taken between the peaks could contain more than one constituent, for Peaks B and C do not completely return to base line, and Peak D shows marked tailing.

Curve *a* of Figure 2 is the infrared spectrum between 2 and 11 μ of the above phospholipid sample before chromatographic separation. Assignment of the absorption bands to specific groups follows that described by Freeman (5). Both phosphate paper and silicic acid paper chromatograms run on this sample revealed intense spots corresponding to phosphatidyl ethanolamine (PE), phosphatidyl choline (PC), and sphingomyelin. There were also distinct spots in the correct positions for cerebroside and lysolecithin, in addition to small amounts of uncharacterized materials that migrated to the solvent front or remained at the origin.

Infrared spectra were similar for corresponding fractions from the two-column chromatographic runs mentioned above. They were compared with those of pure reference compounds presented by Smith and Freeman (24). Curves *b* and *c* of Figure 2 are the spectra of Fractions 12 and 15 from Peak A and its shoulder (Figure 1). Both spectra resemble that of phrenosin, except for considerable absorption in the carbonyl ester region at 5.8 μ in Fraction 12 and a trace in Fraction 15. The paper chromatograms confirmed the presence of cerebrosides and revealed another unknown lipid that could account for the observed absorption at 5.8 μ. This lipid was not apparent on the whole phospholipid runs. The evidence from curves *b* and *c* shows that the peak at A included cerebrosides, together with a lesser amount of an unknown lipid whose presence was not revealed in paper chromatography of the whole lipid.

Curve *a* of Figure 3 is the spectrum of Fraction 23 (Peak B, Figure 1) and is typical of PE (*cf.*, 24). This fraction contained 4.16% of phosphorus and gave a positive anthrone reaction. Paper chromatography revealed only traces of cerebrosides, however, and it was concluded that the fraction was practically pure PE.

Curve *b* of Figure 3 shows that the spectrum of Fraction 32 (Peak C, Figure 1) resembled those of Fraction 23 and pure PE (*cf.*, 24) except that curve *b* had a more pronounced absorption band in the hydroxyl region around 3.0 μ. However, it is known (20, 24) that phosphatidyl serine (PS) has an infrared spectrum similar to that of PE and may be eluted after PE from silicic acid. Paper chromatography of combined Fractions 32 and 33 revealed only small amounts of PE and PC, the principal constituents being PS and phosphatidyl inosi-

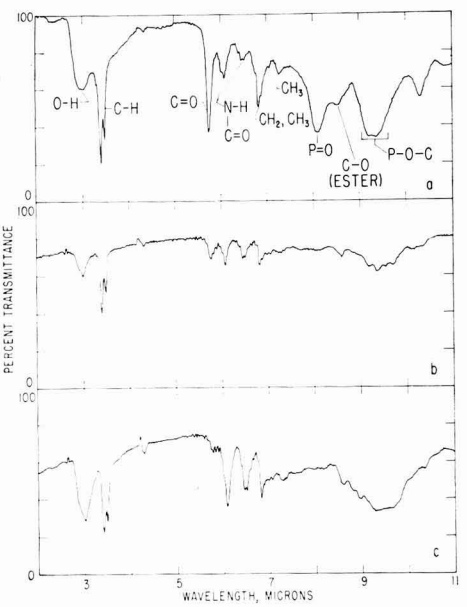

FIG. 2. Infrared spectra of lipid films spread on potassium bromide disks. (a) total milk phospholipids showing absorption band assignments. (b) Fraction 12 of Figure 1, cerebrosides plus unidentified lipid. (c) Fraction 15, mostly cerebrosides.

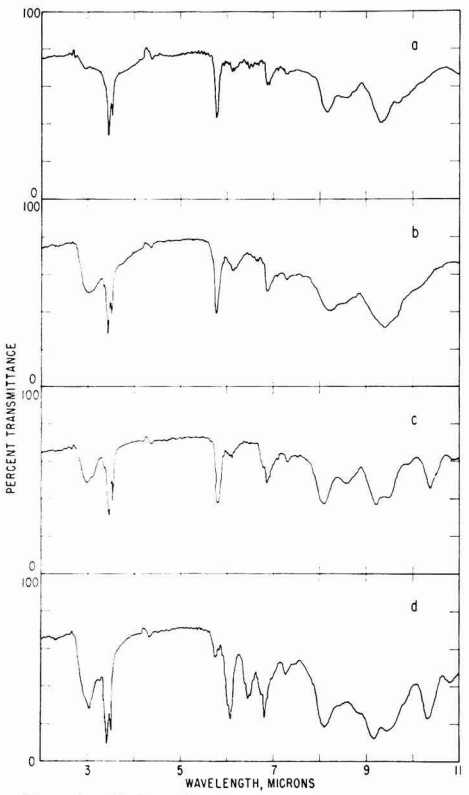

FIG. 3. Infrared spectra of fractions from chromatography of milk phospholipids (Figure 1). Samples run as films on potassium bromide disks. (a) Fraction 23, phosphatidyl ethanolamines. (b) Fraction 32, mostly phosphatidyl serines and phosphatidyl inositols. (c) Fraction 38, phosphatidyl cholines. (d) Fraction 62, sphingomyelins.

tols (PI). PI could account for the greater absorption in the hydroxyl region around 3.0 μ. The presence of PI is also consistent with the fact that it is eluted readily with 40% methanol in chloroform (19). Because Peak C did not begin at or return to the base line, a mixture is not unexpected. The evidence as a whole indicates that the material included in combined Fractions 32 and 33 was largely PS.

The spectrum of Fraction 38 (Peak D of Figure 1) in Figure 3-*c* is characteristic of PC (*cf.*, 24). The paper chromatograms and the phosphorus content of 3.88% confirmed that this fraction was pure PC.

Proceeding from Fraction 42 to 51 (Figure 1), the decreasing absorption at 5.8 μ, together with the increasing absorption at 6.1 μ, was consistent with decreasing PC content accompanied by an increase in sphingomyelin content.

The spectrum of combined Fractions 52 to 54 (Figure 1) corresponded to that of authentic sphingomyelin (*cf.*, 24). Furthermore, the paper chromatograms indicated that only 1-2% PC was present. The sample contained 3.92% phosphorus.

Curve *d* of Figure 3, the spectrum of Fraction 62 (Peak E, Figure 1), corresponds to that of sphingomyelin (*cf.*, 24), except for a small band at 5.8 μ. Paper chromatograms revealed only a trace of PC, however, and it was concluded that this fraction was practically pure sphingomyelin. It contained 3.81% phosphorus.

Fraction 68 (Figure 1), according to its infrared spectrum and paper chromatograms, was mostly lysolecithins plus a small amount of sphingomyelins.

Fatty acid compositions of individual lipid classes. Curves *a* and *b* of Figure 4 are gas-liquid chromatograms of the fatty acid methyl esters from PE (Fraction 23 of Figure 1) before and after hydrogenation. These curves are representative of the GLC separations obtained in this study, except that C_{23} and C_{24}

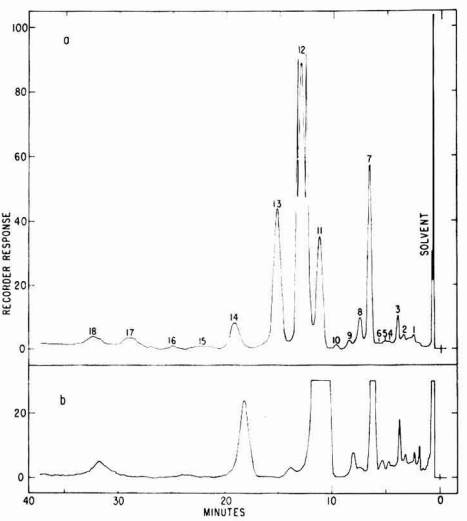

FIG. 4. GLC separation of fatty acid methyl esters of phosphatidyl ethanolamines before (4-a) and after (4-b) hydrogenation. See text for GLC conditions. Hydrogenated sample was approximately twice as large as normal sample. Designation of fatty acids by carbon chain length and number of double bonds: (1) 12:0, (2) 14:0 branched, (3) 14:0, (4) 14:1, (5) 15:1, (6) 16:0 branched, (7) 16:0, (8) 16:1, (9) 17:0, (10) 17:1, (11) 18:0, (12) 18:1, (13) 18:2 and 19:0, (14) 18:3 and 20:0, (15) 20:1, (16) 21:0, (17) 20:4, (18) 22:0. Peak 12 was attenuated ×2.

constituents also were found in the sphingolipids. The hydrogenated esters had elution volumes identical with those of the major saturated constituents, and did not exceed C_{24} in chain length.

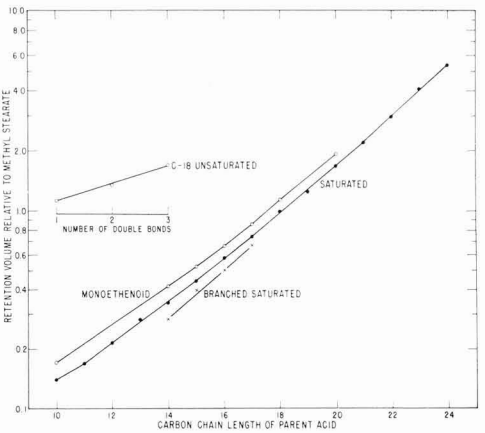

FIG. 5. Semilog plot showing relation between relative retention volumes from chromatograms of major classes of milk phospholipids and: (a) number of double bonds in the C-18 unsaturated esters, (b) number of carbon atoms in parent acid for saturated straight- and branched-chain acids, and for monoethenoid acids.

Figure 5 is a semilog plot of the average relative retention volumes from the GLC chromatograms of the phospholipid and sphingolipid classes vs. the number of carbons in the chain of the parent acid. Retention volumes of the saturated even-numbered homologous series from C_{10} through C_{22}, and of the C_{18} unsaturated esters, corresponded to those for the standard mixtures analyzed with the same GLC column. Retention volumes for the branched-chain, monoethenoid, and C_{20} through C_{24} saturated and unsaturated esters were in close agreement with the data of Hawke et al. (9) and Nelson (18).

Table 1 compares the fatty acid composition of fractions representative of the major classes of sphingolipids and phospholipids in milk with the composition of triglycerides obtained from the same source.

DISCUSSION

PC and sphingomyelins were not sharply separated in the present study, but that appears to be an inherent limitation of conventional silicic acid columns (18, 19). Though infrared spectra of lipid films are useful in identifying most of the lipid classes, they may fail to reveal minor impurities that can be detected by paper chromatography. For this reason, both techniques were used in the present study. No attempt was made to distinguish between PE, PS, and their corresponding plasmalogen forms, which others (14, 19, 22) have reported to be present in small amounts in milk phospholipids.

In agreement with our previous results (24), and with those of other investigators (3, 14, 19), the present results show that the major phospholipids and sphingolipids of milk are PE, PC, and sphingomyelins, with smaller amounts of PS and PI also present. Again, evidence of cerebrosides was strong, but the trace of lysolecithins found may be primarily an artifact. At this stage, attention should be drawn to the results of Billimoria et al., who found, in addition to the PC, PE, PS, and sphingomyelin reported by other investigators, a complex phosphatidic acid, phosphatidylserine galactoside, unknown compound X, phosphatidylcholine galactoside, and a choline lipid containing glucose and galactose. Moreover, in contrast to Billimoria et al., we consider PE as a major and not a minor constituent of milk phospholipids. It is possible that one or more of those previously unreported lipids might have been found in the present study if all fractions had been analyzed by paper chromatography. Nevertheless, our work indicates that any amounts must be very small, since they were not readily detected on paper chromatograms of the total milk phospholipids.

Table 1 shows that there were marked differences in fatty acid composition among the phospholipids and other lipids of milk. The cerebrosides and sphingomyelins were richest in saturated fatty acids, the triglycerides contained somewhat less, and the PC, PS, and PE contained still less. Triglycerides, as compared with the other lipids, contained less of the higher-molecular-weight even and odd C_{20} to C_{24} acids. In contrast to the results for the triglycerides, no appreciable amounts of acids below C_{10} were found in the phospholipids, though traces were present in the cerebrosides.

Palmitic was the principal saturated fatty acid of the cerebrosides, PC, sphingomyelins, and triglycerides, and stearic was the main saturated acid of PE and PS. PE, PS, and PC contained larger amounts of oleic, linoleic, linolenic, and arachidonic acids than did the sphingolipids and triglycerides. Though pentaenoic acid has been found in milk phospholipids by the use of alkali-isomerization (25), this acid was not revealed by GLC in the present investigation. The finding of substantial amounts of higher-molecular-weight saturated

TABLE 1

Fatty acid composition of phospholipids and other lipids in milk [a]

Fatty acid [b]	Cerebrosides (Fraction 12) [c]	Phosphatidyl ethanolamines (Fraction 23)	Phosphatidyl serines (Fractions 32-33)	Phosphatidyl cholines (Fraction 38)	Sphingomyelins (Fractions 52-54)	Sphingomyelins (Fractions 61-63)	Triglycerides
4:0	.....	.....	.....	.....	.....	.....	12.1
6:0	0.5	.....	.....	.....	.....	.....	3.9
8:0	0.2	.....	.....	.....	Trace	Trace	2.1
10:0	6.1	.....	Trace	Trace	0.7	0.3	3.4
10:1	.8	.....	.....	.....	.....	.....	0.4
11:0	.....	.....	.....	.....	0.7	0.2	.....
12:0	5.6	0.3	0.3	0.3	1.1	0.5	4.2
12:1	.6	.....	.....	.....	.....	.....	0.2
13:0	.1	.....	.....	.....	0.6	0.3	0.4
14:0 br	Trace	0.3	0.1	Trace	.....	.....	0.3
14:0	10.8	0.7	0.8	3.1	4.8	6.5	10.4
14:1	1.2	0.1	0.1	0.4	0.4	Trace	2.6
15:0 br	.....	.....	.....	0.2	Trace	Trace	.....
15:0	1.2	0.1	0.1	1.1	1.0	1.4	1.5
15:1	0.2	.....	0.1	.....	Trace	0.1	0.3
16:0 br	.....	0.3	0.1	0.4	0.1	0.1	Trace
16:0	27.6	8.3	7.1	26.1	24.5	58.0	23.5
16:1	1.4	1.9	1.7	3.0	1.7	1.2	3.9
17:0 br	.....	.....	.....	1.3	.....	Trace	0.7
17:0	0.9	0.5	0.4	0.8	0.8	1.2	0.8
17:1	1.1	0.3	0.4	0.3	0.2	0.1	0.6
18:0	3.3	8.5	16.9	8.2	3.3	3.5	7.1
18:1	5.7	53.6	45.8	34.3	6.4	7.1	17.6
19:0	.....	0.6	1.0	Trace	2.2	0.3	2.3
18:2	1.0	13.4	12.0	11.9		2.6	
20:0	1.6	4.3	3.4	0.5	1.4	1.5	1.5
18:3				3.5			
20:1	0.9	1.3	0.4	.....	1.3	0.4	0.1
21:0	0.8	0.5	0.5	0.3	1.8	1.1	.....
20:4	.....	3.2	3.2	1.3	2.0	.....	0.2
22:0	9.4	1.9	2.6	1.1	14.2	5.2	.....
23:0	12.5	.....	.....	.....	21.5	6.2	.....
24:0	6.6	.....	.....	.....	8.6	2.6	.....
Unknown	.....	.....	3.4	1.9	1.2	.....	0.1
Total saturated	86.4	24.1	31.6	43.4	85.5	88.9	72.3

[a] Data are expressed as mole per cent of total fatty acids
[b] Carbon-chain length:number of double bonds (4).
[c] See Figure 1.

acids (above C_{20}) in the sphingolipids is in agreement with the results of other workers for sphingolipids from other sources (13, 18). Paper chromatography indicated the presence in milk phospholipids of some cerebrosides with hydroxy-fatty acids, but these acids were not found by the GLC technique used.

Since only two fatty acids can occur in a single PE, PC, or PS molecule, and only one in a cerebroside or sphingomyelin molecule, each lipid class must be heterogeneous with respect to fatty acids and should be considered as a family of compounds. Furthermore, when a mixture of different lipids is subjected to silicic acid chromatography, the migration rate of molecules in each class down the column is influenced by fatty acid composition (5). In studying the fatty acid composition of lipid classes, it is important to obtain as complete a separation of each lipid class as possible. This was attempted in the present investigation, but the column chromatographic technique imposed limitations. The authors consider that a weighed average of the two sphingomyelin analyses would be more representative of the fatty acid composition of milk sphingomyelins than the data for either sample given in Table 1. With regard to the other lipid classes, the fractions analyzed are considered reasonably representative because they included the middle portions of comparatively sharp peaks.

With reference to the positions of the saturated and unsaturated fatty acids in the glycerol moiety of the glycerophospholipids, we are

TABLE 2

Fatty acid composition of phosphatidyl cholines (PC) and phosphatidyl ethanolamines (PE) from milk and egg [a]

Fatty acids	PC		PE	
	Milk	Egg [b]	Milk	Egg [b]
Saturated	43	43	24	60
Monoethenoid	39	40	57	14
Polyethenoid	18	18	19	27

[a] Data are expressed as mole per cent of total fatty acids.
[b] Data of Hawke (8).

in agreement with the conclusion of Rhodes and Lea (19), that the unsaturated fatty acids cannot all be attached at the β-position, because more than 50% of the total acids are unsaturated. No evidence was obtained as to the possibility that all the saturated acids occur at the α^1-position, as suggested by Hanahan et al. (7).

Eggs are another important source of dietary phospholipids, and the fatty acid composition of egg PC and PE has been examined by Hawke (8). Comparing the fatty acids of the PC fraction with those obtained from milk, the total amounts of saturated, monoethenoid, and polyethenoid acids (43, 40, and 18 mole %, respectively) were similar, but there were differences in the amounts of individual acids within each group. In the PE fractions, however, the differences in composition were marked. The respective amounts (mole %) for egg and milk were: saturated 60, 24; monoethenoid 14, 57; and polyethenoid 27, 19. With regard to the polyethenoid acids, egg PE contained less dienoic and more C_{20} and C_{22} acids with four to six double bonds than did milk PE.

The present investigation has shown that the various lipids of milk differ markedly in fatty acid composition, but the physiological implications of the differences are unknown. In this connection, Rouser and Schloredt (21) showed that the fatty acid composition of PE from various sources is important in the in vitro clotting of blood plasma. Fatty acid composition may also be important in explaining the marked in vitro thromboplastic activity of the unknown lipid X, described by Billimoria et al. However, in vivo feeding studies are needed to elucidate any possible effects of milk phospholipids on the composition and properties of blood phospholipids.

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LIPID EXCHANGE BETWEEN BOVINE SERUM LIPOPROTEINS IN VITRO [1, 2]

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SUMMARY

Lipid incorporation and exchange in whole blood by bovine serum lipoproteins of high ($D > 1.063$) and low density ($D < 1.063$) have been studied in vitro. The high-density lipoproteins evinced a strong binding capacity for cholesterol and phospholipids. Low-density lipoproteins showed a great affinity for cholesteryl palmitate and tripalmitin. Palmitic and linoleic acids were bound almost entirely by serum proteins. Cholesterol exchanged between high- and low-density lipoproteins, rate of transfer being in the direction of the former. Cholesteryl palmitate moved only from low- to high-density lipoproteins. Tripalmitin and phospholipid did not transfer in either direction. Palmitic and linoleic acids transferred from both lipoprotein groups to the serum proteins. Cholesterol and tripalmitin labeled lipoproteins of high and low density lost a small amount of radioactivity to the blood cells.

In a previous report, the major bovine serum lipoproteins of high ($D > 1.063$ g/ml) and low density ($D < 1.063$ g/ml) were analyzed with regard to lipid classes and fatty acid composition (4). Significant differences were noted. Whether these differences predispose preferential udder absorption of a particular lipoprotein group and, thereby, impart to it relative importance in the process of milk fat synthesis has yet to be determined. Udder perfusion studies, utilizing serum lipoproteins selectively labeled in their lipid moiety, would test this hypothesis. As a prerequisite for such studies, however, explication seems in order regarding lipid exchange dynamics in bovine blood per se.

In human and rabbit blood both cholesterol (2) and certain phospholipids (13) exchange between high- and low-density lipoproteins and, in human blood, they have been shown to transfer readily between plasma and blood cells (8, 13). Cholesteryl ester exchange between serum lipoproteins proceeds at a very slow rate in dogs (5). Triglycerides have been demonstrated to undergo no exchange between high- and low-density lipoproteins in ordinary human blood (12), but in post-heparin serum, low-density lipoproteins lose labeled triglyceride to lipoproteins of high density (12).

The present investigation sought to develop a more unified picture of lipid exchange dynamics by including all of the major lipid classes and relating them to one animal species. This would then provide part of the groundwork for later perfusion studies.

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EXPERIMENTAL PROCEDURE

Radioactive material. Tripalmitin-1-C^{14}, cholesterol-4-C^{14}, palmitic acid-1-C^{14}, and linoleic acid-1-C^{14} were obtained from Nuclear-Chicago Corporation. Cholesteryl palmitate-1-C^{14} was prepared by the method of Swell and Treadwell (17), isolated on silicic acid columns according to the procedures described by Hirsch and Ahrens (10) and McCarthy and Duthie (14), and its purity ascertained by infrared spectroscopy. Isotopically labeled phospholipids with a specific activity of 5,131 cpm/mg were obtained from goat blood 24 hr following an intravenous injection of 7 mc of disodium hydrogen phosphate-P^{32}. The phospholipids were isolated on silicic acid columns (10). P^{32}-labeled serum phospholipoproteins of high density were also obtained from the same goat by ultracentrifugation (9).

Methods. Preparation of labeled lipoproteins. Blood was obtained by jugular venipuncture

from normal lactating Holstein cows, and the serum separated. Incorporation of labeled lipid into the serum lipoproteins was achieved according to the technique of Stein and Shapiro (15). Each labeled lipid (containing 1 μc of C^{14} and not more than 0.1 μ mole) was added, in 0.25 ml alcoholic dispersion, to 30 ml of serum and incubated 1 hr at 40 C with gentle shaking. Phospholipids (containing 0.5 μc of P^{32} and not more than 0.25 g) were added in 0.5 ml alcoholic dispersion.

A preliminary centrifugation was performed to remove any labeled lipid that had not been incorporated into the lipoproteins. Seven milliliters of the incubated serum was overlaid with 3 ml of 0.15 M NaCl and centrifuged at 50,000 $\times$ g for 30 min at 11 C. The top 2.5-ml fraction was then removed from each tube with a syringe. No radioactivity was found in these fractions. Control blanks containing 0.15 M NaCl and the appropriately labeled lipid (added in alcoholic dispersion) were subjected to similar incubation and centrifugation; they exhibited a complete floating of radioactivity in the top 2-ml fraction.

Ultracentrifugal separation of lipoproteins. The infranatant serum was adjusted to a solvent density of 1.063 g/ml and ultracentrifuged at 105,000 $\times$ g for 22 hr according to Havel et al. (9). This allowed the low-density lipoproteins ($D < 1.063$) to float to the top of the tube while the high-density lipoproteins ($D > 1.063$) sedimented, with the serum proteins, toward the bottom. For use in lipid-exchange incubations the top 2-ml fraction was removed with a syringe and dialyzed against 0.15 M NaCl at 10 C for 48 hr. The next 4-ml fraction was discarded. The bottom 4-ml fraction, containing high-density lipoproteins and serum proteins, was adjusted to a density of 1.21 g/ml and again ultracentrifuged (9). The top 2-ml fraction, which then contained the high-density lipoproteins, was removed and dialyzed against 0.15 M NaCl at 10 C for 24 hr. For label distribution studies, following lipid-incorporation and lipid-exchange incubations, 1-ml fractions were removed sequentially with a syringe from the ultracentrifuge tubes at density 1.063 g/ml.

When palmitic acid-1-C^{14} and linoleic acid-1-C^{14} provided the isotope, serial 1-ml fractions were also removed at density 1.21 g/ml to enable measurement of nonesterified fatty acid binding by serum proteins. In this case, the bottom 2-ml fraction at density 1.21, containing the serum proteins, had also been dialyzed against 0.15 M NaCl at 10 C for 24 hr and used in the exchange incubations.

Exchange incubations. Following dialysis, the labeled lipoproteins of high and low density (as well as the labeled serum proteins in the nonesterified fatty acid studies) were each divided into two parts of 4 ml. Each part contained that amount of the particular lipoprotein (or protein) found in 14 ml of the original serum. One 4-ml portion was combined with 5 ml of 0.15 M NaCl and served as a control. The remaining portion was added to 10 ml of oxalated fresh whole blood obtained from the same cow that had provided the original serum lipoproteins. Both the control and experimental flasks were incubated at 40 C for 1 hr with gentle shaking.

After incubation, the controls were refrigerated at 0 C while the contents of the experimental flasks were being centrifuged at 5 C for 15 min at 552 $\times$ g to enable removal of the plasma from the blood cells. Aliquots of the plasma were then subjected to ultracentrifugation (9), to separate the high- and low-density lipoproteins. Aliquots from the control flasks were similarly ultracentrifuged. Fractions of 1 ml each were serially removed from the ultracentrifuge tubes, as described above, and analyzed for radioactivity.

Measurement of radioactivity. A 50-μl aliquot was plancheted from each of the 1-ml consecutive ultracentrifuge tube fractions and assayed for radioactivity in a thin window flow counter. Degree of quenching at these low concentrations was examined and found to be negligible. To determine the per cent of activity transferred to the blood cells, similar aliquots were counted from the washed blood cells, 0.15 M NaCl washings, and the nonultracentrifuged plasma remaining after the exchange incubations.

The possibility of isotope transfer between lipid classes was examined and found to be negligible. Lipids from high- and low-density lipoproteins and from proteins (in the nonesterified fatty acid studies) were extracted. Lipid classes were isolated on silicic acid columns (10, 14) and radioactivity measured. In all cases the isotope had remained in its original lipid class. The radioactivity found in the blood cells, however, Table 3, was not delineated as to lipid class, since the prime interest of this experiment was in lipid transfer between serum lipoproteins.

Electrophoresis. Degree of separation between the high- and low-density lipoproteins was determined electrophoretically following the ultracentrifugation of bovine serum at a solvent density of 1.063 g/ml and serial removal with a syringe. From each 1-ml fraction (representing in toto the contents of the ultra-

centrifuge tube from top to bottom) an aliquot of 25 μl was subjected to hanging-curtain paper electrophoresis with a technique similar to that of Straus and Wurm (16). Prior dialysis was found to be unnecessary. A constant current of 160 v was applied for 16 hr, after which the electrophoretic strips were dried in an oven at 130 F for 15 min. Lipids were stained with fat red 7B (16).

Serum and oxalated blood used in this experiment contained penicillin and dihydrostreptomycin (approximately 100 units each/milliliter biological fluid) as well as 0.01% disodiumethylenediaminetetraacetate, to inhibit oxidation of the low-density lipoproteins. All salt solutions contained 0.05% disodiumethylenediaminetetraacetate.

A corollary study was made of lipid exchange in lactating goat blood. Isotopic lipids, however, were limited to cholesterol-4-C^{14}, tripalmitin-1-C^{14}, and P^{32}-phospholipoproteins, the latter class labeled in vivo.

RESULTS

Table 1 shows the distribution of radioactivity in the ultracentrifuge tubes following binding-incubations of labeled lipids with serum, and subsequent ultracentrifugation. Tube fractions 1-4 and 5-10 represent the activity present in low- and high-density lipoproteins, respectively, at density 1.063. At density 1.21 these fractions represent activity present in the high-density lipoproteins and proteins, respectively. The density 1.21 classification was used to differentiate the nonesterified fatty acid binding of serum proteins from that of the lipoproteins. This consideration was unnecessary for the other lipid classes (3, 18). The data, from representative experiments, is expressed as per cent of total ultracentrifuge tube activity at density 1.063. From the distribution of radioactivity it can be seen that tripalmitin and cholesteryl palmitate are preferentially incorporated by the low-density lipoproteins. Cholesterol and phospholipids have a greater affinity for the high-density lipoproteins. Palmitic and linoleic acids are bound almost entirely by the serum proteins.

TABLE 1

Distribution [a] of radioactivity in the ultractntrifuge tube following incubation of labeled lipids with bovine serum

	Tube fractions			
	D 1.063 g/ml		D 1.21 g/ml	
Labeled lipid	1-4 [b]	5-10 [b]	1-4 [c]	5-10 [c]
Cholesterol-4-C^{14}	25	75		
Cholesteryl palmitate-1-C^{14}	76	24		
Tripalmitin-1-C^{14}	78	22		
Phospholipid-P^{32}	0	100		
Palmitic acid-1-C^{14}	1		3	96
Linoleic acid-1-C^{14}	1		3	96

[a] Expressed as per cent of total activity in the ultracentrifuge tube at density 1.063 g/ml.

[b] At density 1.063 g/ml, Fraction 1-4 contains low-density lipoproteins, Fraction 5-10 contains high-density lipoproteins and serum proteins.

[c] At density 1.21 g/ml, Fraction 1-4 contains high-density lipoproteins, Fraction 5-10 contains serum proteins.

Triglycerides synthesized in human blood cells have been demonstrated to be preferentially incorporated by high-density lipoproteins (11), cholesterol (18), and certain phospholipids (7, 11) by low-density lipoproteins, and palmitic and linoleic acids by serum albumin (1) and low-density lipoproteins (7).

Table 2 presents the distribution of radioactivity in the ultracentrifuge tubes following exchange incubations of labeled lipoproteins with unlabeled whole blood and subsequent ultracentrifugation. Tube fraction categories are the same as those described for Table 1. The data again are expressed as per cent of total tube activity at density 1.063 and are taken from representative experiments. It can be seen that cholesterol exchanges between the high- and low-density lipoproteins in whole blood, the transfer from low- to high-density lipoproteins being far the greater. Cholesteryl

TABLE 2

Distribution [a] of radioactivity in the ultracentrifuge tube following incubation of labeled lipoproteins with unlabeled whole blood

	Tube fraction	
	D 1.063 g/ml	
Label	1-4 [b]	5-10 [b]
HDL[c]-cholesterol-4-C^{14}	12	88
LDL[c]-cholesterol-4-C^{14}	50	50
HDL-chol. palmitate-1-C^{14}	0	100
LDL-chol. palmitate-1-C^{14}	67	33
HDL-tripalmitin-1-C^{14}	0	100
LDL-tripalmitin-1-C^{14}	100	0
HDL-phospholipid-P^{32}	0	100
LDL-phospholipid-P^{32}	....[d]	....

[a] Expressed as per cent of total activity in the ultracentrifuge tube at density 1.063 g/ml.

[b] Fraction 1-4 contains low-density lipoproteins, Fraction 5-10 contains high-density lipoproteins and serum proteins.

[c] HDL refers to high-density lipoproteins, LDL refers to low-density lipoproteins.

[d] No activity in any fraction.

TABLE 3

Radioactivity in blood cells following incubations of labeled lipoproteins (and serum proteins) with unlabeled whole blood

Label	Activity [a] in blood cells
HDL[b]-cholesterol-4-C^{14}	5
LDL[b]-cholesterol-4-C^{14}	1
HDL-cholesteryl palmitate-1-C^{14}	0
LDL-cholesteryl palmitate-1-C^{14}	0
HDL-tripalmitin-1-C^{14}	3
LDL-tripalmitin-1-C^{14}	1
HDL-phospholipid-P^{32}	0
LDL-phospholipid-P^{32}	0
HDL-palmitic acid-1-C^{14} [c]	0
LDL-palmitic acid-1-C^{14} [c]	0
P [b]-palmitic acid-1-C^{14} [c]	0

[a] Expressed as per cent of total activity in incubation flask.

[b] HDL refers to high-density lipoproteins, LDL refers to low-density lipoproteins, P refers to serum proteins.

[c] Data are identical to those of bound linoleic acid.

palmitate moved only from low- to high-density lipoproteins. Tripalmitin showed no lipoprotein transfer and phospholipids showed no transfer from high- to low-density lipoproteins. Palmitic and linoleic acids moved from both high- and low-density lipoproteins to the serum proteins. Controls containing the appropriately labeled lipoprotein in 0.15 M NaCl were subjected to the same conditions of incubation and ultracentrifugation. In each case, radioactivity remained with the initially labeled lipoprotein class.

The exchange of cholesterol and nonexchange of tripalmitin is in agreement with studies of human (8, 12) and dog (5) blood. The one-way transfer of cholesteryl palmitate from low- to high-density lipoproteins is in partial accord with dog studies, in which slow in vivo exchange of cholesterol esters was demonstrated. In vitro conditions and/or shorter incubation time (1 hr) used in our studies, to avoid lipoprotein decomposition (7), may have prevented the observation of eventual two-way exchange. The lack of phospholipid transfer is in disagreement with the findings of Eder et al. (2), who demonstrated in vitro phospholipid exchange between rabbit serum lipoproteins. This variance may be the result of species differences, insofar as both cow and goat blood studied in our experiment showed an identical lack of phospholipid transfer.

Radioactivity contained in the blood cells, following the exchange incubations, is presented in Table 3. Data, from representative experiments, is expressed as per cent of total activity within the incubation flasks. It can be seen that tripalmitin and cholesterol from both high- and low-density lipoproteins transferred to the blood cells.

In human blood studies cholesterol (8), certain phospholipids (13), and triglycerides (13) have been demonstrated to exchange between blood cells and plasma. However, nonesterified fatty acids at normal physiological levels, when equilibrated between human erythrocytes and serum albumin, have shown a strong affinity for the latter (6).

Figure 1 illustrates electrophoretically a typical separation between bovine serum lipoproteins of high and low density obtained by ultracentrifugation in a solvent density of 1.063 g/ml. Each electrophoretic paper strip represents a separate 1-ml fraction removed con-

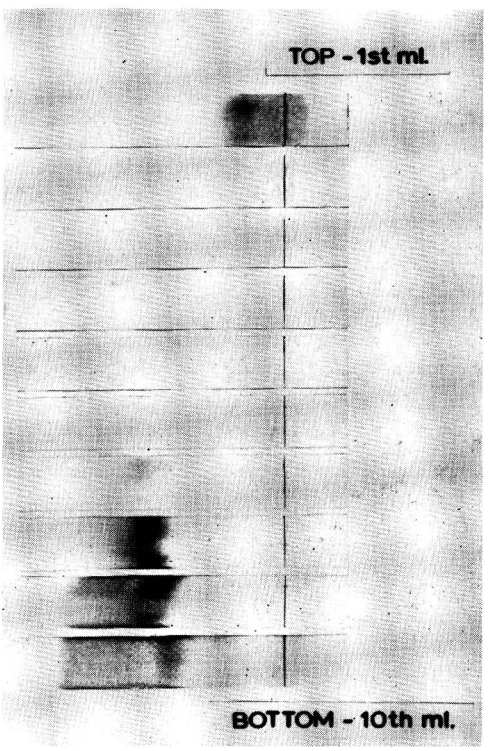

FIG. 1. Electrophoretic strips showing distribution of bovine serum lipoproteins of high ($D > 1.063$) and low density ($D < 1.063$) in the ultracentrifuge tube after spinning at a solvent density of 1.063 g/ml.

secutively from the ultracentrifuge tube from top to bottom. The strips have been lipid-stained. Migration toward the left is in the direction of the positive electrode. The top first milliliter strip shows the low-density lipoproteins which have floated to the top of the ultracentrifuge tube and are characterized electrophoretically by their correspondence in mobility and trailing to β-lipoproteins (7). Residual traces of low-density lipoproteins can be seen in the second milliliter strip, as well. The third through sixth milliliter strips are essentially lipid-free. High-density lipoproteins, which exhibit the characteristic mobility of α-lipoproteins, are contained in the seventh through tenth milliliter strips. Since the lipid stain, fat red 7B, is bound to a slight extent by bovine serum proteins (3, 16), fast-moving serum albumin is evident in the eighth through tenth milliliter strips and β-globulin and non-moving γ-globulin can be seen in the tenth milliliter strip. This lipid-staining pattern of serum proteins is found, as well, in the ninth and tenth milliliter strips after ultracentrifugation at a solvent density of 1.21 g/ml.

In the corollary goat study, using labeled cholesterol, tripalmitin, and phospholipoproteins, incorporation and exchange patterns between high- and low-density lipoproteins were found to duplicate those of the cow. Moreover, electrophoretic analysis demonstrated a similar ultracentrifugal separation of high- and low-density lipoproteins at a solvent density of 1.063 g/ml.

DISCUSSION

Within the limits of in vitro techniques, bovine serum lipoproteins have shown definite patterns of lipid incorporation and exchange. It must be realized, however, that lipid incorporation, depending on the lipid, may actually represent an end result of both incorporation and exchange dynamics. Since a patricular lipid may be bound by several blood components, but preferentially transferred among them to only one, misinterpretations are possible that incorporation occurred only by that one. The current value of lipid incorporation studies, therefore, may be in establishing methods for obtaining labeled blood components, rather than as an index necessarily to their in vivo binding capacities.

The high-density lipoproteins demonstrated a great affinity for cholesterol. This affinity was manifested whether cholesterol was presented as an alcoholic dispersion or as part of a low-density lipoprotein lipid moiety. The low-density lipoproteins, on the other hand [considering that they normally contain less than 4% of the serum cholesterol (4)], evinced an even greater binding capacity for the alcoholically dispersed cholesterol. The incorporated cholesterol, however, tended to be transferred to lipoproteins of high density in whole blood. It would appear that cholesterol, although transported in the blood preferentially by the high-density lipoproteins, may be incorporated initially by the lipoproteins of low density.

The low-density lipoproteins also exhibited a pronounced binding capacity for tripalmitin, although triglycerides in bovine high- and low-density lipoproteins are almost identical in fatty acid composition and weight per unit of serum (4). This would suggest that lipid incorporation is regulated by more than simple diffusion processes. Since no exchange of tripalmitin occurred between the two lipoprotein groups in whole blood, triglyceride reapportionment would seem necessitated in other tissues of the body, a major portion probably in the liver.

Cholesteryl palmitate was bound preferentially by the low-density lipoproteins, but showed some transfer to the high-density lipoproteins in whole blood. A reverse transfer, from high- to low-density lipoproteins, did not occur. Bovine low-density lipoproteins normally contain only 4% of the total serum cholesteryl esters (4). However, the proportion of cholesteryl palmitate within the low-density lipoprotein cholesteryl esters is five times as great as the proportion of cholesteryl palmitate present in the cholesteryl esters of the high-density lipoproteins (4). Thus, a possible significance may be attributable to the influence of individual esterified fatty acids per se on lipid-binding capacities of lipoproteins.

The phospholipids were bound entirely by the high-density lipoproteins and showed no transfer when later incubated with unlabeled whole blood. Phospholipids in the serum of the P^{32}-injected goat showed in vivo incorporation, as well, only into the high-density lipoproteins both 24 hr and eight days following injection. These in vivo-labeled high-density phospholipoproteins similarly showed no transfer of label when incubated with unlabeled whole goat blood. Since phospholipids comprise 32% of the lipid moiety of bovine low-density lipoproteins (4), the demonstrated inability of the latter to bind endogenous or alcoholically dispersed phospholipids may indicate the existence of an alimentary source of phospholipids for this class of lipoproteins in the ruminant.

The transfer of cholesterol, and especially of tripalmitin, to the blood cells from both the high- and low-density lipoproteins remains to be elucidated.

By their differing patterns of lipid incorporation and exchange, the bovine serum lipoproteins of high and low density would appear to perform different, although overlapping, functions. The high-density lipoproteins, very unsaturated and small but exhibiting a great affinity for cholesterol and phospholipids, would appear to be more endogenously transportive. The low-density lipoproteins containing only 5-7% of bovine total serum lipids (4), being considerably more saturated and bulky with glyceride, and demonstrating great binding capacities for tripalmitin and cholesteryl palmitate, may have a propensity for exogenous and early post-hepatic lipid transport. The relative contribution of high- and low-density lipoproteins to ruminant metabolism may be influenced by this difference in emphasis.

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EFFECT OF COMBINING SINGLE-STRAIN CULTURES AS CHEESE STARTER ON BITTERNESS IN CHEDDAR CHEESE AT SIX MONTHS OF AGE

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SUMMARY

Five strains of *Streptococcus cremoris* were used to investigate how bitterness in pasteurized-milk cheese was influenced by different combinations and proportions of paired strains in the starter.

The combination of strains as starter influenced bitterness in cheese at six months of age as follows: (a) The intensity of bitterness decreased as the proportion of the nonbitter-cheese-producing strain in the starter increased; (b) the intensity of bitterness was less in cheese made with Strain ML_1 in the combination than with Strain E_8, even though the intensities of bitterness were low in cheeses made with either ML_1 or E_8 alone; and (c) the combination of two bitter-cheese-producing strains yielded bitter cheese. The results showed that nonbitter cheese could be produced even if one strain produced bitter cheese when used alone as starter. Being able to use a bitter-cheese-producing strain as one of a pair of strains in a starter simplifies the problem of selecting strains for rotation in a phage-control program.

The intensity of bitterness was higher in cheese where the average chain lengths of TCA-soluble peptides and amino acids were higher.

Data presented in a previous paper (4) indicated that the strain of starter culture had a pronounced effect on the development of bitterness in Cheddar cheese. The effect of combining strains on bitterness in cheese is of direct interest in formulating commercial cultures and in selecting pairs of strains for rotation in a phage-control program. The choice of strains would be simplified if it were possible to produce nonbitter cheese by selecting and combining strains on the following basis: one strain for its acid-producing characteristics, even though it produced bitter cheese when used alone; another strain for its nonbitter-cheese-producing characteristics even though its acid-producing characteristics were not entirely satisfactory. Only one of the 11 strains in the previous study combined satisfactory acid-producing characteristics with the production of nonbitter cheese. The following experiment was carried out to test the effect on bitterness in Cheddar cheese of combining single-strain cultures as cheese starter.

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EXPERIMENTAL PROCEDURES

General outline. Five strains of *Streptococcus cremoris* were used. Strains HP, C_{13}, and K had previously produced bitter cheese; Strains ML_1 and E_8 had produced nonbitter cheese (4). These strains were paired to give five combinations: HP:ML_1, C_{13}:ML_1, HP:E_8, C_{13}:E_8, and HP:K. The first four combinations comprised one bitter strain and one nonbitter strain; both members of the fifth pair had produced bitter cheese in this laboratory. In this paper the terms bitter and nonbitter strains refer to the taste characteristics of cheese made with them as starters; milk cultures of these organisms were not bitter.

Each day, the strains of one pair were used to make four vats of cheese. Using the combination of HP and ML_1 as an example, the starter for the four vats or treatments, A, B, C, and D, consisted of: HP alone (A), HP

TABLE 1

Percentage of starter used to inoculate vats for each treatment and combillation

Treatment	A	B	C	D
Ratio of strains	1:0	1:1	1:3	0:1
Strain combination	('Yo)			
HP:ML_1	1.75 (HP)	1.03: 1.03	0.56 :1.69	2.5 (ML_1)
C_{13}:ML_1	2.0(C_{13})	1.11: 1.11	0.59 :1.76	2.5 (ML_1)
HP :E.	1.75(HP)	0.96 :0.96	0.50: 1.51	2.125 (E.)
C_{13}:E_8	2.0 (C13)	1.03 :1.03	0.52: 1.57	2.125 (Es)
HP:K	1.75(HP)	1.17: 1.17	0.70: 2.10	3.5(K)

and ML_1 in a weight ratio of one to one (B), the two strains in a weight ratio of one to three (C), and ML_1 alone (D). The other fonr pairs were combined in the same proportions.

The five combinations of strains were nsed to make cheese ill a series of three replicates, giving a total of 60 vats of cheese. The order of use of the combinations was randomized in each replicate.

Addition of starter to the vats. The strains were grown and added separately to the vat. The percentages of starter used are shown in Table 1. Preliminary experiments were conducted to find the percentage of starter required for each individual strain to give pH 6.05 in the curd at draining the whey, with 130 min cooking time. In Treatment B, where the strains were combined in a 1 :1 ratio, the amount of each was determined algebraically as $\frac{ad}{a+d}$ %, where a and d were the percentages of starter used in the A and D treatments, respectively. In Treatment C, where the strains were combined in a ratio of 1 :3, the percentages were $\frac{ad}{3a+d}$ % and $\frac{3ad}{3a+d}$ %. ThiS assumed that the acid-producing powers of these strains were additive and that neither strain inhibited or enhanced the n('tivit.v of the other strain.

Manufacturing procedures. Pasteurized (161 F, 16 sec) milk from the herds of the Central Experimental Farm was made into cheese in foul' 450-lb lots on each day. This milk was standardized to a casein-fat ratio of 0.70 by the removal of creanl. The vats were set at 88 F, 1 min after adding the starter. The curd was cut at two and one-half times the coagulation time after adding the rennet. Cooking cOllllll1eneed 5 min after cutting, and proceeded gradually until 100 F was reached 30 min later. The whey was drained when the curd reached a pH of 6.05. The eurd was stirred twice, at 4 and at 7 min after draining, then piled. The curd was milled at pH 5.4. Salt was added 10 min later in two applications, 5 min apart, at the normal rate of Van Slyke and Price (10), according to the fat content of the standardized milk. The curd was hooped 30 min later into 20-lb square hoops and pressed overnight at 60 psi, eut into 5-lb blocks, and wrapped in Parakote. The cheese was stored at 60 F for 2 wk, then at 40 F for the remainder of the storage period.

Chemical analyses. Fat (7), moisture (1), salt (10), and pH (small glass electrode, Beckman pH meter model H-2) were determined on cheese from each vat at 2 wk of age. Acid-soluble amino nitrogen and acid-soluble, TCA-soluble nitrogen were determined on the cheeses at six months of age (4).

Evalaation of cheese for bitterness at six months of age. At six months of age, the cheeses were examined for bitterness by a panel of four tasters, using a procedure similar to that dpscrihed previously (4). Each cheese was tasted twice during 1 wk and twice again the following \l'eek, for a total of four eva-Iuabons per taster or 16 evaluations per cheese. Each taster scored the cheese for intensity of bitterness on a scale of 1 to 6, in whole numbers only. The reference standard at 5 on the scale was a ground sample of bitter cheese made with Strain HI> alone. Standard 3 was an equal mixture by weight of Standard 5 and nonbitter cheese made with Strain ML_1 alone. As in the previous study (4), it was the opinion of the panel members that most consumers would not criticize cheese for bitterness with a mean score of less than 3.0. The results of comparison of various solutions of quinine sulfate with Standards 3 and 5 are given elsewhere (5).

RESULTS AND DISCUSSION

Table 2 is a summary of sonle manufacturing data and of the composition of the 60 cheeses at 2 wk of age.

The eheddaring time, or the time from piling to milling, ranged from 42 to 130 min. The

TABLE 2

Summary of manufacturing data and of composition of cheese at 2 wk of age

Treatment Ratio of strains	A [a] 1:0	B [a] 1:1	C [a] 1:3	D [a] 0:1	Mean	Range
Manufacturing data						
Cooking time—*(min)*	131.8	124.5	124.1	131.7	128.0(±6.1) [b]	114–140
pH of curd at time of draining whey	6.05	6.04	6.04	6.05	6.05(±0.010)	6.07–6.03
Increase in acidity of whey during cooking—*(%)*	0.033	0.033	0.032	0.030	0.032(±0.002)	0.028–0.035
Cheddaring time—*(min)*	64.9	64.2	65.2	95.5	72.4(±18.1)	42–130
Milling pH	5.39	5.38	5.39	5.39	5.39(±0.019)	5.41–5.30
Composition of cheese at 2 wk						
Moisture—*(%)*	35.51	35.47	35.63	35.66	35.57(±0.30)	34.88–36.50
Fat—*(%)*	33.83	33.94	33.80	33.65	33.80(±0.43)	33.04–34.93
pH	5.02	5.00	5.00	5.00 [c]	5.01(±0.025)	4.96–5.11
pH	.....	.....	.....	5.24 [d]	5.24(±0.021)	5.22–5.26
Salt—*(%)*	1.84	1.83	1.86	1.90	1.86(±0.083)	1.70–2.11

[a] Mean of 15 vats.
[b] Values in parentheses are standard deviations of the observations for individual vats about the means.
[c] Mean of 12 vats, omitting Strain K.
[d] Three vats made with Strain K alone.

longer times occurred when Strains K and ML_1 were used alone as starters in Treatment D. Where these slower strains were combined with faster strains in the B and C treatments, cheddaring times were very close to those of the faster strains used alone.

The pH of the cheeses ranged from 5.26 to 4.96 at 2 wk of age. The three highest values of 5.22, 5.23, and 5.26 arose from the three cheeses made with Strain K alone. The pH values for the other 57 vats ranged from 5.11 to 4.96. The pH values of the cheeses made by combining HP and K were about the same as those made with HP alone.

Effect on the cooking times of combining cultures. Table 3 shows the cooking times for each combination and each treatment, averaged over the three replicates. Generally, the cooking times in the B and C treatments were the same as or less than those in the A and D treatments. Combining HP and K resulted in the same cooking times as when HP and K were used alone. For combinations of HP and E_8 and of HP and ML_1, the cooking times in the B and C treatments tended to be shorter than in the A and D treatments, but the differences were not significant ($P < 0.05$) for all comparisons. The combinations of ML_1 and E_8 with C_{13} produced significantly shorter cooking times in the B and C treatments than in comparable vats, where they were used alone. These results show that none of the paired strains were inhibitory to each other; the pairing of either ML_1 or E_8 with C_{13} resulted in a stimulatory effect; the

TABLE 3

Mean cooking times for cheese made with various combinations of strains

Treatment Ratio of strains	A 1:0	D 0:1	B 1:1	C 1:3
Strain combination		*(min)*		
HP:ML_1 [b]	128.7	133.3	121.3	125.7
C_{13}:ML_1 [b]	137.7	131.0	120.3	118.3
HP:E_8 [b]	126.3	130.3	125.0	126.3
C_{13}:E_8 [b]	134.7	132.3	123.3	122.7
HP:K [b]	131.7	131.3	132.7	127.7

Standard error for horizontal comparisons = ±1.83, d.f. = 30. Values not joined by underlining are significantly different ($P < 0.05$) by Duncan's multiple range test (3).

TABLE 4

Mean scores for bitterness at six months of age in cheese made with various combinations of strains

Treatment	A	B	C	D
Ratio of strains	1:0	1:1	1:3	0:1
Strain combination				
HP:ML_1	4.6	3.5	2.7	1.8
C_{13}:ML_1				
Omitting Replicate 1	3.9	2.5	2.3	1.5
Including Replicate 1	(4.0)	(2.5)	(2.1)	(1.9)
HP:E_8	4.3	3.9	3.4	2.6
C_{13}:E_8	4.1	3.8	3.2	2.2
HP:K	4.5	4.5	4.3	3.6

Standard error for horizontal comparisons = ±0.13, d.f. = 28. Values not joined by underlining are significantly different ($P < 0.05$); values in parentheses not included.

pairing of the three strains with HP resulted in a variable stimulatory effect, the extent depending on the strain.

Bitterness in cheese made with the combined strains. The mean scores given by the taste panel for bitterness of the cheese at six months of age are in Table 4.

For the first four combinations of strains, there was a progressive decrease in the bitterness score of the cheese as the proportion of the more bitter of the two strains was decreased. The extent of the decrease from A to D was, of course, the difference in bitterness scores for the two strains used separately. Interest naturally lies in the bitterness scores for the intermediate combinations B and C, and in how far they deviate from a strictly additive model. In combination, one strain might in effect dominate another with respect to bitterness in the resulting cheese, or the strain might interact to produce deviations from the linear model. Accordingly, the relation between the bitterness of the cheeses and the proportion (x) of the more bitter strain was studied in detail.

Comparisons between the four treatments were split into three orthogonal components: L_1, given by $9A + B - 3C - 7C$ (capital letters denoting mean scores for the corresponding treatments); L_2, given by $A - 3B + 2C$; and L_3, given by $A - B - 2C + 2D$. L_1 represents the part of the variation in the bitterness scores between A, B, C, and D which is attributable to a simple linear regression on x. L_2 represents the deviation from a linear regression on x for Treatments A, B, and C only, and can also be considered as the amount by which the observed mean score for C deviates from a line through the means for A and B only. Similarly, L_3 gives the amount by which the mean score for D deviates from a straight line fitted to the means for A, B, and C. Positive values for L_2 and L_3 indicate a curvature which is concave upwards when bitterness score is plotted against x; negative values of L_2 and L_3 indicate a curvature which is concave downwards.

Analysis of the bitterness scores for each combination separately showed an outstandingly high error mean square ($P < 0.01$) for the combination C_{13}:ML_1. This high error mean square was due to one unusually high result in the D treatment, believed to be due to an error in the coding of the cheeses from Treatments B, C, and D on that day. This was evident also in the nitrogen analyses. Omission of the one value for Treatment D resulted in an error mean square not significantly different from those for the other combinations. On the basis of this evidence, all the results for that day were omitted; and the statistical analysis completed using missing-value techniques.

The values of L_1, L_2, and L_3 for each strain combination are given in the left portion of Table 5, with their standard errors. For all five combinations, L_1, the linear regression component, was very highly significant ($P < 0.001$), whereas L_2, the curvature component for Treatments A, B, and C, was in no case significant at the 5% level. An interesting difference between the combinations shows up in the values for L_3. Combinations containing E_8 or K as the less bitter component had significant ($P < 0.05$) negative values for L_3; for combinations containing ML_1 as the less bitter component, L_3 was not significant ($P > 0.05$).

The net result of these findings was that in combination with HP or C_{13}, Strain ML_1 moderated the bitterness produced by the other

TABLE 5

Components of the regression of the means of bitterness score and nitrogen ratio on the proportion (x) of the more bitter strain in the starter

Strain combination	Regression components (see text)					
	Bitterness score			Nitrogen ratio		
	L_1	L_2	L_3	L_1	L_2	L_3
HP:ML_1	24.1**	−0.5	−0.7	24.5**	−0.2	−0.9
C_{13}:ML_1						
Omitting Replicate 1	19.6**	0.8	−0.1	15.2**	0.4	0.7
Including Replicate 1	(18.2)	(1.2)	(1.2)	(11.5)	(1.0)	(1.1)
HP:E_8	14.1**	−0.5	−1.3*	12.2**	0.7	−0.3
C_{13}:E_8	15.6**	−0.9	−1.6*	6.2*	−0.8	−0.2
HP:K	6.2**	−0.3	−1.4*	7.3*	−0.1	−0.6
Standard error = d.f. = 28	±1.52	±0.48	±0.41	±2.01	±0.64	±0.54

* = $0.01 > P > 0.001$.
** = $0.001 > P$.
$L_1 = 9A + B - 3C - 7D$.
$L_2 = A - 3B + 2C$.
$L_3 = A - B - 2C + 2D$, where A, B, C, D denote the means for the corresponding treatments.

strain roughly in proportion to the amount of ML_1 present in the starter. The nonbitter Strain E_8, on the other hand, which on its own gave cheese only slightly more bitter than did ML_1, was less effective than ML_1 in moderating bitterness when used in combination with HP or C_{13}. With the combination HP:K, the bitterness-producing characteristics of HP appeared to outweigh those of the slightly less bitter Strain K. However, cheese made with Strain K alone ranged in pH from 5.22 to 5.26, whereas the pH values of all the other cheeses approximated 5.00. If pH has an influence on bitterness (2, 4, 8, 9), cheese made with Strain K would have been more bitter had the pH been at 5.00 rather than at 5.25.

In general, these observations showed that the intensity of bitterness in cheese made by combining one strain producing bitter cheese and one strain producing nonbitter cheese was influenced by: (a) the proportions in which they were combined; and (b) the characteristics of the nonbitter strain. The combination of two bitter strains yielded bitter cheese. From the results of this experiment it appears possible to make nonbitter cheese (score of less than three) by combining a bitter strain and a nonbitter strain in suitable proportions as starter in the vat.

When single strains are used in the commercial manufacture of Cheddar cheese, they are generally used as pairs. It is of interest to note that three pairs (R_6:ML_1; KH:R_1; C_{13}:E_8) used in New Zealand (11) each comprised one strain that had produced bitter cheese and one strain that had produced nonbitter cheese in our laboratory (4).

Protein breakdown in cheese made with the combined strains. The levels of acid-soluble amino nitrogen and of acid-soluble, TCA-soluble nitrogen were similar to those in the previous study (4) ranging from 0.83-1.55 mg and from 5.3-7.2 mg per gram of cheese, respectively. The relationship between the bitterness scores and the ratio of TCA-soluble nitrogen to amino nitrogen is shown in Figure 1; this ratio is a measure of the average length of the TCA-soluble peptides and amino acids in the cheese (4). Generally, the longer the average length of the peptides, the more bitter was the cheese. A regression analysis for bitterness scores on the nitrogen ratios was found to be highly significant ($P < 0.01$); regression accounted for more than 80% of the total variation among the mean bitterness scores for each treatment and each strain combination shown in Figure 1. However, after adjustment for the nitrogen ratio, there remained significant differences ($P < 0.05$) in bitterness scores associated with the various strains and their combinations.

Differences in the relationship between bitterness score and nitrogen ratio may also be assessed from Table 5. The right-hand section of this table gives the values of the components L_1, L_2, and L_3 for the nitrogen ratio for each combination of strains, with their standard errors. For all strain combinations, the nitro-

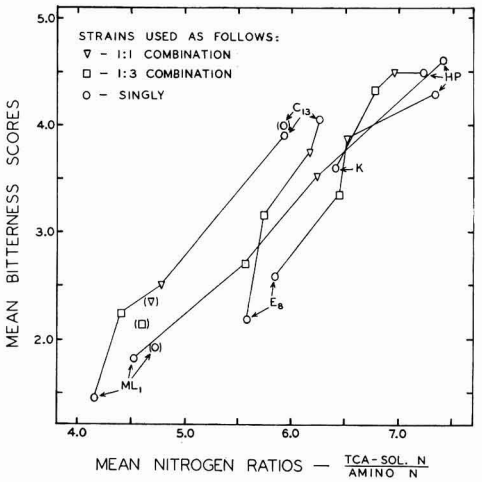

FIG. 1. Relation between bitterness scores and the nitrogen ratios for the different strains and their combinations. Each point is the mean of three replicate cheeses, except for combination C_{13}:ML_1, for which each point is the mean of two replicate cheeses; points in parentheses are means of the three replicates in each treatment for combination C_{13}:ML_1 (see text). Comparable points are joined for the various combinations.

gen ratio dropped as the proportion (x) of the more bitter strain was decreased, in much the same way as for the bitterness scores. The L_2 and L_3 components for the nitrogen ratio were, however, not significant, and did not seem to be consistently related to the corresponding values for the bitterness scores, e.g., L_3 for strain combinations HP:E_8, C_{13}:E_8 and HP:K.

As suggested previously (4), bitterness in the cheese was probably due to bitter-tasting peptides resulting from a deficiency of suitable proteolytic enzymes in the starter organisms. Differences among the strains in the relation between bitterness scores and protein breakdown suggest that patterns of protein breakdown were not identical.

The lower intensities of bitterness when part of the bitter strain was replaced by a nonbitter strain probably resulted from hydrolysis of the bitter-tasting peptides by desirable proteolytic enzymes from the nonbitter strains. It would follow that the presence or addition of nonstarter organisms with suitable proteolytic enzymes might control bitterness. These observations support the suggestions of Moir (6) and Czulak (2), that the lower frequency of bitter flavors in raw-milk cheese than in pasteurized-milk cheese is attributable to natural contaminants.

The data support and extend Czulak's explanation (2) for two types of bitterness in cheese. Where bitterness appears early and lasts throughout the curing period, it would appear that a bitter strain was used as a starter, or a bitter strain predominated in the starter. Where bitterness appears early and later disappears, it would appear that here, too, a bitter strain was used as starter, but that the secondary flora, perhaps of lactobacilli, possessed the requisite proteolytic characteristics to hydrolyze the bitter-tasting peptides. A third type of bitter flavor, which appears later in the curing period and persists, is less easily explained.

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CHANGES IN THE VOLATILE FLAVOR COMPONENTS OF STERILIZED CONCENTRATED MILK DURING STORAGE [1]

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SUMMARY

A characteristic flavor, often described as an old rubber flavor, frequently develops in sterilized concentrated 3 to 1 milk during storage.

Evidence is presented that ethanal, dimethyl sulfide, acetone, 2-pentanone, and a pentyl acetate are present in sterilized concentrated milks and are related to flavor changes in this product during storage. Quantitative data are presented for these and other volatile compounds in sterilized concentrated milk stored at various temperatures and times.

Increases in storage time or in storage temperature gave significant increases in certain volatile components. Three volatile compounds (including one believed to be 2-pentanone) were detected in stored milks but not immediately after processing and before storage. A possible pentyl acetate was found in the largest amount of all of the volatiles detected in the samples.

This investigation was undertaken to examine the effects of processing conditions and of storage temperature and duration upon the formation of certain off-flavors in sterilized whole milk concentrates. The volatile components were selected for study because of the ease of separating and identifying them by means of gas chromatography. It was hoped that a study of the substances in both freshly processed and stored samples would lead to an understanding of the chemical changes responsible for flavor deterioration.

A number of volatile compounds have been shown to be present in processed milks and dairy products which may be related to their flavor. The volatile compounds well-characterized in dairy products include the following: All straight-chain saturated aldehydes from methanal to decanal except butanal have been reported; 2-propenal, isobutanal, isopentanal, 2,4-heptadienal, 2-nonenal, furfural, and benzaldehyde have also been reported (3, 7, 13). All odd-numbered straight-chain saturated 2-ketones from acetone through pentadecanone have been found as well as butanone and 2-hexanone (3, 7, 9). All saturated straight-chain acids from formic through octadecanoic have been characterized with the exception of pentanoic, heptanoic, and nonanoic acids (5, 6, 12). 5-Decalactone and 5-dodecalactone have been characterized from evaporated and dry whole milk (8). Methanethiol, dimethyl sulfide, and dimethyl disulfide have been shown present in irradiated skimmilk (2).

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EXPERIMENTAL PROCEDURE

Processing and storage of sterilized concentrated milk. Samples of sterilized concentrated 3 to 1 milk used in this study were supplied from a research project concerned with this product as described by Swanson (10). The process for preparation of this product was as follows: A high-quality fresh whole milk was forewarmed at 63 C or 85 C for 30 min. It then was cooled to 49 C and evaporated to about 37 to 38% total solids. The product was transferred to a vat and standardized to 36.5% total solids.

The concentrated milk was held for 2 min at 93 C for viscosity control or not held at this stage. It was then pumped through a tubular-type heat exchanger and sterilized at 146 C for 3.5 sec. If it had not been held for 2 min at 93 C before sterilization, it was now held for 4 min at 93 C. The sterilized concentrated milk

was cooled to 66 C and homogenized. The product was cooled to 10-13 C and aseptically canned in 237-ml (8-oz) lacquer-lined cans. About 221 ml were placed in each can.

Three batches were prepared with slight variations in the forewarming temperature and the viscosity control. One hundred cans (22.1 liters of concentrate) from each batch were stored in constant temperature rooms. The forewarming temperature, viscosity control conditions, and storage conditions of these batches are given in Table 1.

Distillation. From Table 1 it may be seen that a total of 16 different batches of product, designated by lot letter and storage number, e.g., A1, B3, etc., were analyzed. Samples from each of these batches were treated by the following procedure: 2.65 liters (12 cans) of concentrate were diluted to 8.0 liters with distilled water in a 12-liter round-bottom flask, and distilled under nitrogen at 15 to 25 mm pressure from a 45 C water bath. Antifoam AF emulsion [3] was used for foam suppression during the distillation. The distillate was passed through a Liebig condenser, then through a trap consisting of a 2-liter round-bottom flask cooled to 1 C with ice water, and finally through two more traps made of one-liter round-bottom flasks which were held at about −73 C with ethanol–dry ice. The characteristic off-odor (resembling that of old rubber) of the stored product was observed in high concentration in the distillation traps. In 2¼ hr 1,100 to 1,200 ml of distillate with an odor similar to the original sample was collected in the first trap, 10 to 12 ml in the second, with a cooked, burnt, and malt-like odor, and 2 to 3 ml in the third, with an odor similar to the second but more intense.

[3] Dow Chemical Corporation, Midland, Michigan.

Paper chromatography. The contents of the three traps were mixed together, about 4 ml of 1% 2,4-dinitrophenylhydrazine in 30% sulphuric acid was added to a 100-ml portion of the combined distillate and this mixture was allowed to stand overnight at about 25 C. The dinitrophenylhydrazones were extracted three times with 100-ml portions of carbonyl-free petroleum ether (b.p. 30-60 C), the extract dried over anhydrous sodium sulphate, and then concentrated to a volume of about 30 ml. Suitable aliquots (ca. 0.1 ml) of this solution were chromatographed on Whatman No. 1 paper with the solvent system methanol:heptane (1:2, by volume) according to the general procedure of Huelin (4).

Gas chromatography. The remaining 1,000 to 1,100 ml of distillate combined from the three traps was concentrated, either immediately or after storage at about 5 C in a closed flask for 12 to 16 hr. For this purpose, helium was bubbled through the distillate at a rate of two to three bubbles per second (30 to 40 ml per minute). The distillate was contained in a 2-liter, two-necked flask held at 30 C and was continuously stirred by means of a magnetic stirrer. To remove excess moisture, the effluent gas stream was passed through a one-liter two-

TABLE 1

Conditions for preparation of sterilized concentrated 3 to 1 milk

Batch no.	Forewarming temperature (30 min)	Holding time at 93 C		Conditions of storage	
		Before sterilization	After sterilization	Time	Temperature
	(C)	*(min)*		*(wk)*	*(C)*
A1	63	0	4	<0.3 [a]	2
A2	63	0	4	3	24
A3	63	0	4	4	2
A4	63	0	4	4	43
B1	85	0	4	<0.3 [a]	2
B2	85	0	4	3	24
B3	85	0	4	4	2
B4	85	0	4	4	43
B5	85	0	4	6	24
C1	63	2	0	<0.3 [a]	2
C2	63	2	0	3	24
C3	63	2	0	4	2
C4	63	2	0	4	43
C5	63	2	0	6	24
C6	63	2	0	8	43

[a] This portion of the batch (A1, B1, and C1) was analyzed within two days after processing and served as control for the remainder of the batch placed in storage.

TABLE 2

Gas chromatographic separation of volatile components of sterilized concentrated 3 to 1 milk

Batch no.	Peak number [a]						
	2	3	4	5	6	7	8
	(parts per billion of original milk)						
A1	1.1	1.2	1.1		22.6		
A2			0.2		9.5	0.4	
A3		0.1	0.4		14.2	1.9	
A4		4.1	1.1	2.1	18.0		
B1			0.6		12.5		
B2			0.7	0.3	13.9		
B3		0.1	1.0		33.7	0.3	0.7
B4	0.2		1.9	0.4	14.7		
B5			1.8		18.9		
C1		0.1	1.1		20.4		
C2		4.2	6.6		34.6	0.8	2.8
C3	0.8	17.9	6.2	0.1	38.8	1.9	2.3
C4		0.1	3.7		10.8		
C5	0.2	0.1	35.2		11.7	0.2	
C6	2.6	5.5	24.4		19.7	4.3	0.9

[a] Peaks are numbered the same as in Figure 2.

necked flask which was cooled with cold water at about 15 C. The gas stream was then passed through a cold finger trap designed by Thomson (11). The finger consisted of 100 mm of 10-mm o.d. pyrex glass tubing into which was sealed a 90-mm-long inlet tube of 5-mm o.d. pyrex glass tubing. The inside tube was packed with pyrex glass wool; inlet and outlet sides were equipped with ℉ no. 1 stopcocks and ℉ 5/20 joints. The trap was immersed in liquid air at −190 C. After helium had been passed through the system for 8 to 9 hr, about 0.5 ml of liquid had condensed in the trap. The stopcocks were then closed and the trap was stored at −10 C until the contents were to be injected into the gas chromatograph.

Gas chromatograms were run on a polyethylene glycol column [4] packed with a mixture of one part of the glycol on four parts of 60-80 mesh fire brick. The column was 5 mm in inside diameter and 200 mm long and an inlet pressure of 3.8 psig was used to maintain a helium flow rate of 11.2 ml/minute. The column temperature varied in different runs from 88 to 96 C. All runs were made on a Model 154B Perkin-Elmer gas chromatograph equipped with a thermoconductivity detector. This instrument is provided with a gas sampling valve which permits the stream of carrier gas to be passed either directly through the column or to be by-passed through the trap containing the sample.

Before injection of the sample, the Thomson trap was slowly warmed to 100 C and held there for 1 to 2 min. The gas sampling valve was then opened and left open until the maximum of the air peak had passed through the column (about 2.6 min), thus permitting the helium carrier gas to flush the volatile portion of the trap contents into the column.

Peaks were collected from the gas chromatograph by passing the effluent from the column through a trap consisting of a U tube, 110 mm long, of 6-mm pyrex tubing immersed in liquid air at −190 C, and equipped with a four-way stopcock to divert the gas flow through the trap when the desired peak emerged (1). This trap is shown in Figure 1. After a peak had been collected, a ground glass blind was placed on the inlet arm of the trap, and it was evacuated to 0.05 mm, taking care to maintain a high liquid air level around the trap. The traps were then submitted for analysis of the contents on a CEC 21-081A mass spectrometer run from M/e (mass/charge ratio of positive ions) 2 to 130 with an ionizing potential of 320 electron volts.[5] From a reference sample of acetone it was estimated that at least 0.1 μl of sample was required for identification under the conditions employed.

RESULTS

The gas chromatography results on the 16 sterile concentrated milk samples are shown in Table 2, and Figure 2 shows a representative gas chromatogram, obtained under the standard conditions described, on Batch C3. Most of the samples (Table 2) did not contain all of the

[4] Perkin-Elmer Company, Norwalk, Connecticut; Carbowax 1500 column Kx.

[5] Consolidated Electrodynamics Corporation, Pasadena, California.

components shown in Figure 2, but in each case the peak numbers are assigned to the components which appeared to have the same relative retention volumes in the various chromatograms.

To obtain relative retention volumes for the individual peaks, Peak 4 was chosen as the internal standard, since it was present in each chromatogram. Thus, for example, if the retention volume of Peak 4 is set equal to one, then Peak 2 was found to have a relative retention volume of 0.46 ± 0.02 in seven samples.

Rigorous proof of the identity of the compounds observed in these samples has not yet been obtained. The indications as to the structure of each component are summarized below. Peak 1 is an air peak in every chromatogram. Its average retention time is 2.60 ± 0.15 min from the time of injection. Since this corresponds to the dead volume of the system, it has been set equal to time zero and all other peaks measured from it. In 11 chromatograms a shoulder was observed on the air peak after 3.09 ± 0.32 min. It was noted that merely by closing the gas sampling valve after equilibrating the system with the valve open, a large enough pressure change was introduced in about 4 min to slowly shift the baseline enough to account for this shoulder. (No. 1A, Figure 2.) Consequently, this shoulder most probably is a part of the air peak and has been disregarded.

Peak 2 was tentatively identified as ethanal on the basis of its relative retention volume (0.46 ± 0.02 as indicated above compared to 0.49 ± 0.02 for an authentic sample). Furthermore, the paper chromatograms of the 2,4-dinitrophenylhydrazones prepared from the mixed volatiles as described above showed a spot with the same appearance and R_f value, 0.42, as authentic ethanal 2,4-dinitrophenylhydrazone. It was noted also that the emerging gas from Peak 2 had a distinct odor of ethanal.

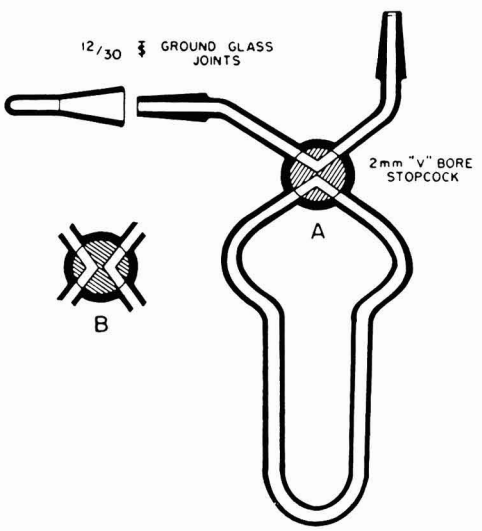

FIG. 1. Trap used for collection of samples for mass spectrometry. From Bazinet and Walsh (1).

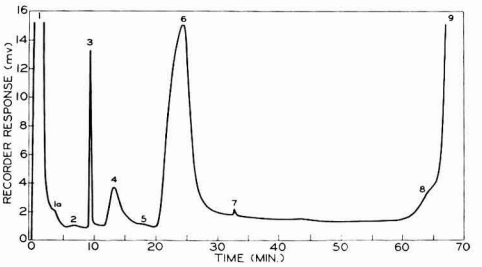

FIG. 2. Gas chromatogram of sterilized milk concentrate Sample C3 run on a Carbowax 1500 column at 90 C and at a flow rate of 11.2 ml of helium per minute.

Peak 3 had a relative retention volume of 0.60 ± 0.05 in nine samples. Dimethyl sulfide injected by syringe also had a relative retention volume of 0.60. The gas chromatographic peak was extremely sharp for this compound just as with an authentic sample of dimethyl sulfide. Furthermore, the emerging peak had an odor identical to that of dimethyl sulfide. For these reasons, Peak 3 is tentatively regarded as being this compound.

Peak 4, chosen as the standard, emerged in an average time of 12.5 min at an average column temperature of 91.7 C. The relative retention volume of this peak had a precision of about 1.00 ± 0.02. Samples of acetone injected in the same manner had an average retention time of 12.7 min at an average column temperature of 91.9 C. The paper chromatograms also showed a spot which agreed in R_f value (0.66) with authentic acetone-2,4-dinitrophenylhydrazone. Peak 4, therefore, presumably corresponds to acetone.

Peak 5, which appeared in only two samples, had a relative retention volume of 1.38 ± 0.01. Ethyl acetate injected by syringe gave a relative retention volume of 1.39, whereas ethylamine gave 1.43. No other information on the possible nature of this component was obtained.

Peak 6 appeared in all 16 chromatograms and had a relative retention volume of 1.68 ± 0.13. The material causing this peak was collected from Samples B1, B2, B5, C3, and C4 and separate mass spectra were run. The mass spectral data, Table 3, indicate that too little

TABLE 3

Mass spectrum of Peak 6

M/e	% Height	M/e	% Height	M/e	% Height	M/e	% Height
15	20	34	5	55	13	69	1
20	10	39	18	56	23	70	11
26	4	41	48	57	58	71	1
27	38	42	28	58	3	73	21
29	78	43	100	59	1	87	1
31	4	45	8	61	1	130	20

sample was collected to permit a clear-cut identification. Peaks wholly or partially attributable to air have been omitted from Table 3. The mass of the parent peak (Table 3) indicates that the molecular weight of this component was 130, a value that would agree, for example, with the formula $C_7H_{14}O_2$. The large base peak, 43, might very well have been due to an acetyl radical, while the 87 peak must represent the fragment of the original molecule remaining after loss of the mass 43 unit.

Since these figures were consistent with the possibility that the material might be a pentyl acetate, the eight isomers of this structure were synthesized and their mass spectra determined. The spectra showed a marked similarity between the unknown and the four branched-chain methylbutanol acetates, but it was not possible to conclude definitely from the evidence obtained that the substance was in fact identical with any one of these isomers.

Peak 7 appeared in eight gas chromatograms and had a relative retention volume of 2.49 ± 0.27. The relative retention volume of 2-pentanone under the same conditions was 2.54. Other compounds with similar relative retention volumes are 3-pentanone, 2,3-butanedione, propanol, pentanal, and propyl acetate. The 2,4-dinitrophenylhydrazine derivatives of the mixed volatiles gave a spot whose R_f value on paper chromatograms corresponded exactly with authentic 2-pentanone, viz., 0.79.

Peak 8, found in five of the gas chromatograms, had a relative retention volume of 4.76 ± 0.16. This material was not identified, although it may be noted that hexanal, 2-hexanone, butyl acetate, and dimethyl disulfide have similar retention volumes.

Peak 9, Figure 2, is due to water. Only the start of this peak is indicated in Figure 2.

The quantitative data for the various components given in Table 2 were calculated from the peak heights. The detector response to the various identified compounds was determined and was found to be 36.1 mV/μl for ethanal, 94.8 mV/μl for dimethyl sulfide, 62.3 mV/μl for acetone, and 35.6 mV/μl for 2-pentanone. The average of these values is 57.2 mV/μl. The values corresponding to the pure compounds were used in calculating the quantitative data for Peaks 2 (ethanal), 3 (dimethyl sulfide), 4 (acetone), and 7 (2-pentanone), whereas the average was used for Peaks 5, 6, and 8. For convenience, the amounts so found have been expressed in parts per billion of the reconstituted milk. It should be noted that complete recovery of all volatiles probably was not attained in any case. However, since a carefully standardized procedure was employed and was the same for all samples, the quantitative results at least should be of relative significance.

DISCUSSION

It may be seen from Tables 1 and 2 that there was an increase of the volatile compounds detected in the concentrated milk samples when these samples were stored for longer times and at higher temperatures. The forewarming and holding treatments appear to have caused an increase in the amount of Peak 3 (probably dimethyl sulfide) in the C batches but not in the A or B batches. Peaks 5, 7, and 8 were never present in the freshly processed samples (A1, B1, C1) but soon appeared during storage.

At the present time, it is difficult to correlate these results with organoleptic observations. The odors of Peaks 2 and 6 closely resembled the odor of the stored samples. Preliminary results from taste panel studies conducted by the Department of Home Economics indicate that when trace amounts of the compounds tentatively identified in these samples are added to Grade A whole milk detectable changes in the flavor are produced. Further work will obviously be required before the exact chemical basis for the deterioration of these concentrated milk samples can be elucidated.

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SYMBIOSIS AMONG LACTIC STREPTOCOCCI

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SUMMARY

Combination of isolates from mixed-strain lactic streptococcus cultures showed different interactions when grown in milk. The effects varied from marked stimulation to inhibition. The interaction between strains could not be predicted from the growth rate of the individual isolates, or their cultural properties on an agar medium designed to measure acid production by bacterial colonies.

The marked acceleration of a faster culture by a slower one was obtained by using filtrate from a milk culture of the slower organism as well as by the organism itself. The active components were present nearly optimally in the slow culture after only one day at 22 C, and 1% of this filtrate was sufficient to stimulate the faster strain. The active material in the filtrate was dialyzable through cellophane. Filtrate of the fast strain was only slightly active for the slow culture.

Activity of the filtrate from the slow culture was adsorbed on Amberlite IRC (H^+) and eluted by a 4% solution of aqueous ammonia. The eluate was observed to contain all of the activity in the original filtrate. Part of the activity was destroyed by acid hydrolysis.

Dairy starter cultures usually contain a variety of strains of lactic streptococci. The changes which these produce when inoculated in milk are the result of their associative growth. Comparatively little is known, however, about the factors which enable strains to exert symbiotic effects during growth in milk.

Marshall (11, 12, 13) and Marshall and Farrand (14) demonstrated the importance of bacterial associations in the souring of raw milk as early as 1903, and found that many bacteria commonly occurring in milk stimulated the growth and fermenting activity of lactic acid bacteria, the effects differing with different bacteria. Hansen (8) observed that extracts of *Streptococcus cremoris* and *Streptococcus lactis* stimulated the development of *Lactobacillus casei* and *Betacoccus cremoris* (*Leuconostoc citrovorum*). Luxwolda (10) was, with one exception, unable to demonstrate stimulation of *Streptococcus lactis acidi* grown in association with other organisms. *Bacterium fluorescens liquefaciens*, however, stimulated the formation of more cells but had no influence on acid development. Certain alkali-forming organisms retarded acid production at low temperatures. Claydon and Koburger (2) observed that bacteria-free filtrates of milk cultures of a strain of *Pseudomonas fluorescens* stimulated acid production by lactic starter cultures. Marshall (15) and Fouassier (4) observed increased acid production by *S. lactis* in the presence of *Bacillus subtilis*. *B. subtilis* was also reported by Rice (18) to stimulate acid production by lactic starters during associative growth. Iya and Frazier (9) observed that at certain temperatures and ratios of organisms, *S. lactis* was stimulated by *Aerobacter aerogenes*. Hall (7) found that *Bacterium coli* (*Escherichia coli*) stimulated acid production of lactic cultures. Nurmikko (16) showed that different strains of lactic acid bacteria could be grown in symbiosis in a synthetic medium incapable of supporting the growth of either strain in pure cultures. He pointed out that each strain produced growth factors required by the other and, hence, suggested that such phenomena might also play an important part in associations of lactic acid bacteria in milk and dairy products. Greene (6) and Olson (17) demonstrated the importance of associative growth of lactic streptococci in the souring of milk. They found that the acid

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production of some strains combined with others may have various effects, ranging from pronounced stimulation of acid to a depressing effect with very slow acid production.

There is, therefore, evidence that definite advantages can be obtained by associative growth of certain single strains of lactic streptococci. However, judicious combinations of strains can be accomplished only when factors promoting symbiotic effects are better understood. The present study was designed to obtain more information on the nature of the symbiotic effects exhibited by certain lactic streptococci during their growth in milk.

EXPERIMENTAL PROCEDURE

All cultures were carried in sterile litmus milk and subcultures were made with 1% inoculum; incubation was at 22 C for approximately 24 hr, unless otherwise noted. Acid production by cultures was tested in sterile reconstituted nonfat milk (NFM) containing 10% solids.

Mixed-strain starter cultures from various sources were used as sources of single-strain isolates. The pure strains of lactic streptococci were isolated by plating the starter cultures with lactic agar (3). The medium was modified so that it contained lactose only as the fermentable sugar; 0.25% calcium carbonate and 0.004% brom-cresol purple were also added. The plates were incubated at 22 C for three or four days. The colonies showing different morphological and acid-forming characteristics were transferred to litmus milk. The single-strain cultures were subcultured at least five times in litmus milk. Litmus milk inoculated with an individual strain was frozen as a stock culture. Mixed cultures were made in litmus milk by use of 1% inoculum from each isolate. The mixtures were subcultured (1% inoculum) three times before further observations were made.

Acid production by single strains and their combinations were observed in sterile 10% NFM. After inoculation the cultures were tempered at 22 C in a water bath and incubated at that temperature 18-24 hr. The tubes were then cooled in ice water and titrated with 0.1 N NaOH to pH 8.3 to determine the amount of acid production.

Filtrates of the culture after growth in sterile 10% NFM were prepared by three different procedures. Before information was obtained on lability of the activity, sterile filtrate was obtained by Seitz filtration. The culture was adjusted to pH 7.0 before filtration. Culture filtrate also was obtained by precipitation of proteins with 10% trichloroacetic acid. The precipitate was removed by centrifugation and the supernatant filtered through paper. The filtrate was extracted with ether to remove the excess trichloroacetic acid. The ether was removed by vacuum condensing at 40 C. For assay purposes the pH was adjusted to 7.0 and the volume made to the volume of the original culture by addition of distilled water. In the third method, four parts of a cold acetone-ethanol mixture (3:1) was added to one part of precooled culture. The precipitate was allowed to develop in a freezer for 4 hr with occasional shaking, after which the culture was centrifuged and the supernatant material was filtered through paper. The filtrate was condensed to dryness under vacuum and its volume made to that of starting culture after adjusting to pH 7.0.

Culture filtrate prepared by acetone-alcohol precipitation was dialyzed under refrigeration in cellophane tubing against three changes of distilled water. The dialysate and nondialyzable portion were concentrated to the volume of the original filtrate, with the pH being adjusted to 7.0.

Culture filtrate (after acetone-alcohol precipitation) was passed through an ion exchange resin (Amberlite IRC 120) in the H^+ cycle. The column was washed with water until the washings showed no lactose by the Molisch test. Both the effluent and washings were condensed separately under vacuum and the volume readjusted to that of the original filtrate. The material held on the column was eluted with 4% aqueous ammonia solution (NH_4OH diluted to contain 4% NH_3). The eluate was concentrated under vacuum to near dryness, distilled water added, and again condensed to near dryness. The volume was then made to that of the starting filtrate by the addition of distilled water. Portions of this eluate and the original filtrate were hydrolyzed with 5 N HCl by autoclaving the mixture (one part eluate or filtrate plus four parts 5 N HCl) at 121 C for 6 hr. HCl was removed by drying and wetting repeatedly on a steam bath four times. The pH was adjusted to seven by addition of 0.1 N NaOH and the volume made up to the original with distilled water.

The various culture fractions were assayed for their stimulatory activity. Sterile 11% NFM was cooled in ice water and inoculated (1%) with the test culture. This was distributed in 9-ml volumes in culture vials. Then 1 ml of the original or diluted test material was added to duplicate vials to obtain desired concentrations in the inoculated milk. Each tube was adjusted, when needed, to a total volume

of 10 ml with distilled H_2O. The vials were then removed from the ice water, their contents mixed well, and tempered to 22 C. Incubation was continued at this temperature for 18 hr, after which the acid production in each tube was determined by titration with 0.1 N NaOH to pH 8.3.

RESULTS

Initial experiments were designed to study interactions among pairs of single-strain lactic streptococci which showed different colony characteristics on modified lactic agar plates. The results of some representative combinations studied are given in Table 1. It was observed that acid production by the colony on lactic agar was no indication of the amount of acid it would produce when grown in milk. Similarly, the amount of acid produced in milk by pairs of single strains could not be predicted from the acid produced by individual strains. The combined growth of different pairs of strains had various effects on acid production, ranging from pronounced stimulation to slight inhibition. Usually, however, the highest amount of acid was produced by mixtures containing a slow-growing culture. In these instances, acid production was markedly greater than that produced by either single component strain. These data indicated that one strain was being markedly stimulated by the other, or that both were mutually stimulated. To study the nature of the stimulatory factor(s) involved, two strains of the lactic streptococci were selected. One (F 1A) was a slow-growing strain and the other (F 2A) a faster-growing strain.

The Seitz filtrates of both strains were obtained after various incubation periods. The filtrates before and after autoclaving 15 min at 121 C were tested for stimulation of the other respective strain. No stimulation of F 2A was obtained until the slow-growing strain (F 1A) had been allowed to grow in milk (Table 2). Most of the stimulation obtained was after the culture had been incubated only one day, although some increased stimulation was noticed after the culture was incubated three, five, and seven days. This increase, however, was progressively less with increased incubation time. In addition, the degree of stimulation obtained was almost maximum with 1% of the filtrate; increasing the filtrate to 10% gave only a small amount of added stimulation. Autoclaving the filtrate had no measurable effect on its activity. The Seitz filtrate of the faster-growing strain (F 2A) was found to be comparatively ineffective in stimulating the slow-growing strain (Table 3). Only the addition of 10% of the filtrate gave appreciable stimulation (approx. 20%) over that of the control. Activity was also present in culture filtrates after protein precipitation by trichloroacetic acid or by acetone and ethanol.

The data in Table 4 give certain descriptions of the nature of the stimulatory factor(s) in the acetone-ethanol filtrate of the slow-growing culture (F 1A). The filtrate of culture F 1A was observed to be as stimulatory to F 2A as was the culture itself. The ability of the active factor(s) to dialyze through cellophane indicated that it was of relatively low molecular weight. The marked loss of activity after hydrolysis with acid suggested that a peptide(s) might be involved in causing activity in the original filtrate. However, since the filtrate had been freed only from proteins, further fractionation was conducted. The filtrate was passed through a resin column (IRC — 120 in H^+ cycle) to free the active material from components such as lactose, and the various fractions were assayed for activity (Table 4). Essentially all of the active material was held on the column, since very little, if any, was present in the effluent and washings. The activity was completely removed by the ammonia solution. The activity of this fraction was partially lost on hydrolysis with acid, but most of the activity remained in the hydrolysate. These data indicated that possibly more than one component was involved in causing the stimulatory effect by culture F 1A.

DISCUSSION

Milk is known to be something less than an optimum medium for the growth of many lactic streptococci. The availability of certain nitrogenous components appears to be the main restrictive property of this medium. It has been shown that enzymatic digests of proteins (1, 5) and extracts of certain plant and animal tissues (19) accelerated the growth of lactic streptococci. Slower-growing cultures responded more to the supplements than did faster strains, indicating an inability of the slower strains to obtain needed nutrients from the milk. The nutritive qualities of the various extracts have been considered to be due to various nitrogenous compounds, including peptides. The present study has indicated that the beneficial interaction between two selected strains of lactic streptococci may involve nitrogenous metabolites. The relative stability of the activity in the resin column eluate to acid hydrolysis suggested that much of the activity in the eluate was caused by compounds stable to acid and heat, and which possibly were not peptide in nature.

TABLE 1

Interactions among single strains of lactic streptococci and their colony characteristics on modified lactic agar

Strain	Acid production [a] by cultures		Colony characteristics on plating medium
	Single	Combined	
	(ml 0.1 N NaOH/10 ml culture)		
1	2.16	7.21	Large, clear acid zone
3	6.21		Lens, clear acid zone
6	2.21	6.17	Small, no acid zone
E	5.97		Small, clear acid zone
3	6.21	6.09	Lens, clear acid zone
6	2.21		Small, no acid zone
Ps 2	2.48	5.32	Small, no acid zone
D 3	4.93		Moderate, clear acid zone
Ps 3	2.46	5.22	Moderate, clear acid zone
D 3	4.93		Moderate, clear acid zone
Ps 3	2.46	7.15	Moderate, clear acid zone
Ps 7	6.91		Lens, clear acid zone
F 1	2.32	7.11	Large, no acid zone
F 5	4.31		Small, clear acid zone
F 2	6.50	7.41	Small, no acid zone
F 4	2.31		Lens, clear acid zone
F 1	2.32	2.31	Large, no acid zone
F 4	2.31		Lens, clear acid zone
F 1A	2.24	7.15	Large, no acid zone
F 2A	4.83		Small, no acid zone

[a] Incubated at 22 C 18-24 hr; 1% inoculum.

TABLE 2

Stimulation of faster-growing strain (F 2A)[a] by Seitz filtrates of medium from slower-growing strain (F 1A)

Time F 1A incubated	0 (control)	Amount of F 1A filtrate added		
		0.1%	1%	10%
(days)		*(ml N/10 NaOH/10 ml culture)*		
0	3.70	3.60	3.70	4.00
1	3.70	4.00	5.60	6.00
3	3.70	4.10	6.00	6.15
5	3.70	4.10	6.20	6.30
7	3.70	4.45	6.30	6.40

[a] Eighteen hours' incubation at 22 C.

TABLE 3

Stimulation of slow-growing strain (F 1A)[a] by Sietz filtrates of medium from faster-growing strain (F 2A)

Time F 2A incubated	0 (control)	Amount of F 2A filtrate added		
		0.1%	1%	10%
(days)		*(ml N/10 NaOH/10 ml culture)*		
0	3.85	3.80	3.90	4.00
½	3.85	3.85	3.85	4.45
1	3.85	3.80	3.75	4.35
2	3.85	3.80	3.90	4.65
3	3.85	3.80	3.80	5.00

[a] Thirty-six hours' incubation at 22 C.

TABLE 4

Stimulation of the fast strain (F 2A) by different fractions of the milk culture of the slow strain (F 1A)[a]

F 1A medium fraction[b]	Incubation at 22 C for		
	16 hr	18 hr	20 hr
	(ml 0.1 N NaOH/10 ml culture)		
Control strain (F 2A)	3.55	3.90	4.34
Combined strains (F 1A + F 2A)	6.40	7.45	7.65
Filtrate (acetone-alcohol)	6.73	7.28	7.58
Dialyzed filtrate	3.78	4.08	4.60
Dialysate	6.35	7.28	7.09
Hydrolyzed filtrate	3.80	4.20	4.65
Effluent from column[c]	3.73	4.25	4.65
Washings from column	3.58	4.00	4.58
Ammonia eluate from column	6.95	7.50	7.75
Hydrolyzed ammonia eluate	6.03	6.50	6.72

[a] Culture incubated 4 days at 22 C before preparing filtrate.
[b] Each added in 1% concentration (based on volume of original culture).
[c] Amberlite IRC-120 in H^+ cycle.

Means are not clear whereby cultures can be selected that will show symbiotic activity. The present study has shown that such interactions cannot be predicted solely on the basis of high acid production by single strains. Rather, strains that grow slowly in milk may be equally as important in symbiotic activities. Although the cause of this slowness in cultures is not understood, the slow culture used in the present study was sufficiently active to allow the accumulation of metabolites stimulatory to the faster strain. The value of such cultures in promoting growth of others is obvious. Study is being continued on the nature of the stimulatory factors produced by various single-strain streptococci.

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DETERGENCY EFFECTS OF TRISODIUM PHOSPHATE WITH AND WITHOUT SODIUM HYPOCHLORITE ON MILK-PROTEIN SOILS [1]

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SUMMARY

A method was developed for evaluating the detergency of trisodium phosphate on milk protein films alone and in the presence of sodium hypochlorite.

Synthetic soil films were prepared by dipping mechanically 22 by 50 by 0.14-0.25 mm micro cover glasses into an aqueous milk plant clarifier slime suspension. Hardness and tenacity were imparted to the films by heating and adding trisodium phosphate, calcium chloride, and sodium hypochlorite in alternate dipping solutions. The films were dried and weighed, then exposed to a detergent treatment. Immediately after the detergent treatment the glass film supports, containing the film residues, were transferred quantitatively into 50-ml micro-Kjeldahl flasks for nitrogen determination.

When maintained at pH 11.5 ± 0.1 at a temperature of 65 to 54 C (beginning at 65 C, at the end of period the temperature was 54 C) for 10 min without agitation, the use of sodium hypochlorite with trisodium phosphate significantly increased the protein removal, using either tap or distilled water. No optimum level of hypochlorite was observed. Increased levels resulted in increased peptization.

Among the more recent detergent introductions are those of the chlorinated type. A survey of the United States detergent manufacturers and distributors revealed that 75 of them were producing or distributing chlorinated detergent compounds. The order of their frequency of use were: (a) chlorinated trisodium phosphate; (b) trichloroisocyanuric acid; and (c) dichloroisocyanuric acid.

Often problems and questions accompany the introduction of new products. In the case of chlorinated cleaners the question arises as to the benefits gained through the use of them. If beneficial, at what concentration do they yield the best results? And also, what is the mechanism by which they accomplish the purpose for which they were designed? Although some data were available (4), answering these questions in part, further studies seemed necessary.

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Visual observation and the assessment of viable bacteria on the milk contact surfaces have been generally employed to indicate the cleaning efficiency of chlorinated compounds (2, 3, 4). However, the determination of the extent of detergency was considered not to be entirely related to measurement by bacteriological methods.

Radioisotope techniques have also been found very useful in providing quantitative data of cleaning efficiency and function by measurement of residual soil. Two disadvantages of the method are the cost of the equipment and certain potenial personnel health hazards.

Maxcy and Shahani (5) evaluated circulation cleaning of a welded pipeline by a kjeldahl analysis of used detergent solution. They reported the method to have a sensitivity of 2 ppm milk solids. Preliminary trials with this method on a micro-Kjeldahl basis resulted in poor recovery of known amounts of nitrogen. Troublesome popping occurred. Another explanation for the nitrogen loss was offered by Cahn and Powell (1), who reported that nitrogen was liberated when ammonia was allowed to react with hypochlorite in an alkaline medium.

This paper reports on the investigations performed to determine the effect on protein removal of subjecting film residues on glass surfaces to detergent solutions.

EXPERIMENTAL PROCEDURE

Film residues on glass micro-cover slides were prepared, using the device shown in Figure 1. Preliminary studies had shown that detergency could not be measured experimentally by available procedures using stainless steel slides. The device was made by modifying a Raytheon curd tension meter (Model 2-505) to permit the attachment of slide racks. Adjustable base supports allowed leveling of the complete dipping assembly. A plumb bob suspended from the center of the slide rack acted as a gauge for dipping depth. Reproducible dipping depths were attained by manually switching the machine to reverse when the plumb bob reached an arbitrary depth in the dipping medium. Micro-cover glasses (22 by 50 by 0.14-0.24 mm) served as the film supports. For the purpose of determining sample size, the film supports were weighed on a Christian Becker (Style AB-2) analytical balance before and after filming.

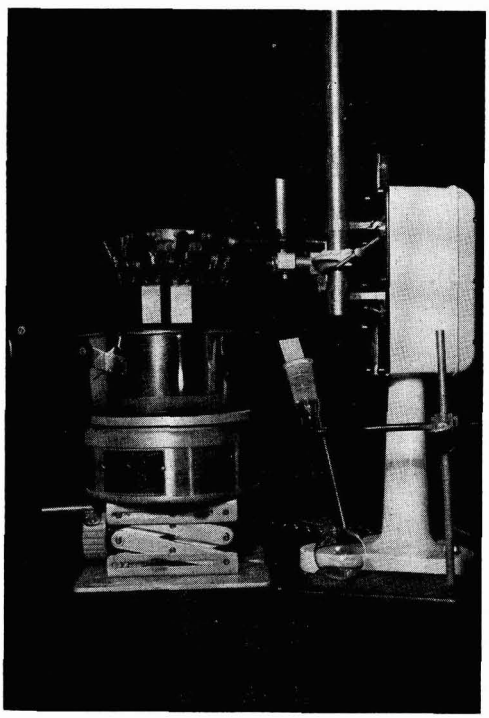

FIG. 1. Temperature-controlled mechanical filming and washing apparatus for micro-Kjeldahl analysis of film residues.

Synthetic soil films high in milk protein, and sufficiently resistant to washing [3] to permit study after the detergency application, were developed in the investigation. The filming procedure consisted of ten complete cycles, of which one is outlined as follows: (a) one mechanical dipping of slides in a 0.5% trisodium phosphate tap water solution containing 2 g calcium chloride and 8 ml sodium hypochlorite per liter of solution; (b) one mechanical dipping in a suspension of 60% clarifier slime, freshly prepared, in tap water; (c) air drying between dips over a hot plate. Following the ten dipping cycles, the films were heated for 13 hr at 80 C in an air controlled circulated oven.

Trisodium phosphate was used in this study for two reasons: (a) The results of a survey of commercial chlorinated detergents showed trisodium to be the most common supporting chemical used; (b) trisodium phosphate had the advantage of being of known composition, which made possible good control of the preparation of the chlorinated product.

The dried films were washed without agitation under conditions of temperature, time, and detergent concentration. Immediately following the washing, two filmed micro-cover glasses, comprising a sample, were positioned in the neck of a 50-ml micro-Kjeldahl flask as shown in Figure 1. Without delay, 4 ml of concentrated sulfuric acid were used to coat the film residues. The film supports and film residues were then quantitatively broken into the neck of the micro-Kjeldahl flask with the aid of a sharp, pointed forcep. Each flask contained two glass beads and approximately 0.5 g potassium sulfate (K_2SO_4). A clean glass rod was used to push the glass fragments down into the bulb of the flask and to break the larger glass fragments. Crushing of the film supports was not desirable because of the resulting violent boiling during the Kjeldahl oxidation with subsequent sample loss.

One milliliter of 10% copper sulfate (w/v, aqueous, $CuSO_4$) in a 5-ml hypodermic syringe served to wash the glass rod before its extraction from the flask. After a 4-hr oxidation period, the samples were distilled into 125-ml Erlenmeyer flesks containing 25 ml 2% boric acid (w/v, aqueous) and five drops of brom cresol green indicator (0.1% w/v, alcoholic).

[3] Washing throughout this paper refers to detergency supplied only by submersion of the micro-cover glasses in a quiescent solution.

Fifty milliliters of distillate were collected and titrated with 0.0142 N sulfuric acid.

Results of the micro-Kjeldahl analysis were expressed as micrograms of nitrogen recovered per milligram of original film sample.

RESULTS

Before the development of more tenacious clarifier slime films, protein solubility (removal) of skimmilk-tap water films was found to increase significantly [4] by addition of sodium hypochlorite to a 0.1% trisodium phosphate tap water solution, as shown in the data of Table 1.

TABLE 1

Solubility of skimmilk films treated in 0.1% Na_3PO_4 with various levels of chlorine, tap water solutions

Sodium hypochlorite	Available chlorine	pH	Residual film [a]	σ
(ml)	*(ppm)*		*(μg N/mg sample)*	
0.00	0.00	9.40	69.14	2.59
2.00	92.00	9.70	50.85	5.52
4.30	191.00	10.00	30.32	8.24
6.60	283.00	10.00	24.71	12.80
12.00	510.00	10.40	7.80	5.74

[a] Represents mean of six trials, micrograms nitrogen removed by washing per milligram film sample (10 min unagitated wash, 65 ⟶ 54 C).

The protein remaining after washing, expressed as μg nitrogen per milligram film sample, was decreased from 69.14 when no chlorine was used to 7.8 with 510 ppm available chlorine added. However, the increase in protein solubility was accompanied by an increase in pH of the trisodium phosphate solution from 9.4 to 10.4 by the addition of sodium hypochlorite and was possibly responsible for the higher degree of peptization.

Experimentation showed that the pH of trisodium phosphate solutions was stabilized at 11.5 ± 0.1 by using a 0.15% concentration of trisodium phosphate in distilled water and 0.5% in tap water of 340 ppm total hardness. When the trisodium phosphate concentration was maintained in this manner, the pH was unaffected by the addition of sodium hypochlorite and the chlorine effect could be studied independently of pH. A highly tenacious film was developed from clarifier slime, from which the following results using stabilized pH were derived.

Data of Tables 2 and 3 show that protein solubility increased significantly when sodium hypochlorite was added to either 0.15% tri-

[4] The word significant as used in this report indicates significance at the 1% probability level as computed by the "t" test.

TABLE 2

Solubility of milk slime films treated in 0.5% Na_3PO_4 adjusted to 156 ppm with NaOCl, tap water solutions

Trial	Residual protein after washing in: Trisodium phosphate	Residual protein after washing in: Trisodium phosphate plus chlorine
	(μg N/mg s) [a]	*(μg N/mg s)* [a]
A	18.68	10.31
B	14.74	10.51
C	16.17	10.74
D	19.25	15.00
E	23.18	17.02
F	30.31	15.71
$\bar{X}$	20.39	13.22
σ	5.71	3.01

[a] Represents micrograms nitrogen remaining after washing per milligram sample (10 min unagitated wash, 65-54 C, pH 11.5 ± 0.1).

TABLE 3

Solubility of milk slime films treated in 0.15% Na_3PO_4 adjusted to 156 ppm chlorine, distilled water solutions

Trial	Residual protein after washing in: Trisodium phosphate	Residual protein after washing in: Trisodium phosphate plus chlorine
	(μg N/mg s) [a]	*(μg N/mg s)* [a]
A	13.17	15.19
B	16.52	16.11
C	15.86	9.62
D	24.46	16.11
E	29.53	14.96
F	22.19	8.88
G	27.22	10.33
H	24.26	9.71
I	6.59	6.36
J	15.22	3.08
K	20.00	3.19
L	7.47	9.17
M	9.01	8.38
N	31.96	3.59
O	10.31	4.32
$\bar{X}$	18.45	9.27
σ	7.65	4.65

[a] Represents micrograms nitrogen remaining after washing per milligram film sample (10 min unagitated wash, 65 to 54 C, pH 11.5 ± 0.1).

sodium phosphate distilled water solutions or 0.5% trisodium phosphate tap water solutions. Both performed at pH 11.5 ± 0.1.

Time of detergent reaction was shown in preliminary studies to be important for obtaining increased protein solubility resulting from chlorination. The data illustrated in Figure 2 show the importance of the time factor. Protein solubility, due to chlorination, increased with time after 1 min of washing. With 1-min reaction time, no difference was found between the protein solubility of solutions containing 0.15% trisodium phosphate with or without hypochlorites at pH 11.5 ± 0.1 and 65 C.

Figure 3 depicts the mean of eight trials performed with 0.15% trisodium phosphate distilled water solution. No optimum level of hypochlorite was found. The lowest increase in rate of protein solubility occurred after the 226 ppm available chlorine level. However, less film was present at the end of the washing period at this level of chlorine than at any other level.

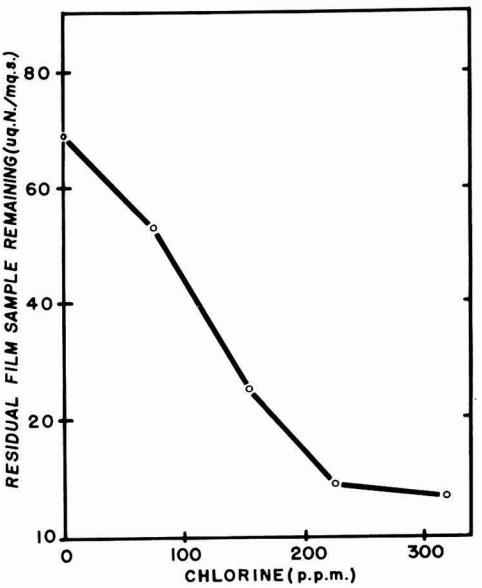

FIG. 3. Evaluation of optimum levels of chlorine on protein solubility of soil films.

DISCUSSION

Of the constituents present in soils of milk products (fat, proteins, lactose, and minerals) the protein-mineral complex is considered the most tenacious and difficult to remove in the cleaning operation. By measuring the difference in nitrogen content of soils previous to and after the washing procedure, the micro-Kjeldahl technique offers a quantitative method of evaluating the removal of the protein-mineral complex. The method is relatively simple, inexpensive, and rapid. In this study significant results were obtained after a comparatively small number of trials.

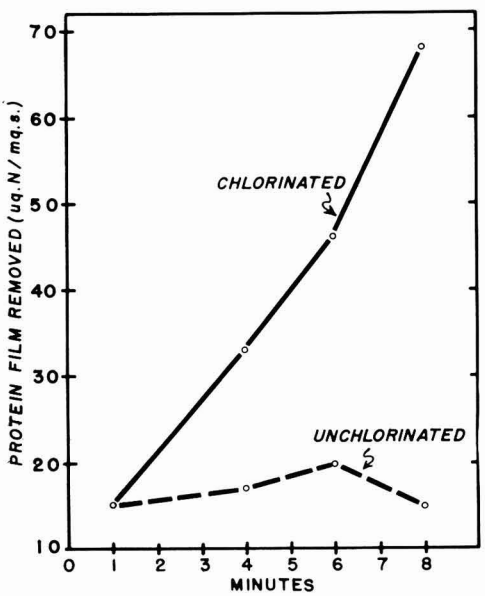

FIG. 2. Effect of time of exposure on the solubility of milk slime films treated with 0.15% Na_3PO_4 and 156 ppm chlorine from NaCl in distilled water.

As the hypochlorite concentration of the trisodium phosphate solutions was increased, more nitrogen was removed from the soiling materials. This result is in agreement with a report in the literature by Cahn and Powell (1), that the peptization of nitrogen compounds occurs in an alkaline medium in the presence of hypochlorites. Many citations in the literature commend the value of adding hypochlorites to alkaline cleaning compounds. However, no quantitative data demonstrating this benefit were found.

Though this study was performed on glass surfaces, the conditions for cleaning stainless steel would appear to be similar. Examples of similar conditions are the composition of the soil, the detergents, and the physical phenomena of time, temperature, and pH.

The results of this investigation indicate a commercial cleaning system employing trisodium phosphate and perhaps other alkaline phosphates would benefit from a high pH, high temperature, extended cleaning solution ex-

posure and generous amount of sodium hypochlorite.

Practical application of this study would seem to lie in the development of a laboratory method which provides quantitative data relative to a detergency function. Quantitative data heretofore unpublished were presented to show the effect on the solubility of protein in the soil when hypochlorite was included in trisodium phosphate washing solution.

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CHEMICAL AND BACTERIOLOGICAL CHANGES IN GRASS SILAGE DURING THE EARLY STAGES OF FERMENTATION. II. BACTERIOLOGICAL CHANGES

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SUMMARY

The results showed that aeration of the forages influenced the bacterial flora and the chemical quality of the silages examined.

The majority of the organisms isolated from the silages during the early stages of fermentation were cocci and Gram-negative rods. A few diphtheroids, aerobic bacilli, and pseudomonads were found, but occurred too infrequently to be of importance. Lactobacilli were usually present in high percentages during the late stages of fermentation.

Only minor differences were observed in the growth rates and total numbers of viable bacterial cells in good and poor silages, and the percentages of acid-producing bacteria in different crops and cuttings were similar.

The streptococci and Gram-negative bacteria were prominent during the initial stages of ensiling. In the poor silages the Gram-negative bacteria reached higher percentages and decreased slower than in the good silages. Streptococci and leuconostocs were observed in the late stages of fermentation. The good silages demonstrated a typical sequence in that the cocci, with the exception of the pediococci, and Gram-negative rods disappeared and the high acid–producing lactobacilli predominated.

The results emphasized the importance of the cocci during the early stages of ensiling. Favorable pH values were obtained in silages when the cocci were in control of the fermentation.

The presence of high numbers of lactobacilli on fresh plants did not necessarily determine the quality of the silage. In the better-quality silages the fermentation was initiated by the streptococci, later supported by the pediococci and leuconostocs, and completed by the lactobacilli.

Since the proportions of acid-producing bacteria were not much different in the silages, it is suggested that the deterioration of the aerated silages was a result of substrate depletion which affected the metabolism of the bacteria. When they were unable to produce enough preserving acids the sporeforming anaerobes were able to compete.

In an accompanying paper (13) chemical changes in orchardgrass and alfalfa silages were reported. Aeration was shown to have a marked effect on the quality of the silages. High temperatures were observed in the aerated silages as a result of oxidation and subsequently the pH, butyric acid and ammonia nitrogen increased while the lactic acid decreased. Nonaerated silages which were packed and sealed were generally of good quality and contained lactic acid as the principal product of bacterial fermentation.

As reported earlier (9, 12) many of the important changes which determine the quality of silage are established during the early stages of the fermentation process. Since little is known of the microbiological changes during the early stages of fermentation, a detailed examination of changes in silage microorganisms during the early ensiling period has been made to determine intergroup relationships. Attempts were also made to relate type and sequence changes to the quality of the silages.

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MATERIALS AND METHODS

The ensiling methods and procedures used in studying the bacteria isolated were reported earlier (9, 12). The large number of bacteria

isolated from the silages were not studied in detail, although enough tests were run to place them in comparatively few groups. Previous experience indicated that the majority of the organisms could be reliably identified by a few tests. However, some overlapping does occur, because in some instances it is necessary to rely on morphology and possibly temperature characteristics.

RESULTS AND DISCUSSION

The total viable counts of bacteria from the silages examined were like those previously described (9, 12) and graphic presentation will not be repeated here. Similar numbers of bacteria were found on plates and roll tubes, regardless of treatment or crop ensiled. Initial plate counts on the fresh forage usually ranged between 10^7 and 10^8 per g and the anaerobic tube counts between 10^4 and 10^5. The difference in the initial plate and tube counts represents the numbers of strictly aerobic organisms which would grow only on the plates. Within a few hours (12 to 24) after ensiling the plate and tube, counts were similar and continued to increase until values between 10^9 and 10^{10} bacteria per gram of silage were reached at about 30 to 40 hr and then showed a tendency to decrease after 48 hr.

No secondary growth was observed, except in the poor silages which contained rather large numbers of sporeforming anaerobes. Seldom were there more than 10^3 anaerobic spores per gram detected on the fresh plant, but when inferior silage was obtained the numbers had increased in some silages to between 10^6 and 10^7 bacteria per gram. Generally, the anaerobic sporeformers were found in highest numbers 48 hr after ensiling and in some silages after six or seven days.

It is surprising that the effects of oxygen and resulting high temperatures in the aerated silages were not more apparent on the growth rates and total number of viable bacteria. But, practically no differences were observed between aerated and sealed silages. One might assume that increased oxygen tension and temperature would either inhibit or stimulate certain groups of bacteria and this would be detectable by differences between plate and anaerobic tube counts in aerated and sealed silages. Although no obvious differences were found in growth rates of the bacteria, the chemical changes of the aerated silages readily pointed out (13) the effects of increased oxygen and temperature on the metabolism of the bacteria and their effects on the final composition and quality of the silages.

Acid production is sometimes used to measure growth of bacteria and in some substrates a fixed relationship exists. Acid production in the silages examined could not be correlated with bacterial multiplication and the majority of the acid produced was formed after active growth ceased. Because of this a considerable lag in acid production was observed. Gibson et al. (3) suggest that acids may be formed in silage by bacteria which have ceased to grow or have lost their viability.

In a study of this type, where different forages are involved and attempts are made to examine representative bacteria, it is necessary to work with large numbers of cultures. For practical purposes, when the numbers to be studied are large, the tests that can be determined on each culture are limited. A total of 5,300 bacterial cultures was isolated from the forages and the majority was picked during the first 48 hr after ensiling. The Gram-positive acid-producing bacteria accounted for 62.1% (3,292) of the total number isolated, the Gram-negative rods 28.1% (1,487), and the diphtheroids and aerobic bacilli 1.47% (78). Some of the cultures failed to grow or were lost during the course of study (443).

Bacilli were found on the fresh forage and occasionally during the first few hours after ensiling, but soon disappeared. The diphtheroids contributed little to the fermentation after about 8 hr. These organisms were rather pleomorphic and quite aerobic.

The Gram-negative rods were variable in size, shape, oxygen requirements, and pigment produced. Pigmentation varied from yellow, orange, and pink to cream and sometimes white. The majority of the colonies from the fresh forage which were able to grow on plates were pigmented. When these colonies were transferred to semisolid agar in tubes many showed facultative growth. Several workers (2, 3, 5, 15) have suggested that these organisms are strict aerobes and it is true that they are replaced by other organisms soon after ensiling. However, of the pigmented colonies examined only about 42% were strict aerobes and although heavy surface growth was observed these organisms were capable of growing throughout the tube. All of the Gram-negative cultures from plates and tubes which showed cream to white colonies were facultative. No doubt most of the pigmented colonies were *Flavobacterium* and many organisms in this group are facultative. The decrease of these organisms during early ensiling may be related more to acid conditions than to their oxygen requirement.

TABLE 1

Presumptive identification of silage organisms

Organism	Morphology	Final pH in glucose broth	Reactions in litmus milk	Production of ammonia from arginine (%)	Growth in 6.5% salt (%)	Growth at 45 C (%)
Streptococci	Cells oval-shaped. Occurred singly, in pairs and short chains. Averaged 0.7 μ by 0.8 μ to 1.2 μ.	3.9 to 4.6	RCA* — 63.5 A — 18.6 Sl.A — 14.2 Pep. — 3.7	96.7	86.4	46.0
Pediococci	Coccus-shaped, occurred singly, in pairs, as tetrads and sometimes short chains. Averaged 0.6 to 0.7 μ by 0.8 to 1.2 μ.	3.7 to 4.1	RCA — 43.7 A — 44.7 Sl.A — 11.6	77.9	85.4	95.1
Leuconostoc	Coccus to rod shapes, occurred singly, in pairs, short chains, and clumps. Some cells slightly pleomorphic averaged 0.4 to 0.7 μ by 0.8 to 4 or 5 μ.	4.1 to 4.7	RCA — 5.4 RA, Sl.A — 94.6	27.9	85.0	88.0
Heterofermentative lactobacilli	Short to medium rods, ends rounded. Occurred singly and in pairs. Occasionally filamentous. Averaged 0.7 to 0.9 μ by 1.3 to 4 μ.	3.8 to 4.3	RCA — 22.3 Sl.A — 77.7	72.4	82.9	4.0
Homofermentative lactobacilli	Variable rods, usually rounded ends. Occurred singly, in pairs and sometimes chains. Averaged 0.7 to 0.9 μ by 1.3 to 8.0 μ. Filamentous cells observed.	3.6 to 4.2	RCA — 55.8 RA — 25.0 Sl.A — 19.2	36.7	79.3	33.5

* RCA = Reduced, curdled, and acid. RA = Reduced, acid. A = Acid. Sl.A = Slight acid. Pep. = Peptonized.

Occasionally in this work yeasts, pseudomonads, and *Serratia* were found. Because they were so uncommon it is doubtful they are important in the fermentation.

The acid-producing bacteria picked were examined in more detail than the ones described above. Some of the tests used and presumptive identification of the cultures may be seen in Table 1. The majority of the acid producing strains studied was streptococci. The data in Table 1 point out variations that occurred in some tests. Some of the streptococci failed to produce ammonia from arginine, grow in 6.5% salt and at 45 C. Failure of the streptococci to grow at 45 C was observed previously (7) and more recent data (6) showed variations in other tests listed. A few cultures tested produced catalase, reduced nitrate, and showed motility (6, 10, 11). The data presented along with previous work suggest that *Streptococcus faecalis, Streptococcus faecalis* var. *liquefaciens,* and *Streptococcus faecium* were the primary streptococci isolated.

The gas-producing leuconostocs which failed to produce dextran on 10% sucrose were combined with the dextran producers. Similar organisms were earlier differentiated as *Leuconostoc mesenteroides* and *Leuconostoc,* type 1 (7).

Although insufficient data were obtained to

precisely classify the homo- and heterofermentative lactobacilli in this study, previous work would suggest their close relationship with *Lactobacillus plantarum, Lactobacillus casei,* and *Lactobacillus brevis* (8).

The distribution of lactic acid bacteria on the fresh forage and in the silages is shown in Table 2. The data include the percentages of organisms in different forages, cuttings, and treatments. It is interesting to note that the proportions of bacteria were not too dissimilar. As might be expected, since most of the samples were taken during the first 48 hr of the fermentation, the cocci predominated.

The highest percentage of streptococci was found in the aerated, first-cutting orchardgrass ensiled in 1957 and, with the exception of the first-cutting alfalfa 1957, these organisms were obtained in about the same percentages in most silages. When they decreased the pediococci, leuconostocs, and homofermentative lactobacilli increased.

Probably the most noticeable variation in the distribution of the cocci was in the 1957 first-cutting alfalfa (S-5 and S-8). The leuconostocs predominated and accounted for over 40% of the total lactic acid bacteria isolated.

The heterofermentative lactobacilli occurred in the silages in low percentages and at about the same magnitude, but the homofermentative ones showed rather wide variations from 1.3 to 17.6%.

Neither the type of forage nor the high temperatures which resulted in the forages because of oxidation greatly affected the percentage distribution of the acid producing bacteria.

Orla-Jensen, Orla-Jensen and Kjaer (17), and other workers (1, 4, 16, 18) have shown in the silage fermentation that the Gram-negative bacteria are gradually replaced by spherical acid-producing ones and subsequently by the slower-growing, high acid-producing lactobacilli. The data in Table 3, based on detailed changes during the early stages of fermentation, support this earlier work. The data from individual silages were combined and Table 3 shows the average percentages of bacteria in the aerated and sealed silages at each sampling period.

It should be borne in mind that although some individual silages within each group showed chemical and bacterial (Table 2) variations (13), they were not great enough to critically influence the over-all percentage averages of organisms given in Table 3.

The development of organisms (Table 3) in the aerated and sealed silages showed some differences, but the changes were not as great as might have been expected. The microorganisms on the fresh grass were composed primarily of Gram-negative rods (aerobic and facultative) and streptococci.

During the first 8 to 10 hr after ensiling, the Gram-negative bacteria and streptococci predominated. However, the streptococci did not compete as well in the aerated as in the sealed silages. Although the numbers of streptococci were similar on the fresh plant, they decreased

TABLE 2

Distribution of lactic acid bacteria in aerated and sealed silages

Type of forage ensiled	Treatment	Strepto-cocci	Pedio-cocci	Leuco-nostoc	Hetero-fermen-tative rods	Homo-fermen-tative rods
Orchardgrass 1st cutting 1957	Aerated S-6	84.5	7.2	1.5	3.2	3.4
	Sealed S-4	74.2	16.1	0.0	2.0	7.6
Orchardgrass 2nd cutting 1957	Aerated S-6	63.1	19.0	5.1	2.0	10.7
	Sealed S-4	44.8	26.7	7.4	3.2	17.6
Alfalfa 1st cutting 1957	Aerated S-5	23.1	30.4	42.4	2.1	1.9
	Sealed S-8	31.2	19.5	42.8	2.0	4.4
Alfalfa 2nd cutting 1957	Aerated S-5	69.4	10.3	3.4	1.3	15.5
	Sealed S-8	66.4	9.7	12.7	3.0	8.2
Orchardgrass 2nd cutting 1959	Aerated S-6	64.8	25.2	8.3	0.3	1.3
	Sealed S-4	68.6	12.1	7.9	2.7	8.6

TABLE 3

Development of microorganisms in the silages

Time after ensiling	Strepto-cocci	Pedio-cocci	Leuco-nostoc	Heterofer-mentative lacto-bacilli	Homofer-mentative lacto-bacilli	Diph-theroids	Gram-nega-tive rods
	Aerated silages						
(hr)	(%)						
0	28.1[a]	2.0	1.0	0.0	5.2	2.0	61.5
2	38.4	4.4	0.0	1.1	0.0	3.3	52.7
4	32.9	9.1	7.9	0.0	0.0	0.0	50.0
6	27.1	5.5	9.7	0.0	1.0	3.2	53.2
8	10.8	7.6	6.5	1.1	2.1	3.2	68.4
10	15.4	0.0	12.1	0.0	1.1	0.0	71.4
12	24.4	0.0	11.1	0.0	4.4	1.1	58.9
14	29.4	0.0	11.5	0.0	1.0	1.0	56.9
16	35.5	2.2	11.1	0.0	0.0	1.1	50.0
18	40.0	0.0	18.9	1.0	0.0	0.0	40.0
20	45.0	2.2	14.2	1.1	1.1	1.1	35.1
22	48.9	7.4	13.8	0.0	3.1	0.0	26.6
24	42.0	12.5	12.5	0.0	1.1	0.0	31.8
26	46.7	6.5	15.2	1.0	0.0	0.0	30.4
28	35.7	23.1	21.0	0.0	3.1	0.0	16.9
30	42.4	16.3	8.6	0.0	1.0	0.0	31.6
32	47.8	9.7	15.2	0.0	3.2	0.0	24.0
34	48.9	27.1	7.6	1.1	2.1	0.0	13.1
36	47.3	15.0	9.7	2.1	7.5	0.0	18.3
38	41.9	11.8	5.4	4.3	3.2	0.0	33.3
40	37.2	11.6	9.3	0.0	11.6	0.0	30.2
42	35.2	20.4	6.8	1.1	1.1	0.0	35.3
44	46.3	16.8	9.5	0.0	5.2	0.0	22.1
46	47.9	28.1	4.1	0.0	3.1	0.0	16.7
48	33.3	27.9	4.3	3.2	5.3	0.0	25.8
7 days	10.0	85.0	0.0	0.0	5.0	0.0	0.0
14 days	26.1	38.6	1.1	15.9	18.2	0.0	0.0
30 days	40.0[b]	20.0	5.0	0.0	35.0	0.0	0.0
62 days	0.0[c]	0.0	0.0	0.0	0.0	30.0	70.0
	Sealed silages						
0	26.6	2.1	1.0	1.0	11.7	5.3	52.1
2	29.9	10.3	1.0	0.0	3.1	6.0	49.4
4	35.5	2.1	1.1	1.0	1.0	5.3	53.7
6	30.2	0.0	1.0	1.0	5.2	4.1	58.3
8	43.5	11.7	2.3	0.0	1.2	2.3	38.8
10	40.2	2.1	4.3	0.0	1.1	0.0	52.5
12	54.3	3.2	7.6	0.0	2.2	0.0	32.7
14	62.1	1.1	1.1	2.2	3.4	0.0	29.8
16	62.1	1.1	11.5	0.0	2.3	1.1	21.8
18	57.0	4.6	18.6	0.0	0.0	0.0	19.7
20	44.8	5.7	19.5	2.3	5.6	1.1	21.8
22	50.0	10.0	16.6	5.5	3.3	0.0	14.4
24	55.5	11.1	12.3	0.0	1.2	0.0	19.7
26	42.5	13.7	11.3	0.0	5.0	0.0	27.5
28	41.2	11.2	22.5	3.7	6.3	0.0	15.0
30	44.3	6.8	17.0	0.0	4.6	0.0	27.2
32	40.0	9.4	14.9	1.0	5.3	0.0	29.4
34	43.9	8.8	28.5	2.2	2.2	0.0	14.2
36	46.5	16.2	17.5	0.0	2.3	0.0	17.4
38	31.8	27.3	12.5	2.3	7.9	0.0	18.1
40	36.9	28.2	14.1	0.0	7.6	0.0	13.0
42	41.4	21.2	9.5	3.2	12.0	0.0	12.7
44	35.6	25.2	26.4	1.1	7.0	0.0	4.5
46	41.4	25.5	15.9	0.0	7.5	0.0	9.5
48	32.9	29.6	17.5	2.2	6.6	0.0	10.9
7 days	25.0	30.0	0.0	0.0	45.0	0.0	0.0
14 days	3.3	45.5	1.1	10.0	36.7	0.0	3.3
30 days	0.0	5.0	0.0	10.0	85.0	0.0	0.0
62 days	0.0	15.0	0.0	45.0	40.0	0.0	0.0

[a] = Average percentages of all silages through 14 days (ten silages).
[b] = Average percentages of 1957 and 1959 second-cutting orchardgrass (four silages).
[c] = Average percentages of 1959 second-cutting orchardgrass (two silages).

to about 11% in the aerated silage at 8 hr, but had increased to 43.5% in the sealed silages. The increase of Gram-negative bacteria in the aerated silage points out the influence of oxygen on their growth and competitive ability. The other groups of organisms appeared to impart little influence during the first 8 to 10 hr of the fermentation.

The changes which occurred after 12 through about 48 hr showed a more balanced flora and attempts of the pediococci and leuconostocs to compete with the streptococci and Gram-negative bacteria. During this period the pediococci increased to about the same extent in both aerated and sealed silages. The leuconostocs were also similar through about 32 hr. After this period they decreased in the aerated silages but maintained rather high percentages in the sealed silages. Both the hetero- and homofermentative lactobacilli were able to survive in the silages in small percentages but showed no superior competitive ability. The data show, however, that the homofermentative lactobacilli were more evenly distributed than the heterofermentative ones and occurred in slightly higher percentages. It was of interest to follow changes in the Gram-negative bacteria during the 12- to 48-hr fermentation period. They disappeared more rapidly in the sealed silages and at 48 hr only 10.9% were found as compared to 25.8% in the aerated silages.

At seven days the pediococci and, to a lesser extent, the streptococci had completely dominated the fermentation process in the aerated silages. These results are not consistent with earlier work (9), but do emphasize the competitive ability of the pediococci. Unlike the aerated silages, the sealed ones contained a high percentage of homofermentative lactobacilli and the presence of this high acid-producing group is indicated by the low pH values of the silages (13).

The flora in the aerated silages at 14 days was more balanced. The pediococci decreased and fair percentages of hetero- and homofermentative lactobacilli appeared. The chemical changes in the silage showed, however, that they occurred too late to prevent deterioration. The sealed silage, on the other hand, had developed a flora indicative of a normal fermentation.

The final stages of the fermentation (30 and 62 days) showed that the flora in the aerated silages had not stabilized and although sizable percentages of lactobacilli and pediococci were found the streptococci and leuconostocs increased, which indicated a lack of high acid conditions. The 62-day sample contained non-lactic acid bacteria. This material could not be considered silage, because it decomposed and had a pH of 7.0. The sealed silages contained primarily lactobacilli and some pediococci. The appearance and proportions of these groups are similar to those previously obtained in good-quality (sealed) silages (9).

These results point out the importance of the cocci in the fermentation process. The streptococci were especially significant in their ability to compete with the Gram-negative bacteria during the initial ensiling period. As shown in an accompanying paper (13), many of the silages studied were not superior in quality. Nevertheless, the pH values in the sealed silages were in a range which showed that in the absence of high initial proportions of lactobacilli satisfactory silage could be obtained. Development of lactobacilli occurred at earlier stages in silages previously studied (9), which shows inherent differences in the ability of organisms to compete. It should be noted, however, that these silages were of better quality when the initial flora consisted primarily of cocci.

In all of the good-quality silages studied the fermentation was initiated by the streptococci, later supported by the pediococci and leuconostocs, and completed by lactobacilli. Stirling (19) and others (3, 15, 20) have emphasized a need for high numbers of lactobacilli on forage and their relationship to silage quality. Obviously, if these organisms are present initially in high numbers and can control the fermentation, the pH of a silage should drop quickly because of their acid-producing ability. However, the results presented in this paper showed that although few lactobacilli were present on the fresh plant they were able to exist in low numbers during the early ensiling period, and were unable to increase greatly, but did eventually dominate the fermentation. These results and other work (9) would suggest that the initial numbers of bacteria on the fresh plant is not the deciding factor in silage quality but that a sequence of events is desirable in which the cocci are finally replaced by the high acid-producing lactobacilli.

The exact role played by the cocci is not clearly understood, but it is possible that they are setting the stage for subsequent growth of lactobacilli.

The presence or absence of certain groups of bacteria does not necessarily define the problem of quality differences which we observed, because the poor silages contained sufficient numbers of lactic acid bacteria. In the poor silage, however, the lactic acid was destroyed

and butyric acid and ammonia were formed. It would appear that although enough acid-producing bacteria were present in poor silage some of the nutrients necessary for their metabolism had been destroyed as a result of forage treatment (aeration).

The chemical changes in the silages studied (13) showed that the amount of sugar in the plant did not limit acid production. The large amount of acid produced in relation to the low initial sugar points out that when certain minimum requirements are satisfied the lactic acid bacteria are extremely versatile. At limiting concentrations the amount of sugar did not control multiplication of bacteria. It is generally true, however, that an organism will grow about as well at low sugar levels as at high, but growth ceases earlier. The continued acid production in the silages showed that although multiplication had ceased, substrates more complex than simple sugars were being utilized and Lockhart (14) has shown that metabolism without cell division is a common phenomenon.

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EFFECT OF STAGE OF MATURITY ON THE NUTRITIVE VALUE OF ATLAS SORGHUM SILAGE FOR LACTATING DAIRY COWS [1]

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SUMMARY

Atlas sorgo silages harvested at the milk, soft dough, hard dough, and mature stages have been compared as roughages for lactating cows. Two experiments were conducted, each using 12 cows in a switch-back design. With advancing maturity, consumption of dry matter increased and 4% fat-corrected-milk (FCM) per pound dry matter intake decreased; consumption of silage (as fed), milk fat percentage, and body weight change were not significantly affected by maturity at harvest. FCM production was slightly higher for the two more mature stages, but differences among maturities were significant only in the first experiment, in which the soft dough stage was inferior to the more mature stages. Since daily performance of the lactating cow was not appreciably influenced by the stage of maturity, it appears advantageous to harvest Atlas sorghum when acreage yields are near maximum, usually at the hard seed stage. At this station dry matter yields of Atlas sorghum increased 33% from the milk stage to the mature stage. Delaying harvest an additional ten days resulted in a 57% increase in dry matter yield, compared to that at the milk stage. The differences found in quality of the silage dry matter favoring early-cut silage appear insufficient to compensate for the usual yield advantage of harvesting Atlas after reaching maturity.

Differences in the nutritive value of silages made from sorghums cut at various stages of maturity are suggested by their chemical composition and by the relation of maturity to the proportion and hardness of sorghum seed.

Brief reports of Texas experiments (2, 5) indicate higher milk yields from Tracy sorghum silage cut when the oldest seed is in the soft dough stage and from Hy Hegari harvested in the bloom stage compared to the hard seed stages.

Soft dough stage Atlas silage was practically equal to mature and superior to boot stage Atlas in terms of weight gains of wintering beef cattle (3). Feeder calves produced slightly better gains on soft dough Atlas than mature Atlas silage (3). In both beef cattle experiments, more gain per acre was realized from Atlas harvested at the mature stage.

Ramsey et al. (8) found practically no difference in average dry matter digestibility of Tracy sorghum ensiled at four stages ranging from fully flowered to the ripe seed stage. In contrast, other experiments (2, 5) resulted in lower digestibility values for silages made from the dough or hard seed stages of sorghum than at more immature stages. It is evident that the present knowledge in this subject is limited and inconclusive.

Therefore, this study was undertaken to evaluate the influence of stage of maturity at harvest on the nutritive value of Atlas sorgo silage for lactating dairy cows.

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EXPERIMENTAL PROCEDURE

Experiment I. Nine Jersey and three Guernsey cows were used as experimental animals in a switch-back design (6). Cows were kept in stanchion stalls to facilitate collection of individual feed consumption data, but were turned into a concrete lot for about 1 hr daily for exercise.

During the first seven days of a 14-day preliminary period, hay was gradually eliminated from the ration; and, during the final seven days the entire roughage portion of the ration was composed of a mixture of the experimental silages. Then cows were fed specific silages during three 21-day experimental periods. The final 14 days of each period were used for evaluation of silage consumption and milk production.

The four experimental silages used were Atlas sorghum cut at the milk, early dough, medium dough, and mature stages. The silages were harvested from two fields in the same area. A portion from each field was cut at each stage and preserved in an upright tile silo. Silages were fed ad libitum as the sole roughage three times daily (6 AM, 11 AM, and 5 PM). The amounts offered at successive feedings were adjusted to approximate a 3 to 5 lb refusal per feeding. The concentrate ration used was composed of rolled yellow corn, 700 lb; rolled oats, 700 lb; wheat bran, 700 lb; soybean oil meal, 100 lb; bone meal, 18 lb; and salt, 18 lb. The level of concentrate feeding during the first week of the first experimental period was based on Morrison's (7) optimum TDN requirements for maintenance and production for cows consuming Atlas sorghum at the average rate determined during the preliminary period. Concentrate feeding levels were adjusted each week by multiplying the average persistency of milk production for all experimental cows during the preceding week by the amount of concentrate currently allowed each cow.

Milk weights were recorded and the silages were sampled daily, butterfat tests were made weekly (24-hr composite), and cows were weighed on two successive days, at the beginning and end of each experimental period. Standard methods were used for silage analyses.

Experiment II. This experiment was to evaluate, again, Atlas silages of the same stages of maturity under very similar conditions to those of Experiment I. Eight Jersey and four Guernsey cows were used in the same design and were housed and managed in the same manner as cows in Experiment I.

During the first seven days of a 14-day preliminary period hay was gradually eliminated and during the final seven days of this period the entire roughage was silage. Due to the limited amount of sorghum silage available for feeding, corn silage was fed to all cows during all except the last four days of the preliminary period. During the last four days a mixture of the experimental sorghum silages was fed. The length of experimental periods was also 21 days in this study; however, the supply of silage reduced the final period to 10 and 12 days for the medium dough and the other silages, respectively. A separate analysis of the milk production data was made using only the 8th-12th (and 8th-10th for medium dough, Period III) days for all periods. Using this analysis the experimental error was larger, but results were very similar to those obtained when data from the last 2 wk for Periods I and II were used with the available data from Period III. Therefore, the latter data seemed justified and are those which are presented in this paper.

The four silage stages were cut from a common field which appeared to produce reasonably uniform sorghum. The data collected and the method of feeding silage was the same as for the preceding experiment. A concentrate mixture composed of rolled corn, 800 lb; wheat bran, 500 lb; soybean oil meal, 400 lb; salt, 8 lb; and dicalcium phosphate, 8 lb was fed. During the first period concentrate feeding was in accordance with Morrison's table (7) for average roughage consumption. For subsequent periods the amount was reduced for each cow by multiplying the previous allowance by the average milk production persistency for all experimental animals.

RESULTS AND DISCUSSION

Composition of silages. The content of crude fiber, crude protein, and ether extract decreased and nitrogen-free extract (NFE) increased abruptly between the milk and soft dough stages. With further advancement in maturity the trend continued, at a reduced rate (Table 1).

TABLE 1

Proximate composition of silages

Stage of maturity	Dry matter	Crude fiber	Crude protein	Ether extract	Ash	NFE
Experiment I	*(%)*			*(% of dry matter)*		
Milk	22.1	34.3	9.10	3.53	8.22	44.85
Soft dough	24.8	26.9	8.10	3.18	8.40	53.42
Medium dough	26.0	26.7	7.53	3.09	7.76	54.92
Mature	27.4	25.6	7.46	3.10	6.68	57.16
Experiment II						
Milk	20.5	29.1	9.52	3.78	8.96	48.64
Soft dough	23.2	26.8	7.55	3.39	8.01	54.25
Medium dough	27.0	25.9	7.30	3.33	8.47	55.00
Mature	29.0	27.2	7.53	3.38	8.63	53.26

TABLE 2

Mean performance of lactating cows fed Atlas silage cut at four stages of maturity

Stage of maturity	Silage consumed: As fed	Silage consumed: Total D. M.	Silage consumed: D. M/ cwt	FCM	FCM per lb D. M.[a] eaten	Milk fat	Body wt change
	(lb/day)				*(lb)*	*(%)*	*(lb/21 days)*
Experiment I							
Milk	55.0	12.2	1.25	27.5	1.17	6.04	−1.5
Soft dough	55.8	13.8	1.41	26.7	1.07	5.87	−2.2
Hard dough	54.5	14.0	1.43	28.4	1.13	6.20	−5.1
Mature	55.1	15.2	1.55	28.2	1.07	5.89	+8.8
Standard error	±1.17	±0.28	±0.037	±0.33	±0.0187	±0.168	±12.1
Significance of difference	NS	P<1%	P<1%	P<1%	P<1%	NS	NS
Experiment II							
Milk	66.2	13.5	1.46	30.6	1.24	5.36	+20.5
Soft dough	64.2	14.7	1.60	31.0	1.19	5.21	+10.4
Hard dough	61.3	16.5	1.75	31.3	1.14	5.34	+ 5.9
Mature	60.1	17.2	1.84	31.5	1.11	5.09	− 5.6
Standard error	±3.40	±0.79	±0.092	±0.63	±0.052	±0.126	±12.9
Significance of difference	NS	P<1%	P<5%	NS	NS	NS	NS

[a] Silage plus concentrate dry matter.

The mature stage of Experiment II was higher in dry matter than the mature silage in Experiment I, suggesting that it was physiologically older than the latter. The range in silage dry matter content was greater in the second experiment. Although the intention was to harvest the silage at comparable stages for the two experiments, the dry matter values suggest that a wider range of maturities was compared in Experiment II. It is recognized, however, that variations in moisture content result from other factors as well as from maturity.

Silage consumption. Performance data for both experiments are presented in Table 2. Consumption of silage was not significantly different among treatments in either experiment. However, in Experiment II silage intake was pregressively lower with advancing maturity. Others (3) observed a similar influence of maturity on silage intake. Average consumption was 55.1 and 60.4 lb for the first and second experiments, respectively. The reason for greater intake in Experiment II is unknown. When compared on the basis of dry matter consumed the maturities differed significantly. There was an increase in intake with each more advanced maturity stage. In both experiments, about 25% more dry matter was consumed of the silage in the mature stage compared to the milk stage. Silage dry matter intake per hundredweight corresponded closely with total dry matter intake and was below that expected for average-quality roughage in all cases except for the hard dough and mature stages fed in Experiment II. In contrast, Hilston and Gifford (3) found that dry matter consumption by beef cattle from soft dough and mature Atlas sorgo silage was almost identical. The present study confirms their finding that the dry matter of early-cut Atlas is higher in nutritive value than mature Atlas.

Milk production. The average daily yields of FCM differed little among silage maturity stages. Considering both experiments, milk production averaged 0.9 lb/day higher for the hard dough and mature silages compared with the milk and soft dough silages. Differences in milk production among maturities were significant only in the first experiment. In an earlier, unpublished experiment at this station slightly lower milk yield resulted from feeding Tracy sorgo ensiled at the early dough stage compared to the hard dough stage. Similarly, small differences were reported in daily milk yields of cows fed corn silages harvested at various maturities (1, 4, 9). However, the results of the present report are in contrast to those of Texas experiments (2, 5), in which higher milk yields resulted from feeding immature compared to hard seed sorgo silage.

The yield of FCM per pound of dry matter consumed (from both grain and silage) decreased progressively with advancing maturity, with the exception of the soft dough stage in Experiment I. This exception may have been due to the low milk production on this treatment and the higher proportion of dry matter

required for maintenance. Since production of FCM (within experiments) was very similar for other treatments, differences in the FCM produced per pound of total dry matter consumed reflect the influence of the silage on the quality of the complete ration. However, difference in silage dry matter consumed would appear to be a more accurate measure of the relative value of the dry matter of the different silages. No relationship between treatments and milk fat percentage was revealed.

Body weight changes. Statistical analysis of body weight changes revealed high variation among individuals and no significant differences among treatments. Experiment II results suggest that as sorghum matures, it progressively diminishes in value for body weight gain. Except for the mature stage, a similar trend was observed in the first experiment.

Conclusion. The stage of maturity at harvest of Atlas sorgo for silage had little apparent effect on its value when full-fed as the exclusive roughage for lactating cows. The value of the dry matter of Atlas sorgo silage decreased with maturity. Unpublished yield data from this station reveal increases in Atlas dry matter production of 33% from the milk to the mature seed stage, and a 57% increase by delaying harvest an additional ten days. Therefore, it appears that the yield advantage of harvesting Atlas in the mature stage may more than compensate for its apparent lower quality compared to more immature stages. The yield advantage and the negligible seepage loss appear to favor harvest of Atlas for dairy cattle in the mature stage.

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ESTIMATED NET ENERGY AND TOTAL DIGESTIBLE NUTRIENT RELATIONSHIPS OF CLASSES OF FEEDS

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SUMMARY

The advantages and disadvantages of net energy and total digestible nutrient (TDN) methods of measuring the nutritive value of foods were discussed briefly. Several procedures for converting TDN into estimated net energy (ENE) were reported. The current objective was to determine the effect of the substitution of feed class regressions of ENE on TDN for a general regression of the combined feed classes, on the amount of error involved in converting TDN to ENE values.

Using Morrison's data on ENE and TDN, 21st edition, it appeared that all the feed classes had regression coefficients that differed significantly from the general regression coefficient for the combined classes. The feed classes, silage excepted, apparently had similar slopes for their ENE/TDN relationships and the reason why the general regression line was significantly different was because the feed classes operated at different levels of energy.

Data in Morrison's 22nd edition were treated in the same manner, and results agreed satisfactorily with the previous edition except for silage and green roughage classes. Due to the many changes between editions in TDN or ENE values, the regression lines of these two classes differed significantly from those of the earlier edition. Regression equations of the feed classes developed from data in each edition are given, along with their errors of the estimate.

In converting TDN to ENE values using Morrison's data, the appropriate feed class regression appears to be more accurate than a general regression.

Net energy, theoretically, is a better measure of feed values than TDN, since it represents the energy actually used by the animal for both maintenance and productive purposes. By definition, net energy is the difference between the total energy of a feed and the sum of the energies of the feces, urine, combustible gases, and heat increment. Since it varies with the level of intake, animal species used, and combination of feeds with which it is fed, the net energy value of a specific feed should not be considered a constant, but an average value. Net energy values of feeds are desirable, since this quantity minus energy for maintenance is directly proportional to the level of production. However, due to the difficulty and expense involved in determining net energy, the absolute net energy of only a few feeds is known, and it appears that even with automation considerable time may elapse before the relationship of net energy and TDN can be established on an absolute basis.

The TDN system of feed evaluation is subject to several sources of error. It overevaluates forages, its analytical methods are empirical, and its energy value assignments to components are questionable. In spite of these disadvantages TDN continues to be used within the United States. Specifically, TDN is the sum of the digestible percentages of protein, carbohydrates, and 2.25 times the ether extract of the ration. The two main reasons for the continued use of TDN are: its ease of determination and the large mass of feed data accumulated in terms of TDN.

Estimated net energy (ENE) values, according to Morrison (6) are based on a study of all available data on the relative productive value of feeds, with chief reliance placed on results of hundreds of feeding trials conducted by experiment stations comparing the values of different feeds. While Morrison admits judgment was used in computing ENE values, and that ENE values are only approximate and subject to revision as additional data become available, he believes it possible to evaluate the most important feeds [(2), Appendix Table II] more correctly on a net energy basis than by TDN.

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TABLE 1

Regression of estimated net energy (Y) on per cent TDN (X) (dry basis)

Feed class	No. of samples	Equation	r [a]	s
Concentrates	86	$Y = 4.40 + 0.933\ X$	0.88	5.4
Roots	8	$Y = 1.000\ X$	1.00	....
Roughage:				
Green	38	$Y = -6.66 + 0.958\ X$	0.95	1.8
Dry	61	$Y = -11.57 + 0.965\ X$	0.81	3.4
Silages	23	$Y = 22.43 + 0.442\ X$	0.59	2.8
Straws	9	$Y = -18.79 + 0.875\ X$	0.81	6.9
Combined	225	$Y = -31.48 + 1.346\ X$	0.96	5.9

[a] All significant at 1% level; r = sample correlation coefficient; s = standard error of the estimate.

Several plans for converting TDN into ENE have been proposed. Forbes and Kriss (2), by adopting the value of 3.563 therms of metabolizable energy for a kilogram TDN of mixed rations, and 57.5% as the fraction of net availability of metabolizable energy for fattening, arrived at a value of 2.049 therms net energy per kilogram TDN. Haecker's (3) standard and Morrison's (6) table of requirements gave a value of 1.870 therms net energy per kilogram TDN [cited by Blaxter (1)]. Leroy (4) published a table for interchanging values of different systems of feed evaluation. In this table he reported 1 kg TDN of roughage as equivalent to 1.65 kcal net energy for fattening, and 1 kg TDN concentrates equal to 2.18 kcal. Data presented in Leroy's table appear to be in error from values reported by other workers. It is assumed that Leroy meant therms instead of kcal. Moore et al. (5), using a different approach, developed a general regression equation of the ENE/TDN relationship for all classes of feeds. These authors state that while the use of a regression equation to calculate the energy value of a specific feed was open to question, the regression of ENE on TDN offers a means of approximation and might serve as a guide to establishing more accurate productive values.

The purpose of this paper was to find out if the substitution of feed class regression equations for a general equation would significantly affect the amount of error involved in converting TDN to ENE values.

MATERIALS AND METHODS

Data used were Appendix Tables I and II of the 21st edition of Morrison's Feeds and Feedings, where feeds are reported in both TDN and ENE values. With the intent of reducing error, only feeds with nine or more TDN determinations were selected. When two ENE values for a feed were given, the ENE value for dairy cows was the one utilized. All feeds were handled on a dry matter basis.

Feeds were separated into the four main feed classes of Morrison; namely, dry roughages, green roughages, silages, and concentrates. On inspection of ENE/TDN ratios of the feeds, it was found that both roughages contained two groups whose regression coefficients differed significantly from each other. The subdivision of dry and green roughages made a total of six feed classes, when straws and hulls from dry roughages, and roots from green roughages were added. It was observed that roots could be classified with concentrates, since on a dry basis both have similar high energy and low crude fiber contents, and their ENE/TDN regression coefficients show no significant difference.

RESULTS AND DISCUSSION

Regression equations of ENE on TDN for the six classes of feeds are shown in Table 1, as well as a general equation for the combined classes. All six feed class regression coefficients were found to differ significantly from the general regression coefficient at the 1% level. Correlation coefficients of the ENE/TDN relationships and errors of the estimate of the feed classes are also reported in Table 1. The combined feeds had highest correlation coefficient and next to the highest error of estimate. The high correlation coefficient of the combined feeds is explained in part by the length of its regression line. In predictability, however, the error of the estimate is the important factor.

In Figure 1 the regression lines of the feeds are given. In descending order of energy content, the feed classes were found to be in the following order: roots, concentrates, green roughages, silages, dry roughages, and straws and hulls. The average ENE/TDN ratios of

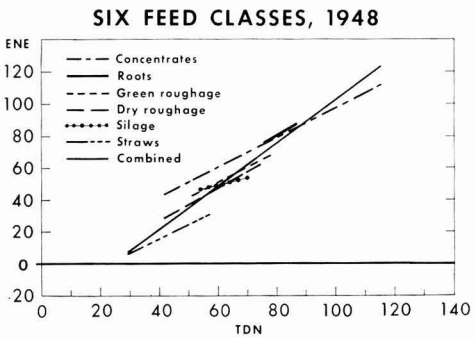

FIG. 1. Feed class regressions of ENE (Y) on per cent TDN (X).

the feed classes were also in the same order, having the following respective values: 1.000, 0.987, 0.854, 0.813, 0.761, and 0.501. It appears that the general regression line is composed of a combination of feed classes that operate at different levels of energy. The slopes of the feed class lines, with the exception of silages and straws, are similar, since the regression coefficients of their lines are contained within a 0.067 range. It would appear that the significance of the general equation line from the feed class lines is due mainly to the shifting of the slope of the general regression line to accommodate feed classes of different energy levels.

The removal of feeds with less than nine TDN determinations evidently had little effect on the accuracy of the general equation. The combined equation, with less than nine TDN feeds excluded, was $Y = -31.48 + 1.346\ X$ where Y = ENE and X = TDN. Moore et al. (5), with no exclusions, had a general regression equation of $Y = -34.63 + 1.393\ X$.

The ENE and TDN data of feeds in Morrison's 22nd edition were treated in the same manner, and the feed class regression equations, correlation coefficients, and errors of the estimate are shown in Table 2. Results obtained with the latter edition agreed satisfactorily with previous edition, except for silage and green roughage classes. These differences probably were due to the many changes in the TDN and ENE values of the feeds, and to additions of feeds to the list. For example, the changes in green roughage equation were caused by alterations in energy value in half of the 38 items of the earlier edition and the addition of 30 new feeds. These alterations apparently resulted in the green roughage regression coefficient becoming similar to, and not significantly different from, the general regression coefficient of the combined feeds. The slopes of the class and combined feed regressions of the 22nd edition are shown in Figure 2. In the silage feed class the change in regression coefficients between editions was probably because all silages except two had altered values of TDN, ENE, or both in the later edition.

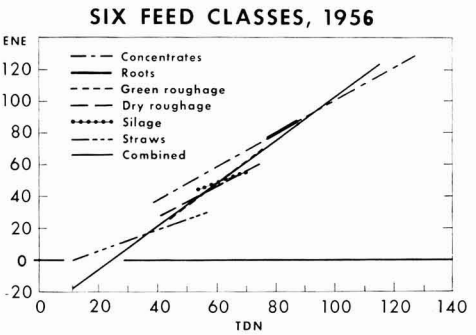

FIG. 2. Feed class regressions of ENE (Y) on per cent TDN (X).

TABLE 2

Regression of estimated net energy (Y) on per cent TDN (X) (dry basis)

Feed class	No. of samples	Equation	r [a]	s
Concentrates	92	$Y = -4.59 + 1.040\ X$	0.91	5.1
Roots	8	$Y = 1.000\ X$	1.00	....
Roughage:				
Green	69	$Y = -32.66 + 1.356\ X$	0.85	2.5
Dry	70	$Y = -10.56 + 0.945\ X$	0.86	3.3
Silages	20	$Y = 3.07 + 0.753\ X$	0.74	3.0
Straws	14	$Y = -7.55 + 0.668\ X$	0.92	4.0
Combined	273	$Y = -32.15 + 1.353\ X$	0.96	5.4

[a] All significant at 1% level; r = sample correlation coefficient; s = standard error of the estimate.

The agreement of the ENE/TDN ratios of the feed classes in the two editions was close. Reported in the same order as in the 21st edition, they were: 1.000, 0.983, 0.859, 0.805, 0.758, and 0.501.

The errors of the estimate of the feed classes for the later edition were slightly lower than those of the earlier edition, except for green roughage and silage. It is the author's opinion that the equations of Table 2 are to be preferred, but the only reason for this choice is that they are based on data of a more recent edition.

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MAINTENANCE OF PREGNANCY IN OVARIECTOMIZED CATTLE WITH PROGESTIN COMPOUNDS AND THEIR EFFECT ON PROGESTIN LEVELS IN THE CORPUS LUTEUM [1, 2]

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SUMMARY

Bilateral ovariectomy was performed on 22 dairy animals at 55-143 days of pregnancy. Exogenous progesterone in the form of Delalutin (17-alpha-hydroxyprogesterone-17-n-caproate) and repositol progesterone (progesterone in propylene glycol) was administered at various dosage levels and time intervals to try to maintain pregnancy. Pregnancy was maintained following ovariectomy after 88 days with daily injections of 125 mg of Delalutin when the treatment was started at least eight days before surgery. Daily injections of 50 mg of repositol progesterone beginning 0-12 days before ovariectomy maintained pregnancy after the 66th day. Smaller daily injections of either repositol progesterone or Delalutin were more satisfactory in maintaining pregnancy than larger, less frequent injections. Neither repositol progesterone nor Delalutin maintained pregnancy when 500 mg were injected weekly. When repositol progesterone was withdrawn more than six days before parturition (expected at 279 days), the placentae were retained in all cases and were very difficult to remove 48 to 60 hr later. When repositol progesterone or Delalutin was withdrawn at 274-278 days, normal parturition occurred in two to six days.

Total progesterone and its concentration in the corpus luteum was significantly increased ($P < 0.005$) by administering progestins one to 12 days before ovariectomy. Δ^4-pregnene-20-β-ol-3-one was unaffected, being slightly higher in the untreated cows. There was also a consistent increase in progesterone concentration in the ovaries minus the corpus luteum for the treated cows, as compared with untreated cows ($P < 0.10$).

The corpus luteum (CL) containing principally progesterone is an essential structure in the orderly regulation of the sex cycle. Although the length of time the CL is essential for pregnancy varies between species, the requirement for progesterone or compounds with similar activity remains.

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Progesterone is predominantly a hormone of pregnancy and is required for the preparation of the endometrium for implantation of the ovum. It is necessary for the nutrition and protection of the embryo during the time it is lying free in the uterus. Progesterone causes growth of the uterine glands, changes the contractions of uterine muscle, and because of these effects and the necessity for maintaining the endometrium it is essential for the continuation of pregnancy.

The CL are essential throughout all or most of pregnancy and apparently constitute the principal source of progesterone in the rabbit (3), goat (4), and rat (13).

In the mare (9), monkey (10), guinea pig (14), and man (1) the CL are not essential after mid-pregnancy, since at that time the ovaries can be removed without interfering with

the continuance of pregnancy. These species presumably possess extra-ovarian sources of progestins.

In sheep, two out of six maintained pregnancy following ovariectomy at 54-77 days of pregnancy, as compared with two out of three when ovariectomy was delayed until 99-103 days of pregnancy (2).

In cattle, it is generally agreed that an active CL is required at least during the first 200 days of pregnancy (8, 12, 17, 18, 23). The results are, however, inconclusive and varied, depending on whether the CL or the entire ovary bearing it is removed. It also has been observed that progestin content of the CL is higher after 180 days, as compared with 90-180 days of pregnancy (26).

Measurable amounts of progestins have been found in adrenals and ovaries minus their CL of pregnant and nonpregnant cows (6, 27). Thus, other variable endogenous sources of progestins remain to assist in pregnancy maintenance even when pregnant cows are ovariectomized.

As earlier reviewed (5), exogenous progestins are associated with varied effects on the CL, depending on dosage levels, stages of the estrous cycle, and pregnancy when administration starts, and time for observation following progestin withdrawal. In general, exogenous progesterone appears to have a greater inhibiting effect on swine CL (25) than for sheep (28) or cattle (15, 20). A single dose of progesterone on Day 1 of the estrous cycle in cows significantly reduced CL weight and progesterone concentration, as observed on Day 14 (16). The size of the CL of pregnancy was decreased at 56 days, when a single dose of progesterone was given on the 35th, 42nd, or 49th days of pregnancy (15). In none of the experiments reviewed (5) was weight and progestin concentration of the CL determined immediately following varying periods of progestin administration.

The major purpose of this study was to determine the dosage level and frequency of progestin administration necessary to maintain pregnancy in cattle ovariectomized at 55-143 days of pregnancy. The excised ovaries and CL were weighed and assayed for progestin content to estimate the effects of presurgical administration of progestins.

MATERIALS AND METHODS

Nineteen 18-month-old 950-1,000-lb Holstein heifers, one 10-year-old 950-lb Jersey, one 5-year-old 1,700 lb Holstein, and one 3-year-old 1,400-lb Holstein were used in this investigation. The animals received the same grain and alfalfa hay ration as the regular dairy herd heifers. The investigation was conducted over a 30-month period. The progestins were administered by intramuscular injections into the thigh muscle. The progestins used were Delalutin (17-alpha-hydroxy-progesterone-17-n-caproate), referred to hereafter as Delalutin, and repositol progesterone (progesterone in propylene glycol), referred to hereafter as progesterone. In the Clauberg bioassay (rabbits), Delalutin is five times as active as progesterone (29).

Bilateral ovariectomy was performed on 55-143 days of pregnancy by entering the peritoneal cavity through an incision in the abdominal wall. Daily observations were made for abortions or discharges of a macerated fetus. Periodic rectal examinations of each fetus were made during the experiment. Dosage levels of the progestins and intervals of administration were varied, as outlined in Table 1.

Immediately following surgical removal, the CL was removed from the ovary bearing it and the tissues were separately frozen in solid CO_2 and stored at -25 C until assayed. Progesterone and Δ 4-pregnene-20 β-ol-3-one (20 β-ol) were separately determined in the CL and the combined ovaries of individual animals, according to the method of Stormshak et al. (27).

RESULTS AND DISCUSSION

Delalutin. Control animals were not used in this investigation, since others (12, 17, 18, 23) have conclusively shown that CL removal during early pregnancy is followed by abortion.

Table 1 shows that when Delalutin injections began on the day of ovariectomy, pregnancy was not maintained (Cows 1 and 2). Cow 2, ovariectomized on Day 94 of pregnancy, aborted in eight days. Cows 14 and 15 did maintain pregnancy when daily injections of 50 mg of progesterone were started immediately following surgery. These results suggest that availability of Delalutin is delayed either from slow absorption or slow metabolism to a progestationally active compound as compared with progesterone.

When Delalutin therapy was started eight to 12 days before surgery (Cows 3-8), pregnancy was successfully maintained in five of six cases. Cow 6, pregnant 76 days at ovariectomy, aborted in 20 days. The five other cows were pregnant from 89 to 143 days at ovariectomy. This suggests that 125 mg of Delalutin daily may be borderline at the 76th day stage of pregnancy. In contrast, Cows 11, 13, and 14, in similar stages of pregnancy at ovariectomy,

TABLE 1

Effect of exogenous progestins on maintenance of pregnancy in ovariectomized cattle

Cow no.	Treatment level	Days of gestation: Ovariec-tomized	Injection began	Last injection	Parturition
1	125 mg daily (D)	60	60	103	Resorption discharges 103
2	125 mg daily (D)	94	94	102	Aborted 102
3	125 mg daily (D)	143	131	274	Live calf 280
4	125 mg daily (D)	89	80	273	Live calf 276
5	125 mg daily (D)	110	102	278	Live calf 288 R. P.
6	125 mg daily (D)	76	68	96	Aborted 96
7	125 mg daily (D)	89	78	273	Live calf 275
8	125 mg daily (D)	96	85	275	Live calf 279
9	75 mg daily (P)	89	84	277	Live calf 280
10	50 mg daily (P)	82	76	274	Live calf 277
11	50 mg daily (P)	77	71	272	Live calf 278 R. P.
12	50 mg daily (P)	84	81	258	Live calf 265 R. P.
13	50 mg daily (P)	67	61	275	Live calf 277
14	50 mg daily (P)	78	78	252	Live calf 265 R. P.
15	50 mg daily (P) until 103 then 125 mg daily (D) until 253, then 125 mg (D) on 256, 260	92	92	260	Live calf 273 R. P.
16	250 mg twice weekly (D)	62	57	64	Aborted 65
17	250 mg every other day (D)	75	73	111	Resorption discharges 110
18	125 mg daily until 99, then 500 mg weekly (D)	92	85	116	Aborted 116
19	125 mg daily until 91, then 250 mg twice weekly (D)	91	84	123	Aborted 123
20	50 mg daily until 58, then 100 mg 60, 62, 150 mg 64, 66 250 mg 70, 73 500 mg 80 and weekly (P)	55	49	108	Aborted 109
21	50 mg daily until 69, then 150 mg twice weekly (P)	62	51	106	Aborted 109
22	50 mg daily until 94, then 150 mg twice weekly until 273	87	76	273	Live calf 279 R. P.

D = Delalutin; P = Progesterone; R. P. = Retained placenta.

maintained pregnancy from daily injections of 50 mg of progesterone.

Progesterone. In the next phase of the investigation, repositol progesterone was used as the source of exogenous progesterone. The results are shown in Table 1.

Cow 9, given daily injections of 75 mg progesterone beginning five days before surgery on the 84th day of pregnancy, carried to term. Pregnancy was successfully maintained in four cows (No. 10-13) 67-84 days pregnant with daily injections of 50 mg of progesterone beginning three to six days before surgery. Cows 14 and 15, 78 to 92 days pregnant, were successfully carried to term with daily injections of 50 mg of progesterone, beginning on day of surgery. These results would indicate that progesterone becomes available soon after administration.

Frequency of therapy. Since pregnancy could be maintained with daily injections of either Delalutin or progesterone, the next phase of the investigation was to use larger doses, given less frequently.

Pregnancy was not maintained in four cows (No. 16-19, Table 1) on either 250-mg injections of Delalutin every other day or 500 mg injected weekly, even though therapy was started two to seven days before surgery.

Cow 20, ovariectomized on Day 55 of preg-

nancy, received daily injections of 50 mg of progesterone from the 49th to 62nd day, then gradually changed to 500 mg weekly on Day 80, aborted on the 109th day of pregnancy.

Pregnancy was maintained in Cow 22 with 150 mg of progesterone twice weekly after the 94th day, whereas pregnancy was not maintained in Cow 21 with this level beginning on Day 69. These results indicate that less progesterone is required to maintain pregnancy at 94 days than at 69 days. Stormshak and Erb (26) had earlier observed that the CL of pregnancy in the cow contained significantly less progestins from 90-180 days than during the first and last third of pregnancy. This suggests that the minimal requirement for progesterone varies during pregnancy.

Incidence of retained placenta. When progesterone was withdrawn more than six days before parturition (expected at 279 days), the placenta was retained in all cases and was very difficult to remove 48 to 60 hr later. McDonald et al. (18) also reported retained placentae in seven of eight cases in which the cows had been subjected to CL removal at approximately the 60th day of pregnancy and had received exogenous progesterone up to 25-110 days before parturition.

Influence of exogenous progestins on corpus luteum size and content of progestins. Ovaries and the CL from 20 of the Holsteins shown in Table 1 were analyzed for progesterone and 20 β-ol. Eighteen were heifers and two were cows (3 and 5 yr of age), pregnant 55-96 days at ovariectomy.

Twenty untreated cows pregnant 58-107 days at time of slaughter (17 cows) or ovariectomy (three cows) were used to make statistical comparisons. Six were heifers and the remaining cows were under 6 yr of age. Four dairy breeds were involved, but this should not cause bias, since Stormshak and Erb (27) found no significant difference between breeds relative to CL size or progestin content of pregnant cows. Evidence is scanty, but age of cow does not appear to be a factor, although values for some cows over 10 yr are higher than the population range of younger cows (5).

Delalutin-treated cows showed increased levels of progesterone and 20 β-ol in the CL, as compared with those treated with repositol progesterone (Table 2), but the differences were not significant ($P < 0.25$).

Two factors, namely, variable stage of pregnancy and variable treatment periods, could bias the analysis. However, in this study stage of pregnancy (55-96) days was not related to weight or progestin content of the CL as determined by a multiple regression analysis ($r = -0.12$ between weight of the CL and stage of pregnancy within treatments). The relationship between stage of pregnancy and prog-

TABLE 2

Influence of administering Delalutin or repositol progesterone on the average progestins in the corpus luteum and ovary of the pregnant cow

	Corpus luteum							Ovary	
		Total			Concentration				Concentration progesterone
Measurement	Wt	Prog.	20 β-ol	Total	Prog.	20 β-ol	Total	Wt	
	(g)	*(μg)*			*(μg/g)*			*(g)*	*(μg/g)*
125 mg Delalutin one to 12 days before ovariectomy (ten cows)									
Average	6.2	230[a]	64[a]	294[a]	38.5	10.3	48.8	15.0[a]	2.6
50 mg repositol progesterone one to 12 days before ovariectomy (ten cows)									
Average	5.8	187	46	233	33.1	8.3	41.3	11.8	3.8[a]
Total treatment (20 cows)									
Average	6.0	208[b]	55	263[b]	35.7[b]	9.3	45.0[b]	13.4	3.2[d]
Standard error	0.3	18	7	20	3.3	1.0	3.8	1.0	0.5
Untreated cows—17 sampled at slaughter and three by ovariectomy (20 cows)									
Average	5.5	91	61	152	16.6	11.2	27.8	15.9[c]	1.8[c]
Standard error	0.5	17	10	24	3.1	2.0	4.1	1.1	0.2

[a] Exceeds other treatment mean at $P > 0.10 < 0.25$.

[b] Treatment mean exceeds untreated mean at $P < 0.005$.

[c] Data for ten cows.

[d] Treatment mean exceeds untreated mean at $P < 0.10$.

estins in the CL was even less. Stage of pregnancy did not have a significant effect on the CL weight or progestin content in the 20 untreated cows ($r = -0.30$).

The correlation between days treated and CL weight was significant ($r = 0.46$, 18 d.f.). The regression was 0.19 g/day and its standard error was 0.09 g/day. The correlation was unaltered by removing the effects of stage of pregnancy. Thus, there were no biasing effects due to stage of pregnancy by treatments. Therefore, stage of pregnancy was not considered further in the analysis.

Delalutin and repositol progesterone were associated with nearly identical average effects on CL weight over the treatment period of one to 12 days. Length of treatment was not significantly correlated with the concentration of progesterone or 20 β-ol in the CL. For every such comparison, the average linear regression coefficient was less than its standard error. Since time of treatment had no significant effect on progestin levels in the CL, this variable was not included in the statistical comparison of treated and untreated cows (Table 2).

Total progesterone and its concentration in the CL was significantly increased ($P < 0.005$) by administering progestins one to 12 days before ovariectomy; 20 β-ol was unaffected, being slightly higher in the untreated cows. There was also a consistent increase in progesterone concentration in the ovaries minus the CL for treated cows, as compared with untreated cows ($P < 0.10$).

Compared with untreated cows, total progestin and concentration per gram of CL was more than doubled by treatment. An average concentration of 45.0 μg/g for the CL of treated cows approximates levels found only in the 14-16 day CL [44 μg/g Loy et al. (16); 35 μg/g Foote et al. (7); and 40 μg/g—Erb and Stormshak (6)].

Loy et al. (16) have shown that if progesterone is administered as a single dose on Day 1 or 5 after estrus the CL is reduced in size and progestin content at Day 14 (16).

General discussion. Zimbelman et al. (29) have observed that exogenous progesterone does not cause regression or impair apparent function of the CL in pregnant ewes. In contrast, swine CL are rather easily damaged by progesterone, and embryonic mortality is high when dose levels exceed 80 mg/day (22, 25). The results of others cannot be compared with the present study, since intervals of time after last administration were longer. The quantitative effect (more progestin and larger CL), as it relates to estrous synchronization studies, appears to have considerable importance. The significant increase of progesterone in the CL during presurgical therapy suggests that exogenous sources tend to accumulate in the CL or create higher blood levels which slow down the release of the endogenous supply from the CL.

Reifenstein (21) found Delalutin to have long progestational effects in women. He reported the fetal salvage rate rose from 17.1 to 70.7% with Delalutin and from 13.8 to 70.0% with free progesterone. The improvement was accomplished with an average of one 250 to 375 mg injection per week with Delalutin, in contrast to an average of five injections of 100 mg per week with free progesterone.

The slow response of Delalutin for pregnancy maintenance as compared with progesterone is puzzling, since progesterone level in the CL was higher ($P < 0.25$) when Delalutin was administered. This increase occurred even when Delalutin was given less than two days before CL removal. This suggests that absorption is not this slow but, rather, that metabolism to a progestationally active material is slowed. The greater requirement for Delalutin (125 mg progesterone equivalent) vs. 50 mg for progesterone suggests that either more progesterone is destroyed in the metabolism of the Delalutin compound or that a less active compound than progesterone results. This area needs more careful study.

The necessity of progesterone or progesterone-like substances for the maintenance of pregnancy during the first half of gestation is substantiated by early termination of pregnancy following the removal of the ovaries when insufficient exogenous progesterone is administered. The fetus apparently dies within hours when the level of progesterone is inadequate.

It appears that pregnancy can be maintained with daily injections of 125 mg of Delalutin in 1,000-lb heifers (0.125 mg per pound body weight), if the treatment is started at least six days before ovariectomy in animals 89 to 143 days pregnant. Pregnancy cannot be maintained when treatment begins on day of ovariectomy. Apparently, the absorption rate of Delalutin is not fast enough or metabolism of the ester is too slow to prevent the progesterone from dropping below the critical level. Daily injections of 50 mg (0.05 mg per pound body weight) of progesterone, beginning on day of ovariectomy, maintained pregnancy in animals starting at 67 to 92 days of pregnancy.

Hawk et al. (11) maintained pregnancy in five of six dairy animals ovariectomized five,

six, or seven days after breeding with daily injections of 25 mg of progesterone and 6.25 μg of estrone per 100 lb of body weight, beginning on the day prior to surgery.

Smaller daily injections of either progesterone or Delalutin are more satisfactory in maintaining pregnancy than larger, less frequent injections. Neither progesterone or Delalutin maintained pregnancy when 500 mg was given weekly; 250 mg twice weekly appears to be a threshold level as to dosage and time interval for progesterone to maintain pregnancy. Pregnancy was not maintained with 250-mg injections of Delalutin twice weekly.

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TECHNICAL NOTE

EFFECT OF STAGE OF LACTATION ON ACCURACY OF ESTIMATING MILK PROTEIN USING ORANGE G DYE BINDING[1]

Dye binding is used for estimating the protein in milk of individual cows. Generally, milk is sampled at monthly intervals, beginning as early as the seventh day following calving. In this regard, two questions arise relative to accuracy of estimation of the protein. Can the dye-binding method of Ashworth et al. (1) be used to estimate protein in milk samples early in lactation? Is milking-to-milking variation in protein following calving so erratic that yield of protein during the first test period following calving cannot be reliably estimated? Some of the data of Ashworth et al. (1) were re-examined for possible stage of lactation effects on the accuracy of determining protein using dye binding.

It is known that per cent fat in milk from an individual milking of a cow may vary considerably throughout lactation (3). This variation is most extreme early in lactation (4). Garrett and Overman (4), summarizing the results of 25 investigators, observed that milk fat varied less (0.15-9.55%) than protein (4.80-27.35%) in colostrum at calving. By the fourth milking, protein varied less (4.27-6.52%) than milk fat (1.70-6.06%). Additional work (4) involving one Holstein and Ayrshire cow showed that protein declined very rapidly during the first 24 hr (four milkings) following calving. Though Larson and Kendall (5) did not report averages for seven cows, it appears from graph interpolation that the first milking colostrum averaged 11% protein and declined to an average of 5% during the next 24 hr.

In the present study, three Jerseys and nine Holsteins calving from November, 1959, to February, 1960, were used. Colostrum was taken at the first milking after calving and at 12-hr intervals for the next 19 milkings. Total protein was estimated by dye binding as described earlier (1). Macro-Kjeldahl determinations were made on milkings 1-4, using two Jerseys and three Holsteins (20 determinations). An additional ten determinations were made from these same cows, using scattered milkings from 4-11. Protein was determined for the remaining samples, using only the dye-binding method. In addition, 176 milk samples taken at known stages of lactation were evaluated for possible effects of stage of lactation on accuracy of the dye-binding test. These latter data were a part of the total data in an earlier report (1).

Protein determined by dye binding averaged 0.23% less than the Kjeldahl method for the 30 comparisons involving milkings 1-11. Protein content was 0.54% lower (range −0.19 to −1.13%) using dye binding for five colostrum samples taken at calving and 0.17% lower (range +0.23 to −0.69) for the remaining 25 comparisons. The total correlation between the dye binding and Kjeldahl methods was 0.997. The regression coefficient was 1.029% and its standard error was 0.015%, using the Kjeldahl method as the dependent variable. Though dye-binding results were lower, particularly for initial colostrum, a relatively constant linear relationship exists between protein determined over a range of 3.65-18.63% by the dye-binding and Kjeldahl methods.

Dolby (2) found that Orange G and Amido black dyes reacted with protein in the same molar ratio, though the latter was the more sensitive optical indicator. Using Amido black, the dye-binding capacity of the milk protein was constant from three or four days after calving until the last month of lactation (2).

TABLE 1

Average protein in colostrum and milk from early lactation of three Jerseys and six Holsteins using the dye-binding method

Milking	Protein		
	Jerseys	Holsteins	S.D.[a]
(no.)		*(%)*	
1	11.21	14.63	2.20
2	7.63	9.10	2.60
3	5.25	5.84	1.06
4	4.46	5.12	0.89
5-6	4.32	4.21	0.56
7-8	4.22	3.96	0.48
9-10	4.10	3.90	0.44
11-12	4.04	3.82	0.34
13-14	3.84	3.89	0.54
15-16	4.04	3.65	0.24
17-18	3.98	3.47	0.15
19-20	3.96	3.38	0.20
13-20	3.95	3.60	0.35[b]
305-day lactation	3.63	3.13	0.18

[a] Standard deviation using milking number or lactation within breed of cow.

[b] Standard deviation within cow and milking number was 0.32%.

Table 1 shows that protein declines very rapidly during the first two days (four milkings), which is in good agreement with earlier work (4, 5). Milking 13, or the beginning of the seventh day after calving, is the earliest a cow can be sampled under recognized testing programs. For Milkings 13 to 20 the standard deviation for protein was 0.32% for within

[1] Scientific paper No. 2199, Washington Agricultural Experiment Stations, Pullman. Project 1378.

cow and milking number. The average per cent protein between Milkings 13 to 20 was not significantly different, but the averages for individual cows were ($P < 0.01$). Using 95% confidence limits, it was estimated that the random milking-to-milking variation exceeds 0.64% and a two-milking composite sample exceeds 0.45% protein one time in 20. As judged from the random variations in per cent fat (3), this amount of variation is not excessive.

Table 2 shows that dye binding per cent protein was −0.034% from five samples taken seven to 22 days in lactation, as compared with −0.17% for 25 comparisons from Milkings 2-11. From 46-225 days, dye binding per cent protein was from 0.022 to 0.033% higher than the corresponding Kjeldahl values. The differences were on an average less in late lactation, but there was a slight increase in variability. Using Amido black, Dolby (2) observed that dye binding was 0.025% higher in late lactation milk. The cows involved were sampled during a single month and were in lactation eight to nine months (2). The milk samples shown in Table 2 were taken during January to September and the stage of lactation was essentially random during each of these months.

TABLE 2

Influence of stage of lactation on relative accuracy of dye binding–original data from Ashworth et al. (1)

Stage of lactation	Comparisons	Average difference dye binding—Kjeldahl	Standard deviation of the differences
(days)	*(no.)*	*(%)*	
7- 22	5	−.034	.107
23- 45	8	.008	.082
46-105	23	.024	.083
106-165	27	.033	.087
166-225	30	.022	.110
226-285	45	.005	.112
286-345	33	.018	.115
Over 345	5	−.024	.125
Total	176	.015	.103

As compared with macro-Kjeldahl, protein determined by dye binding (Orange G) average 0.54% low in first-milking colostrum. For Milkings 2 to 11, dye binding averaged 0.17% lower. At consecutive periods of lactation starting with the seventh day, average deviations ranged from −0.034 to 0.033%. Random variation in two-milking composites taken between 13 and 20 milkings after calving exceeded 0.45% protein one time in 20 observations. From this study it is concluded: (a) That dye binding can be used to estimate per cent protein in milk sampled early in lactation; (b) that testing as early as the seventh day after calving is practical when protein is being estimated during the lactation period; and (c) that stage of lactation has no practical effect on accuracy of per cent protein determined by dye binding (Orange G).

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OUR INDUSTRY TODAY

HOW THE DAIRY INDUSTRY CAN PROFITABLY USE THE SERVICES OF THE DAIRY TECHNOLOGY SPECIALIST[1]

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A glance at the contents listed on the cover of recent issues of the JOURNAL OF DAIRY SCIENCE staggers the imagination of the man in industry. The range, scope, depth, complexity, and sheer volume of research being done and reported is such that it is beyond the capabilities and time of the man involved in processing and distribution.

Even abstracts and summaries cover too wide a field to be read. When the man in industry seeks knowledge on a certain problem he can look it up, of course. But the real problem to my mind is this—How can he become acquainted with new discoveries which could revolutionize his business, plant, product, or methods of distribution?

From our Industry journals I learned of what's being done in Dairy Science and the Dairy Industry Newsletter by Olivia Nicol and DISA bulletins. But all these reach journalists—not the harried, hurried milk plant operator. In addition to communicating to the man in industry, the results of this wide field of research in terms readily understood by the layman, perhaps a reverse system which would acquaint the dairy scientist with industry's most pressing problems would stimulate investigation and research in those fields.

One of the biggest problems facing today's milk distributor is marketing and distribution. At the Milk Industry Foundation Convention in November, 1960, the president of a supermarket firm said that when the supermarket could process and distribute its own milk more efficiently than is being done by the dairy industry, the supermarket would operate its own milk plants. He was not too complimentary regarding the manner in which the industry is performing today. Over the nation there are already several supermarket-owned milk plants, or captive creameries, as they are called on the West Coast.

Retail milk delivery to the home is, in the opinion of many dairy leaders, doomed. Yet surveys show that milk consumption is higher when milk is delivered to the home. One survey indicated 10 qt per week consumed by the average family against 6 qt when bought at the store. This trend to out-of-store milk distribution will cut consumption.

The research and interest in sterile milk is important. However, the economics of sterile milk, particularly when concentrated, depends on the raw milk being bought at Class II or III (manufacturing milk) prices. This same price advantage given to fresh whole milk would save the consumer the same amount and would make the new process unnecessary. The more producer milk sold in Class I, the higher the farm dairy income. Is it wise to encourage the sale of manufactured milk products to compete with Class I?

An astute friend told me not long ago that, in any crowd, he can pick out the dairymen. "How do you do it?' I inquired.

"I can tell by the gleam in their eyes," he replied.

"Really? Explain."

"The gleam is caused by the reflection of light shining through the holes in their heads."

This facetiously points up the greatest problem in our industry today—break-down of our human relationships within the industry—lack of confidence in competitors. And it has resulted in price wars which have cost the industry millions of dollars, and the end is not yet. Can the dairy technologist help us solve this problem? Should the dairy curricula be broadened to include whatever courses might help solve this human friction? Should students be encouraged to join clubs, fraternities, living units which condition them for bumping elbows? The National Cotton Council has for 20 yr maintained its own permanent market research staff, with its efforts about equally divided between research and promotion. This market research staff guides technical research into areas where it is needed, and follows work under way.

Perhaps a committee or board made up of dairy scientists, operators of the various dairy product plants, and writers could review research reports as made available by the present abstract and reporting agencies, and discuss them. Then this scientific information must be translated into lay language. It must be boiled down to its essentials. Then its practical application must be recognized and this information communicated to the people who need it and can apply it to practical use in daily plant operation. Then the flow should be reversed and the problems and interests of the industry communicated to the scientist. To summarize it in a word—a much overworked word—what we need is better communication.

At the Symposium on Basic Research of the American Association for the Advancement of Science in 1959, Dr. W. O. Baker said, "Man's curiosity and the satisfaction he gets out of exercising it according to his own bent, can be coupled with the needs and ambitions of the human race." This should be our guide and goal.

[1] Presented to Dairy Manufacturing Extension Section, A.D.S.A., Madison, Wisconsin, June 13, 1961.

ASSOCIATION AFFAIRS

AMERICAN DAIRY SCIENCE ASSOCIATION REVIEW[1]

E. L. JACK, President, A.D.S.A.
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The Executive Board meeting at Beltsville, Maryland, on February 26, 1962, on recommendation of the Journal Management Committee, directed that the A.D.S.A. Review be discontinued.

This action was based on:

1. Cost of publication in the face of an Association financial deficit, increasing publication costs, and the financial inadvisability of duplication of summaries.
2. Summaries in the JOURNAL are, or should be, so similar that duplicate publication seems unjustifiable regardless of finances.
3. Unfavorable outlook for a separate popular journal in the near future makes the A.D.S.A. Review unnecessary as a preliminary step in this direction.
4. Lack of any expressed enthusiasm among the membership for the Review. The sentiment, in fact, is in opposition.

This action means that the Editor-in-Chief and the Secretary-Treasurer in the future will not send to trade journals and to industry organizations popularized versions of research papers published in the JOURNAL OF DAIRY SCIENCE.

It was agreed that many summaries and research papers could be improved by the authors properly informing the readers about the objectives of the work. A sentence or two at the beginning of the summary and the text of the paper, showing the general importance of the field of research, the significance of the specific work, and the objectives sought will make the paper meaningful without unduly burdening the writer. The attention of authors and reviewers to these points is requested.

[1] For the origin of this publication, refer to J. Dairy Sci., 43: 1538. 1960.

ABSTRACTS OF PAPERS TO BE PRESENTED AT THE FIFTY-SEVENTH ANNUAL MEETING

of the

AMERICAN DAIRY SCIENCE ASSOCIATION

University of Maryland
College Park
June 17–21, 1962

MANUFACTURING SECTION

* Designates author who presented paper.

M1. Study of the mechanism of sunlight flavor development in milk. J. A. SINGLETON,* L. W. AURAND, AND F. W. LANCASTER, North Carolina State College, Raleigh.

Riboflavin has been implicated as one of the major reactants in the development of sunlight flavor in milk. The electron acceptor and donor properties of riboflavin have been well established, as well as its ability to react with amino acids and other nitrogenous compounds. Samples of milk were exposed both to solar radiation and to approximately monochromatic light of successively variable wave lengths for different time intervals (0, 1, 5, and 10 hr). Quantitative determinations of riboflavin and tryptophane were made on all samples of milk. Flavor scores were obtained on samples of milk subjected to monochromatic light of several wave lengths (424, 450, and 479 mμ). Other factors considered in this study were oxygen tension and inhibitors of autoxidation. Results of the study were as follows: (a) A direct relationship existed between riboflavin destruction and the intensity of the off-flavor; (b) a direct relationship existed between loss of tryptophane and the intensity of off-flavor development; (c) maximum flavor development occurred at 450 mμ; (d) oxygen was necessary for flavor development; and (e) chain stoppers, pyrogallol and 2,4-dinitrophenol, were effective inhibitors of flavor development, indicating a free radical mechanism was involved.

M2. Dihydroquercetin as an inhibitor of spontaneous and copper-induced oxidation in fluid and dry milk products. T. S. RAJAN* AND G. A. RICHARDSON, Oregon State University, Corvallis.

Dihydroquercetin was found to be a suitable and effective antioxidant for milk. Spontaneous milk was characterized by the slow oxidation of its reduced ascorbic acid and a corresponding rate of increase of dehydroascorbic acid. The inhibition of spontaneous oxidation by dihydroquercetin involved a rapid oxidation of reduced ascorbic acid to dehydroascorbic acid. Copper-sensitive milk contaminated with copper was characterized by a rapid rise in Eh and oxidation of its reduced ascorbic acid. Dihydroquercetin did not alter the changes in Eh and ascorbic acid, but inhibited oxidation. The effectiveness of dihydroquercetin was not impaired during spray-drying of milk and buttermilk as detected organoleptically and by the TBA test.

Amperometric titration of copper with dihydroquercetin in acetate-buffer indicated a molar ratio of the dihydroquercetin-copper complex of 2:1, whereas more copper was complexed when dihydroquercetin was dissolved in milk instead of buffer. Whey proteins possessed an affinity to copper 8.32 times that of casein. It is postulated that although dihydroquercetin is capable of complexing copper, its role as an antioxidant in copper-contaminated milk is that of a free radical acceptor.

M3. Influence of linoleic acid content of milk lipids on susceptibility of milk to oxidized flavor. L. M. SMITH, W. L. DUNKLEY,* AND M. RONNING, University of California, Davis.

The concentration of linoleic acid in milk lipids was increased by infusing a cow with a cottonseed oil emulsion and the change in oxidative stability of the milk and milk fat was determined. Infusing 150 g of cottonseed oil (as Lipomul I.V.) tripled the linoleic acid content of the milk triglycerides in the first milk following the infusion. A smaller increase in linoleic acid was found in the milk phospholipids, but the increase was not obtained until the second milking after the in-

fusion. Little change was noted in the concentration of other fatty acids. The oxidative stability of the milk fat decreased after the infusion and the change in stability appeared to be related to the increase in linoleic acid content. In contrast, an increase in susceptibility of the milk to copper-induced oxidized flavor, as measured by the thiobarbituric acid test, appeared to be dependent on changes in the linoleic acid content of the phospholipids.

M4. Xanthine oxidase in milk and factors affecting its activity. R. R. PEREIRA,* T. KRISTOFFERSEN, AND W. J. HARPER, The Ohio State University, Columbus.

Xanthine oxidase activity in milk was related to the source and various treatments of the milk. Enzyme activity was determined according to the triphenyl tetrazolium chloride method of Zittle (J. Dairy Sci., 1956), with a xanthine concentration of 2.94×10^{-5} moles per liter. The results are expressed as units of xanthine oxidase per milliliter of product.

Milk from morning and evening milkings yielded essentially the same results, with a morning range of 33-121, average 62, and evening range 38-100, average 61. The activity of herd milks collected on an every-other-day basis ranged from 26 to 58.

Incubation of fresh individual cow's milk at 40 to 95 F for 0 to 48 hr, resulted in an early decrease in enzyme activity, followed by an increase to approximately the original level.

Xanthine oxidase activity in various products was: milk—37; cream (gravity separated, range 61% fat)—232; skimmilk—20; rennet whey—24. Minimum pasteurization treatments of the milk did not affect the enzyme activity. However, heating to 170 F for 18 sec resulted in a 27% decrease, and this heat treatment plus homogenization at 2,000 lb/square inch, caused a 45% decrease.

M5. Observations on the development of oxidized flavor in multi-vitamin mineral milk. R. A. SCANLAN* AND W. F. SHIPE, Cornell University, Ithaca, New York.

Flavor studies were conducted to determine effects of processing on the flavor of multi-vitamin mineral (MVM) milk. MVM milk processed by high-temperature short-time (HTST) pasteurizer and homogenizer units developed no off-flavor. However, when MVM milk was subjected to steam-vacuum treatment (200 F) in addition to the HTST and homogenization treatments, an oxidized flavor developed. A double-chamber Cherry-Burrell Aro-Vac unit was used for the steam-vacuum treatment. Oxidized flavor developed, regardless of whether the MVM preparation was added before or after processing. This indicates that alteration of the milk was involved in the development of the off-flavor, rather than alteration of the MVM preparation. Special batches of MVM preparations were made, each preparation lacking one ingredient. Addition of these preparations to milk subjected to the steam-vacuum treatment indicated that the iron compound was responsible for development of the oxidized flavor. The type of the iron compound seems to be critical. Recently, it was observed that MVM milk subjected to a steam-vacuum treatment in another plant, using a double-chamber vacuum unit, did not develop the oxidized flavor.

M6. Purification and characterization of milk lipase. R. C. CHANDAN* AND K. M. SHAHANI, University of Nebraska, Lincoln.

Since milk lipase is known to be associated with casein, a concentrated source of the enzyme-casein complex from slime was used as the starting material. Fat and moisture from the source were removed by preparing acetone powders. Lipase was extracted with water and precipitated with ammonium sulfate (50% saturation). Further purification was effected by fractional precipitation by varying the saturation of ammonium sulfate, treatment with acetone at −10 C, and Sephadex filtration. Lipase was assayed by silicic acid chromatography and potentiometrically, using milk fat as the substrate. Protein estimation was carried out by Lowry's method and by ultraviolet absorption. Stepwise purified fractions examined by ultracentrifugal and electrophoretic studies indicated most pure lipase fraction to be a low molecular weight and homogeneous protein. Starch gel electrophoresis in Tris-EDTA, pH 9.0 buffer, gave only one band. Specific activity of the lipase fraction gave an over-all purification of 88, 250, and 2,600-fold on the protein, milk solids, and milk basis, respectively.

The purified enzyme showed little activity against esters, indicating it to be a true lipase. Also, the enzyme exhibited a single pH optimum of around 9.0.

M7. Relationships between certain short-chain fatty acids and rancid flavors in milk. C. W. KOLAR, JR.* AND J. B. MICKLE, Oklahoma State University, Stillwater.

The objectives were to determine what relationships might exist between the amounts of water-soluble, free fatty acids present in milk and the rancid flavors of that milk. Rancid flavors were developed in fresh milk by mixing raw and homogenized-pasteurized herd milk, by using a temperature-activation treatment on herd and individual cow's milk, and by cooling individual cow's milk. Free fatty acids were extracted from the milk samples after 0, 24, 48, and 72 hr of storage by an ether extraction procedure, and were analyzed using column chromatography. The chromatographic results were compared to storage times, organoleptic scores, and acid degree values.

No consistent patterns were observed when comparing the amounts of C_1, C_2, or C_3 acids to any of the other variables measured in this study. The results from individual cow's milk indicated that the water-soluble C_1-C_8 acids, measured as a group, increased with time. In two experiments these increases were related to similar increases in acid degree values [$r = 0.95$ and 0.94 ($P < 0.01$)] and flavor scores [$r = 0.74$ and 0.53 ($P < 0.05$)]. Similar correlation coefficients calculated for the data obtained from herd milk were not significant ($P > 0.05$).

M8. Factors affecting distribution of fatty acids hydrolyzed from milk fat by milk lipase. J. A. ROBERTSON* AND W. J. HARPER, The Ohio State University, Columbus.

The effect of substrate concentration, pH, and selected inhibitors on the relative concentration of individual fatty acids hydrolyzed from milk fat by milk lipase was investigated. The free fatty acids were obtained by silica gel extraction, concentrated as sodium salts, and prepared as methyl esters with diazomethane. The esters (C-6 to C-18) were gas-chromatographed, using an Apiezon-L column (220 C) with a thermal conductivity detector.

When lipase activity was determined at pH 8.6 with 4.4% (fat) substrate, the concentration of fatty acids (area per cent) released by hydrolysis was: C-6, 5.4%; C-8, 2.64%; C-10, 4.11%; C-12, 3.88%; C-14, 10.88%; C-16, 31.3%, C-18[1=], 18.14%; C-18, 8.5%; and unidentified peaks, 15.13%. Different distribution of free fatty acids hydrolyzed from milk fat was obtained at pH 7.0 and at pH 8.6. Decreased substrate concentration at pH 7.0 or 8.6 produced an increased area per cent of C-6, C-8, C-10, and C-12. Ferric chloride, a competitive inhibitor, increased the longer chain length unidentified acids resulting from hydrolysis at pH 8.6 in proportion to C-6, C-8, and C-10 acids. Diisopropyl fluorophosphate, a noncompetitive inhibitor, had an insignificant effect on free fatty acid distribution.

M9. Lipolysis of glyceryl 1-oleate 2,3-dicaproate by a B-esterase concentrate from milk. R. G. JENSEN* AND J. SAMPUGNA, University of Connecticut, Storrs, and T. L. FORSTER, Washington State University, Pullman.

Glyceryl 1-oleate 2,3-dicaproate was synthesized and subjected to the action of a B-esterase concentrate prepared from bovine milk, to determine if B-esterase preferentially released short-chain fatty acids from triglycerides. Free fatty acids (FFA) and mono- (MG) and diglycerides (DG) were obtained with the aid of an ion exchange resin and thin-layer chromatography. The fatty acids were identified by gas-liquid chromatography. The following concentrations of oleic and caproic acids ($M\%$) were found; FFA—46.9, 53.1; MG—9.5, 90.5; DG—25.7, 74.3, and triglyceride (TG)—34.0, 66.0. If short-chain specificity did not exist, theoretical quantities of oleate and caproate should have been: FFA—50, 50; MG—0, 100; DG—25, 75; TG—33, 67. Therefore, in this system there was no short-chain specificity.

M10. Lipase activity of fractionated casein extracts. P. GAFFNEY,* W. J. HARPER, AND I. A. GOULD, The Ohio State University, Columbus.

An investigation was conducted of the distribution of lipase activity in various fractions of casein extracts. Protein concentrations were determined by the Phenol-Biuret method and lipase activity was assayed at pH 8.6.

Rennet-coagulated casein was extracted with an equal weight of water or a water-lysozyme (20-2,000 μg/ml) solution by high-speed mixing for 5 min at 2 C, followed by centrifugation. The lipase specific activity of the water extract was seven to ten times that of the original skimmilk, whereas that of the water-lysozme extract was 11-13 times greater.

Three fractions of casein water extract obtained by elution at pH 7.0 from Sephadex 25 columns (gel filtration) exhibited slight increases of specific activity of lipase in the first and second fractions. With DEAE cellulose (ion exchange), an eight-step sodium chloride (0-1 M) gradient at pH 7.0 resulted in separation of eight protein fractions. Fractions 2-3 and 4 contained about 80% of the total protein, with a lipase specific activity of less than one. Fractions 5, 6, 7, and 8 all displayed higher specific activity than the starting material, and the specific activity of Fraction 6 was 80- to 140-fold greater.

M11. Consumer preferences of creamed Cottage cheese with different sugar and salt levels. G. W. GROSS, E. J. FINNEGAN,* AND J. J. SHEURING, University of Georgia, Athens.

Creamed Cottage cheese containing five levels of sucrose (0.0, 0.5, 1.0, 1.5, and 2.0%) and creamed Cottage cheese with 1.0% sucrose and different levels of salt (0.50, 0.75, 1.00, 1.25, and 1.50%) were evaluated by two consumer surveys, consisting of 925 and 862 individuals, respectively, employing the Dykstra method of paired comparisons. Analyses were made of treatment ratings for flavor and texture and of pairwise preferences for flavor and texture, excluding no-preference responses. In the first survey of five sugar levels in creamed Cottage cheese, the 1.0% sugar additive was preferred ($P < .05$) by the consumer over all other samples for flavor and texture. In the second survey, of five salt levels in creamed Cottage cheese with 1.0% sugar added, the 1.25% salt additive was preferred ($P < .05$) by the consumer.

M12. Effects of gelatin level and homogenization temperatures on the viscosity and thixotropic properties of dressing for Cottage cheese. N. F. OLSON* AND W. V. PRICE, University of Wisconsin, Madison.

Viscosity and thixotropic properties of 12.5% dressing were determined by a Brookfield viscometer. A control and samples containing 0.15, 0.30, and 0.45% gelatin (250 Bloom) were pasteurized at 160 F for 30 min and homogenized at 3,500 psi at 141, 121, and 92 F (temperature from homogenizer).

Homogenization at 92 F immediately caused marked increases in viscosity of samples without gelatin, lesser increases in samples with 0.45%, and only slight increases in samples with 0.15 and 0.30% gelatin; this was associated with differences in fat clumping. Storage for 48 hr at 40 F caused viscosity increases in all samples except in the control homogenized at 121 or 141 F and in samples containing 0.15% gelatin. The viscosity varied directly with increased amounts of gelatin above 0.15%. Agitation after 48 hr of storage decreased viscosity; samples with the highest viscosity showed greatest decreases. Samples varied in ability to regain this lost viscosity during subsequent storage; the control homogenized at 92 F and samples with 0.3% gelatin showed the best regain, but samples containing 0.45% gelatin regained only a small part of this former viscosity. The data indicate the value of viscosity and thixotropic measurements for viscosity control and for predicting changes in characteristics of dressing which might occur during creaming and packaging.

M13. Formation of curd by direct addition of acid to skimmilk. T. F. MCNURLIN* AND C. A. ERNSTROM, University of Wisconsin, Madison.

The addition of concentrated lactic or hydrochloric acid to skimmilk at 40 F, followed by warming without agitation to 70-80 F, resulted in a firm curd suitable for Cottage cheese manufacture. The use of 1 ml of rennet per 1,000 lb of milk improved the body of the finished curd. The amount of acid needed was determined by acidifying a small volume of milk at 70 F to the desired pH, and adding a proportional amount to the cheese milk. Electrical resistance heating was used to warm, without agitation, the acidified skimmilk to the setting temperature. Studies on acid curd between pH 4.5 and 5.0 indicated that curd firmness increased with decreasing pH, increasing setting temperature, and with time at the setting temperature.

A test was devised which measured coagulation time after the milk reached the setting temperature. As the pH decreased and the setting temperature increased, the coagulation time became shorter. Increasing the solids in the milk from 9 to 16% resulted in longer coagulation times at all pH values and setting temperatures. However, once coagulated, the high-solids curd quickly became firm enough to cut.

M14. Effect of stabilizer on body of cultured cream. E. S. GUTHRIE, Cornell University, Ithaca, New York.

Besides several alginates used as stabilizers in cultured cream, the following stabilizing products were investigated: agar, gelatin, locust bean, pectin, maize starch, tapioca starch, potato starch, rennet, and rice flour. Employed in this study of 20 stabilizing agents were 960 samples of cultured cream.

The data show that the stabilizers studied in this research increased the plasticity, solidity, and viscosity of the cultured creams as shown by the Hilker-Guthrie Sour Cream Body Tester; and that the amounts of extraneous water and graininess, also, were increased.

M15. Initial syneresis rates of milk gels. W. F. STOLL AND H. A. MORRIS,* University of Minnesota. St. Paul.

Several methods for determining the extent of syneresis occurring in milk gels have been investigated. A method based on the change in specific gravity of the gels during syneresis has been developed and applied to several milk gel systems. A series of bottles containing monochlorobenzene and kerosene in different proportions were prepared, so that a specific gravity gradient of 1.0316 to 1.0542 was obtained. The specific gravity of the gel was determined by finding the mixture in which the gel just floated. The specific gravity of the milk gel was determined at intervals after cutting and compared to the original specific gravity of the milk, to determine the amount of syneresis.

The rate of syneresis was very rapid immediately after cutting the gel, followed by a slower rate of syneresis. Initial rapid syneresis rate was more evident in low fat milk gels than in whole milk gels.

Syneresis during practical cheese making has also been determined. Curves showing rate and extent of syneresis during manufacture of Cheddar and Blue cheese will be shown.

M16. Calcium and phosphorus retention studies in Cheddar cheese. R. W. WEIK* AND H. A. MORRIS, University of Minnesota, St. Paul.

Cheddar type cheeses of controlled composition were made to obtain information about hardness and Ca and P retained by the curd after dialysis. Equilibrium dialysis has been used to determine the rate and amount of Ca and P that could be depleted from these cheeses. Cheese hardness was also determined.

An inverse relationship was found between Ca retained and cheese hardness. Calcium was found to be more tenaciously retained by soft cheese than by hard cheese, with values

ranging from 3.8 to 1.7 mg Ca retained/gram cheese after dialysis. No marked trend was observed for P; thus, a higher Ca/P ratio, both initially and during depletion, was found for the soft cheese. The initial ratios for most cheeses were between 1.46 and 1.52. Soft cheese ratios dropped to as low as 1.32, then increased slightly during dialysis, whereas hard cheese ratios showed a steady decrease to values below 1.0. Modified Cheddar cheese (i.e., USDA Modification, Dariworld, eac.) retained less Ca than normal Cheddar cheese and the Ca/P ratio was slightly lower, both initially and after depletion.

M17. Role of thiamine disulfide reducing substances and uric acid in Cheddar cheese. T. KRISTOFFERSEN, Ohio Agricultural Experiment Station, Columbus.

The possible relationship of thiamine disulfide (TDS) reducing substances (Harland and Ashworth—J. Dairy Sci., 1945) and uric acid (Bergman—J. Biol. Chem., 1954) to Cheddar cheese quality was investigated. Results for 24 commercial cheeses were: TDS—range 2.3 to 30.2 and average 16.5 mg cysteine · HCl equiv/100 g; uric acid—range 0.127 to 0.375 and average 0.269 mg/g. Corky and curdy body was associated with relatively low concentrations of uric acid and short and crumbly body with relatively high concentrations. No definite relationship was apparent between flavor and body and TDS values.

Uric acid and TDS determinations were conducted on six paired lots of Cheddar cheese, three made from strictly fresh raw milk (I) and three from the same raw milk incubated 5 hr at 37 C (II). After two and six months, respectively, the average results were: I—4.7 and 3.5 mg cysteine · HCl equiv/100 g, and 0.172 and 0.332 mg/g uric acid; II—5.5 and 13.0 mg cysteine · HCl equiv/100 g, and 0.164 and 0.363 mg/g uric acid. The flavor of I was metallic and lacking character, whereas that of II was typical. The body characteristics of I and II were similar. The development of characteristic Cheddar cheese flavor appears to relate to the formation of TDS reducing substances.

M18. Heat-treated and hydrogen peroxide–treated milks for Cheddar cheese. P. F. FOX* AND F. V. KOSIKOWSKI, Cornell University, Ithaca, New York.

Raw milks purposely contaminated with coliform organisms were treated with H_2O_2-catalase or heat-treated to observe effects on coliform destruction and cheese quality.

Eleven strains of *Escherichia coli, Aerobacter aerogenes,* and *Aerobacter cloacae* (ATCC) were inoculated into 7,000-lb lots of cold milk. Following storage, separate portions of the high-count milk were heated at 135, 138, 140, and 142 F for 16 sec. A portion was treated with 0.02% H_2O_2 at 130 F—16 sec, followed by catalase addition. Cheddar cheese was made by standard procedure. Raw milk and properly pasteurized milk cheese served as controls.

One raw milk with a coliform count of 4.8 million per milliliter showed a coliform count in resulting curds at time of milling of 600 million per gram. Pasteurized milk and hydrogen peroxide milk and cheese were almost devoid of coliforms. Heat-treated milks showed a coliform bacterial destruction of from 50 to 90%, but in the resulting curds and cheese coliform destruction as high as 99.9% was evident.

Qualities of Cheddar cheese varied considerably with treatment, but in all instances improvement over the high coliform count raw milk cheese was noted. Most typical cheese flavor was observed in cheese from heat-treated milks. Cheese manufacturing time was delayed 1½ to 2 hr, using H_2O_2-catalase milk, and the resulting cheese displayed a bland, foreign flavor.

M19. Method for enumerating nonlactic organisms in fresh Cheddar cheese. O. R. IRVINE AND M. E. BEACH,* Kemptville Agricultural School, Kemptville, Ontario.

Standard plate count agar to which was added 3.75 ppm of brom cresol purple, was found to be sufficiently inhibitory to starter bacteria to permit counts to be made of contaminating organisms in month-old Cheddar cheese made from both raw and pasteurized milk. A 0.1-g sample of cheese was macerated in 3-4 ml of sterile 2% sodium citrate solution which, after further dilution, was plated. Plates were incubated at 32 C 24-36 hr.

The great majority of colonies appearing on the plates were small, round, acid-producing colonies of the *Micrococcus* genus. Numbers per gram of cheese ranged from practically zero in cheese made from pasteurized and heat-treated milk to above 500 million in raw milk cheese with fruity and similar undesirable flavors. Magnitude of the count appeared to be related to the strictness of milk grading practiced by the various factories. Larger, alkaline-producing colonies of small rod-shaped organisms were also frequently observed and high counts of these are believed to be indicative of unhygienic factory conditions.

M20. Methods to improve the quality of reconstituted milk Cheddar cheese. I. I. PETERS* AND J. D. WILLIAMS, A. & M. College of Texas, College Station.

Sixteen lots of cheese were made in four vats. Lactic starter and peptonized milk culture of *Lactobacillus casei* were added to each milk. Milk with 0.02% $CaCl_2$ added served as control (Treatment 1). Treatments 2, 3, and 4 consisted of acidifying milk to 0.225% with lactic, citric, and hydrochloric acid, respectively. Half the curd of each lot was salted

with NaCl, the other half with NaCl plus trace quantities of KCl and $MnCl_2 \cdot 2\ H_2O$. Wheels from each split lot were ripened for (a) six months, 10 C, (b) three months, 16 C plus three months, 10 C, and (c) six months, 16 C.

At three months moisture contents of Treatments 1 and 4 were significantly higher than 2 and 3. Body scores for ripening (b) were significantly higher that (a) and (c). At six months moisture content of Treatment 1 was significantly higher than for 2, 3, and 4. Moisture content of ripening (a) and (b) was significantly greater than for (c). Body scores for ripening (a) were significantly greater than (b) and (c). At three and six months flavor scores were significantly greater for ripening (a) than for (b) and (c).

M21. Spray drying Cheddar cheese and Blue cheese. R. L. BRADLEY, JR.* AND C. M. STINE, Michigan State University, East Lansing.

Cheddar and blue-veined cheeses were spray dried in a commercial type horizontal, co-current dryer. The cheeses were diluted to 40% total solids, homogenized at 2,000 psig and spray-dried at exit air temperatures ranging from 155 to 190 F. Other trials involved the heating of cheese slurries containing added salt and emulsifiers to 150 to 170 F, prior to homogenizing and drying.

Low exit air temperatures (160 F) reduced the oxidation of the lipids during spray drying. Inert ingredients such as crystalline cellulose, starch, protein hydrolysate, whey and gum arabic, when added to the slurries, failed to improve the quality of the dry cheese.

Cheese slurries atomized with low-pressure nozzles designed to produce a large particle showed better retention of volatile flavors and less oxidative deterioration than corresponding powders of smaller particle size. The addition of various blends of antioxidants and gas packing of the powders contributed significantly to the storage stability of the spray-dried cheeses.

M22. Metering device for inoculating Blue cheese curd. J. J. JANZEN, Clemson College, Clemson, South Carolina.

In the continuous hooping operation for Blue cheese the curd and whey mixture is pumped into the upper end of a slightly inclined, rotating, perforated, cylindrical drum. The whey drains freely while the curd is propelled toward the lower end of the drum. At this point the curd is deposited onto a sloping chute. The chute contains a specially designed auger which mixes the curd and also moves it downwards toward a funnel-shaped hopper. The hoops are filled by passing them under the hopper and manipulating the valve closure as required. The mold powder is accurately metered onto the curd as it leaves the rotating drum. The metering device is a specially mounted Vibra-Flow vibratory type feeder. This device permits one to regulate the inoculation level consistent with rate of curd flow. For best results the mold powder should be maxed with some salt. The salt acts as an extender and also prevents undue clumping of the mold powder.

This system of mold dispensing has been found to be superior to sieve methods tried earlier. The auger mixing, coupled with the metering device, makes it possible to uniformly distribute the mold throughout the curd. In addition, it offers the distinct possibility of salting the curd during the same operation.

M23. Amperometric titration of sulfhydryl and disulfide groups in milk. U. YOSHINO, H. K. WILSON,* AND E. O. HERREID, University of Illinois, Urbana.

Sulfhydryl (–SH) and disulfide (–SS–) groups in milk proteins were estimated, amperometrically, with silver nitrate. Cysteine, β-lactoglobulin, milk, and whey were titrated at different pH levels in sodium acetate, ammonium nitrate, and tris buffer solutions. This was done to develop a procedure that might be used to study the fate of these sulfur groups in fluid milk products heated at high temperatures and their probable relationship to physical defects that appear in these products during storage. Cysteine yielded results 5 to 50% higher than the theoretical values, depending on the titrating conditions. The cysteine content of β-lactoglobulin in sodium acetate buffer at pH 10.2 was 96% of the theoretical amount. Adding 1% sodium lauryl sulfate to the titrating medium made more –SH groups available, whereas 8 M urea decreased them. Decreased titers of –SH groups in heated milk coincided with the denaturation of serum proteins; they were probably oxidized to –SS– groups. The disulfide was stable at the temperatures used. Sterilization of concentrated milk decreased titratable –SH and increased –SS–.

M24. Cellulose acetate electrophoresis of milk serum proteins. N. S. MHATRE* AND J. G. LEEDER, Rutgers—The State University, New Brunswick, N. J.

Using a cellulose acetate membrane as a stabilizing medium, the electrophoresis of serum proteins was carried out in a veronal buffer of pH 8.6 and an ionic strength of 0.05, using a current of 200 v for 2 hr at room temperature. The strips were stained with Ponceau S dye and analyzed in a Spinco Analytrol or eluted and read in a Coleman Jr. spectrophotometer. Five distinct fractions were obtained and identified as blood serum albumin, beta-lactoglobulin, alpha-lactalbumin, pseudo-, and euglobulin in their decreasing rate of migration. The quantitative estimation of all the fractions was found to be statistically reliable. The micro-Kjeldahl analysis for protein nitrogen showed that the amount of dye uptake by each protein fraction was propor-

tional to its concentration. It is believed that the method offers significant advantages in its application for research purposes.

M25. Lability of sulfur in casein during peptization with sodium hydroxide. A. M. El-Negoumy* and E. W. Bird, Iowa State University, Ames.

Casein sol from 1 g was prepared by dropwise addition of 1 N NaOH, during stirring for 30 min; longer periods were required below pH 8.0. The mixture was heated to 90-95 C, acidified with 1 N HCl (pH 2.0-3.0) and H_2S swept by nitrogen into a starch-NaOH solution. This solution was acidified and titrated with 0.03 N KI-KIO_3. The milligrams of sulfur released per 100 g casein were 1.21, 2.04, 2.09, 2.40, 3.01, 8.36, and 21.8, at pH's 6.4, 7.5, 8.5, 9.48, 10.7, 11.6, and 12.4, respectively. Dialysates from peptized casein sols showed 7.6, 12.32, 13.5, and 25.69 mg sulfur at pH's 8.0, 9.43, 10.6, and 12.04, respectively. Dialysis seems to disturb an equilibrium and causes greater sulfur release. Neither decinormal NaOH nor 4.4 C peptizing temperature prevents this sulfur loss.

No sulfur was released at pH 11.70 using concentrated NH_4OH, suggesting pH is not the only factor involved.

Among cysteine, cystine, and methionine only cysteine released sulfur (1.38 mg/g) by the method described. Glutathione released 1.25 mg sulfur per gram.

M26. Physical-chemical state of the nonmicellular proteins in raw skimmilk. P. M. T. Hansen, M. B. Rao, and R. McL. Whitney,* University of Illinois, Urbana.

Electrophoretic patterns of the nonmicellular proteins in their natural environment were obtained by equilibrium dialysis of the whey secured by ultracentrifugation of raw skimmilk against an ocean of the same skimmilk, followed by electrophoresis with the natural protein-free milk system from the same milk as a buffer. Comparison of these patterns with those obtained with the same milk protein fraction in veronal buffer pH 8.6, $\Gamma/2 = 0.1$, indicated the same number of electrophoretic components. From a comparison of the relative areas in the two sets of patterns, a tentative identification of the components of the natural system was obtained, and the absence of any association of these components with each other was indicated. With a new technique called electrophoretic boundary elimination these components were identified in the natural nonmicellular fraction in order of increasing mobility: immune globulins, β-casein, α-lactalbumin, β-lactoglobulin, and serum albumin. A Rowland precipitation of the casein from this fraction, followed by electrophoretic analysis of the precipitate in veronal buffer pH 8.6, $\Gamma/2 = 0.1$, indicated the presence of β-casein, γ-casein, and another component with a mobility of 4.73×10^{-5} cm^2 volt^{-1} sec^{-1}. The β-casein and γ-casein in this fraction constituted only a part of these components in skimmilk.

M27. Procedure for isolation of κ-casein by use of sulfuric acid. C. A. Zittle, Eastern Regional Research Laboratory, Philadelphia, Pennsylvania.

κ-Casein has been isolated from whole acid–precipitated casein by the precipitation of the other caseins with sulfuric acid. The method is very simple, is consistent in good yields of κ-casein of high stabilizing activity, and gives a readily soluble κ-casein of better than 90% purity.

The procedure is as follows:

A frozen block of acid-precipitated whole casein weighing about 350 g (60 to 95 g of protein) is dissolved in one liter of 6.6 M urea. This solution is acidified with 200 ml of 7 N H_2SO_4 (one part concentrated to four parts water). After acidification two liters of water are added. The pH of the mixture is 1.3 to 1.5. No precipitation is apparent at first, but it gradually forms and becomes flocculent. After standing for 2 hr the precipitate is filtered off and discarded. The κ-casein in the filtrate is precipitated by the addition of 132 g (1 M) ammonium sulfate to each liter of filtrate. The precipitate is collected, suspended in water, and dissolved by the addition of 1 N NaOH to a final pH of 7.5. The solution is dialyzed and freeze-dried. The yields of κ-casein have been 7 to 12% of the whole casein used.

M28. Casein variants in the milk from individual cows. M. P. Thompson,* C. A. Kiddy, L. Pepper, and C. A. Zittle, Eastern Regional Research Laboratory, Philadelphia, Pennsylvania.

α_s-Casein from the milks of several individual cows at the USDA dairy cattle herd at Beltsville shows definite heterogeneity. Starch-gel-urea-electrophoresis at pH 8.6 reveals two α_s bands in some individual caseins, whereas the pattern for most α_s-caseins contains only one band. To date the α_s variants have been observed only in milk from the daughters of a single sire, suggesting a genetic basis; thus, the variants will be referred to as α_s-A and α_s-B, in order of decreasing electrophoretic mobility. Most individual milks and all pooled milks thus far examined contain α_s-casein comparable to α_s-B. α_s-Casein of the A type has not yet been observed singly.

The α_s-A/B band has been isolated by the urea fractionation method of Hipp et al., followed by calcium ion precipitation at 0-4 C. α_s-A/B has a phosphorus content of 0.90%, whereas pooled α_s-casein has a phosphorus content greater than 1%. α_s-A/B requires additional amounts of κ-casein to become fully stabilized against Ca^{++} precipitation. In addi-

tion, α_s-A/B has been found to sediment as a two-component system of 5.45 and 4.40 S, the smaller value approximating that for pooled α_s-casein. Similarly, α_s-A/B casein is resolved into two components at pH 8.6, veronal buffer, $\Gamma/2 + 0.10$, in free-boundary electrophoresis.

M29. Some optical properties of casein-lactose interaction products. P. KLIMAN* AND M. J. PALLANSCH, Eastern Utilization Research and Development Division, USDA, Washington, D. C.

The optical properties of neutral solutions containing lactose and sodium caseinate subjected to a variety of heat treatments were studied in the visible and ultraviolet regions by use of a standard double-beam spectrophotometer and a spectrophotofluorimeter. Mild heating resulted in the formation of calories interaction products which emitted energy adsorbed at 325 mμ in the form of fluorescence at 420 mμ. With increased heat treatment, changes occurred which led to the development of a brown color in the solution. The subsequent increase in browning on increased heat input was accompanied by destruction of the fluorescent moiety adsorbing at 325 mμ and the development of a new structure which radiated the nergy adsorbed at 390 mμ in the 460 mμ region. Materials with optical properties similar to those formed in the model system have been observed in sterile evaporated milk and milk powders. The actual relationship between the fluorescence exhibited by these products and their flavor remains to be established.

M30. Electrophoretic properties of the proteins in Cottage cheese curd. J. C. COLMEY,* R. McL. WHITNEY, AND S. L. TUCKEY, University of Illinois, Urbana.

Electrophoretic analyses of Cottage cheese curd were made. The effects of four variables on the physical and electrophoretic properties of the curd were determined. 1. Breed—skimmilk from Holstein and Jersey cows was used. 2. Heat treatment—skimmilk was pasteurized, respectively, at either 143, 150, or 175 F for 30 min. 3. Time of setting or incubation—both long and short sets were used. 4. Method of coagulation—both rennet extract and acid coagulation were studied.

The action of rennet extract with mixed lactic culture causes an asymmetry in the alpha-casein component and is more evident in Cottage cheese curd manufactured by the long-set method. Patterns of acid type curd manufactured from milk heated at 143 F for 30 min showed no asymmetry of the alpha-casein component. However, the alpha-casein component of acid type curd manufactured from Holstein skimmilk heated at 150 F for 30 min was separated into two components. This phenomenon was not evident in rennet type curd made by the short-set method from the same milk. However, a separation of the alpha-casein component did occur in rennet type curd manufactured from Holstein skimmilk (150 for 130 min) by the long-set method. Jersey skimmilk appeared to be more resistant to the effects of heat treatment and the action of rennet extract with mixed lactic culture than Holstein skimmilk. The separation of the alpha-casein component was correlated with a weak-bodied curd.

M31. Chemical and physical properties of several protein particle size fractions of raw and heated skimmilk. C. V. MORR,* I. A. GOULD, AND Q. VAN WINKLE, The Ohio State University, Columbus.

Separate aliquots of raw and heated skimmilk (88 C—10 min) were subjected to centrifugal treatments calculated to sediment protein particles of 55, 83, and 135 mμ diameter. These skimmilks and each of the supernatants and sediments were examined for nitrogen, calcium, and phosphorus content. The raw and heated skimmilks contained 42.5 and 71.2% of their total nitrogen in the 55-mμ and larger diameter range, respectively. Only a slight dependence of chemical composition of the sedimented proteins to particle size was exhibited.

Aliquots of each of the above were solubilized with oxalate and analyzed ultracentrifugally (pH 6.98); 90.7% of the protein (raw skimmilk) had an $S_{25} = 1.02$ S, whereas 49.1% of the protein (oxalate-treated raw skimmilk) had an $S_{25} = 1.85$ S.

Electrophoretic mobility and relative protein distribution for each component (pH 6.98, ionic strength 0.10) was determined for each above oxalate-treated sample. Protein sediments (135 mμ and above) from raw and heated skimmilk contained 72.5 and 69.5% of their protein in electrophoretic component 1 (desc. mobility = 5.5); whereas, protein sediments (55 mμ and above) contained 78.2 and 81.6% of their protein in this component, respectively.

M32. Fluorimetry as a method of determining the protein content of milk. V. H. HOLSINGER,* K. K. FOX, AND M. J. PALLANSCH, Eastern Utilization Research and Development Division, USDA, Washington, D. C.

A study of the ultraviolet fluorescence of milk, and the factors influencing it, was made to corroborate the claims of Konev and Kozunin [Dairy Sci. Abstr., 23:103 (1961)] that this property could be used to determine the protein concentration of milk. Using a commercially available spectrophotofluorimeter, it was found that the fluorescence displayed at 340 mμ was activated principally by adsorption at 280 mμ. The fluorescent efficiency of the proteins was found to be influenced by the extent of aggregation of the casein, concen-

tration, temperature, and length of exposure to short wave radiation. Moderate heat treatment and pH changes had no detectable effect on protein fluorescence. Considering these facts, a fluorimetric analytical procedure was developed for milk proteins which gave excellent agreement with results obtained by Kjeldahl analysis when tested on dilutions of milk. However, results became more erratic when milks of individual cows were analyzed. These deviations, amounting to ± .2% protein, were thought to arise from variations in the turbidity of the samples. Precise determination of proteins in milk by fluorimetry may depend on developing suitable corrections for this parameter.

M33. Activation of prorennin. A. G. RAND, JR.* AND C. A. ERNSTROM, University of Wisconsin, Madison.

Prorennin, extracted from calves' stomachs, was purified by two precipitations from saturated sodium chloride, followed by clarification with aluminum hydroxide. The purified material was free from pepsin activity and contained about 0.1% of its potential activity as active rennin. Activation was carried out at 25 C between pH 2.0 and 5.5.

The conversion of prorennin to rennin appeared to be autocatalytic between pH 4.0 and 5.0. The rate of activation below pH 4.0 was very rapid, and above pH 5.0 it was extremely slow. The presence of sodium chloride in activation mixtures below pH 4.5 reduced the rate of activation and the total recovery of active enzyme in relation to the amount of salt present. At pH values of 4.7 and 5.0 an increase in the sodium chloride concentration up to 1.7 M increased the rate of activation. When the salt was raised from 1.7 to 2.6 M the rate of activation as well as the total recovery of active enzyme was decreased.

M34. Intra-species variations in the germination response of Bacillus licheniformis spores to various amino acids. J. H. MARTIN* AND W. J. HARPER, The Ohio State University, Columbus.

Experiments were conducted to determine the effect of various amino acids on the germination of *Bacillus licheniformis* spores. Stimulation of germination by 0.1% amino acid at 35 C was determined by (a) following the changes in optical density of spore suspensions in phosphate buffer (pH 7.2), and (b) quantitative estimation of the changes in the heat-resistant spore population by plate counts.

Of 14 different amino acids tested, including both the L- and D-forms of ten of these, only L-alanine, L-cysteine, aand L-valine greatly stimulated germination of spores of a selected strain of *B. licheniformis*: the rates of germination after 0.25, 0.5, and 1.0 hr of incubation were: L-alanine, 94, 98, and 99%; L-cysteine, 86, 90, and 93%; and L-valine, 74, 76, and 77%. All other amino acids produced less than 20% germination.

The germination response to these amino acids varied with different strains of *B. licheniformis*. For example, after 1 hr of incubation, spores of *B. licheniformis* Strain 1 had germinated to the extent of 99 (L-alanine), 93 (L-cysteine), and 77% (L-valine), whereas the spores of *B. licheniformis* Strain 4 had germination percentages of 96 (L-alanine), 12 (L-cysteine), and 41% (L-valine).

M35. Preventing penicillinase production in cells of antibiotic-sensitive Bacillus subtilis. J. M. PALMER* AND F. V. KOSIKOWSKI, Cornell University, Ithaca, New York.

Spores of *Bacillus subtilis* (6633) from commercial sources, when stored at warm room temperatures in buffered, nonnutrient agar, display slight germination with time. The resulting, dormant, vegetative cells in three to seven days at 30 C elaborate penicillinase into the agar after induction by penicillin. Washed spores prepared in the authors' laboratory are less prone to this condition.

Penicillinase induction can reduce sensitivity of reverse-phase penicillin disc assays of milk through neutralization of the antibiotic when discs wetted with test milk contact the contaminated agar.

To minimize such a condition attempts were made to prevent penicillinase production by mild heat, chemical and gas treatments of the spores residing in plain agar. Some success was obtained using 8-hydroxyquinoline and versene, but required concentrations were too critical. Preheating seeded agar or spores was unsuccessful.

Prevention of penicillinase production in agar was attained through storing spores in carbon dioxide atmospheres under slight pressure. Direct gassing gave good results, but a simple, effective method was to add small amounts of solid carbon dioxide to pouches holding plates of seeded agar prior to sealing.

Maximum sensitivity to penicillin of *B. subtilis* spores stored in gas was extended many days at 30 C over controls and growth of the test organism was rapid during assay incubation. Such results may be due to an inhibiting effect on spore germination.

M36. Effect of skimmilks cultured with different strains of Leuconostoc citrovorum in growth of some microorganisms associated with Cottage cheese spoilage. E. H. MARTH* AND R. V. HUSSONG, Research and Development Division, National Dairy Products Corporation, Glenview, Illinois.

Skimmilks were fermented with four cultures of *Leuconostoc citrovorum* according to procedures of Mather and Babel (J. Dairy Sci., 42:1045. 1959.). Fermented milks were filtered, the filtrate from each subdivided, and portions adjusted to pH values of 4.5, 4.7, 4.9,

and 5.1. Each filtrate was evaluated by disc assay procedures for inhibitory effect on growth of microorganisms.

When undiluted filtrates were tested, those fermented by two cultures of *L. citrovorum* regularly inhibited two strains of *Aerobacter aerogenes,* two of *Escherichia coli,* five of *Pseudomonas fluorescens,* and two of *Pseudomonas fragi.* Often an increase in pH was accompanied by a decrease in frequency of inhibition or in zone size. The other cultures displayed less inhibitory activity throughout and, sometimes, none at higher pH levels. No filtrates inhibited *Torula glutinis, Saccharomyces cerevisiae, Saccharomyces fragilis,* or *Mycotorula lipolytica.*

Filtrates were diluted with cream or water to levels present in Cottage cheese if cultured skimmilks were used in a creaming mix. Diluted filtrates failed to inhibit two strains of *A. aerogenes,* two of *E. coli,* four of *P. fluorescens,* and one of *P. fragi.* One strain of *P. fluorescens* was inhibited by three series of filtrates, and inhibition of one strain of *P. fragi* was erratic.

M37. Changes in the characteristics of staphylococcus aureus during cheese ripening. M. E. STILES,* L. D. WITTER, AND S. L. TUCKEY, University of Illinois, Urbana.

Five different cheese types wer prepared from pasteurized milk containing *Staphylococcus aureus* strain M.F. 31 (100,000 per milliliter). Changes in the *S. aureus* population were followed by surface streaking on a control medium (trypticase Soy agar) and selective media (mannitol salt agar and Staphylococcus Medium #110).

During ripening, three changes were observed in the characteristics of the test organism. In the early stage of ripening, a significant number of white colony variants appeared on all plates, but they decreased considerably with further ripening. There was a decrease in the percentage of organisms recovered on the selective media as the ripening progressed.

Further investigation of the variants indicated that they had developed a salt preference and no longer beta-haemolysis, concurrent with the demonstration of achromogenesis. The observation of sectored colonies with entire edges gave strong evidence of the close relationship between the white and yellow colonies.

White variants arose from yellow clones and yellow variants from white clones, when they were grown in a continuous culture system. Data substantiating the nature and implications of this variation in *S. aureus* will be presented.

M38. Influence of increased antibiotic resistance on heat resistance of staphylococci. A. N. MYHR, University of Saskatchewan, Saskatoon, Canada.

Thirty-three cultures of coagulase positive staphylococci isolated from milk drawn aseptically from cows in five herds and 20 cultures from human sources were studied. Antobiotic resistance was induced by consecutive daily transfers in serial double dilutions of the desired antibiotic in Brain Heart Infusion broth. Resistance was developed to 2,000 units of penicillin, 200 μg aureomycin, and 4000 μg streptomycin per milliliter. The parent cultures, carried in broth without antibiotics, served as controls. Antibiotic-sensitive cultures usually developed resistance to the antibiotics within three or four transfers in the broth containing antibiotics.

Thermal death times of the antibiotic-resistant cultures and the nonresistant parent strains were determined by suspending the organisms in skimmilk (approximately 50,000 per milliliter) and heating the suspensions in flame-sealed tubes immersed in water at 140 F. After heat treatment, the milk cultures were incubated at 35 C for five days, then streaked on plate count agar to ascertain survival. No significant change in heat resistance occurred with increased resistance to the antibiotics studied. Destruction times at 140 F ranged from 1-35 min, with most of the cultures being destroyed within 5-20 min.

M39. Arylesterase activity of bovine milk as related to incidence of mastitis. R. R. MARQUARDT* AND T. L. FORSTER, Washington State University, Pullman.

Comparisons of catalase, chloride, and C.M.T. values with those of arylesterase (A-esterase) for milk samples from 104 quarters indicated that level of A-esterase activity is highly associated with severity of mastitis. Milk with high chloride, catalase, or C.M.T. values had an average A-esterase activity of approximately 10 to 12 times greater than that of normal milk.

Three of the four quarters of two Holstein-Friesian cows were infected four times over an 18-day period with varying levels of staphylococcal microorganisms. The A-esterase activity increased markedly 4 hr. after infection, reaching a peak 16 hr. after infection, followed by a gradual decrease. Catalase and C.M.T. values corresponded with those of A-esterase, but the response 4 to 12 hr. after infection was not as great as that of A-esterase. Chloride values in most cases did not vary greatly from normal values.

The results from 16 milk samples showed that A-esterase activity of refrigerated milk is not decreased during four days of storage.

M40. Host-bacteriophage relationship as influenced by penicillin. E. M. MIKOLAJCIK AND I. A. GOULD, The Ohio State University, Columbus.

Single-step growth curves were determined in a hydrolyzed milk protein broth for bacteriophage active against penicillin-susceptible

Streptococcus lactis C_{10} in (1) control, (2) with 0.05 IU penicillin/milliliter added just prior to absorption, and (3) with induced penicillin-resistant host cells. Plaque counts were run at 2-min. intervals as outlined by Delbruck and Luria *(Arch. Biochem.,* 1:111. 1942).

Per cent absorption of bacteriophage by host cells at 5 min was 66 for the control, 59 with penicillin added, and 58 for penicillin-resistant cultures. The latent period (min) for bacteriophage averaged 15.9 for the control, 20 when penicillin was present, and 19 with induced-resistant cells. Rise periods for bacteriophage were the same for the control and for the system with penicillin added (19 min), but with antibiotic-resistant host cells the rise period was 15 min.

The presence of penicillin doubled the burst size of bacteria susceptible to this antibiotic (84 new particles), in comparison to 42 for the control, and 38 for the induced-resistant cells.

These results may explain the earlier observation that the increased incidence of bacteriophage and the higher bacteriophage titers were due to the presence of penicillin.

M41. Inhibitory effects of fatty acids and other surface-active compounds on Streptococcus lactis. R. B. Maxcy* and R. C. Chandan, University of Nebraska, Lincoln.

Free fatty acids in milk inhibit the development of *Streptococcus lactis.* The mechanism of the inhibition is not understood. Studies were conducted to elucidate the nature of the inhibition by categorizing it into a physical phenomenon (expressed by surface tension) or a direct toxic effect through interference with metabolism. Media containing 0.01% skimmilk powder and media containing 0.01–4.0% micro-inoculum broth were used. These media accentuated the effect of the fatty acids and other surface-active materials. Fatty acids of various chain length were used. Capric acid was found to be most effective and caused complete inhibition at 0.05% concentration, with a partial inhibition at 0.01%. The extent of inhibition was related to the concentration of the fatty acid and to the surface tension of the medium. The critical value of surface tension was approximately 35 dynes/cm at 25 C. Other surface-active materials, e.g., decyl alcohol, Nacconol, and Tig (a commercial cleaner) gave the same pattern of inhibition with comparable surface tension depression. Neither butyric acid, ethyl decanoate, nor Tween 40 was inhibitory. The surface tension was not reduced to approximately 35 dynes, the apparent critical level in this medium.

M42. Inhibition of lactic starter cultures by selected spore-forming organisms. J. H. Martin,* D. B. Kenkare, and W. J. Harper, The Ohio State University, Columbus.

Studies were conducted on the effect of spore-forming organisms on the acid production by *Streptococcus* starter cultures in sterile skimmilk.

Each sterile skimmilk sample was inocluated with 50,000 spores per 100 ml, heated at 190 F for 30 min, colled, inoculated independently with 1% of a skimmilk culture of *Streptococcus lactis, Streptococcus cremoris,* and three commercial lactic cultures, and incubated for 16 hr at 72 F.

All 13 strains of *Bacillus licheniformis* and all eight strains of *Bacillus cereus* caused slight inhibition of lactic acid production. The amount of inhibition varied both in relation to the *Bacillus* strain and in respect to the starter culture used with a given *Bacillus* strain. Maximum inhibition was 12.1% with *B. licheniformis,* and 7.8% with *B. cereus.*

The inhibition effect was somewhat more marked when a mixture of spores consisting of *B. licheniformis* (48.7%), *B. cereus* (42%), *B. pumilus* (3.8%), *B. cereus* var. *mycoides* (1.9%), *B. brevis* (1.7%), *B. laterosporus* (1.1%), and *B. circulans* (1.0%) was added. The inhibition of acid production ranged from 10.4 to 14.4%, averaging 11.8%.

M43. Injury and death of Streptococcus lactis due to freezing and storage. C. W. Moss* and M. L. Speck, North Carolina State College, Raleigh.

Streptococcus lactis was grown into the early stationary phase, centrifuged, washed, and resuspended in either skimmilk (10% NFMS) or buffered distilled water. Aliquots of each suspension were frozen and stored at −20 C for various intervals up to 28 days. Colony counts of the culture were made periodically, using a maximal and minimal agar medium to determine injury and death. The maximal medium was lactic agar (J. Dairy Science, 39: 1611. 1956). The minimal medium had the same composition as lactic agar, except the concentration of tryptone was reduced to 0.5% and yeast extract to 0.1%. Death was determined by the difference in plate counts on the maximal agar medium before and after freezing. Injured cells were determined by the difference in plate counts on the maximal and minimal media.

The culture before freezing gave rise to the same number of colonies on both plating media, although the colonies were smaller on the minimal medium. Greatest injury of the cells occurred during early stages of storage and decreased with time, while death continuously increased. Injury and death were more pronounced when cells were frozen in water than when frozen in 10% NFMS. Certain cultures survived better when frozen rapidly whereas, with others, survival was greater when freezing was slow. Subculture of thawed cells eliminated those which showed injury after subsequent growth and freezing.

M44. Proteolytic activity and acid production of lactic streptococci after refrigerated storage. R. A. COWMAN* AND M. L. SPECK, North Carolina State College, Raleigh.

Cultures of *Streptococcus lactis* were grown in a casein, yeast extract, glucose medium. Incubation was at 32 C to the late log phase and cells were harvested by centrifugation. The cells were washed and resuspended in 0.05 *M* phosphate buffer (pH 7). The suspension was added to steamed reconstituted milk (11% NFMS), and stored at 2–4 C. After various intervals, an aliquot was removed from storage to determine colony count, acid production, and proteolytic activity. Colony counts were made using lactic agar (J. Dairy Sci. 39: 1611. 1956). Acid production was determined by inoculating 1% of the cell suspension into steamed 11% NFMS, incubating at 32 C, and making pH measurements at intervals. At these same intervals, proteolysis (J. Dairy Sci., 30: 881. 1947) in the milk was measured. In addition, proteolytic activity of the stored cells was measured by adding toluene to the milk suspension and incubating 4 hr at 37 C.

During storage, colony counts remained relatively constant, decreasing only slightly. There was a pronounced reduction in proteolytic activity of the growing and toluene-treated cells after the first one to two days of storage. The cells showed a decreased rate of acid production after one day of storage; thereafter, they produced acid at comparable rates. These data indicate that rapidity with which lactic streptococci grow in milk after extended refrigerated storage is not dependent on their total proteolytic activity.

M45. Certain enzymes of glycolytic and hexosemonophosphate shunt pathways of Streptococcus lactis. K. M. SHAHANI* AND J. R. VAKIL, University of Nebraska, Lincoln.

Previous studies revealed that while metabolizing carbohydrates *Streptococcus lactis* produced acetic and formic acids, carbon dioxide, ethanol and glycerol besides lactic acid, indicating that the organism functioned as an heterofermenter. Also, the organism could metabolize lactobionate and gluconate. Studies using cell-free sonic extract revealed that the organism possessed hexokinase, aldolase, lactic dehydrogenase, zwischenferment, and 6-phosphate gluconic dehydrogenase, indicating that the organism behaved as a facultative homofermenter. Penicillin, streptomycin, aueromycin, and terramycin (10 units/milliliter) inhibited the five enzymes to varying degrees. Hexokinase was not sensitive to the antibiotics. Penicillin had no effect on aldolase, but showed marked inhibitions (51–81%) of the enzymes. Streptomycin, aureomycin, and terramycin inhibited aldolase by 20–46% and the other three enzymes by 62–100%.

The cells of *S. lactis* grown both in the presence and absence of the antibiotics and harvested at different periods of incubation (18, 24, 36, and 48 hr) possessed all the above enzymes, indicating the enzymes to be the constitutive ones. However, the production of these enzymes was inhibited significantly when the antibiotics were present in the growth medium.

M46. Different pathways of lactose metabolism of Streptococcus lactis and their sensitivity to antibiotics. J. R. VAKIL* AND K. M. SHAHANI, University of Nebraska, Lincoln.

The objective of this study was to investigate the pathway(s) of lactose degradation by *Streptococcus lactis*. Previously, it was reported that sonic cell-free extracts of the organism did not possess the lactase activity. However, the cell-free extracts prepared by sand trituration revealed the presence of lactase, but its concentration was very low (0.093 units/gram dry cells). This low level of lactase did not account for the observed rapid utilization of lactose. Using β-0-nitrophenyl galactoside as the substrate, it was possible to establish the presence of β-galactosidase activity (0.94 units/gram dry cells) in the extracts. The organism also possessed lactose dehydrogenase, which oxidized lactose to lactobionate. Lactose dehydrogenase was assayed in the extract spectrophotometrically by measuring the rate of reduction of 2,6-dichlorophenolindophenol in presence of lactose. The presence of lactose dehydrogenase (0.20 units/gram dry cells), lactase, and β-galactosidase, and complete absence of any enzyme similar to sucrose or maltose phosphorylase in *S. lactis*, indicated that the organism metabolized lactose partly by hydrolytic cleavage via lactase to galactose and glucose and partly by direct oxidation to lactobionate via lactose dehydrogenase. Penicillin, streptomycin, aureomycin, and terramycin inhibited these enzymes to varying degrees.

M47. A selective medium for detecting Leuconostoc organisms in mixed-strain starter cultures. J. V. MAYEUX, W. E. SANDINE, AND P. R. ELLIKER,* Oregon Agricultural Experiment Station, Corvallis.

The *Leuconostoc* medium of Mayeux and Colmer, containing 1% tryptone, 0.5% yeast extract, 10% sucrose, and 1.5% agar (J. Bacteriol., 81: 1009. 1961), was modified by adding 0.1% sodium citrate, 0.5% glucose, and 0.25% gelatin to better support growth of *Leuconostoc* species found in mixed-strain starter cultures. To the melted, cooled agar was added sterile aqueous sodium azide to a final concentration of 75 ppm. Plates were poured, dried, spread with suitably diluted cultures, and incubated at 21 C four days. *Streptococcus lactis, Streptococcus cremoris,* and *Streptococcus diacetilactis* were inhibited and colonies which were opaque and white to yellow in color appeared only after four days. Col-

onies of *L. citrovorum* were 0.5 to 2.0 mm in diameter, translucent, and exhibited a bluish iridescence. *L. dextranicum* colonies were large (1 to 5 mm), transparent, and slimy due to dextran production. Comparative studies were made of single-strain lactic streptococcus, *Leuconostoc,* and mixed-strain starter cultures using the basal medium with and without sodium azide. The method was useful in enumerating and isolating *Leuconostoc* species from mixed-strain starter cultures.

M48. A selective plating medium for Leuconostoc in mixed lactic cultures. F. E. McDonough,* R. E. Hargrove, and R. P. Tittsler, Eastern Utilization Research and Development Division, USDA, Washington, D. C.

The need for a differential plating medium to determine the *Leuconostoc* content of mixed lactic starters has been well established. *Leuconostoc* (17 strains) was found to be much more resistant to small amounts of tetracycline than either *S. lactis* or *S. cremoris* (19 strains), particularly *S. cremoris.* Consequently, a simple selective plating medium consisting of a tomato juice agar base containing 0.15 μg/ml of tetracycline has been developed. It inhibits the growth of most lactis-cremoris strains but permits normal growth of the *Leuconostoc* strains. Five experimentally mixed starters of known composition and seven commercial mixed lactic starters were used to further evaluate the effectiveness of the medium. These mixed starters were plated on the control medium to determine total count and on the experimental medium for *Leuconostoc.* The plates were incubated 24, 48, and 72 hr at 30 C. The optimum incubation period was 48 hr because a few *S. lactis* strains began to grow thereafter. A representative number of colonies (140) were isolated from the tetracycline agar and identified by growth in litmus milk and by other biochemical tests. Of those isolated after 48 and 72 hr of incubation, 99 and 86%, respectively, were identified as *Leuconostoc.*

M49. An agar medium for differentiating Streptococcus lactis and Streptococcus cremoris. Nikki Turner, W. E. Sandine,* and P. R. Elliker, Oregon Agricultural Experiment Station, Corvallis.

The ability of *S. lactis* to produce ammonia from arginine was used to develop an agar medium to distinguish colonies of this bacterium from those of *S. cremoris.* Medium containing 0.5% tryptone, 0.5% yeast extract, 0.3% L-arginine-HCl, 0.05% glucose, 0.2% K_2HPO_4, and 1.5% agar was adjusted to pH 6.0 with HCl and autoclaved for 15 min at 121 C. To each 100 ml of melted, cooled (48-50 C) agar was added 1.0 ml of aqueous, filter-sterilized 0.5% 2, 3, 5-triphenyltetrazolium chloride (TCC). Petri plates were poured and allowed to dry at 30 C for 24 hr. Cultures to be tested were grown at 30 C in broth of the same medium lacking TCC and diluted to about 10^3 cells per milliliter in serile distilled water. Aliquots (0.1 ml) were spread on the agar plates, which were incubated at 30 C in a candle-oats jar for 24 to 48 hr. Colonies of *S. lactis* appeared bright red, whereas those of *S. cremoris* were white. Mixtures of the two species produced red and white colonies which proved to be *S. lactis* and *S. cremoris,* respectively, upon subculturing. Ultraviolet light irradiation of *S. lactis* induced mutations such that white colonies were produced on the TCC-arginine agar; these mutants were unable to produce ammonia from arginine.

M50. Effect of large variation in the milk fat, solids-not-fat, liquid fat, and air contents of butter on its appearance, spreadability, and hesion. H. Grill, Jr.* and S. T. Coulter, University of Minnesota, St. Paul.

A central, composite, rotatable second-order design experiment was arranged in which products were prepared using conventionally churned unsalted butter as a base and varying the total fat content from 62 to 78%, added liquid fat as corn oil from 0 to 12%, added milk solids-not-fat from 0 to 9%, both with and without the addition of 1% of an emulsifier-stabilizer combination, all worked in a vacuum-sealed, water-jacketed mixer but at pressures of atmospheric and 7, 14, 21, and 28 inches of vacuum. Spreadability was assessed subjectively and by sectility and apparent viscosity using a Brookfield Viscosimeter at both 50 and 60 F.

Sectility and apparent viscosity decreased significantly (0.01 level) linearly with decrease in milk fat, liquid fat (corn oil), and in working pressure. There was no significant interaction among the variables. The addition of the emulsifier-stabilizer combination reduced the hardness by about 28%. Hardness at 60 F was about half that at 50 F. Hesion was significantly decreased by increase in liquid fat. Samples worked to contain the least air were the most smooth and waxy.

In a separate experiment the addition after churning of a milk fat fraction liquid at 55 F reduced the hardness and apparent viscosity values to a greater degree than when added before churning.

M51. Effect of forewarming temperatures on some properties of sterilized concentrated milks. M. E. Seehafer,* A. M. Swanson, and H. E. Calbert, University of Wisconsin, Madison.

In the manufacture of sterilized concentrated milks the preheat treatments (forewarming) have definite effects on the stability both during processing and storage. From previous work it was observed that certain forewarming treatments produced finished products with high solubility indices which, on storage, developed considerable sediment. This study on the sta-

bility of sterilized concentrated milks covers two consecutive three-month periods, November-April, 1960-61. Data presented show the relationship between forewarming treatments, solubility index, and storage stability at 45 and 75 F.

Sterilized concentrated milks (36.5% T.S.) were manufactured using forewarming treatments of 145, 165, and 185 F for 30 min each, plus variations in concentrate holding treatments.

Products made from 145 F forewarmed milks showed an average of 87.6% denaturation of the whey proteins based on raw milk vs. finished product values. Forewarming treatments of 165 and 185 F resulted in 89.8 and 94.1% denaturation, respectively.

The 145 F treatments yielded the highest solubility indices in the finished products, and 185 F treatments showed the lowest.

Sedimentation was most severe in the 145 F forewarmed samples, and the 185 F samples had the least.

Solubility index vs. sedimentation showed a correlation of .93 for both periods at 75 F storage.

M52. Effect of forewarming treatments on heat stability. G. H. HARTMAN, JR.* AND A. M. SWANSON, University of Wisconsin, Madison.

The term heat stability describes the abiilty of milks and milk concentrates to withstand heat treatments, particularly temperatures above 212 F. Forewarming treatments have become standard practice as a means of stabilizing concentrated milks during sterilization. This study was made to determine the effects of forewarming treatments on the heat stability of various heated milk, concentrates, and finished products. Culture tubes containing 15 ml of sample and a short glass rod were rotated in silicone oil held at 140 C. Heat stability values were recorded as number of elapsed minutes before coagulation was observed. Mixed herd milk samples were forewarmed at 145, 165, and 185 F for 30 min with a standard come-up time, then concentrated to 26.0% total solids.

In original fluid form, the milks forewarmed at 145 F were the most heat stable, while the 185 F samples were the least stable. After concentrating, the positions were reversed. In the finished products, the 145 F forewarmed samples were again the most stable.

M53. Casein precipitation from concentrated milk during frozen storage. P. R. WELLS* AND J. G. LEEDER, Rutgers—The State University, New Brunswick, New Jersey.

The influence of prefreezing handling and storage temperature upon protein precipitation and related changes in frozen concentrated milk has been studied.

Dissolved lactose apparently exerts a protective influence upon the protein, for precipitation in the frozen product was invariably preceded by extensive crystallization of lactose measured polarimetrically. Crystallization and precipitation occurred more rapidly in samples cooled to 40 F and held before freezing than in samples frozen immediately after condensing without cooling. Samples cooled and held were more stable at 5 F than at 15 F, but those frozen without cooling showed a marked delay in lactose crystallization and protein precipitation at the higher temperature.

By means of cellulose acetate electrophoresis in veronal at pH 8.6, the precipitated material was identified as casein, with apparently normal proportions of the alpha and beta fractions. The precipitate also contained fairly constant amounts of Ca and PO_4, and precipitation was accompanied by a marked decrease in these components in the supernatant of the reconstituted product.

Studies on the protective action of carbohydrates against protein precipitation indicate that effectiveness is related to ability to increase the solubility of Ca in high concentrations of PO_4.

M54. Process for making puff spray-dried nonfat dry milk and related products. F. P. HANRAHAN, R. W. BELL,* AND B. H. WEBB, Eastern Utilization Research and Development Division, USDA, Washington, D. C.

In a pilot plant operation skimmilk was concentrated to the highest total solids content consistent with essential fluidity, lactose present in excess of its solubility was quickly crystallized, and the product was spray-dried following the injection of a compressed inert gas immediately ahead of the atomizer. The powder consisted of round, opaque individual and clumped particles having a diameter of up to 200 μ. It had excellent dispersibility and a bulk density of 0.35 to 0.40. Approximately 25% of the lactose was the crystalline alpha hydrate isomer. Thus, the steps of agglomerating the spray-dried particles and redrying, normally used in the preparation of instant powders, were eliminated and the output of the dryer was increased. The process is applicable to the drying of whole milk, cream, buttermilk, and whey.

M55. Influence of drying methods on porosity of whole milk powder granules. ELLIOTT BERLIN* AND M. J. PALLANSCH, Eastern Utilization Research and Development Division, USDA, Washington, D. C.

Evidence for marked differences in granule porosity was obtained by a study of the true densities of whole milk powder solids in a variety of powders; gas was used as a displacing agent for volume measurements. Helium penetrated all powders rapidly. Using this gas, true densities averaging close to 1.300 g/cm^3 were obtained for all powders studied. When hydrogen or nitrogen was used as the displacing gas, a marked difference was

observed not only in the rate of penetration but also in the calculated densities of the various powders. Evidence is presented to show that molecules having diameters larger than helium cannot readily penetrate the entire structure of conventional spray-dried powders. The development of foam structure in powders by gas injection prior to either vacuum or spray drying increases powder porosity, thereby creating conditions conducive to the rapid removal of water vapor and occluded gas from the powder granules. Foam drying can be expected to be a more efficient operation, producing powders of greater flavor stability when gas packed. In consideration of the relative magnitude of the porosity in milk powders, it is anticipated that both drying and degassing of foams may be more efficiently carried out at pressures approaching atmospheric.

M56. Flavor stability of dry milks prepared by vacuum shelf-drying and spray-drying procedures. A. TAMSMA, Eastern Utilization Research and Development Division, USDA, Washington, D. C.

Foam-dried milks were prepared by vacuum shelf-drying and spray-drying procedures from milk pasteurized at low temperature, packed in nitrogen, and stored at 40 and 80 F for a period of four months. Flavor was evaluated by a ten-man taste panel (J. Dairy Sci., 44: 1644. 1961). At 40 F the vacuum-dried product as a rule was more stable than the spray-dried foam, scoring three to six points above conventional spray-dried milks; whereas, the spray-dried foam scored one to three points above the conventional spray-dried milk. Both foam-dried products developed stale flavor to a varying extent at 0 degree; the principal flavor which developed at 40 was described as stale; a combination of heated and stale flavors developed at 80. Flavor stability of foam-dried milks is superior to conventional spray-dried milks and may be improved by optimizing factors such as oxygen content and moisture level.

M57. Effect of residual oxygen on flavor stability of dry milk foams. N. HOWARD* AND A. TAMSMA, Eastern Utilization Research and Development Division, USDA, Washington, D. C.

The influence on flavor of oxygen levels in the range of 0.001 to 0.1% in the interstitial gas of milk foams dried by vacuum-shelf or spray-drying procedure and canned in nitrogen were evaluated by a ten-man taste panel (J. Dairy Sci., 44: 1644. 1961). Vacuum-shelf dried foam milk did not change in flavor during six months of storage at 40 F; quantitative measurement of oxygen content before and after storage indicated that some oxygen was used by the powder. Foam spray-dried powder retained more oxygen after evacuation than the vacuum-dried product. Flavor stability also depends on moisture level; for example, spray-dried foam was very sensitive to oxygen at low moisture level (2 to 3%), but not at higher moisture level (4 to 5%). Low levels combined with higher moisture improved flavor stability of both products. Differences in stability between dry milk samples indicate that other factors in addition to oxygen and moisture are involved in flavor stability.

M58. Observations on the emulsifier content and fatty acid distribution in the lipids associated with the air cell structure in ice cream. P. G. KEENEY, The Pennsylvania State University, University Park.

Pint samples of ice cream separated into foam and serum layers when held for 24 hr at 0 C. The foam portion recovered from the melted ice cream was considered to be representative of the film which surrounds the air cells in the frozen product. The quantity of foam recovered was influenced by the type of emulsifier used in the mix and by the output rate at which the continuous freezer was operated.

The fat content of the foam was always higher than that of the serum and the greatest differences were obtained in those samples that contained an emulsifier. However, the emulsifier level of the foam fat was almost always less than that of the serum. The difference was particularly noticeable in ice cream when the freezer was operated at below its maximum capacity.

The fatty acid distribution in foam fat was slightly different from that of serum fat. The significance of these findings will be discussed.

M59. Consumer preferences for emulsifier-stabilizer levels in vanilla ice cream. E. J. FINNEGAN* AND J. J. SHEURING, University of Georgia, Athens.

To determine the reaction of consumers to varying emulsifier-stabilizer levels in vanilla ice cream, a household consumer panel with 955 individuals was conducted during May, 1961. Five vanilla ice creams which had emulsifier-stabilizer levels of 0.14, 0.19, 0.24, 0.29, and 0.34% were tested.

The ice creams were dispensed as they would have been in conventional supermarkets. Panelists were asked to fill out questionnaires relative to flavor and texture preferences for paired samples.

Resulting preferences, excluding no-preference responses, were analyzed statistically using the method of rank analysis of incomplete block designs. Unequal repetitions on pairs indicated that the consumers' flavor preferences (5% level) were for the three highest emulsifier-stabilizer levels (0.24, 0.29, and 0.34%) over the lowest emulsifier-stabilizer level (0.14%). Texture preferences showed that the four higher emulsifier-stabilizer levels were preferred (5%

level) over the 0.14% emulsifier-stabilizer level. The 0.29% level was preferred (5% level) over the 0.19 and 0.34% levels.

M60. Dried buttermilk and its properties in ice cream. K. A. ZIEMER,* C. H. AMUNDSON, W. C. WINDER, AND A. M. SWANSON, University of Wisconsin, Madison.

Reports from other laboratories indicate that the relatively poor flavor stability of dried buttermilk has limited its utilization. To determine the extent of this problem, a survey of commercial powders was made. It showed pronounced variations in fat content, moisture content, and flavor. After less than 6 wk of storage, 11 out of 14 samples were unacceptable in flavor by both sensory and TBA (2-thiobarbituric acid) evaluations. However, since three of the samples retained good flavor quality for more than one year, it is evident that flavor need not be a problem.

Fluid buttermilk was subjected to heat treatments ranging from 145 to 185 F, spray-dried, and used as a source of serum solids in flavored ice cream mixes. Similar mixes were prepared as controls, using condensed buttermilk and nonfat dry milk. After freezing and storing they were evaluated by trained judges. It was found that fresh dry buttermilk can be used in ice cream without adding to or detracting from the flavor. No flavor differences which could be attributed to heat treatment were found. Similarly, no differences were noted in the ice creams which contained dried vs. concentrated buttermilk as the source of serum solids.

M61. Effect of heat treatment on some chemical and physical properties of ice cream mix. R. J. ANDERSON* AND E. L. THOMAS, University of Minnesota, St. Paul.

A pilot tubular heat exchanger, previously described by Harland et al. (J. Dairy Sci., 38:1199. 1955), was utilized for subjecting standard ice cream mixes to various heat treatments. In each of two trials, 50 samples of ice cream mix were secured, representing ten temperatures ranging from 150–280 F and five holding times ranging from 5–108 sec. In addition to measurements of apparent and basic viscosity on each sample of mix, analyses were made to determine the extent of serum protein denaturation and of protein-lactose interaction during the various heat treatments.

Analysis of the data for viscosity revealed a distinct curvilinear relationship between temperature and the logarithm of the time required to produce a given increase in viscosity. Initial increases in viscosity did not occur until 50% or more of the serum proteins were denatured. No appreciable protein-lactose interaction (as reflected by increased acid ferricyanide reducing capacity) occurred until heat treatments necessary to denature approximately 70% of the serum proteins were employed.

M62. Isolation and identification of sulfur compounds resulting from various heat treatments of skimmilk. C. W. DILL,* W. M. ROBERTS, AND L. W. AURAND, North Carolina State College, Raleigh.

Skimmilk was heated to temperatures ranging from 190 to 300 F by direct steam injection with holding times of either 2 or 150 sec, then cooled to 130 F by flash evaporation, and finally cooled to 40 F by a plate cooler. Measurement of heat-activated sulfhydryls was accomplished by amperometric titration using silver nitrate. The volatile sulfur compounds, liberated as a result of the above heat treatments, were collected as precipitates in mercuric chloride solution. Gas chromatography was used to resolve and identify the volatile materials. Volatile components were formed at heat treatments greater than a critical treatment (Dill et al., J. Dairy Sci., 44:1154. 1961), concomitant with a loss in titratable sulfhydryl groups in the milk. The volatile fraction consisted primarily of hydrogen sulfide. The appearance of methyl sulfide in the volatile fraction appeared to correlate more closely to the presence of a cowy odor in the raw skimmilk than to heat treatment.

M63. Gas chromatographic analysis of ethyl ether–extractable flavor and odor compounds from evaporated milk. W. Y. COBB* AND S. PATTON, The Pennsylvania State University, University Park.

The solvent extraction technique (J. Dairy Sci., 44:207. 1961) for milk flavor analysis was employed. Slight modifications included ethyl ether as extracting solvent and longer extracting periods.

Gas chromatographic analysis of the solvent extract residue from evaporated milk on a phosphoric acid–treated diethylene glycol adipate column at 175 C revealed 20 peaks during a 120-min elution time.

Free fatty acid peaks were identified by two means. First, as methyl esters of acids separated from neutral material by the column adsorption method of McCarthy and Duthie [J. Lipid Research, 3(1):117. 1962]. Correlation of these with known methyl esters of C_4–$C^{2=}_{18}$ acids was observed on adipate- and succinate-packed columns and an Apiezon L capillary column. Second, as free acids by correlation of acidic peaks with known C_4–C_{14} acids at 175 C and C_{16}–$C^{2=}_{18}$ acids at 225 C on the phosphoric acid–adipate column. Treatment of the extract with base either eliminated or greatly reduced these peaks.

Peaks, the odors and retention times of which showed similarity to those of -decalactone and -dodecalactone, were observed from the adipate column and an Apiezon L packed column at 225 C; similarly, an eluant corresponding to maltol was detected from the adipate column. Material collected from this peak yielded a positive $FeCl_3$ test.

M64. Some observations on the volatile flavor compounds of ripened cream butter. E. A. DAY,* P. B. LARSEN, R. C. LINDSAY, AND P. R. ELLIKER, Oregon Agricultural Experiment Station, Corvallis.

The volatile fractions of selected starter cultures and of ripened cream butters made with the starter cultures were isolated by low-temperature, reduced-pressure distillation techniques. The volatile fractions were separated by gas chromatography at 70 and 100 C on both polar and nonpolar columns. Tentative identification of compounds represented by the chromatographic peaks was based upon comparison of relative specific retention volumes with values for authentic compounds. Compounds tentatively identified in starter culture volatiles were: ethanal, propanal, n-butanal, 2-methyl butanal, n-pentanal, acetone, butanone, diacetyl, acetoin, ethanol, n-butanol, ethyl acetate, methyl sulfide, and acetic acid. Compounds tentatively identified in ripened cream butter include ethanal, propanal, n-butanal, 3-methylbutanal, n-pentanal, acetone, butanone, 2-pentanone, diacetyl, acetoin, ethanol, n-butanol, ethyl formate, ethyl acetate, ethyl butyrate, methyl sulfide, and acetic acid.

M65. Preliminary observations on the volatile fraction of Cheddar cheese. L. M. LIBBEY,* D. D. BILLS, E. A. DAY, AND J. O. YOUNG, Oregon State University, Corvallis.

Cheddar cheese fat was obtained by centrifugation of cheese at 31,000 × g for 15 min. The flavor volatiles were isolated by passing the fat through a Rota-Film molecular still at 40 C and at lessthan 0.5 μ Hg pressure. The volatiles were trapped in a glass bead trap immersed in liquid nitrogen. Sensory evaluations of the fat revealed that the typical Cheddar aroma was removed by molecular distillation.

The volatiles were transferred from the glass bead trap to a 1/16-inch i.d. stainless steel U-tube and injected into a Barber-Coleman Model 20 gas chromatograph. Packed 1/16-inch i.d. columns were used at temperatures of 55, 70, and 100 C; both polar and nonpolar stationary phases were used. Evaluation of the chromatographic data indicated that there were at least 30 components in the volatile fraction from Cheddar cheese of excellent flavor. Comparison of the relative specific retention volumes of the unknowns with those of authentic compounds enabled tentative identification of over half of the chromatographic peaks. The families of compounds tentatively identified, which include carbonyls, esters, alcohols, and thiols, will be discussed.

M66. Analysis of the free fatty acids of fresh milk fats and ripened cream butters. L. L. KHATRI AND E. A. DAY,* Oregon State University, Corvallis.

The ion exchange resin procedure of Hornstein et al. (Anal. Chem., 32: 540. 1960.) was used to isolate and methylate the free fatty acids from the fat of fresh pasteurized cream and ripened cream butter. The methyl esters were extracted from the methylation reaction mixture with ethyl chloride. Upon removal of ethyl chloride the esters were weighed and aliquots were separated by gas chromatography, using a thermal conductivity detector. The amount of each ester in the mixture was ascertained by determining the percentage of the total peak area of the chromatogram attributable to each ester. Corrections for detector response and efficiency of the ion exchange resin procedure for isolation and methylation of free fatty acids were accounted for in calculating the weight of each methyl ester in the weighed ester mixture. The mean concentration of free fatty acids for ten samples of fresh milk fat, expressed as mg/kg of fat, were C-4, 98.3; C-6, 3.5; C-8, 21.8; C-9, 8.2; C-10, 117.8; C-10$^{=}$, 17.3; C-12, 154.9; C-12$^{=}$, 17.3; C-14, 324; C-14$^{=}$, 88.2; C-16, 819.9; C-16$^{=}$, 126; C-18, 347.6; C-18$^{=}$, 1118.3; C-18$^{2=}$, 151.4; C-18$^{3=}$, 60.2; C-20$^{4=}$, 30.6. Values for ripened cream butters were comparable. Pronounced variations in the concentration of the free fatty acids were evident between milk fat samples.

M67. Flavor threshold values of certain carbonyl compounds in milk. D. A. LILLARD,* M. W. MONTGOMERY, AND E. A. DAY, Oregon State University, Corvallis.

The flavor threshold values (FTV) for some of the carbonyl compounds of oxidized milk lipids were determined in pasteurized, homogenized milk by the method of Patton and Josephson (Food Research, 22: 316. 1957.). When necessary, the carbonyls were purified by gas chromatography prior to use. The average FTV of replicate analysis for each of five judges was used in determining the average FTV of each carbonyl.

The FTV of the carbonyls expressed as ppm were: propanal, 0.43; n-butanal, 0.19; n-pentanal, 0.13; n-hexanal, 0.049; n-heptanal, 0.12; n-octanal, 0.46; n-nonanal, 0.22; n-decanal, 0.24; n-hex-2-enal, 0.067; n-hept-2-enal, 0.077; n-non-2-enal, 0.0042; n-dec-2-enal, 0.092, and n-hepta-2,4-dienal, 0.049.

An additive effect was observed where the FTV was determined for a mixture of equal amounts of two compounds having comparable FTV.

M68. Direct chromatographic analysis of milk. R. Bassette,* S. OZERIS, AND C. H. WHITNAH, Kansas State University, Manhattan.

A method has been developed for the direct analysis of volatile constituents of milk. By using a chromatograph equipped with a hydrogen flame detector and a modified electrometer, and introducing the sample in the form of

head-space vapor, milk volatiles were easily detected. The head-space vapors, taken from above milk in a vial sealed with a rubber serum cap, were enriched by saturating the sample with Na_2SO_4. The sample in the sealed serum vial was warmed to 60 C and 1.0 ml of head-space gas taken with a gas-tight syringe. By using this method on an aqueous solution containing several carbonyl compounds, it was possible to demonstrate these components at the 10 ppb level. With the instrument employed, and the technique described above, samples of milk with oxidized, rancid, high acid, and normal flavors were analyzed. Differences in chromatographs could be associated with the development of various defects in milk. Identification of some of the chromatographic peaks was accomplished by reacting the sample with specific qualitative reagents and noting changes in the chromatograms.

M69. Composition of the free fat of spray-dried whole milk. KARIN LINDQUIST AND J. R. BRUNNER,* Michigan State University, East Lansing.

For the purpose of this study, free fat was defined as the lipid fraction extracted from whole milk powder with a 50/50 (v/v) mixture of petroleum and ethyl ethers. The lipid components of the free fat and total lipid fractions were resolved by adsorption chromatography on a silicic acid column. The glyceride fatty acids were determined by gas chromatography. Data obtained from the analyses of seven samples of fresh powder, both low- and high-heat processed, indicated that the free fat fraction was not essentially different from that of the total lipid. However, some minor dissimilarities between the two fractions were observed: (a) The free fat fraction contained a slightly higher concentration of neutral glycerides and lower concentrations of free fatty acids, monodiglycerides and phospholipids; (b) the free fat contained slightly more C_{10}-C_{18} saturated fatty acids and less C_{18} unsaturated fatty acids.

M70. Seasonal and breed variation in the fatty acid content of milk fat. J. W. STULL* AND W. H. BROWN, The University of Arizona, Tucson.

Milk fat from monthly composite milk samples from approximately 30, 60, and 20 animals of the Guernsey, Holstein, and Jersey breeds, respectively, was analyzed over a period of 2 yr by gas liquid chromatography. The animals received a uniform diet of alfalfa hay and a concentrate mixture of barley, milo, and cottonseed meal.

For the Guernsey, Holstein, and Jersey breeds, respectively, the following fatty acid percentages were found: C_6—1.04, 0.89, 0.97; C_8—0.86, 0.77, 0.89; C_{10}—2.33, 2.15, 2.89; $C_{10}^{1=}$—0.21, 0.25, 0.33; C_{12}—2.99, 2.78, 3.67; C_{14}—10.32, 9.88, 10.62; $C_{14}^{1=}$—1.35, 1.56, 1.48; C_{15}—1.23, 1.37, 1.31; C_{16}—33.13, 30.99, 32.56; $C_{16}^{1=}$—2.63, 2.80, 2.40; C_{17}—0.95, 1.13, 1.09; C_{18}—15.43, 14.61, 14.45; $C_{18}^{1=}$—23.39, 26.41, 23.30; $C_{18}^{2=}$—3.47, 3.48, 3.46, and $C_{18}^{3=}$—0.63, 0.95, 0.56. It should be noted that the methods used to separate the component acids of milk fat did not allow for the detection of butyric acid.

The results show that, in general, Guernsey and Jersey breeds as compared with the Holstein had greater contents of the C_{16} and lower acids. The Holstein breed had a higher $C_{18}^{1=}$ content. There was no consistent variation with season.

M71. Further developments in sediment testing of bulk tank milk on the farm. B. J. LISKA, Purdue University, Lafayette, Indiana.

There is considerable interest in developing tests to determine the quality of bulk tank milk on the farm before it is picked up by the tank truck. The sediment test can serve as one part of this milk quality evaluation of performed on the farm. Recent surveys have indicated the need for more use of the sediment test on bulk tank milk to keep milk high in sediment out of the milk supply.

A simple durable, inexpensive tester for performing the sediment test on bulk milk on the farm has been developed. It requires about 3 min to complete a sediment test using this tester. The milk is heated by the circulation of hot water through a water jacket on the unit. A 1-pt mixed milk sample is used and the sediment is collected on an area 0.4 inch in diameter. Field testing has indicated that the tester is more rapid and easier to use than those now available and is acceptable to bulk tank truck drivers. Several improvements have been made in the tester as a result of the field-testing program.

M72. Characterization of the substances in raw and heated skimmilk responsible for reducing acid potassium ferricyanide. W. E. HOBBS* AND S. T. COULTER, University of Minnesota, St. Paul.

Paper chromatography, ion-exchange resins, and protein-reducing capacity were used to characterize the substances responsible for the reducing action in raw and heated skimmilk. The washed acetic acid precipitates from both raw and heated (180 F/10 mon) skimmilk were dispersed in urea and dialyzed against distilled water for 48 hr at 40 F. Following concentration and deionization (by passing through a monobed) of the dialysates, paper chromatograms of the material obtained revealed the presence of lactose but not of glucose or ascorbic acid. In the undeionized concentrated dialysates the protein-reducing value of the raw skimmilk was 1.15 and that of the heated skimmilk 0.86 (expressed as $MgK_4Fe(CN)_6$ per 100 ml material) whereas,

in the deionized concentrated dialysates, the protein-reducing value was zero in both raw and heated hilk. Data are shown indicating that the materials responsible for the reduction of acid ferricyanide in raw skimmilk are anionic as evidenced by absorption by anionic but not by cationic resins. The addition of glucose, mannose, galactose, and ascorbic acid to raw skimmilk in concentrations normally found in milk had no material effect on protein-reducing values under the conditions studied.

M73. Method for removing Iodine^{131} from milk. G. K. MURTHY,* J. E. GILCHRIST, AND J. E. CAMPBELL, Robert A. Taft Sanitary Engineering Center, U.S.P.H.S., Cincinnati, Ohio.

Milk containing I^{131} (labeled in vitro, in vivo, or from fallout) is passed through a column containing Dowex 1-X8 resin. The resin was previously charged at pH of milk with chloride, phosphate, and citrate prepared using formula:

$$X = 4.03 \text{ (mM Cl/l)} + 4.64 \text{ (mM inorg P/l)} + 20.4 \text{ (mM Cit/l)}$$

where X is one liter, numericals represent ratio of respective anions in charging solution to that in average milk, and expressions in parentheses are the anionic composition of milk to be treated. Analysis of original and treated milks showed removal of 98% I^{131} up to 120-130 and 95% from 230 resin bed volumes of milk. The I^{131} removal did not vary with flow rates of 5-20 ml/min or with temperatures of 0-30 C. All the inorganic I^{131} was removed from in vivo milk except protein-bound I^{131}. The PBI^{131} in individual cow's milk may vary 0-10%; however, in market milk it is low or negligible. The anionic composition of milk is essentially unchanged and the organoleptic quality of milk was comparable to that of pasteurized whole milk. The absorbed I^{131} on resin may be stripped with 2 M HCl prior to resin regeneration.

M74. Effect of temperature and flow rate of milk through columns of ion exchange resins on the removal of strontium^{85}. D. G. EASTERLY,* J. Y. HARRIS, L. A. BUNCE, AND L. F. EDMONDSON, Eastern Utilization Research and Development Division, USDA, Washington, D. C.

Milk in vivo labeled with Sr^{85} was obtained from a cow dosed orally. Samples were passed through cationic exchange resin columns in the CaKMgNa form. One series of experiments was made at temperatures of 40, 60, and 80 F for milk at a pH of 5.4 and at 40, 60, 80, 140, and 180 F for normal milk. A constant flow rate of 0.5 resin bed volumes (rbv) per min was maintained. Flow rate studies were carried out at 0.25, 0.5, 1.0, and 2.0 rbv per minute.

The temperature did not affect the amount of strontium^{85} removed when the pH was maintained at 5.4. The average amount removed from the first 25 rbv at this pH was 82%. The amount removed increased with increasing temperature at the pH of normal milk, averaging 30% at 40 F and 38% at 180 F.

The amount removed decreased from 88 to 68% as the flow rate was increased from 0.25 to 2.00 bv per minute at pH 5.4, and from 40 to 30% for normal milk.

There was a linear decrease in the removal of the radiostrontium with successive fractions collected up to 50 resin bed volumes at both pH levels.

M75. Estimating solids-not-fat in bulked herd milk using the plastic bead method of Golding. R. E. ERB, Purdue University, Lafayette, Indiana, and L. J. MANUS, Washington State University, Pullman.

Comparative studies involving milk samples from individual cows and herds show that a single regression formula for indirectly estimating per cent solids-not-fat (SNF) is unsatisfactory. Inherent differences between breeds and among cows and herds of the same breed in ratios of fat to SNF and in ratios of protein to lactose are the principal reasons. Estimating per cent SNF from per cent fat is unsatisfactory except for pooled samples from many herds of several breeds. Percentage protein more accurately estimates per cent SNF than per cent fat. In bulk milk from individual herds the standard error of estimate exceeded one time in 20 determinations (S.E.) is approximately 0.28% SNF when using per cent protein as compared with 0.37% SNF when using per cent fat. Using a single formula involving density and per cent fat, the S.E. was 0.29% SNF. Similarity, use of individual breed formulae showed S.E. of 0.27, 0.31, 0.26, and 0.28% SNF, respectively, for herds of Guernseys, Holsteins, Jerseys, and mixed breeds. Among per cent fat ranges (breed ignored) the S.E. ranged from 0.23 to 0.27% SNF. When per cent protein was also included in the multiple regression equations the S.E. were 0.26% SNF for an all-breed equation and ranged from 0.22 to 0.28% SNF for individual breed equations and 0.21 to 0.24% SNF between per cent fat ranges.

M76. Significance of the Rechnagel effect in determining the density of whole milk. N. S. GOLDING, Washington State University, Pullman.

In the hydrometric determinations of the density of whole milk for estimating the solids-not-fat (SNF) content, a large proportion of the time is used to overcome the Rechnagel effect. At least three authorities have described different methods to overcome this effect. Therefore, when determining the density of milk it is desirable that it be clarified by close density measurements. These measurements show: (a) The Rechnagel effect is associated

only with the fat of milk. (b) Heating cold samples of milk (below 40 F) to 104 F and cooling to 68 F reduces the density over just warming to 68 F in proportion to the percentage of fat. For each increase of one per cent in fat the density is lowered .20 to .25 of a lactometer reading. (c) Fresh whole milk samples held at 68 F over night have the same density as the same milk held cold and heated to 104 F, then cooled back to 68 F. The determinations were made with Holstein, Jersey, and Guernsey individual cow's milk. These findings suggest that in testing for SNF much time can be saved by holding samples of fresh milk over night at 68 F and omitting the heating up and cooling down. This procedure could be especially helpful in mass-testing daily composite milk samples from individual cows.

M77. Effects of added iodine on milk and some of its components. C. S. GELDA,* E. L. THOMAS, AND J. J. JEZESKI, University of Minnesota; W. G. MIZUNO AND E. D. BERGLUND, Research and Development Department, Economics Laboratory, Inc., St. Paul.

The effect on the flavor of milk of additions of iodine with a typical surfactant as the carrier (iodophor) and with KI was studied. Determinations of possible association of iodine with milk components were made using organoleptic and radioactive tracer techniques utilizing I^{131}.

The smallest concentrations of iodine that could be detected organoleptically were: 0.06-0.08 ppm in tap water; 6-8 ppm in skimmilk; and 10-14 ppm in whole milk. Threshold concentrations were similar for chlorine alone and for total chlorine and iodine when combined in solution.

Organoleptic evaluation of fractionated components of iodinated milk revealed that a typical chemical flavor was associated to a greater degree with the buttermilk and whey fractions than with any other fraction. Through further studies, using I^{131} as a tracer, it was shown that iodine or iodophor added to milk exists mainly as water-soluble iodide ion. However, a small fraction of the total iodine was strongly bound to both the phospholipids and serum protein fractions.

M78. Mechanization in dairy plant accounting. L. C. DODGE, Michigan State University, East Lansing.

Office accounting equipment was studied to determine the units available to the industry, their practical applications in dairy plant accounting, and the development of criteria for judging the feasibility of the systems to various size operations.

Costs vary directly with the flexibility, speed, and extent of mechanization in the system. Most machines are available for rent or purchase. Maintenance is usually included in the rental fee. Costs include a preliminary systems study and training for operators conducted by the equipment manufacturer.

The dairy plant will probably get better systems analysis if it obtains competitive proposals from different manufacturers. Frequently, the service available from the manufacturer is the only significant difference between brands of equipment.

Some of the systems available include: accounting machines, add-punches for paper tape, punched card tabulating equipment, and electronic computers. Service organizations are available on a fee basis to do accounting work.

Several factors other than cost efficiency frequently contribute in the decision to purchase mechanized accounting equipment. Some of these factors include: speed necessary to get up-to-date operating data, increased number of reports required of the accounting department, increased clerical requirements based on planned expansion, and high clerical turnover.

PRODUCTION SECTION

* Designates author who presented paper.

P1. Chemical fractionation and bioassay of extracts from toxic tall fescue. D. R. JACOBSON,* AND W. M. MILLER, University of Kentucky, and S. G. YATES, H. L. TOOKEY, AND I. A. WOLFF, Northern Regional Research Laboratory, Peoria, Illinois.

The first fraction of toxic tall fescue, an alcoholic extract, was administered via rumen fistula at the rate of 2 lb 2 × daily for 37 days beginning January 23, 1959. The extract was prepared from a pure stand of tall fescue that contained no seed heads and showed no evidence of sclerosia of *Claviceps purpurea* (ergot). A complete picture of the toxicity syndrome, including dry gangrene in the distal portion of the tail, was obtained. The animal died March 5, 1959. In January, 1960, an aqueous phase prepared from such an alcoholic extract proved toxic. After extracting with chloroform at pH 11, the residual aqueous phase was still toxic. A bioassay technique has been developed in which the toxic effect (as manifested by a reduction in the skin temperature of the distal portion of the tail in a cold environment) is monitored periodically through the use of a skin thermocouple and a telethermometer before and after administration of the fescue extracts. Where the room temperature ranged from 0-10 C, the seven-day mean differences between room temperature and skin-of-tail tem-

perature were 8.6, 21.2, 20.4, and 21.7 for the toxic fraction, control, and two other nontoxic fractions, respectively. The difference was significant ($P < 0.01$).

P2. An in vitro enzyme digestion technique to predict the nutritive value of forages. E. DONEFER,* P. J. NIEMANN, E. W. CRAMPTON, AND L. E. LLOYD, Macdonald College (McGill University), Quebec, Canada.

Preliminary observations have been made on the development of a simple and rapid technique to predict the digestible energy potential of a forage as expressed by its Nutritive Value Index (J. Animal Sci., 19:538. 1960.). A 1-g forage sample was placed in a 250-ml screw-cap centrifuge bottle containing 75 ml of potassium hydrogen phthalate buffer solution (pH 4) in which was dissolved either 100 mg of Cellulase 36 (Rohn and Haas) or 100 mg of Cellulase 36 + 150 mg pepsin (1:10,000). Bottles containing only forage sample and buffer were used as controls. The samples were agitated 24 hr in an A.O.A.C. pepsin digester (modified to hold the 250-ml bottles) placed in an incubator held at 40 C. Following digestion, the bottles were centrifuged at 1,900 rpm for 10 min and the residues transferred to preweighed Selas filtering crucibles for dry matter determinations. The ultimate usefulness of this technique is indicated by the following highly significant correlations found between the Nutritive Value Indices of 14 forages as determined in vivo with sheep and per cent dry matter disappearance with (1) buffer alone, 0.91; (2) cellulase, 0.92; and (3) cellulase + pepsin, 0.93.

P3. Estimation of forage protein digestibility and determination of the effects of heat-drying upon forages by means of the nitrogen content of acid-detergent fiber. P. J. VAN SOEST, Dairy Cattle Research Branch, Beltsville, Maryland.

Studies have been conducted on the composition of the residue obtained by refluxing forage material with 2% hexadecyltrimethylammonium bromide in 1 N H_2SO_4 for 1 hr. Results on unheated forages reveal that this residue, termed acid-detergent fiber, contains 2-20% of the total forage nitrogen. This nitrogen appears to have a low in vivo digestibility. The proportion of plant nitrogen dissolved by the acid-detergent reagent on 16 forages is highly associated with digestible nitrogen ($r = +0.94$). It appeared to be a much better indicator of nitrogen digestibility than the forage nitrogen content ($r = +0.81$).

Laboratory studies show that while many factors are involved, such as moisture and acidity, heat-drying forage below 80 C has only slight effects on the detergent solubility of the forage protein. However, this effect is of analytical significance for fiber and lignin. At higher temperatures under certain conditions there can be a serious effect on analytical results and a masked decline in in vivo digestibility.

The damage to forage protein appears to involve the nonenzymic browning reaction. Water is essential for the reaction which involves the condensation of carbohydrate degradation products with protein. The detergent solubility of the protein is a valuable tool for determining heat effects.

P4. Relationship between chemical composition of orchardgrass forage and the chemical quality of the resulting silage. W. C. JACOBSON AND H. G. WISEMAN, Dairy Cattle Research Branch, Beltsville, Maryland.

Liberal application of nitrogen fertilizers to forage grasses may double the crude protein content. This increases the difficulty of converting forage into good silage. The quantitative aspects of the sugar-protein ratio to chemical quality was investigated in 1959-1960. Forty-six lots of ground orchardgrass were ensiled in quart jars equipped with traps for carbon dioxide collection. Chemical analyses were made on the forage as ensiled and on the resulting silage after 30 days of storage. The range of these various determinations was as follows: dry matter, 13.7 to 32.8%; crude protein, 8.2 to 34.8%; total sugars, 0.97 to 11.3%; pH, 3.75 to 5.95. The following correlation coefficients were calculated: protein with pH +.567, sugars with pH −.282. The ratio of sugar to crude protein × 100 for the forages was plotted against the pH of the silage. The critical value of the sugar-protein ratio versus silage pH was 35. All forages which had a sugar-protein ratio greater than 35 made silages with pH values lower than 4.00; with smaller ratios the pH values were extremely variable, but generally above pH 4.0. This suggests that good-quality silage can be made if the sugar-protein ratio of the forage is adjusted so that it is greater than 35.

P5. Storage losses, chemical quality, and feeding value of low-moisture silage stored in conventional silos. J. C. DERBYSHIRE,* C. H. GORDON, H. G. WISEMAN, AND C. G. MELIN, Animal Husbandry Research Division, and J. R. MCCALMONT, Agricultural Engineering Research Division, USDA, Beltsville, Maryland.

First-cutting alfalfa was mowed, conditioned, and harvested May 19-21, 1960, as baled, barn-dried hay or stored as silage in two 10 by 40 ft upright tile silos containing up to 65% dry matter (avg 42%). Silages were chopped (¼-inch theoretical), tramped, topped with two loads of unwilted forage, and sealed with weighted plastic. Silo 1 received the additional sealing precautions of rubber gaskets around silo doors, plus a load of unwilted forage on top of each day's filling. Data indicating excellent dry matter preservation for both Silos 1 and 2, respectively, were: per cent fed, 91.2, 95.4; per cent spoiled,

1.3, 3.0; and average temperature, 92, 93 F. Values suggesting a limited fermentation of low-moisture material in the respective silos were: pH, 4.8, 4.6; ammoniacal nitrogen as per cent of total nitrogen, 11.8, 10.8; butyric acid, 0.7, 0.1; acetic acid, 2.9, 2.4; and lactic acid, 4.7, 5.2.

Forages were fed to milking cows in a 120-day switchback trial, to sheep in a cross-over digestion trial, and to heifers in a 120-day continuous trial. A highly significant positive correlation ($r = 0.86$) existed between dry matter consumed by heifers and per cent dry matter in the silage (range 29 to 68%). Silage was equal or superior to hay with respect to dry matter intake, milk production, body weight gains, and energy digestibility.

Similar results are being obtained in subsequent trials with the 1961 crop.

P6. Effect of nitrogen fertilization on chemical quality and feeding value of orchardgrass silage. C. H. GORDON, J. C. DERBYSHIRE, E. A. KANE, W. C. JACOBSON, AND C. G. MELIN, Dairy Cattle Research Branch, Beltsville, Maryland.

First-cutting orchardgrass containing 23% crude protein was harvested May 15-17, 1961, from areas fertilized with 400 lb ammonium nitrate per acre on April 27 (+N). Unfertilized control forage (−N) contained 13% crude protein. Forages were direct-cut harvested and stored in upright silos. Resulting silages were fed as the only forage to milking cows in a switch-back trial and to sheep in a cross-over digestion trial. Average daily values obtained from the cows for −N and +N silages, respectively, were: FCM 18.8 and 16.5 lb per cow; silage dry matter consumed 1.78 and 1.37 lb per hundred pounds liveweight; liveweight change +1.0 and −1.4 lb per cow. These differences were statistically significant ($P < 0.01$). Dry matter digestibilities in the respective silages were 63.8 and 65.6%.

Chemical quality of both silages was poor, but +N silage had distinctly higher values for pH (5.4 vs. 5.0), butyric acid (7.3 vs. 5.0), acetic acid (7.9 vs. 5.3), and ammoniacal nitrogen as per cent of total nitrogen (51.7 vs. 39.0).

A similar procedure in 1960 resulted in silages of 14 and 24% crude protein, but with no apparent difference in feeding value, although the same type of chemical quality distinctions was common to both years. An earlier 1960 harvest date (May 5-6), as well as year-to-year differences in chemical composition may have contributed to the year-by-treatment interaction. The results of 1961 show that nitrogen fertilization may produce a silage of poorer chemical quality and lower feeding value.

P7. Palatability studies of organic acid mixtures characteristic of good- and poor-quality grass silages. L. L. RUSOFF AND P. F. RANDEL, Louisiana State University, Baton Rouge.

Twelve dairy heifers and four dairy steers were used to test the palatability of mixtures of the major organic acids usually present in good-quality grass silage (71.1% lactic, 23.1% acetic, 3.8% succinic, and 2.0% propionic acids) and in poor-quality silage (46.1% butyric, 36.5% acetic, 9.6% propionic, 5.8% succinic, and 2% lactic acids). Four preference trials using four different animals per trial with six-day experimental periods were conducted. Each animal had a choice of low-quality hay sprinkled with the two organic acid mixtures or water. More hay treated with the mixture of organic acids typical of poor-quality silage was consumed than hay treated with the mixture of organic acids typical of good-quality silage or water. The differences among consumptions of the three treated hays and between the hays treated with acid mixtures were highly significant ($P < .01$). Any decrease in palatability of poor-quality silage does not appear to be due to its characteristic content of volatile fatty acids, especially butyric, but to some other constituents.

P8. Effectiveness of sealed storage and enzymes on alfalfa-brome haylage preservation and feeding value. S. PERRY AND H. VOELKER, South Dakota State College, Brookings.

Alfalfa-brome haylage, 36-46% moisture, was preserved in two concrete stave silos and in two sealed storage units and fed experimentally during the next 84 days to four groups of six cows each, and four groups of ten heifers each. An enzyme culture of *Aspergillus oryzae* was added at 0.5% to one concrete and one sealed unit as filled. Results (all not statistically significant) respectively for sealed, enzyme, sealed, no enzyme, unsealed, enzyme, unsealed no enzyme were: pounds haylage dry matter consumed per cow daily, 38.9, 35.8, 35.3, 36.4; average pounds liveweight gain in 84 days, 83, 60, 75, 61; 4% F.C.M. daily, 34.7, 32.4, 34.1, 32.1; per cent persistency of production, 92.5, 91.6, 91.3, 93.0; average pounds dry matter consumed by heifers daily, 20.2, 20.1, 19.2, 19.9; average dry gains, 1.81, 1.93, 2.17, 1.90, with no significant differences in any observations on the heifers. The pH of enzyme haylages dropped slightly faster than untreated haylages, but all reached pH 5.6 at 5 wk. Lactic acid was predominant in all haylages, acetic moderate, with no butyric. Temperatures of unsealed untreated haylage reached 125 F two days after ensiling; the enzyme, unsealed haylage, 115 F. Temperatures rose negligibly in sealed units.

P9. Storage losses and feeding value of red clover haylage and direct-cut silage. W. H. CLONINGER* AND E. M. KESLER, Pennsylvania State University, University Park.

During two seasons, first-cutting red clover was harvested as haylage or direct-cut silage and stored in gas-tight steel silos. In 1960, the haylage averaged 49.1% moisture as stored, the silage, 76.6%. In 1961 the percentages were 45.3 and 79.5, respectively. The silos, size 14 by 45 ft, were completely filled each year. In 1960 34.4 tons of D.M. were stored in the form of haylage, in 1961, 34.6 tons. Comparable tons of silage D.M. were 35.8 and 29.4. In 1960, losses due to fermentation and seepage were 0.5% of the haylage D.M. and 18.5% of the silage. Respective top spoilage losses were 8.4 and 1.9%. The forages were fed to two groups of ten lactating cows in cross-over trials. Haylage or silage was offered ad libitum and constituted over 50% of the nutrient intake. Limited amounts of hay were fed, plus concentrates according to production. In 1960 no differences in milk production, body weight change, or nutrient intake were noted. The second trial is in progress.

P10. Effect of moisture content on the value of alfalfa-cracked corn silage for lactating cows. W. T. HOWARD* AND F. G. OWEN, University of Nebraska, Lincoln.

Silage moisture levels of 68% (H), 53% (M), and 47% (L) were obtained by field-wilting of alfalfa before mixing with corn and ensiling. Cracked corn was added to constitute about 50% of the dry matter in the silage. Twelve Holstein cows were used in a switch-back design with 21-day periods. Cows were assigned to two equal blocks of the design. One block received an alfalfa-corn pellet supplement with the silage; the other block received no pellets. In addition, experimental animals received only water and minerals. Digestibilities were determined by the total collection technique.

There were significant differences in performances for (H), (M), and (L) silages, respectively, in: daily milk yield, 49.2, 52.5, and 53.6 lb; milk fat, 3.73, 3.42, and 3.31%; and daily dry matter intake, 28.5, 40.5, and 42.3 lb. Daily body weight gains were: .02 (H), 1.56 (M), and 0.72 (L) lb. Dry matter digestibilities and daily FCM production were practically equal among silage moisture treatments. Supplementation with pellets significantly increased weight gains and decreased milk fat per cent, but did not affect, significantly, FCM, dry matter consumption, or dry matter digestibility.

P11. Effect of stage of maturity on the digestibility of sorghum silages. J. W. KUHLMAN AND F. G. OWEN,* University of Nebraska, Lincoln.

Atlas and Rox sorghum each was harvested at the milk, medium-dough, and hard-dough stages and ensiled in sealed metal drums. Digestibilities were determined by the Cr_2O_3, grab-sample technique, using Holstein heifers. Calves received 20 lb of silage and 3 lb of a corn-soybean oil meal concentrate (containing Cr_2O_3) daily. The experimental design consisted of a balanced pair of 3 × 3 Latin squares for each sorghum variety. Ration digestibilities for the milk, medium-dough, and hard-dough stages, respectively, were: dry matter, Atlas, 61, 56, and 52%; Rox, 67, 67, and 66%; energy, Atlas, 63, 58, and 56%; Rox, 68, 68, and 67%; and crude protein, Atlas, 56, 57, and 45%; Rox 60, 58, and 63%. Differences among maturities were significant for the digestibility of dry matter and crude protein of Atlas.

Removal of the influence of the concentrate fed, by calculation, reduced the digestibility values, but the relationships were similar to those presented above for the entire ration.

P12. Chemical, bacteriological, and nutritive value of high-moisture corn. W. G. SCHMUTZ,* R. S. EMERY, AND D. CARPENTER, Michigan State University, East Lansing.

Twenty-six experimental silos, 5 by 7 or 7 by 10 ft, were filled with corn at 24, 32, or 45% moisture, obtained by adding water or picking at the desired moisture Urea, $CaH_4(PO_4)_2 \cdot H_2O$, or H_3PO_4 were sometimes added. Lactobacilli and anaerobes were about 10^9/ml after ten days. Yeasts gradually increased to 10^7/ml for the wetter and 10^8/ml for the drier silages at 60 days. Urea was 50% degraded within 20 days and 80% within 60 days. Ethanol averaged 0.2%; treatment effects were minor. Lactic plus acetic acid concentrations for medium and higher moisture silage were 18 and 100 μM/g, respectively, and for 1% $CaH_4(PO_4)_2 \cdot H_2O$ vs. none, these concentrations were 47 and 59 μM/g, respectively. Acetic accounted for one-third and two-thirds of the total acid. Calcium phosphate and higher moisture depressed pH ($P < .01$). Weight gains tended to increase with lactic acid concentrations. The mean gains in pounds/day and feed efficiency (dry matter/pound gain in brackets), for the dry corn, medium, and wet silages were 2.2 (5.3), 2.0 (6.1), and 2.1 (4.4), respectively. Significance ($P < 0.05$) was attained for moisture level in only one of the three trials. Calcium phosphate increased gain 19% ($P = 0.08$).

P13. Organic solvents for forage preservation. R. O. THOMAS,* L. D. BROWN, AND R. S. EMERY, Michigan State University, East Lansing.

Early-bloom alfalfa was ensiled in six 10- by 40-ft concrete stave silos with the following treatments: Silo 1, control; Silo 2, 0.15% formalin + 0.5% acetone; Silo 3, wilted to 50% DM; Silo 4, 0.15% formalin; Silo 5, 0.25% chloroform and Silo 6, 0.5% ethanol. Core samples were taken approximately ten days after ensiling, to determine any apparent

difference in fermentation due to treatment. The comparative feeding value of the silages was determined by heifer growth studies. Fifty-six heifers (average weight 499 lb) were divided into seven comparable groups on the basis of breed and body weight and randomly assigned to one of the six silages or hay for 87 days. No supplemental feed was offered. The average daily gains and dry matter consumption values were 1.73, 13.9; 2.01, 15.9; 2.16, 17.6; 1.83, 14.7; 1.91, 14.2; 1.67, 13.5; 1.73, 15.1 lb for silo groups 1, 2, 3, 4, 5, and 6 and the hay group, respectively. In the same order, the pH and DM percentages were 5.0, 22.8; 4.3, 23.9; 4.7, 53.3; 4.5, 24.9; 4.8, 24.3; 4.5, 24.2; and ....., 83.4. Differences in DM consumption among treatment groups were significant ($P < 0.01$).

P14. Comparison of quality of hay harvested by various methods as measured by chemical composition, physical properties, and animal acceptability. D. W. WITTNAM, H. L. PORTZ,* AND H. F. BENSON, Southern Illinois University, Carbondale.

Four methods of harvesting were used on forage mixtures containing Vernal and Buffalo alfalfa; mowed-uncrushed, mowed-crushed, rotary-flailed, and cylinder-flailed. Second-crop alfalfa was baled and compared by a modified crude fiber test as used by Meyer and Lofgreen of California, to predict apparent digestibility. Samples were visually graded by a Federal hay grader and by the authors, the latter using a revised Hay Score Card based on one developed by Werner of Wisconsin. Lactating dairy animals were used to determine acceptability of conditioned hays.

Chemical analyses of hay samples showed: (1) Vernal alfalfa was slightly superior to Buffalo alfalfa in calculated TDN; (2) mowed-uncrushed and mowed-crushed averaged from 3 to 5% higher in TDN than the cylinder-flailed, the apparent digestibility of rotary-flailed hay slightly exceeded hay conditioned by other methods; (3) pelleted samples averaged 2.5% higher in calculated TDN than the unpelleted samples. Visual estimates of quality indicated the Hay Score Card method was similar to U. S. federal grading; however, both methods underestimated the value of rotary-flailed hay.

When field drying time was considered, the mowed-crushed hay reached baling moisture from 3 to 5 hr sooner than any other method.

P15. Remote observations of temperature by implant telemetry. VEARL R. SMITH* AND HOWARD A. BALDWIN, University of Arizona, Tucson.

The transmitter used operates in a V.H.F. range between 95-100 mc. The device is sensitive and is capable of monitoring temperature change of 0.1 of a degree C. The sensor-transmitter is a temperature-dependent multivibrator circuit. The tone generated by this circuit varies 200 cycles per 1 C. The circuit is relatively insensitive to power supply and can be operated from a single mercury cell. The batteries presently used provide power for continuous operation of the sensor-transmitter for about 36 hr.

The receiver recorder system is designed to detect and display the modulation signal continuously on a small recorder. The signal is received and detected by a commercially available FM receiver.

The vagina is presently used as a site of implant. Exercising by walking at an atmospheric temperature of 60 F for 30 min caused an increase of internal temperature of 1 C. The temperature returned to pre-exercising levels in approximately 45 min after cessation of exercise.

Spraying with tap water at a temperature of 70 F caused an initial rise in temperature of 0.3 C and a decrease in temperature to below normal in approximately 30 min.

P16. Intraruminal temperature changes as recorded by radio telemetry. A. E. DRACY* AND J. R. JAHN, South Dakota State Agriculture Experiment Station, Brookings.

A radio transmitter was constructed small enough to pass through the esophagus of a mature sheep. The transmitter was weighted to insure its location in the reticulum. The unit operated over a frequency of 300-500 kilocycles.

Recordings indicated that the reticular temperature ranged between 102 and 103 F for the resting sheep.

Measured quantities of water, at known temperatures, were given free choice. Regardless of the temperature of the water, within minutes after drinking the reticular temperature dropped to its minimum and then started on a slow return to normal. When water of 5 C was administered the ruminal temperature dropped from 102.8 to 92.7 F within 15 min. It remained at this temperature 3 min, then started to rise and in 70 min it had reached a temperature of 103.1 F. Water at 29 C initiated a temperature drop from 103 to 99.3 F within 15 min. The temperature then rose in the following 50 min to 103.1 F. When water at 22.5 C was introduced into the rumen at 102.5 F the temperature dropped within 18 min to 97.5 F and returned to 102.1 F within 52 min.

P17. Use of electrocardiographic radio telemetry, in ruminants, to determine the heart rate before, during, and after parturition. J. R. JAHN* AND A. E. DRACY, South Dakota State College, Brookings.

A transistorized, implantable transmitter was constructed. Two stainless steel electrodes extended from the unit, a predetermined length, depending on the size of the thoracic cage of the animal. A 1.3-v mercury battery

furnished power for the transmitter. The unit, excluding the contact ends of the electrodes, was dipped four times in a liquid plastic and allowed to dry to insure against the entrance of body fluids. The transmitter was surgically implanted subcutaneously in a pregnant Holstein cow in the depression posterior to the left shoulder. The right electrode extended dorsally over the spinal column to a point behind the right shoulder, the left electrode extended down behind the left leg on the chest. Using a loop antenna, the signal from the transmitter was picked up by a converted BC-453-B Signal Corps receiver and transferred through a frequency meter to an electrocardiographic recorder. Recordings were taken pre- and post-partum and were compared with the heart rate recorded during the actual parturition. The average heart rate over a four-day period prepartum was 92.6 beats, the average heart rate over a 13-day period post-partum was 66.2 beats and the high reached at parturition was 144 beats per minute.

P18. Nucleic acid content of mammary glands of virgin, pregnant, and lactating rats. H. A. TUCKER* AND R. P. REECE, New Jersey Agricultural Experiment Station, New Brunswick.

Deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) determinations, which estimate growth and metabolic activity, respectively, were made on mammary glands of virgin, pregnant, and lactating rats. During the first four days of pregnancy little if any mammary growth occurred, but by Day 8 of pregnancy development had increased 20.2% over virgin controls. Proliferation of the gland continued throughout pregnancy and by 20 days of pregnancy DNA had increased 184.8% over virgin glands. The increase in cell numbers from the 20th day of pregnancy to Day 1 of lactation was only 9.8%, whereas the increase from Day 1 of lactation to Day 4 of lactation was 25.9%. Maximum mammary development occurred at eight days of lactation. Little involution was noted until Day 24 of lactation. Total milligrams DNA and total milligrams RNA increased proportionately during pregnancy, but with the onset of lactation RNA increased much more rapidly than did DNA. Throughout pregnancy there was a gradual increase in the RNA/DNA ratio. The RNA/DNA ratio increased rapidly during lactation and it is proposed that this curve represent the normal lactation curve of the rat which attained its maximum at Day 21 of lactation.

P19. Production of anti-tadpole antibody in the bovine mammary gland and in the rabbit. G. D. MARX* AND E. V. CARUOLO, University of Minnesota, St. Paul.

All tadpoles were produced by artificially induced ovulation and subsequent fertilization. An injectable antigen of eight-day-old tadpoles was prepared by blending, alternate freezing and thawing, and filtration. Rabbits were injected intravenously and subcutaneously; cows were infused intramammarily. Five injections were made at four-day intervals. Rabbit blood serum and milk gamma-globulin (prepared by alcohol precipitation and subsequent freeze-drying) served as the sources of antibody. Specific and nonspecific rabbit serum and milk gamma-globulin was diluted with pond water containing antibiotic. The final dilutions represented were 1:25, 1:50, and 1:75. Trial I used seven-day-old and Trial II used 35-day-old tadpoles.

The 1:75 dilutions of specific rabbit blood sera and milk gamma-globulin caused an 80% death loss in Trial I. Five per cent died in the control group. Trial II tadpoles were resistant to the 1:25 dilution of milk gamma-globulin; however, the same dilution of rabbit sera caused 100% mortality with no control loss. All deaths occurred within 24 hr. An agglutinated mass surrounding each tadpole at death suggested respiratory involvement. Oxygen utilization was measured by a Warburg respirometer. The results indicate progressive decrease in oxygen uptake when incubated with specific rabbit anti-sera.

P20. Determination of total body water using tritiated water. P. W. ASCHBACHER,* T. H. KAMAL, AND R. G. CRAGLE, UT-AEC Agricultural Research Laboratory, Oak Ridge, Tennessee.

Four dairy cows (two lactating) were injected with 10 mc of tritiated water per 1,000 lb body weight. Blood samples were collected at 8 AM, 2 PM, and 8 PM for ten days. The radioactivity in the samples was assayed with a liquid scintillation spectrometer. Blood was prepared for counting by precipitating the proteins from plasma with equal volumes of trichloroacetic acid. Standards were assayed by adding an aliquot of the dosing solution to plasma-trichloroacetic acid filtrate from animals not receiving radioisotopes.

A plot of log activity against water intake was linear over a five-day period. This indicates that the tritiated water was in equilibrium with total body water. Linear regressions were fitted to these points and extrapolated to zero water intake. A ratio of activity in blood at zero water intake compared to total activity injected was used to calculate the body water estimates of 69.8, 69.0, 67.6, and 71.9, expressed as per cent body weight.

P21. Chemical and physiological properties of the cervical and uterine fluids of repeat and normal breeding cows during estrus. H. C. GUPTA, C. BRANTON,* D. L. EVANS, AND W. H. WATERS, Louisiana State University, Baton Rouge.

After developing a suitable technique for collecting cervical and uterine fluids from intact cows during estrus, 61 samples, including 40 cervical and 21 uterine secretions, were obtained for analyses from 38 dairy cows in the University herd. The average pH values for the cervical and uterine fluids were 7.81 and 7.91, respectively. The average concentrations in milligrams per 100 ml of the various constituents in cervical and uterine fluids, respectively, were as follows: Na—315.8 and 371.7; K—75.6 and 77.5; Ca—10.9 and 17.3; Mg—2.7 and 2.4; inorganic P—3.1 and 6.6; reducing sugars—44.3 and 41.8, and protein N—97.0 and 497.0. The fluids from repeat breeding cows had higher concentrations of electrolytes than did the fluids from normal cows.

Chromatographic studies indicated the presence of complex polysaccharides (muco-polysaccharides) in both types of luminal fluids. The presence of simple sugars could not be demonstrated.

Both the cervical and uterine fluids showed spermatozoan-preservation capacity which varied among the fluids. However, fluids from repeat-breeding cows tended to have lower spermatozoan-preservation capacity than did the fluids from normal cows.

P22. Effect of artificial light, temperature, and humidity on physiological response of dairy bulls. J. D. ROUSSEL,* T. E. PATRICK, H. C. KELLGREN, AND J. O. SHELWICK, Louisiana State University, Baton Rouge.

Twelve mature dairy bulls were employed at random into three groups to evaluate the effects of controlled artificial light, temperature, and humidity on semen characteristics and physiological response. Bulls in Group 1 were housed in one-half of a large barn (control) and Group 2 was housed in the remaining half of the barn with the addition of control artificial light. Bulls in Group 3 were housed in an environmental chamber at 64 F, 15 mm Hg vapor pressure and control artificial light. Animals in Groups 2 and 3 were exposed to three periods of light which were 14, 15, and 16 hr for 5, 6, and 6 wk, respectively.

The corrected mean progressively motile spermatozoa values for Groups 1, 2, and 3 were, respectively, 46.1, 49.3, and 50.1% ($P = <0.01$) and between periods values were 50.1, 49.0, and 46.7%, respectively ($P = <0.05$). Also, significant differences ($P = <0.01$) between groups were obtained on percentage of morophologically abnormal spermatozoa, livability of spermatozoa, and methylene blue reduction time (modified). No significant differences ($P = >0.05$) were observed on concentration of spermatozoa and volume of semen. A higher percentage of shippable ejaculates was attained in favor of Group 3 over 2 and 1, with Group 2 being better than 1.

Protein-bound iodine for these periods (14, 15, and 16 hr light) showed a downward trend at 15 hr of light, with a return to the same for 14 and 16 hr of light. Artificial light caused a decrease in respiration rates in bulls at the ($P = <0.01$). However, there was no significant difference ($P = >0.05$) in body temperature.

P23. Effect of thermal conditions on adrenocortical, thyroidal, and metabolic response of dairy heifers. R. D. THOMPSON,* J. E. JOHNSTON, C. P. BREIDENSTEIN, A. J. GUIDRY, AND W. T. BURNETT, Louisiana State University, Baton Rouge.

Adrenocortical, thyroid, and metabolic response was studied using ten yearling Holstein heifers for 48 days under cool conditions (40–65 F) and for 72 days under controlled hot conditions (75–90 F). The mean plasma level of 17-hydroxycorticosterone increased from 1.7 μg per cent during cool conditions to 4.5 during heat exposure. A temporary eosinophilia occurred immediately upon exposing the animals to heat, which was indicative of an adrenocortical insufficiency. The eosinophilic condition rapidly disappeared and was not followed by an eosinopenia. Serum PBI, thyroxine utilization ($-\log_e b \times 10^{-3}$), and secretion rates (mg/cwt/day) were significantly lower for hot conditions. The mean daily weight gain (pounds) declined from 1.8 to 1.1 for cool and hot conditions, respectively, with the most marked reductions occurring during the second and sixth 12-day period of hot conditions. Rate of weight gain and dry matter intake were highly correlated. Respiration rate and body temperature showed highly significant adverse effects from hot conditions, but were not highly correlated with rate of weight gain. Heat production (kilocalories/cwt$^{.72}$/hr) was highly correlated with serum PBI and tyroxine secretion rate. Measures of adrenocortical response were not significantly associated with any measure of thyroid activity.

P24. Physical factors affecting thermal insulation properties of hair coats of dairy cattle. I. L. BERRY,* Agricultural Engineering Research Division, USDA, Beltsville, Maryland; M. D. SHANKLIN AND H. D. JOHNSON, University of Missouri, Columbia.

Methods and techniques for measuring the thermal insulation and physical properties of hair coats of live dairy animals were devised. The hair-coat weight, depth, bulk density, hair diameter, hair length, hair orientation, and the numerical density of hair were correlated with the hair insulation to determine their effect on the thermal properties of hair coats.

Preliminary data indicated that the total hair-coat insulation of dairy calves was directly and linearly related to the weight of hair per unit area of skin. Further correlations, with more thorough measurements of the physical hair characteristics, indicated that hair

orientation, as approximated by the ratio of hair depth to hair length, affected hair-coat conductivity more than did the other measured variables.

Increasing ratios of hair depth to hair length were associated with decreasing insulation for unit thicknesses of hair and increasing insulation for the entire hair coat. Increasing numbers of hairs per unit area were associated with increasing heat transfer, which indicated that heat transfer along the hair fibers was a major portion of the total heat transferred. The factors of bulk density and hair diameter did not appear to significantly affect hair conductivity.

P25. Causes of fertilization failure in repeat-breeding cows. A. P. GRADEN,* D. OLDS, C. R. MOCHOW, AND J. R. ROONEY, University of Kentucky, Lexington.

Among 101 cows bred early or late in the heat period, 35.6% had fertilized ova when slaughtered three days later. Of the 65 cases of fertilization failure, 27.7% had mislocated (lost in peritoneal cavity?) ova, 18.5% had oviduct obstructions, 15.4% failed to ovulate, 6.2% produced abnormal ova, and 33.8% could not be explained. Considering only the 63 cows from which ova were recovered, cows bred early appeared to have about ten percentage units lower fertility (not significant). More ova appeared to be lost among cows bred twice (early and late). Histological sections of uterine tissue revealed that about 45% appeared to be normal. Among the abnormalities, elastosis of mucosal arteries occurred in 31.8%, cystic glands in 18.2%, and mucosal atrophy in 9.1%. Endometritis was found in only 1.5%. While numbers are still small, cows with histologically normal uteri appeared to have a higher fertilization rate (71.4%) than those having abnormalities (43.8%). Unfertilized ova had outside diameters of 181.6 $\pm$ 21.8 μ. The zonas averaged 13.0 $\pm$ 1 and the vitelli 122.2 $\pm$ 6.3 μ. Fertilized ova became smaller as the number of segments increased (165 μ for eight-cell ova). It was found that vitelli shrink and swell quite readily with osmotic pressure changes.

P26. Progestins in ovarian and peripheral blood of cows during late pregnancy. W. R. GOMES,* O. L. FROST, AND V. L. ESTERGREEN, JR., Washington State University, Pullman.

Progesterone and Δ^4-pregnene-20-β-ol-3-one (20-β-ol) were estimated in ovarian vein and peripheral blood of cows pregnant 250 to 282 days, using a modification of the method of Short (J. Endocrinol., 16:415. 1958.). Recovery was determined with progesterone-4-C^{14}.

For three cows pregnant 250-254 days, whole blood from the ovaries with the corpus luteum averaged 2.2 μg progesterone/milliliter. Two plasma samples obtained during the same period averaged 3.9 μg progesterone/milliliter and in one of these 0.5 μg 20-β-ol/milliliter was also detected. Progestins were not detected (< 2.0 μg/ml) in two other ovarian venous plasma samples. Blood from the ovary of a cow pregnant 282 days contained 1.2 μg progesterone/milliliter of whole blood and 1.4 μg progesterone and 1.3 μg 20-β-ol per milliliter of plasma. No progestin was detected in the blood leaving the ovary without a corpus luteum of another cow pregnant 281 days at the time of surgery.

Peripheral blood plasma from two cows pregnant 250 and 251 days averaged 0.0092 μg/progesterone/milliliter. No progestin was detected in as much as 1,400 ml of peripheral plasma taken within 1 hr after ovariectomy from four cows at 250-254 days of pregnancy.

P27. Relationship of some blood constituents to heat stress in cattle. J. R. WELDY,* University of Maryland, College Park; and R. E. MCDOWELL AND P. J. VANSOEST, Dairy Cattle Research Branch, USDA, Beltsville, Maryland.

To determine the relation of blood ketones, blood glucose, and blood cell volume to changes in rectal temperature under heat stress, 12 mature nonlactating Holsteins fed at 115% of maintenance were paired by weight and placed on experiment in October. One of each pair was kept in a psychrometric chamber for alternating 2-wk periods at 90 and 70 F with 60% relative humidity, while pairmates were kept under prevailing ambient temperatures (40-78 F).

All three blood constituents declined during the first week at 90, rose the second week, and returned to the initial levels in the 70-degree interim period. There was no clear evidence of carry-over effects from the 90-degree periods. The values for the controls showed no significant changes during the trial. The interrelationships of the blood constituents were consistently low ($r = -.16$ to $.01$) and only blood cell volume showed a significant correlation ($-.21$) with rectal temperature. In other studies with Angus heifers kept at 100 F and Holstein and Brown Swiss cows at 90 F for 7 wk, similar results were obtained.

P28. Some changes in digestive physiology of the bovine associated with various feeding frequencies. L. D. SATTER* AND B. R. BAUMGARDT, University of Wisconsin, Madison.

Three mature fistulated cows were fed high-quality chopped alfalfa hay 2×, 4×, and 8× daily in a 3 × 3 Latin-square designed experiment. Feed intake was fixed throughout the experiment at 90% of ad libitum consumption (as determined in a preliminary period) to insure immediate consumption of feed. Digestion coefficients were determined from a ten-day collection period using the Cr_2O_3 indicator method, and urine was collected with catheters during a five-day period. Chromic oxide impregnated in paper was administered

twice daily through the rumen fistula, and rumen samples were taken every 2 hr during a 24-hr period.

A nonsignificant trend for increased digestibility of dry matter, energy, and nitrogen with more frequent feeding was noted. Increased feeding frequency increased nitrogen retention ($P < 0.05$) and decreased the amount of nitrogen excreted in the urine. Relative amounts of VFA, rumen pH, and fecal Cr_2O_3 excretion patterns were not affected by feeding frequency. Fecal Cr_2O_3 content varied between 91.0-114.2% of the mean excretion rate for all feeding frequencies. Animals fed more frequently had slightly higher average ammonia and VFA concentrations. Ruminal ammonia and VFA levels, as well as pH values, fluctuated much less when animals were frequently fed.

P29. Effect of crossbreeding on milk production of dual-purpose cattle. V. C. BEAL, JR.* AND T. G. MARTIN, Purdue University Agricultural Experiment Station, Lafayette, Indiana.

Three breeds of dual-purpose cattle were mated to produce all possible types of purebred and crossbred progeny. The three purebreeds were Red Dane, Red Poll, and Milking Shorthorn. There were three two-breed crossbred groups and three three-breed crossbred groups.

Milk production records were recorded on all cows. This study includes 246 lactation records from 1954 through 1961.

The data were analyzed using the method of least squares with unequal subclass numbers to provide unbiased estimates of the effects of breed of dam, breed of sire, sire in breed, breed of dam by breed of sire and type of dam (purebred or crossbred) on total milk production, persistency, and part-lactation production.

For total production, breed of dam, breed of sire, and the breed of sire by breed of dam interaction, which measures the effect of crossbreeding, were all significant at the 1% level. Sire in breed was also significant at the 1% level. The effect of type of dam was not significant.

P30. Effect of inbreeding on production in different lactations. R. F. GAALAAS,* W. R. HARVEY, AND R. D. PLOWMAN, Dairy Cattle Research Branch, and Biometrical Services, USDA, Beltsville, Maryland.

Multiple covariance analyses were made using 111 cows having four lactation records. These were daughters of six sires. The first four lactation records and the average of these four were analyzed separately. Variables were inbreeding of the cow, inbreeding of her dam, and production.

Reduction in sums of squares due to inbreeding of the dam was small and nonsignificant. Reduction in sums of squares due to inbreeding of the cow accounted for most of the total reduction, but was significant ($P < 5\%$) for first lactation milk and fat only.

Simple covariance analyses were also made of these data. Intra-sire regression of pounds milk per 1% inbreeding of cow were: −105.3, −41.9, −18.0, −26.2, and −47.9 for 1st, 2nd, 3rd, 4th, and average records, respectively. Corresponding regressions of pounds fat were: −3.62, −1.06, −1.32, −0.86, and −1.69. Simple correlation coefficients for milk and fat in the first lactation were highly significant ($P < 1\%$), but none of the other correlations was significant.

No significant differences between sire regressions (linear) nor between different lactations within sires were found.

P31. Interaction of genotype and environment in sire evaluation. L. D. VANVLECK, Cornell University, Ithaca, New York.

First- and second-lactation records of artificially sired Holstein daughters were grouped according to level of production of their stablemates. The four stablemate levels were (1) greater than 1,000 lb milk above season average, (2) between season average and 1,000 lb plus, (3) between season average and 1,000 lb minus, and (4) more than 1,000 lb below season average. A total of 45,876 first lactation and 39,261 second lactation records were included. An analysis of deviations of daughter records from stablemate averages among and within sire groups was used to estimate genetic variances in each of the four environments. Heritability estimates of milk yield for first-lactation records were .28, .28, .25, and .19 for high to low herd levels, respectively. Corresponding estimates for second lactation records were .29, .26, .21, and .19, again from high to low levels. Heritability estimates of fat yield were similar. The sire component of variance in low-level herds was about half that found in high-level herds. The residual component of variance was about 25% lower in low-level than in high-level herds. Both sire and residual components of variance were greater in second-lactation records than in first-lactation records.

P32. Estimating improvement in genetic merit for lactation yield when selecting on production in early lactation. S. R. SEARLE, New Zealand Dairy Production and Marketing Board, Wellington.

The rate of progress in improving additive genetic merit for lactation yield when selecting on a part record, relative to the rate of progress when selecting on lactation yield itself, is measured by the genetic correlation between part record and lactation yield, multiplied by the square root of the ratio of the heritabilities. A study of the milk fat yield of 2,252 daughter-dam pairs in 654 herds indicates that the

first one, two, and three months of lactation are 26, 43, and 41% efficient in this respect, in selecting for lactation yield. These values are noticeably less than those previously reported.

The heritability of lactation yield is estimated as 0.12 and that of production in the first one, two, and three months as 0.11, 0.10, and 0.09 with standard error 0.05. The genetic correlation estimates have standard error 0.30 resulting, in part, from the low heritability values. These, together with the relatively large standard errors, may mean that the estimated selection efficiency differs markedly from its true value. Extremely large quantities of data may rectify this situation, but otherwise the discrepancy between estimated and true values may be quite large. Decisions based on estimated values, therefore, require careful consideration.

P33. Analysis of variation in some factors affecting multiple ovulations in Holstein cattle. A. P. LABHSETWAR,* W. H. TYLER, AND L. E. CASIDA, University of Wisconsin, Madison.

A total of 3,076 ovulations determined by palpation in 728 animals covering six sire lines, two systems of mating outbreds and inbreds, with inbreeding coefficient not exceeding 40%), and three parities were analyzed. The study was limited to observations during 90 days from the day of calving in cows or from 12 months of age in heifers. Ovulations which occurred in service periods characterized by cystic ovaries (cystic service periods) were studied separately. The incidence of multiple ovulations in noncystic and cystic service periods was 4.2 and 12.9%, respectively. Analysis of ovulations in noncystic service periods by the method of least squares showed that outbreds had a significantly higher percentage of multiple ovulations than inbreds (5.2 vs. 3.2%, $P < 0.05$). There was a significant increase in the incidence of multiple ovulations with parity (from 2.9% in heifers to 5.9% following the second calf, $P < 0.05$). The influence of sire line was nonsignificant. Multiple ovulations were not significantly associated with expression of heat (silent or expressed) or with interval since calving in cows or since 12 months of age in heifers. Intracow correlation of multiple ovulations within line, system, parity, and season was found to be 0.074 ($P < 0.01$).

P34. Effects of stage of lactation on type evaluation in first-lactation Holstein cows. H. C. HINES,* T. M. LUDWICK, D. O. RICHARDSON, E. R. RADER, E. W. BRUM, AND A. K. FOWLER, Ohio Agricultural Experiment Station, Wooster.

A group of 200 first-lactation Holstein cows, in three different Ohio herds, was evaluated for type on a complete breakdown basis including ten categories. Evaluations were made independently and simultaneously by two persons three months after calving, eight months after calving, and at approximately the middle of the dry period following the first lactation. For each evaluation period the scores of the two classifiers were averaged. Data were analyzed by the analysis of variance technique to determine the relative importance of stage of lactation, and correlation values were used to estimate repeatabilities.

Categories significantly affected by stage of lactation were middle and loin, rump and thigh, udder attachment, and breed character. As lactation progressed, type scores for these categories increased. For the category dairy character, there was a gradual decrease in type score in all herds as lactation progressed. This decrease, however, was not significant at the .05 level of probability.

P35. Heritabilities, phenotypic and genetic correlations between the components of type, final type rating, and milk fat production in Ayrshire cattle. D. F. BUTCHER,* R. G. MITCHELL, AND I. D. PORTERFIELD, West Virginia University, Morgantown.

Intra-sire, intra-herd variances and covariances of daughters and dams were used to estimate heritabilities, phenotypic and genetic correlations on 8,165 daughter-dam pairs located in 2,924 sire-herd groups. The average of all available type and production records was used. The heritabilities and phenotypic correlations were converted to a single-record basis. Heritabilities for the components of type ranged from 0.05 to 0.27; milk fat production, 0.15; and final type rating, 0.18; $\pm$ 0.02. Phenotypic correlations between type and milk fat production ranged from 0.03 to 0.13, with a standard error of < 0.01. Genetic correlations between type and production ranged from -0.15 to $+0.17$, with a standard error of < 0.10. Genetic correlations between the components of type and final type rating appear to be larger than their respective phenotypic correlations. The amount of progress that could be expected in milk fat production when selecting on final type rating or any one of the ten components of type ranged for -16% to $+23\%$ of the progress expected if selection was based in milk fat production alone.

P36. Selecting young dairy bulls on differences between relatives and their contemporaries. G. R. BARR* AND A. E. FREEMAN, Iowa State University, Ames; J. C. RENNIE, Ontario Agricultural College, Guelph.

Data for this study consisted of milk production information for the parents, grandparents, and daughters of 28 Holstein-Friesian bulls used artificially in Ontario. The 18,675 lactations were expressed as deviations from herd-year-season averages. Adjustments were

made for number of stablemates and estimated genetic differences among herds.

An index was derived to predict a bull's breeding value using the information on his parents and grandparents. The correlation between these predictions and those obtained from the daughters for the 28 bulls was 0.32. Estimates from daughters were about one and a half times as efficient as those from parents and grandparents. Estimates of heritability and repeatability for single lactations were computed from the data. Values of 0.21 for heritability and 0.48 for repeatability were used to calculate the expected correlations for the index. Assuming that the correlation among paternal sisters was all additively genetic, breeding worth could be predicted from pedigrees about as efficiently as from eight or nine daughters.

Causes of variation in milk production were investigated in 43,498 lactations. Components of variance for bulls, herds, years, seasons and their first-order interactions were estimated by the analysis of variance for cross classification.

P37. Anaerobic gas production at 37 C of semen extended in CUE and stored at 5 C. LINDA GRAY AND M. H. EHLERS,* Washington State University, Pullman.

Warburg manometric techniques were adapted to the measurement of sperm metabolism in extended semen previously stored up to seven days at 5 C. Lactic acid, produced by glycolysis, reacts with sodium bicarbonate to form carbonic acid, with the subsequent liberation of carbon dioxide. This carbon dioxide, together with any other gases, contributes to the total gas production. Manometric observations were made at 20-min intervals during 37 C incubation. Eleven ejaculates, initially 71% motility, averaged 180 μl gas per 10^9 sperm in 1 hr when the extended semen was immediately incubated, and 165 μl when cooled and stored two days at 5 C prior to incubation. Twelve ejaculates, initially 58% motility, averaged 118, 100, and 80 μl gas per 10^9 sperm in 1 hr at 37 C following cooling to 5 C and subsequent storage at 5 C for zero, two, and seven days. Gas production in 2 hr at 37 C was 197, 177, and 138 μl per 10^9 sperm for these corresponding storage periods. The usefulness of these experiments is the demonstration of a convenient means for measuring sperm metabolic activity for semen stored in a bicarbonate medium.

P38. Technique for the simultaneous study of metabolic activity and survival of spermatozoa under storage conditions. F. D. BARTLETT, JR.* AND N. L. VANDEMARK, University of Illinois, Urbana.

Studies of sperm metabolism with the Warburg respirometer usually have an error of approximately 10%, are of short duration, and restricted to one temperature. A technique was developed which allows the simultaneous measurement of the effect of different treatments, temperatures, and storage periods on metabolic activity and survival of sperm. This procedure was used for studying the metabolism of sperm in different diluents during storage at three temperatures and for collecting data of a physical nature. Sperm extended in IVT diluents were stored in 10-ml serum bottles with a 1:1 fluid-to-gas ratio at 5, 15.5, and 26.5 C. Initially and after two, four, seven, and ten days of storage, the samples were analyzed for pO_2, pCO_2, total CO_2, lactic acid, glucose, pH, and percentage of motile cells. Gas chromatography was used to quantitatively measure pO_2 and pCO_2 in the gas phase and total CO_2 after acidification of 0.1 ml fluid. Measurement of pCO_2 and total CO_2 allowed the calculation of bound CO_2. Results show that changes as small as 5 μl pO_2 and 1 μl pCO_2 can be detected with an error of less than 5%. Oxygen uptake and CO_2 production were demonstrated during storage at 26.5 C which were correlated with lactic acid production. These changes were less at lower temperatures. As the temperature was decreased, the ratio of the total CO_2 to pCO_2 increased.

P39. Effects of hydrolysis time, age of stain, and freezing of the cells on the Feulgen-DNA content of bovine spermatozoa. G. W. SALISBURY AND F. N. BAKER,* University of Illinois, Urbana.

Twenty Feulgen-stained sperm per slide were measured cytophotometrically in a factorial test of hydrolysis time, stain aging, semen freezing, and sample difference.

Hydrolysis (in HCl, 60 C) for 6, 8, and 10 min averaged 8.01, 8.73, and 8.69 units of deoxyribonucleic acid (DNA) per sperm, respectively. Differences among hydrolysis times were highly significant. Eight minutes appears to be optimum for fresh or frozen sperm.

Stock solutions of stain, prepared alike from the same dye, but used after periods of 180, 103, 74, and 68 days, averaged 8.58, 8.84, 8.24, and 8.29 DNA units per sperm, respectively. Differences among dye solutions were significant, older solutions giving higher DNA values.

DNA content of fresh or frozen sperm from the same or different ejaculate of the same bull did not differ significantly. None of the treatments significantly affected the area of the sperm nuclei (assumed to be proportional to mass) used in conjunction with light extinction values to calculate DNA per sperm.

There were highly significant regressions ($-.008138$, $-.009531$, $-.006504$) of extinction values on nuclear area within the hydrolysis treatments. Within the 8-min hydrolysis, 51% of the variation in extinction values could be explained by this regression.

P40. Ultrastructure of bovine spermatozoa freeze-thawed with and without glycerol. R. G. SAACKE* AND J. O. ALMQUIST, The Pennsylvania State University, University Park.

Previous electron microscope studies using thin sections suggested alteration of the cell membrane as one possible injurious effect on undiluted sperm either freeze-thawed or freeze-dried and reconstituted in the absence of glycerol. The post-thawing ultrastructure of skimmilk diluted sperm from pooled ejaculates slowly frozen to −79 C with and without 10% glycerol was compared to unfrozen aliquots maintained at 5 C. When stained with buffered osmium tetroxide, sperm frozen without glycerol usually showed loss or damage of cell membranes of the midpiece but not the principal piece. Control and glycerol treated cells generally had intact membranes. With buffered potassium permanganate stain, cristae within the mitochondrial helix were observed as highly organized and distinct in control cells but completely disorganized and granular in cells frozen without glycerol. Cristae of glycerolated frozen sperm varied from the normal pattern of controls to the disorganized appearance of unglycerolated frozen cells. Thus, cristae disruption may represent another injurious effect of freezing sperm without glycerol and may explain the failure of some sperm to survive freezing with glycerol. Ultrastructural changes observed in frozen unglycerolated sperm cannot be confined to freeze-thawing injury until cells subjected to other lethal conditions are examined.

P41. Fertility of bovine spermatozoa frozen concentrated, thawed, and re-extended. C. DESJARDINS* AND H. D. HAFS, Michigan State University, East Lansing.

Successful utilization of frozen semen depends upon storage at −79 C or lower until the semen is thawed immediately before insemination. As an alternative, it would be desirable to use thawed semen for at least 60 hr.

Two ejaculates from each of eight bulls were frozen with 200 × 10^6 sperm/ml in 1:4 yolk-citrate-glycerol, thawed, and re-extended to 15 × 10^6 sperm/ ml in either 1:4 YC or CUE and distributed to the breeding technicians to be used as unfrozen semen for 60 hr after thawing. Another two ejaculates from each bull were extended to 15 × 10^6 motile sperm/ml in either 1:4 YC or CUE and distributed.

The average 60- to 90-day nonreturn percentages for these four treatments were 52, 63, 74, and 77, respectively, for 5,918 inseminations. The fertility of 675 cows bred with control semen thawed just prior to insemination was 66%. The fertility of the sperm frozen concentrated, thawed, and re-extended in CUE declined 1% from Day 1 to Day 2 of storage, considerably less than the 11% for sperm re-extended in 1:4 YC ($P \cong .12$). In contrast, the decline for unfrozen CUE was 4%, insignificantly different from the 2% for unfrozen 1:4 YC.

P42. Effects of the temperature and the length of storage on the motility and metabolism of frozen semen. J. J. SULLIVAN* AND J. P. MIXNER, New Jersey Agricultural Experiment Station, Sussex.

Twenty-three semen samples from 11 bulls were diluted with an egg yolk–citrate–glycerol diluent to a concentration of 250 × 10^6 spermatozoa per milliliter. Subsamples were frozen and stored at −196 C in liquid nitrogen (LN) or at −79 C in dry ice–alcohol (DI). After 1 wk, three, six, nine, 12, 15, and 18 months of storage the semen samples were evaluated. The percentage of motile spermatozoa was estimated immediately after thawing. Sugar utilization and lactic acid production of the spermatozoa were measured during 3 hr of incubation at 37 C. Motility, sugar utilization, and lactic acid production were greater for semen stored in LN over all periods of storage ($P < .01$). With increasing time of storage, motility, and sugar utilization of the semen declined linearly and the decline was greater for semen stored in DI ($P < .01$). For every three months of semen storage, motility decreased 1.33% in LN and 2.02% in DI, and sugar utilization decreased 0.03 mg in LN and 0.09 mk in DI. Each of these regressions was significant ($P < .05$). With lactic acid production a highly significant interaction was found between temperature and storage time.

P43. Rapid method of carbon analysis of feeds and excreta. L. W. SMITH, D. R. GILLIAM, P. J. VAN SOEST, AND W. P. FLATT, Animal Husbandry Research Division, USDA, Beltsville, Maryland.

The wet combustion method of carbon analysis of feeds, feces, and urine collected from animals on carbon-nitrogen-energy balance experiments is slow and tedious. An induction furnace and volumetric analyzer designed for measuring the carbon content of metals has been adapted for the analysis of carbon in biological materials. The number of samples which can be routinely analyzed for carbon in an 8-hr day has been increased from eight by wet combustion to 52 by the induction furnace method.

This has been accomplished by the use of an induction furnace, appropriate catalysts, and a semiautomatic burette system. The catalysts used were Fe, CuO, MoO_3, and silicic acid (2:1:1:2). Samples of feeds, dried feces, and wet feces (182 samples 150-510 mg each) have been successfully analyzed with less than ± 0.25% deviation from the mean. Sodium carbonate, glucose, and potassium acid phthalate were used as standards with 99.44, 99.92,

and 99.97% recovery, respectively, using the induction furnace method.

P44. Significance of carry-over effects with the extra extra period Latin-square change-over design. A. C. LINNERUD,* C. E. GATES, AND J. D. DONKER, University of Minnesota, St. Paul.

By extending the final period of a balanced orthogonal Latin-square design two extra periods, estimates of one and two period carry-over effects may be obtained. An experiment with six yearling dairy heifers was planned so that carry-over effects should occur. Three rations were selected on the basis of their diverse quality and physical characteristics. Rations which were fed ad libitum, per cent TDN of ration dry matter, ration consumption as pounds dry matter and pounds TDN/cwt of animal, and body weight change (average difference between last three days of preceding period and first three days of period divided by three) are as follows, respectively, for the three rations: alfalfa hay, 67, 2.6, 1.7, 4.0; corn silage, 73, 2.2, 1.5, 0.6; blue grass straw pellets, 54, 1.5, 0.8, −6.2. Experimental periods lasted 1 wk, with no change-over period. Heifers previously were fed average-quality alfalfa hay ad libitum. Analysis of variance disclosed no significant carry-over effects for pounds of dry matter or TDN consumed/cwt of body weight/day. There was a significant one-period carry-over effect for change in body weight. Highly significant treatment effects were noted for each character.

P45. Net energy of barley and alfalfa hay for milk production as determined by the feed increment method. MAGNAR RONNING, University of California, Davis.

An adaptation of the Scandinavian group feeding method for the energy evaluation of feeds has been studied. A basal ration consisting of 70% alfalfa hay and 30% barley was fed to first-lactation cows at full and restricted levels of intake. The full level was calculated for each cow on the basis of milk production predicted from an indexing period using Morrison's estimated net energy values. The second level of intake was restricted to 75% as much as the full intake. Increments of barley and of alfalfa hay were added in turn to the restricted basal in amounts estimated to restore production to that observed on full basal. The extra-period, Latin-square experimental design was used.

Energy production was estimated by regression of the energy content of total milk solids on milk fat and appropriate adjustments for liveweight change. The net energy of the increments of feed were determined from the corresponding increments in energy production when specific feeds were added to the restricted basal. Thus, the net energy values expressed as megacalories per 100 lb of dry matter were as follows: basal, 63.9; barley, 77.3; alfalfa, 54.9.

P46. Nutrient intake and efficiency of feed utilization in lactating dairy cows. H. G. GRAY,* G. W. TRIMBERGER, L. D. VANVLECK, AND C. R. HENDERSON, Cornell University, Ithaca, New York.

Forage dry matter (DM) intake was determined on four consecutive days monthly during the six-months barn-feeding season for all milking cows in Cornell University's McDonald Farms Guernsey herd, starting November, 1958. The experiment was continuous, with standardized conditions for optimum results.

Average daily forage DM intake for 147 lactating cows during the third month after calving was 19.8 lb, with 11.1 lb grain DM and 36.2 lb actual milk production. The 147 cows, averaging 1,152 lb, were divided by age: 2-yr-olds, 3- and 4-yr-olds, and 5 yr and over. Body-weight averages were 1,022, 1,171, and 1,246 lb; forage DM intakes were 19.1, 20.0, and 21.4 lb, respectively; with grain, the total DM intake was 30.0, 31.8, and 32.8 lb, and actual milk production 31, 37, and 42 lb, respectively. The within-age and within-season-of-calving correlation between forage DM intake and milk production was 0.23 and body weight 0.24.

Average daily pounds dry matter intake for 20 wk by 65 cows from second lactation up, starting 30 to 90 (average 57) days after calving, was: hay 12.8, silage 7.4, grain 11.1, and 31.3 total for average body weight of 1,220 and daily gain 0.55 lb. The average of 40.2 lb of 4% FCM production daily required 49 lb TDN per 100 lb FCM, with maintenance requirements (23.6 lb TDN) included.

P47. Urea phosphate as a source of phosphorus and nitrogen for growing dairy heifers. L. L. RUSOFF, R. T. LOVELL,* AND W. H. WATERS, Louisiana State University, Baton Rouge.

The effect of urea phosphate (17.2% N and 19.9% P) in a grain ration to supply the required level of phosphorus and at the same time replace a part (15%) of the protein level was studied.

Twelve dairy heifers, weighing from 350 to 600 lb, were assigned on a paired basis to two grain rations. The control ration contained steamed bonemeal while the experimental ration included urea phosphate and oyster shell flour. Both rations supplied approximately equal levels of calcium (.52%), phosphorus (.61%), crude protein (14.75%), and TDN (68%). Alfalfa hay was fed with each grain mixture at a ratio of 2:1 on a dry matter basis.

At the end of the 90-day feeding trial, the urea phosphate-fed animals had gained slightly more (1.95 lb/day) than those on the control

ration (1.84 lb/day), but the difference in gain was not significantly different. No difference in values for the digestibility coefficients of both rations were found. Using current feed prices, the urea phosphate ration was cheaper by approximately $0.50 per ton over the control ration.

P48. Effect of feeding various levels of fluorine, calcium-phosphorus mineral, and concentrate mix to dairy females from weaning to mature age. I. Growth and feed consumption. G. E. STODDARD,* L. E. HARRIS, G. Q. BATEMAN, J. L. SHUPE, AND D. A. GREENWOOD, Utah State University, Logan.

Thirty-two Holstein heifers were assigned to eight treatments in a factorial experiment including 10, 28, 55, and 109 ppm fluorine (levels adjusted with sodium fluoride) on a hay basis; 2 and 4 lb of concentrate daily during nonlactating periods and 0.75 and 1.0 lb of concentrate daily for each 1 lb milk fat produced weekly during lactation; and 1 and 3% of a Ca-P mineral in the concentrate mix.

Cumulative daily hay dry matter intake for the first nine 112-day periods averaged 14.0, 13.5, 13.4, and 13.4 Kg for the four fluoride treatment groups; 14.1 and 13.1 Kg for the concentrate groups; and 13.9 and 13.7 Kg for the mineral groups. For the entire experiment cumulative daily hay intakes averaged 20.5, 19.8, 19.6, 17.7; 19.6, 19.3; 19.1, 19.7 Kg, respectively, for the eight treatments.

Cumulative daily body weight gains averaged 1.32, 1.30, 1.33, 1.36; 1.29, 1.37; 1.34, 1.32 Kg, respectively, for the eight treatments for the first five 112-day periods. Cumulative increase in height at withers averaged 0.28, 0.29, 0.27, 0.27; 0.27, 0.28; 0.28, 0.27 cm., respectively, for the eight treatments for the first five 112-day periods and 0.08 cm. average for each group for the entire experiment. Feed/growth relationships and hay refusals showed some treatment influence.

P49. Effect of feeding various levels of fluorine, calcium-phosphorus mineral, and concentrate mix to dairy females from weaning to mature age. II. Milk production. G. E. STODDARD,* G. Q. BATEMAN, L. E. HARRIS, J. L. SHUPE, AND D. A. GREENWOOD, Utah State University, Logan.

Thirty-two Holstein heifers were assigned to eight treatments in a factorial experiment including 10, 28, 55, and 109 ppm fluorine (levels adjusted with sodium fluoride) on a hay basis; and 2 and 4 lb of concentrate daily during nonlactating periods and 0.75 and 1.0 lb of concentrate daily for each 1 lb milk fat produced weekly during lactation; and 1 and 3% of a Ca-P mineral in the concentrate mix.

Kilograms of 4% FCM produced during total lactation periods for eight treatments in order listed above was:

First lactation, 4,200, 3,463, 3,752, 3,796; 3,484, 4,122; 3,890, 3,716. Second lactation, 4,865, 4,049, 3,678, 4,187; 3,942, 4,448; 4,437, 3,952. Third lactation, 5,190, 3,887, 4,604, 3,664; 3,821, 4,841; 4,600, 4,073. Fourth lactation, 4,793, 4,706, 3,607, 3,808; 4,099, 4,358; 4,086, 4,371. Average daily milk production and 305-day total production showed about the same relative treatment effects.

Feed efficiency based on grams of 4% FCM/100 Kcal digestible energy was more related to level of production than fluoride treatment. Efficiency appeared to be greater for higher concentrate level and 1% Ca-P than for lower concentrate level and 3% Ca-P.

Fluorine content of milk increased with time and level of fluorine intake, but average levels were all less than 0.2 ppm well within safe limits.

P50. Dry matter disappearance in nylon bags suspended in rumen as affected by heat, time, and rations and its use in forage evaluation. M. G. YANG,* J. R. INGALLS, AND J. W. THOMAS, Michigan State University, East Lansing.

The per cent dry matter disappearance (per cent DMD) of alfalfa and brome hay heated at temperatures of 0, 80, and 100 C for 24 hr was determined by suspending the hays in nylon bags in the rumen of a fistulated cow for 44, 54, and 64 hr. Heating the hays at either 80 or 100 C significantly decreased the per cent DMD ($P < .01$). When the bags remained in the rumen for 64 hr the per cent DMD was significantly greater than those that were in the rumen for 44 or 54 hr ($P < .01$). Pure stands of first, second, and third cuttings of alfalfa, brome, reed canary, and trefoil and the first cutting of timothy were used to compare the per cent DMD with the dry matter digestibility as determined with a conventional digestion trial using sheep. The correlation coefficient for the per cent DMD and the per cent dry matter digestibility determined with sheep was .79. Between per cent DMD and body weight gain, it was .62. The per cent DMD determined at 54 hr with the nylon bags was always 12 to 24 percentage units lower than the per cent dry matter digestibility determined with sheep. Preliminary studies showed that the per cent DMD for silage or hay substrate in silage-fed animal was less than in a hay-fed animal. When shredded newspaper or nylon cloth was placed in nylon bags the per cent DMD was always negative.

P51. Factors affecting persistency and its importance in 305-day lactation production. J. W. SMITH* AND J. E. LEGATES, North Carolina State College, Raleigh.

Persistency, as measured by the ratio of production for the last 215 days to the production for the first 90 days of the 305-day lactation, was studied in 1,667 first and 3,873 later records from nine North Carolina Insti-

tutional Holstein herds. First calvers were more persistent, with a mean persistency value of 1.844 ± 0.006, whereas the mean for later records was 1.588 ± 0.005. Persistency decreased with age (months) in first records (b = −0.088 ± 0.003) and increased with age in later records (b = 0.0012 ± 0.0004). Age, however, accounted for only 0.8 and 0.4% of the variation in persistency for first and later records, respectively. The number of days from calving to conception (days open) accounted for 7 and 5% of the variation in persistency for first and later records, respectively.

Half-sib analysis of first records within herd-year-season gave heritability values of 0.18, 0.26, and 0.33 for 90-day, 305-day milk yields, and persistency. The genetic correlations were 0.79 between 90-day and 305-day milk, −0.07 between 90-day milk and persistency, and 0.55 between 305-day milk and persistency.

In the later records the heritabilities were 0.38 and 0.36 for 90-day and 305-day milk and their genetic correlation was 0.92. The sire component of variance for persistency in the later records was a small negative, indicating sampling near zero; although the regression of daughter on dam from 963 pairs indicated a heritability of 0.1 for persistency in later records.

P52. Reliability of an estimate of maximum milking rate, and its relationship with production traits. B. T. McDaniel,* E. B. Burnside, and J. E. Legates, North Carolina State College, Raleigh.

This study was undertaken to evaluate the reliability of two measurements of maximum milking rate (pounds milk in four consecutive 15-sec intervals) taken under field conditions, in establishing a cow's phenotype for maximum rate. The relationship between rate and certain production characteristics was also considered.

Data consisted of 996 measurements in 498 lactations of 254 Holstein cows in three herds in the North Carolina Institutional Breeding Program. Two observations were taken on each cow at the PM milking during the second month of lactation.

The within cow and lactation correlation between maximum rate measurements was 0.83. PM milk yield on the day of measurement significantly influenced rates (b = 0.239 ± .087). The effect of PM yield on rate was similar between cows within lactation-herd-season subclasses (b = 0.274 ± .093). Variation in milking rates, either between or within cow, was not affected by days in milk.

The repeatability of lactation milking rates based on the average of two measurements per lactation, adjusted for age of cow and PM yield, was 0.73. The correlation of this lactation measure of rate with age-adjusted 305-day milk yield was 0.05, and the correlation of rate with a persistency index, [(305-90) day milk/90-day milk] was −0.04.

These results indicate that two measurements of maximum milking rate taken under field conditions in early lactation characterize a cow's phenotype, and that the phenotype of milking rate is apparently independent of the phenotypes of 305-day milk yield and persistency.

P53. Milk yield associations with body weight and metabolic size in two Holstein herds. R. T. Starkenburg, Agricultural Experiment Station, Purdue University, Lafayette, Indiana.

Body size and production data on 141 Holsteins from Washington State University and 69 Holsteins from Purdue University were used in this study. Production was expressed as 2×, 305-day, actual 4% FCM. Twelve-month and first-lactation body weights were recorded. Yield was expressed on the basis of metabolic size to determine its applicability in the correction of yield differences associated with differences in body weight. Metabolic size, base 0, was calculated as $W^{0.7}$ and metabolic size, base 1,000, was calculated as $1{,}000 + (W\text{-}1{,}000)^{0.7}$ where W was body weight.

Marked similarity between correlations derived from the two herds was noted. Intra-herd correlations, using body weight at 12 months as the independent variable and the following as dependent variables, were: actual FCM (−0.06); first-lactation weight (0.32); and age at first calving (0.02). Intra-herd correlations, using first lactation weight as the independent variable and the following as dependent variables, were: actual FCM (0.19); FCM per 1,000 lb body weight (−0.29); FCM per unit metabolic size, base 0, (−0.13); and FCM per unit metabolic size, base 1,000, (0.10).

P54. Sources of variation in DHIA records related to proving AI sires. B. Bereskin,* A. E. Freeman, and J. L. Lush, Iowa State University, Ames.

The basic data were 39,000 lactation records of Holstein cows made over 3.5 yr in centrally processed DHIA herds in Iowa. Some of the analyses that were computed follow.

Components of variance for milk production were estimated. As percentages of the total variance they were: bulls, (B) 7%; herds (H), 27%; year-seasons (YS), 2%; BXH, 4%; HXYS, 3%; and residual, 57%. Analyses of herds, year-seasons within herds, cows within herds, and within cow effects, gave the following within herd-year-season repeatability estimates: milk, 0.51; milk fat, 0.47; and test, 0.72.

Twice the regression of daughters on their dams, using averages of records, gave heritability estimates, on a within herd-year-season

basis, of 0.26 for milk, 0.21 for fat, and 0.58 for test. Adjusted to a single-record basis, these were: 0.22 for milk, 0.18 for fat, and 0.52 for test. The year-season effect for older cows was about twice that for younger cows, although the seasonal division was the same for both groups. Deviations from regressed adjusted stablemate averages for both daughters and their dams were studied to determine the magnitude of the dams' contribution to AI sire evaluation.

P55. State, herd, and yearly effects on milk and fat yields of dairy cows. N. R. Thompson,* V. L. Baldwin, and G. C. Graf, Virginia Agricultural Experiment Station, Blacksburg.

A sample survey was made on lactational milk and fat yields of dairy cows in eight regions of the United States. Totals of 8,959 Guernsey, 14,593 Holstein-Friesian, and 5,537 Jersey records were analyzed for effects of regions, states in regions, herds in states, and years in herds. The effects of states, herds in states, years in herds, and cows within years and herds were estimated as components of variance.

The variance components for states and for years in herds were relatively small, with ranges in size of 3.3-10.0% and 0.02-5.3%, respectively, of the total variance. The component for herds was relatively large, ranging from 32.1 to 35.5% of the total variance. These results suggest that much more attention should be paid to herd environment than to either geographical location or year of calving, in the evaluation of milk and fat yields of dairy cows.

P56. Influence of month of calving on lactation milk yield. R. V. Johnson* and R. W. Touchberry, University of Illinois, Urbana.

Centrally processed records from 306 Illinois DHIA herds were used to investigate the influence of month of calving on milk production. The data included 8,704 274-day actual lactation records completed within a 3-yr period. Herd means were established for test-day milk production, and individual test-day records in each herd were expressed as deviations from these herd means to eliminate herd differences. Records were separated by breeds and then grouped by month of calving and age at calving to determine their effects on lactation milk yield.

Holstein cows calving in December, January, and February had the highest milk production (11,101 lb); those calving in June, July, and August the lowest production (9,193 lb) in 274-day lactations. Similar results were observed for three other breeds. Lactation curves varied markedly with month of calving and age of cows. The greatest persistency was shown by cows calving in the fall, but spring calvers had the highest initial milk yield. Cows in their first lactation had greatest persistency but the lowest initial and lactation milk yield. The month of calving accounted for approximately 1% of the variation of milk yield of cows within a herd.

P57. Relationships among udder height, age, and milk production. E. B. Burnside,* B. T. McDaniel, and J. E. Legates, North Carolina State College, Raleigh.

This study was undertaken to evaluate the effects of age and milk yield on two measures of udder height: (1) Height of the lowest point of the udder floor; (2) height of the lowest point of the udder floor minus the hock height. Consideration was given to the relationships between the udder height measurements and production traits, as well as to the repeatability of udder height measurements.

Data consisted of measurements taken during the second month of 657 lactations of 319 Holstein cows in three herds in the North Carolina Institutional Breeding Program.

Ninety-day milk, age, and 305-day milk, listed in order of importance, accounted for 31% of the variation in udder height and 29% of the variation in udder-hock height between cows in herd-season subclasses. In the covariance analysis of udder height the two production traits accounted for twice as much variance as age, while in a similar analysis of udder-hock height, the production traits removed an even larger proportion of the variance relative to age. The age-adjusted correlation between 305-day milk yield and udder height was -0.29; and the correlation between 305-day milk and udder-hock height was -0.44. Correlations between 90-day milk and the two udder height measurements were lower, being -0.24 and -0.28, respectively. Repeatabilities of the two measurements were 0.71 and 0.70, respectively.

P58. Relationship between quality of udder and milk production. W. J. Brakel,* E. L. Akings, E. W. Brum, W. M. Etgen, E. R. Rader, D. O. Richardson, and H. E. Rickard, Ohio Agricultural Experiment Station, Wooster, and The Ohio State University, Columbus.

Quality evaluations of the udders of 52 cows (15A, 7BS, 9G, 15H, and 6J) were made visually and by physical manipulation. Each cow had completed one or more previous lactations. An evaluation of each udder was made 30 days prior to the expected date of calving and on the 30th and 60th day following parturition. Quality of udder scores ranging from one to five were assigned independently by each of the four inspectors. The intra-breed correlation of each inspector's scores with those of the other inspectors ranged from $+0.50$ to $+0.80$ ($P < 0.01$) and the maximum difference between the average scores of the individual inspectors was less than twice the standard error.

When the lactation records for the experimental period were converted to a 305-day,

M.E., 4% FCM basis, the range was from 7,671 to 22,121 lb and the average was 12,080 lb. The regression of milk production, both in the first 60 days and 305 days, on the inspectors' average quality scores at the three different intervals was computed on an intra-breed basis. None of the regression coefficients was significantly different from zero.

P59. Effectiveness of Furaltadone against Salmonella typhimurium in dairy calves. A. S. WOOD,* K. G. RAJU, AND J. B. WILLIAMS, University of Minnesota, St. Paul.

Twelve four-day-old colostrum-fed Holstein calves were divided into three replicates of four and fed for 21 days. Group 1 calves received 6 mg Furaltadone daily per pound body weight plus a single dose of 6 million *Salmonella typhimurium* organisms; Group 2—single dose of 6 million *typhimurium* organisms; Group 3—6 mg Furaltadone daily per pound body weight; Group 4—no drug or inoculum. One calf from each group was housed in a common pen and fed from individual open pails. Feeding utensils were stored in a 200-ppm chlorine solution. Utensils and feed remained free of *S. typhimurium* and *Escherichia coli.* Furaltadone and inoculum were administered in reconstituted skimmilk. Organism concentration was determined initially by MacFarland's Nephelometric Test and verified by Salmonella-Shigella Agar plate count. Minnesota Public Health Department procedure was used to determine fecal flora prior to and during experimental period. Pre-experimental fecal flora contained Coliform, Aerobacter, and Proteus species. Medicated calves, inoculated and noninoculated, had diarrhea of short duration. Nonmedicated calves died within five days. Cross contamination occurred in noninoculated calves within 48 hr.

P60. Thiocyanate disappearance rate from the cerebrospial fluid in control and vitamin A deficient calves. M. OKAMOTO,* J. BITMAN, M. R. CONNOLLY, AND R. W. MILLER, Agricultural Research Service, USDA, Beltsville, Maryland.

Six two-month-old calves were depleted of vitamin A and carotene body reserves, and a similar group was retained as controls. The deficient and control calves were then supplemented with alfalfa leaf meal to provide 10 and 75 μg carotene per pound of body weight, respectively. Mean plasma vitamin A and carotene values during the experimental period were 6.8 and 15.0 μg/100 ml (deficient) and 13.2 and 49 μg/100 ml (control).

Thiocyanate (SCN) was injected into the cisterna magna and the concentration of this marker remaining in the cerebrospinal fluid (CSF) was estimated at 30, 60, 90, 120, 180, 240, and 300 min post-injection.

Comparison of the SCN concentrations during the first 90 min. showed similar values. Samples taken thereafter exhibited higher values in the deficient animals. Graphical interpretation of these data resulted in SCN disappearance rates of 3.02×10^{-3} mg/ml/min for the deficient group and 5.44×10^{-3} mg/ml/min for the control animals. This difference would indicate a lesser reabsorption of the CSF in the deficient calves and could also explain the higher CSF pressures in the deficient animals (225 mm as compared to 96 mm).

P61. Replacement and absorption of cerebrospinal fluid in normal and vitamin A deficient calves. R. W. MILLER,* H. CECIL, T. R. WRENN, AND M. OKAMOTO, Agricultural Research Service, USDA, Beltsville, Maryland.

This investigation was undertaken to determine whether the primary cause of increased cerebrospinal fluid (CSF) pressure in vitamin A deficient calves was overproduction or underabsorption of the CSF.

The CSF pressure was measured, 3.0 ml of fluid were removed, and the length of time necessary for the CSF pressure to return to the original level was determined. This return time was an estimate of replacement. When a stable pressure was re-established, the previously removed 2.0 ml of fluid were injected and the CSF pressure increased. The time taken for the pressure to fall to the original level was an estimate of the capacity of absorption.

Thirty-five minutes were required to replace the 3.0 ml of CSF in the normal animals as compared to 15 min in the deficient calves. When estimating absorption capacity in the deficient calves, the CSF pressure was highly stable in 11 of the 12 experiments and the trials were terminated between 25–60 min. With normal calves the pressures returned to the original level in 17 min.

The results suggest that underabsorption represents a greater factor than overproduction as a cause of increased CSF pressure in vitamin A deficient calves.

P62. Effect of diet on the in vitro metabolism of VFA and glucose by rumen epithelium from young calves. J. D. SUTTON, A. D. MCGILLIARD,* AND N. L. JACOBSON, Iowa State University, Ames.

Three Holstein calves were fed milk, hay, and grain (MHG) and three received milk only (M). The calves were sacrificed at 16 wk of age. Epithelium from the anterior dorsal sac was stripped from the muscle and placed in ice-cold Ringer. The maximum time required after sacrifice to get the tissue into ice-cold Ringer was 45 min; incubation was begun 40 min thereafter. Two-gram samples were incubated for 3 hr in Krebs bicarbonate Ringer buffered to pH 7.2 in a 95:5 atmosphere of oxygen and carbon dioxide. Two hundred micromoles of acetate, propionate, butyrate, an equimolar mixture of these three, or glucose were added to the medium. Mean uptake of

these substrates was (micromoles/ 100 mg dry tissue/ 3 hr) 5.9, 29.6, 44.1, 31.5, and 3.8, respectively, for MHG calves, and 2.9, 5.8, 4.7, 5.8, and 4.2, respectively, for M calves. Ketones were produced from acetate, butyrate, and the equimolar mix. Percentage conversion of acetate and butyrate to ketones was 72 and 88, respectively, for MHG calves, and 17 and 29, respectively, for M calves.

P63. Responses of Holstein calves to dietary calcium, phosphorus, and vitamin D_3. P. T. CHANDLER* AND R. G. CRAGLE, UT-AEC Agricultural Research Laboratory, Oak Ridge, and University of Tennessee, Knoxville.

Thirty male calves were fed 2 lb per day per 100 lb body weight of a semi-purified diet, consisting of 25% casein, 30% cerelose, 15% lactose, 10% lard, 5% coconut fat, 2% lecithin, and 3% vitamins and minerals (exclusive of D_3, calcium, and phosphorus) from 3 to 9 wk of age. Calcium intake was varied from 1.25 to 20 g, phosphorus from 1 to 16 g, and vitamins D_3 from 3 to 30,000 I.U. Blood and growth responses were measured during the 6 wk, as well as oral Ca^{45} and P^{32} uptake during the terminal day. Animals on rations with abnormally low Ca/P ratios (2.5 g/8 g and 5/16) died in 1 to 2 wk. The same ratio with a low total amount (1.25/4) supported normal growth. Medium ratios (5/8 and 2.5/5.66) resulted in normal growth. High ratios (20/4) produced no abnormal effects. Plasma phosphorus was the highest (18 to 22 mg.%) in calves on low Ca/P ratios and was the lowest (10 to 14 mg. %) in calves on high Ca/P ratios. Interactions between ratio, intake, and vitamin D_3 affected Ca^{45} uptake. Urinary excretion of calcium and phosphorus was directly dependent upon the dietary level.

P64. Comparison of alfalfa, beet pulp, or soybran flakes as the basic roughage in high-roughage pellets for dairy calves. ADDANKI SOMASUNDARAM,* J. W. HIBBS, AND H. R. CONRAD, Ohio Agricultural Experiment Station, Wooster.

Eighteen Holstein and 12 Jersey calves were fed to 16 wk in a comparison of high (67%) roughage pellets in which either alfalfa (OA), beet pulp (OB), and soybran flakes (OS) was the basic roughage. Per cent cellulose in the three rations was 24.1, 24.9, and 28.8, respectively. Per cent TDN, measured in five-day digestion trials, was 59.1, 75.9, and 70.6, respectively. Both dry matter and cellulose digestibility were higher in the OB and OS groups. Protein digestibility was similar in all three groups. Jerseys consumed more TDN and gained more in body weight and withers height when fed the OB or OS pellets. TDN intake and gains in Holsteins did not vary markedly among the three rations. Pellets eaten/pounds gain, 8-16 wk, was highest in the OS pellet group. Biweekly in vitro cellulose digestion and volatile fatty acid production plus pH, total and individual volatile fatty acid content of strained rumen juice and glutamic oxalacetic transaminase (GOT) activity in sonically oscillated rumen juice were used to follow development of rumen function. Both beet pulp and soybran flakes were found to be promising sources of highly digestible roughage for use in high-roughage calf rations.

P65. Natural radioactivity provides a method for studying body composition in young calves. E. G. STANT, JR.,* T. G. MARTIN, W. V. KESSLER, J. E. CHRISTIAN, AND F. N. ANDREWS, Purdue University Agricultural Experiment Station, Lafayette, Indiana.

A large-volume liquid scintillation counter was used to determine the amount of naturally occurring K^{40} of live calves and the component parts of the calves following slaughter. Eight live calves were counted within 16 days following birth. Their average activity was found to be 33.1 cpm/lb. Four calves were slaughtered immediately, while the remaining four were fed a high-energy diet to three months of age, at which time they were slaughtered. The average activity of the older calves was 18.0 cpm/lb, respectively. The older calves had values of 17.9 and 31.1 cpm/lb, respectively, for offal and carcass. The carcasses were divided into bone and meat portions. The activity of the bones was 43.6 and 27.7 cpm/lb, respectively, for newborn and older calves. The meat portion, which included the fat, had activities of 46.7 and 39.7 cpm/lb, respectively, for newborn and older calves. The average counts per minute given above were corrected for variation due to counter efficiency. Geometry and background depression corrections are being developed.

P66. Studies on the physiological effects of anemia in newborn calves. C. T. SETTLEMIRE,* J. W. HIBBS, AND H. R. CONRAD, Ohio Agricultural Experiment Station, Wooster.

In a previous report (Hibbs et al., J. Dairy Sci., 44:1184. 1961.) it was shown that iron deficiency anemia in newborn dairy calves (oxyhemoglobin $< 9\%$) did not limit postnatal growth, nor was growth enhanced by the increased hemoglobin per cent resulting from single, intramuscular injections of 500 mg of Fe (dextran). In this study the basal metabolic rate (BMR), pulse rate, and respiration rate of six anemic calves (oxyhemoglobin 5.4 to 8.9%) were compared with 16 normal calves (oxyhemoglobin 9.0 to 15.6%) between three and 22 days of age, fed only whole milk. The anemic calves had a small but statistically insignificant elevation in pulse rate. In both normal and anemic calves linear correlations of BMR with pulse rate and respiration rate were high, 86.9 and 91.4%, respectively. BMR was not altered by lowering oxyhemoglobin level in one calf by bleeding or by raising the

oxyhemoglobin level in two anemic calves by Fe injections. A comparison of heart, liver, and spleen weight/kg of body weight of nine anemic calves at birth (oxyhemoglobin 4.4 to 8.7%) with 22 normal calves (oxyhemoglobin 9.2 to 17.3%) showed no compensatory changes under the environmental conditions that existed.

P67. Abomasal hydrolysis of milk fat by pregastric esterase in the calf. D. E. OTTERBY,* H. A. RAMSEY, AND G. H. WISE, North Carolina State College, Raleigh.

The hydrolysis of milk fat in the abomasum by pregastric esterase was studied in four calves fitted with rumen and duodenal fistulae. Whole milk was either nipple-fed (oral feeding) or was placed directly into the abomasum (abomasal feeding) to by-pass the area of pregastric esterase secretion. Subsequently, serial collections of the abomasal digesta, which passed into the duodenum, were made for 10 hr. Each sample of digesta was analyzed chromatographically for free and total fractions of butyric and of higher acids. When the milk was fed orally, 68% of the total butyric acid in the 10-hr collection of digesta was present as free acid, as compared to 16% for abomasal feeding. Similarly, 21% of the total higher fatty acids was present as free acids for the oral feeding, as contrasted to 9% for abomasal feeding. In the orally fed calves, 85% of the free butyric acid appeared in the digesta during the first 2 hr of collection, as compared to 35% for abomasal feeding. Results of this study suggest that an appreciable quantity of butyric acid is released from milk fat by pregastric esterase in the abomasum.

P68. Effect of b-estradiol and b-estradiol plus oxytocin on established lactation in the rat. R. R. GALA AND R. P. REECE, New Jersey Agricultural Experiment Station, New Brunswick.

Twenty lactating hooded Norway rats, divided into four groups of five each, had their litters adjusted to ten pups per litter on Day 2 of lactation and injected to Day 13 as follows: (1) control—corn oil; (2) 1 μg estradiol/day; (3) 10 μg estradiol/day; and (4) 10 μg estradiol/day + 1 IU oxytocin 3× daily (IP). Litter weight and mother body weight gains were recorded throughout lactation and on Day 13 the mothers were injected subcutaneously with 0.1 mg of colchicine per 100 g body weight (BW) and sacrificed 10 hr later. The right inguinal mammary gland was fixed, sectioned, and stained, and the number of mitatic figures per 1,000 cells recorded. Anterior pituitaries (AP) were assayed for lactogen content by the pigeon intradermal crop-gland test. Average values and their standard errors are: litter weight increases (g) — (1) 118.6 ± 3.0, (2) 103.1 ± 1.9 ($P > .05$), (3) 74.9 ± 5.9 ($P > .01$, and (4) 61.9 ± 7.6 ($P > .01$); milligrams of AP/100 g. BW—(1) 3.73 ± 0.17, (2) 4.14 ± 0.08, (3) 5.66 ± 0.46 ($P > .01$), and (4) 6.43 ± 0.35 ($P > .01$); IU of lactogen/AP—(1) $0.876 \pm .054$, (2) 1.434 ± 0.270 ($P > .05$), (3) $1.422 \pm .125$ ($P > .05$), and (4) $1.959 \pm .169$ ($P > .01$); mitotic figures/1,000 cells—(1) 10.1 ± 2.3, (2) 22.8 ± 2.3 ($P > .01$), (3) 27.0 ± 1.7 ($P > .01$), and (4) 11.1 ± 1.5. Statistical analyses indicated no significant differences among groups of mother's BW, mother BW increases, and IU of lactogen/mg of AP. The data suggests that estrogen alone inhibits lactation by stimulating mammary growth; however, when combined with oxytocin the inhibitory effect appears to be acting on the anterior pituitary.

P69. Effect of bovine endometrial extracts, vasopressin, and oxytocin on luteal function in rats. P. V. MALVEN* AND WILLIAM HANSEL, Cornell University, Ithaca, New York.

Hysterectomy (Day 2) significantly prolonged the period of pseudopregnancy from 13.7 ± 0.3 (19 rats) to 17.1 ± 0.5 days (20 rats). Daily subcutaneous injections begun on Day 8 of aqueous or ether extracts of bovine endometrium, collected from heifers at the following stages of the estrous cycle, had no significant effect on the prolonged pseudopregnancy. (1) Day 15, normal heifers, (2) Day 18, normal heifers, (3) Day 4–8, oxytocin-treated heifers.

In contrast, vasopressin injections significantly shortened the length of pseudopregnancy in hysterectomized rats. The vasopressin treatments and their respective means were as follows: (1) eight units/day begun on Day 8, (two replicates) 14.7 ± 0.7 days (20 rats) and 12.4 ± 0.5 days (nine rats); (2) eight units/day begun on Day 1, 12.6 ± 0.7 days (ten rats); (3) 4 units/day begun on Day 8, 13.1 ± 0.9 days (eight rats); (4) eight units/day synthetic lysine vasopressin begun on Day 8, $13.9 \pm .0.6$ days (six rats). Vasopressin injections also significantly ($P < 0.02$) shortened the period of pseudopregnancy in intact rats from 12.8 ± 0.3 (ten rats) to 11.5 ± 0.4 days (ten rats). Oxytocin injections (12 units/day) had no significant effect in hysterectomized rats (16.7 ± 0.8 days, 20 rats).

P70. Method for the quantitation of bovine urinary estrogen levels using isotope dilution. T. N. MELLIN, R. E. ERB, AND V. L. ESTERGREEN, JR., Washington State University, Pullman.

Aliquots of bovine uring collected at 8-hr intervals two days before and after parturition were boiled 10 min to destroy enzymatic and bacterial activity and deep-frozen until assayed. The thawed samples were hydrolyzed using beef-liver betaglucuronidase. Maximum levels of free estrogens were observed when using 200 Fishman units/ml of urine, incubated at 37 C for 2 days. Total yields were

further increased by acid hydrolysis of the ether-extracted enzyme hydrolysate. Before initial extraction estradiol–17 β–C^{14} and estrone–C^{14} were added. The hydrolyzed urine (70 ml) was extracted with peroxide-free ether (4 × 40 ml). The ether extract was washed with 9% $NaHCO_3$, dried down, and the residue partitioned between ether: CCl_4 (1:18) and 1 N KOH. The KOH was acidified to pH 3, extracted with ether, and the ether fraction washed with Na_2CO_3 (pH 10.5). Estrogens were separated by paper chomatography, using the formamide/Skellysolve B-benzene and benzene/55% methanol systems.

Based on isotope dilution, recoveries range from 35–70%. Recovery of urinary estradiol–17α, the principal estrogen present, is assumed equal to that for estradiol–17β. Fluorimetric quantitation shows that the decline in estrogens by 10 hr postpartum is due principally to estradiol–17α.

P71. Inhibition by ovarian hormones of the inflammatory response in the sheep uterus. T. H. BRINSFIELD,* H. F. RIGHTER, AND H. W. HAWK, Dairy Cattle Research Branch, USDA, Beltsville, Maryland.

Sixty estrual, luteal-phase and ovariectomized ewes, approximately 12 months of age, were inoculated in utero with *Escherichia coli* and killed 2, 4, 8, or 16 hr later (five ewes per subgroup). Infiltration of the endometrium by polymorphonuclear leukocytes, migration of large numbers of leukocytes into the uterine lumen, and clearance of *E. coli* from the uterine lumen occurred in each endocrine group, but at different rates. The inflammatory response was earliest in ovariectomized ewes, latest in luteal-phase ewes, and at intermediate intervals in estrual ewes. At 4 and/or 8 hr each endocrine group differed significantly ($P < 0.01$ or $P < 0.05$) from each other group in numbers of leukocytes in the uterine endometrium and lumen and numbers of live *E. coli* in the lumen. It appears that both progestins and estrogens, progestins to a greater degree, inhibit the inflammatory response in the sheep uterus. There was an over-all association between numbers of leukocytes and bacteria in the uterine lumens ($r = -0.714$, $P < 0.01$). This correlation did not differ significantly among endocrine groups. Endocrine action on the leukocytic response in the sheep uterus appears to be effected through control of factors influencing the rate of leukocytic diapedesis.

P72. Effect of ovarian status on the acute inflammatory response in the sheep mammary gland. H. W. HAWK,* H. F. RIGHTER, AND T. H. BRINSFIELD, Dairy Cattle Research Branch, USDA, Beltsville, Maryland.

Progesterone delays the leukocytic response to experimental infection in the uterus. Similar effects in the mammary gland could influence the incidence and course of mastitis. Nonlactating estrous and luteal-phase ewes were each inoculated in one gland with *Escherichia coli* (12 pairs of ewes) or *Staphylococcus aureus* (three pairs) and killed 0.5 to 5 hr later. Inflammatory responses were measured by edema, leukocytic infiltration of interstitial tissue, and ducts as determined histologically and bactericidal activity as shown by culture and microscopic examination of cistern and duct fluids. Chemical determinations were also made (Wrenn, Cecil, and Bitman, Abstract, J. Dairy Science, 1962).

Edema and tissue infiltration by neutrophilic leukocytes occurred throughout the inoculated glands within the first hour. By 3 to 4 hr edema was severe (weight increases of 28 to 470%), ductules were filled with leukocytes, and most bacteria had been cleared from the gland. In 15 comparisons, the inflammatory response developed faster in estrous ewes in six pairs and in luteal-phase ewes in three pairs; no differences were apparent in six pairs. The acute inflammatory response to induced infection in mammary glands of cycling ewes is probably independent of ovarian status.

P73. Chemical composition of mammary gland during experimental mastitis in the ewe. T. R. WRENN,* H. C. CECIL, AND J. BITMAN, Dairy Cattle Research Branch, USDA, Beltsville, Maryland.

Mastitis infection was induced experimentally in sheep with *Escherichia coli* and *Staphylococcus aureus*. Inoculations were made on one-half of the udder and chemical changes determined during initial stages of inflammation. Histopathological studies were also made on these tissues (Abstract—Hawk et al., J. Dairy Sci., 1962.). One-half to five hours after inoculation treated udder halves showed great increases in size and weight. This inflammatory response resulted in dilution of adipose tissue from 20% of total weight in the control to 10% in mastitic glands. Water content increased markedly, from 83 to 88%. The response appeared to be very similar in glands from either estrous or luteal phases of the reproductive cycle, whereas ovariectomized ewes showed a lesser response. Na and Cl increased greatly and K decreased, indicating an inflammatory edema. Histamine concentration of the inoculated glands decreased. The most dramatic change occurred in glycogen content, which increased three- to fourfold in inoculated glands. This glycogen response appeared to be correlated with leukocytic invasion. Chemical changes induced by *S. aureus* were not as marked as those occurring after *E. coli* infection.

P74. Response of the lactating goat to intramammary infusions of an adjuvant with Salmonella pullorum. H. E. STRUSS,* W. E. PETERSEN, AND L. SWANSON, University of Minnesota, St. Paul.

Four goats in the third month of lactation

were infused intramammarily with equal amounts of *Salmonella pullorum,* 20 billion cells, and varying quantities of adjuvant (a compound that enhances antibody response in immunization). One goat received only *S. pullorum,* the others received 10, 100, and 400 mg of adjuvant in addition. The adjuvant, CIBA compound 20150-B-59-R-12, and the *S. pullorum* were mixed 1 hr prior to infusion. Three infusions at 3-wk intervals were planned. The maximum rectal temperature of the goats receiving 0 and 10 mg was greater than 105 F but decreased following subsequent infusions; that of the goat receiving the 100-mg quantity was 105 F, but increased on subsequent infusions. The Whiteside test values remained above preinfusion level for 3, 5, and 7 days, respectively, for the control, 10-mg, and 100-mg treatments. The milk production rate of the does remained relatively constant during the 9-wk period except for the doe receiving 400 mg of adjuvant, which stopped secretion after the first infusion. The maximum antibody titer obtained was 1-100. This adjuvant increased antibody titer but at larger doses was depressing to gland secretion.

P75. Some effects of ionizing irradiation on milk enzymes in vivo. K. L. KNOX* ANL J. R. LUICK, University of California, Davis.

The mammary glands of four lactating dairy cows were subjected to x-irradiation ranging from 100 to 300 roentgens surface dose. Whole-milk lipase, esterase, acid, and alkaline phosphatase activities were determined at 8, 12, 24, and 36 hr intervals post-irradiation. Dose rates of 100, 200, or 300 roentgens had little or variable effects upon acid or alkaline phosphatase activity. Whole-milk lypolytic enzyme activity appeared unaffected by a 100-roentgen dose of ionizing radiation. At higher rates of 200 and 300 roentgens, lipase activity dropped to 35.6% of the pre-irradiation level. In one case there was no activity observable for 36 hr post-irradiation. In all cases the activity returned to the pre-irradiation levels in 48 to 72 hr. Esterase activity was unaffected by a 100-roentgen surface dose, but dropped to 42.2% pre-irradiation value at dose rates of 200 and 300 roentgens. It was observed that in vitro irradiation had no effect on any of the enzymes assayed.

P76. Effect of nitrate on the in vitro conversion of beta-carotene to vitamin A. B. S. REDDY* AND J. W. THOMAS, Michigan State University, East Lansing.

In vitro incubation of Tween 80, Na-glycoholate, and 112 μg beta-carotene/g of Holstein cow duodenal tissue homogenized with aqueous sucrose produced 1.06 $\pm$ 0.16 μg of vitamin A/g tissue. The Carr-Price reaction and absorption spectra were used for identification and quantitative analysis. The vitamin A formed amounted to 2.57 $\pm$ 0.05% of the carotene unrecovered. Further trials showed that when carotene:tissue ratio increased the vitaman A formed per microgram of unrecovered carotene decreased, and vitamin A formed per gram tissue increased. The addition of 671 to 34 μM of NO_3 per gram homogenate decreased vitamin A formed to 30-60% of control; 7 to 3 μM to 70-90%; 1 μM to 97% and 0.1 μM to 100% of control. When the duodenal homogenates of heifers fed 130 mg KNO_2/lb body weight and a calf fed 1% $NaNO_3$ were incubated, conversion was reduced to about 45% of control animals. Addition of 322 μM of NO_3/g homogenate of KNO_2 fed heifers reduced conversion to only 66% of its control. Conversion was greatly inhibited by heating homogenates. Conversion by ileum was 54% of that for duodenum, but carotene unrecovered was 62% vs. 23% for duodenum.

P77. Vitamin A and carotene concentrations of various organs and glands of cattle fed controlled levels of carotene and/or vitamin A. I. R. JONES,* P. H. WESWIG, AND J. F. BONE, Oregon State University, Corvallis.

At the conclusion of experiments, conducted over 3 yr and during which blood, liver biopsy, and milk samples from the cows were analyzed for carotene and vitamin A, five sets of identical twin bulls, one additional bull, and six cows were slaughtered and samples of internal organs and glands were taken for analyses. The basal hay ration containing less carotene than 1 mg/lb was supplemented with natural or synthetic carotene or vitamin A palmitate at rates of 50 to 500 γ/kg, body weight.

The mean values (γ/g fresh tissue) obtained for liver, pituitaries, adrenals, thyroids, testes, and ovaries, respectively, averaged for carotene 0.99, 0.72, 1.59, 0.23, 2.29, and 1.78, and for vitamin A 58.82, 0.75, 0.40, 0.39, 0.49, and 0.56.

The glands reflected the level and source of supplements fed but to a lesser degree than the liver. Thus, the liver values for carotene and vitamin A, respectively, with carotene supplements averaged 1.77 and 15.91 and with vitamin A palmitate 0.30 and 96.95. In comparison, the respective values for the pituitaries were 0.87, 0.28, 0.63, and 1.09, and for the thyroids 0.38, 0.23, 0.15, and 0.48.

P78. Studies of calcium homeostasis in the bovine. G. N. CURRIE* AND B. R. POULTON, University of Maine, Orono.

These studies were initiated to determine the response of serum calcium, magnesium, and phosphorus to the intravenous infusion of chelating compounds. Bull calves (125-150 lb) were used and subjected to jugular infusion of the disodium salt of ethylenediaminetetraacetic acid (Na_2EDTA) at a level of 25 mg/kg of body weight. The infused Na_2EDTA

was given in a 0.1 M solution in saline at a rate of 6-8 ml/minute.

Blood samples were taken from the opposite jugular, both before and after infusion following a set periodic schedule. Results showed a precipitous drop of serum calcium of approximately 2.5 mg per cent. Rate of return curves showed serum calcium values had returned to normal in approximately 4 hr.

Additional studies were conducted to evaluate the effects of parathyroid extract on this experimental hypocalcemia. Five hours previous to Na_2EDTA infusion, animals were treated with approximately 7.5 U.S.P. units of parathyroid extract per kilogram of body weight. As expected, the pretreatment with parathyroid extract reduced the severity of the hypocalcemia and decreased the length of time required for serum calcium levels to return to normal.

P79. Ratios of serum phosphorus to calcium or total reducing substances in parturient dairy cows. K. A. KENDALL AND K. E. HARSHBARGER, University of Illinois, Urbana.

Levels of calcium, inorganic phosphorus, and total reducing substances (T.R.S.) in the blood serum of dairy cows during the first 24 hr following parturition were studied. Eighteen cows which were considered normal and seven which developed parturient paresis were included in the study. The average serum inorganic phosphorus to calcium ratio in the normal cows was 1:2.12, while in the cows developing parturient paresis the ratio was 1:4.14. When the relative levels of inorganic phosphorus to T.R.S. were compared, the average P:T.R.S. ratio in the normal cows was 1:11.4, and in the parturient paresis cows the ratio was 1:35.3. The data further emphasize the possible importance of an imbalance between blood serum inorganic phosphorus and calcium or T.R.S., or both, in the symptomology of parturient paresis in dairy cows.

P80. Relationship between skinfold thickness and fatness, production, dairy character, and roughage intake in dairy cows. A. A. RIMM,* R. E. MATHER, AND P. F. RANDEL, New Jersey Agricultural Experiment Station, Sussex.

Skinfold thickness was measured with the Lange skinfold caliper to the nearest 0.1 mm at the shoulder, rib, flank, and udder. Subcutaneous tissue plus skin were also measured at the shoulder and flank. Thickness of subcutaneous tissue was determined by difference. During the past 3 yr a total of 99 Holstein cows were measured twice between 107 and 121 days after calving while they were on an ad lib. hay ration. Roughage dry matter intake, weight, and milk production data were obtained during the period. Fatness or condition of the cow was visually estimated to the nearest tenth according to a system where 3.0 was average, 4.0 was fat, and 2.0 was thin.

Among-cow-within-year correlations of condition score measurements with shoulder skin plus tissue (0.26), flank skin plus tissue (0.54), tissue at shoulder (0.31), and tissue at flank (0.52) were highly significant ($P < .01$). There was no correlation between skinfold measurements and production. Roughage dry matter intake was significantly correlated ($P < .05$) with flank skin plus tissue (-0.21) and flank tissue (-0.23). The correlation of dairy character type score with flank skin plus tissue was -0.22 ($P < .05$) and with condition score was -0.30 ($P < .01$).

P81. Some aspects of the regulation of feed intake in dairy cows. H. R. CONRAD,* A. D. PRATT, AND J. W. HIBBS, Ohio Agricultural Experiment Station, Wooster.

This study was undertaken in an effort to quantify the relationship of feed (dry matter) intake to certain physical and physiological factors considered to regulate nutrient consumption in cows. Voluntary feed intake and (dry matter) digestibility were determined in 82 trials, with rations ranging in digestibility from 53 to 80%. A multiple regression analysis revealed that digestibility, fecal dm per 1,000 lb (rate of passage of indigestible residue), and body weight (BW) accounted for the variation in feed intake between 53 and 65% digestibility. $R = 1.01$ ($P < .01$). The terms; $BW^{0.96}$, $Dig.^{1.64}$, and fecal $dm^{10.2}$; expressing these relationships were approximately identical to those predicted of capacity limited feed intake. Between 66 and 80% digestibility, intake decreased with increasing digestibility. In this region intake was also directly related to the chemical energy of production and $BW^{0.62}$ which did not differ significantly from $BW^{0.73}$. It was concluded that physical and physiological factors regulating feed intake change in importance with increasing digestibility. At low digestibility they were: body weight (reflecting roughage capacity) indigestible residue (reflecting rate of passage), and dry matter digestibility. At higher digestibilities intake followed a pattern predictable from Kleiber's law with respect to metabolic size and production.

P82. Effect of rate of hay and concentrate supplementation on milk production of cows fed high moisture grass silage. F. R. MURDOCK* AND A. S. HODGSON, Western Washington Experiment Station, Puyallup, and D. R. WALDO, Dairy Cattle Research Branch, USDA, Beltsville, Maryland.

Sixteen Holstein cows in mid-lactation were fed direct-cut grass silage ad libitum during a 20-wk period. This ration was supplemented with mixed grass-clover hay at two levels (0.5 and 1.0 lb per 100 lb body weight) and two levels of concentrates (0.3 and 0.6 lb per

pound of milk over 18 lb) in an extra-period Latin-square change-over design with four cows on each treatment. Each period comprised 4 wk.

Daily milk weights were recorded and weekly composite milk samples were tested for milk fat and SNF. Two consecutive milkings were sampled weekly for protein analysis. Feed intakes were measured and summarized weekly.

Production of FCM averaged 51.7, 47.8, 51.6, and 48.0 lb daily for high hay–high concentrate, high hay–low concentrate, low hay–high concentrate and low hay–low concentrate groups, respectively. Differences in production were significant between concentrate levels ($P < 0.01$), whereas differences between levels of hay were small and nonsignificant.

Even though production was higher on the high concentrate rations, returns over feed costs were higher on the low concentrate feeding level.

Percentage of milk protein was significantly higher ($P < 0.01$) while cows were fed high concentrates.

P83. Fat percentage and milk production of cows grazed on Sudan grass, pearl millet, and a Sudan grass × sorghum hybrid. R. W. Hemken,* J. H. Vandersall, and N. A. Clark, University of Maryland, College Park.

In a 2 × 3 factorial design, two levels of grain and three summer annual pastures, Sudan grass (S), pearl millet (PM), and a Sudan grass × sorghum hybrid (H) were compared during the summers of 1960 and 1961. Twelve cows were grouped and randomly assigned to treatment each year. The average grain intakes per day were 20.5 lb for the high level and 7.3 lb for the low level.

Fat percentage during the experimental period was compared with the fat percentage produced during a preliminary period when all cows were grazing an orchardgrass-Ladino pasture. The average change of fat percentage was +0.08% for S, −0.27% for PM, −0.05 for H, −0.16 for the high grain level group, and −0.02% for the low grain level group. Pearl millet differed significantly ($P < .01$) from the other forages.

Average daily pounds of milk produced and body weight changes per day were 42.9 and −0.44 for S, 44.4 and −0.26 for PM, 43.8 and −0.44 for H, 45.8 and 0.00 for high grain, and 41.4 and −0.75 for low grain. Grain level influenced both the milk production ($P < .05$) and body weight changes ($P < .01$).

Rumen fluid samples were collected in 1961 for volatile fatty acids determination. No major differences were found; however, the molar per cent of acetic was slightly lower (62.4 vs. 64.4) and propionic slightly higher (24.5 vs. 21.7) for PM when compared with the other forages. These differences were statistically significant at the 5% level of probability.

P84. Effect of feeding various hay-concentrate ratios for short periods on milk yield, SNF, and protein. L. J. Boyd and K. C. Mathew, University of Tennessee, Knoxville.

A 3 × 3 Latin-square experiment using six Holsteins and three Jerseys was conducted for 12 wk. The three rations were: no grain (hay ad libitum), normal (1 lb grain per 3.5 lb 4% FCM plus hay ad libitum), and high grain (unlimited grain with 5 lb hay daily).

Cows on the normal ration consumed slightly more hay than when fed the no grain ration. Daily grain consumption averaged 34.7 lb on high grain. Dry matter intake was singificantly different for all three rations, averaging 24, 31, and 36 lb daily for the no grain, normal, and high grain rations, respectively.

The cows produced slightly more milk on the normal ration than on the other rations. Milk fat tests averaged 3.48, 3.38, and 3.11% for no grain, normal, and high grain, respectively. The 4% FCM averaged 19, 22, and 21 lb daily and was significantly different between all treatments. Average per cent SNF was 8.1 for the no grain, 8.3 for normal, and 8.3 for high grain. SNF on the no grain was significantly less than on the other two rations. Per cent protein averaged 3.15, 3.29, and 3.38 on the no grain, normal, and high grain rations, respectively. Protein per cent was significantly lower on the no grain ration.

P85. Effect of high level grain feeding on milk production. L. D. Brown,* D. V. Armstrong, and C. A. Lassiter, Michigan State University, East Lansing.

Eighteen Holstein cows were divided into three experimental groups approximately 36 days postpartum on the basis of milk production, age, and body weight. During the subsequent 260-day period all cows were fed 40 lb of corn silage per day. In addition, Group 1 cows received alfalfa hay free choice and grain at the rate of 1.0 lb for each 3.5 lb of milk. Group 2 cows were limited to 15 lb of alfalfa hay/cow/day and grain increased to 1.0 lb for each 2.5 lb of milk. Group 3 cows were limited to only 5.0 lb of alfalfa hay/cow/day and grain ad libitum. The average pounds of grain consumed per cow were 2,998, 4,392, and 9,262 lb for Groups 1, 2, and 3, respectively. In the same order, the average pounds of milk and body weight gains per cow for the 260-day period were 9,862, 167; 10,650, 236; and 12,543, 245 lb, respectively. The average fat, protein, and SNF percentages of milk for the 260-day period were 3.9, 3.7, 8.9; 3.9, 3.6, 8.9; and 3.8, 3.7, 9.0 for Groups 1, 2, and 3, respectively. Differences among treatment groups in milk composition were not statistically significant.

P86. Unlimited vs. limited roughage feeding of dairy cows. H. H. OLSON,* L. D. STEWART, M. L. DAHNCKE, AND H. F. BENSON, Southern Illinois University, Carbondale.

A 180-day feeding trial was conducted utilizing the entire dairy herd which was divided into two similar groups of 22 cows each (Holstein, Guernsey, and Jersey). Controls were fed unlimited amounts of hay, 3 lb of corn silage per hundredweight, and 1 lb of a 15% protein grain mixture per 3.5 lb FCM. The experimentals were restricted to 1 lb of hay and 1.5 lb of corn silage per hundredweight, unlimited corn and cob meal plus a limited amount of a 36% protein concentrate.

Average daily consumption per cow was: controls—9.3 lb grain, 23.8 lb hay, and 39.6 lb corn silage; experimentals—2.6 lb concentrate, 21.6 lb corn and cob meal, 6.2 lb hay, and 18.7 lb corn silage. Average daily FCM production for the controls was 28.2 lb vs. 27.8 lb for the experimentals. Due to low corn prices a greater return over feed cost was obtained from the experimental group. Average gain of body weight was 83 lb for the controls and 124 lb for the experimentals. A statistical analysis of nine closely matched pairs indicated the experimentals produced significantly more milk with a higher milk fat and solids-not-fat content.

P87. Observations on free-choice grain and hay feeding of dairy cattle. H. H. OLSON* AND H. F. BENSON, Southern Illinois University, Carbondale.

During a 56-day period, the University dairy herd was permitted free access to alfalfa-grass hay and a 13.5% protein grain mixture. The grain mixture contained corn and cob meal, soybean meal, minerals, and vitamins. The amount of feed fed, milk, milk fat, and solids-not-fat production was recorded for each group. Composition of the ration and the nutrient requirements for maintenance, growth, reproduction, and production were calculated using the feeding standards of Morrison.

One group of Holsteins representing 1,641 cow days averaging 1,336 lb of weight and producing 37.3 lb of FCM daily consumed 4 lb of hay and 42.7 lb of grain. This represented a daily TDN intake of 33.1 lb, contrasted to the calculated requirements of 24.6 lb. The average body weight gain was 70.6 lb. The average milk fat test was 3.2%.

A group of Guernseys and Jerseys representing 1,407 cow days averaging 1,046 lb of weight and producing 24.6 lb of FCM consumed 3.5 lb of hay and 32.6 lb of grain. This represented a daily TDN intake of 25.9 lb contrasted to the calculated requirements of 18.6 lb. The average body weight gain was 53.1 lb. The average milk fat test was 4.6%.

P88. Influence of breed and variations in percentage fat of individual cows when estimating solids-not-fat with the plastic bead method of Golding. R. E. ERB, Purdue University, Lafayette, Indiana.

Erb et al. (J. Dairy Sci., 43: 607. 1960.) in the interest of simplicity suggested a single formula for estimating per cent solids-not-fat (SNF) using the plastic bead method of Golding. Subsequent work showed that formulae were significantly different for Guernseys, Holsteins, and Jerseys when derived from milk samples of individual cows or bulked milk. The standard error of estimate exceeded one time in 20 determinations (S.E.) was 0.35% SNF for an all-breed formula based on per cent fat and density and derived from 1,388 samples of milk from individual cows. Similarly, the S.E. for the individual breed formulae were 0.34, 0.33, and 0.41% SNF, respectively, for Guernseys, Holsteins, and Jerseys. The S.E. for separate formula derived within 0.5% fat intervals (breed ignored) ranged from 0.20% SNF for milk with 3.0 to 3.4% fat to 0.43% SNF for milk with 4.5 to 4.9% fat or more than 6.4% fat. The accuracy of equations for indirectly estimating per cent SNF can be further improved by using equations derived from per cent fat intervals within breed. Under these conditions additionally including per cent protein in the prediction equations results in little practical improvement in accuracy. Prediction equations based on samples from individual cows are unsatisfactory for application to sample of bulk milk from herds.

P89. Experiences with solids-not-fat testing in the Virginia D.H.I.A. program. N. R. THOMPSON, Virginia Polytechnic Institute, Blacksburg.

The need arose, in our research with milk constituents, to obtain SNF records on individual cows. Two lactometric methods, Watson (initially) and Golding (later), were compared with the official gravimetric method and found sufficiently accurate for individual cow recording. Sampling one day each month was found fully as accurate for SNF as for fat. Water baths, glassware, and other items were purchased or fabricated. Tentative procedures for sampling and analysis were tried, in cooperation with the local D.H.I.A. Supervisor, and found satisfactory. The first regular monthly SNF test was run on the V.P.I. herd in August, 1958. Nineteen other herds came onto SNF test in this program at later dates.

The Watson lactometer readings and Golding bead counts have been obtained by the D.H.I.A. Supervisors. All monthly and lactational calculations, through 1961, were done at V.P.I. on the IBM 650 computer. However, beginning in January, 1962, the calculations and bookkeeping for six herds have been done

by the Supervisor at the farm. Monthly and lactational reports are provided to cooperating dairymen. SNF testing is as practical as is milk fat testing. It is now available as an optional part of the Virginia D.H.I.A. program.

P90. Protein, solids-not-fat, and fat variations in lactation milk records. S. N. GAUNT,* A. R. CORWIN, H. C. GILMORE, E. H. BIRDSONG, AND S. RUSSELL, University of Massachusetts, Amherst.

Analyses of milk samples taken monthly from 1,000 cows of the five major breeds in 27 herds for 20 months were made for per cent protein, solids-not-fat (SNF), and milk fat as a first phase of a 5-yr study. Averages of lactations completed to date of 130 Ayrshires, 150 Guernseys, 130 Holsteins, 129 Jerseys, and 36 Brown Swiss were, respectively, for protein 3.38, 3.52, 3.19, 3.80, and 3.59; for SNF 8.71, 9.08, 8.51, 9.30, and 9.04; for fat 4.2, 4.9, 3.8, 5.4, and 4.3 with standard deviations for protein ranging from 0.176 to 0.229, for SNF from 0.250 to 0.306, and for fat from 0.295 to 0.510. Weighted monthly averages on per cent composition on 16,377 samples were almost identical to the lactation averages.

Gross correlations for fat and protein per cent were Ayrshire 0.498, Guernsey 0.514, Holstein 0.621, Jersey 0.485, and Brown Swiss 0.657; for fat and SNF per cent, respectively, 0.534, 0.620, 0.566, 0.575, and 0.735; for protein and SNF per cent 0.565, 0.712, 0.791, 0.682, and 0.595; milk pounds and protein per cent −0.453, −0.246, −0.205, −0.200, and −0.095; for milk pounds and SNF per cent −0.249, −0.127, −0.194, −0.199, and −0.111.

P91. Evaluation of protein and solids-not-fat testing for field use. S. N. GAUNT, University of Massachusetts, Amherst.

Various possibilities for conducting testing programs in the field are outlined. For efficiency and economy the program should emphasize the two most important components of milk. Fat is still of economic value, so the question becomes whether to test for protein or solids-not-fat (SNF).

Two general types of testing programs are a central laboratory serving several testers and the individual tester, or modifications. Sufficiently accurate tests are available for testing of protein and SNF: for protein, the dye binding tests using Orange G or Amido Black and for SNF the Watson Lactometer and Plastic Bead Hydrometers. All these tests have critical points requiring training and supervision of testers. The SNF methods are practical portable field tests. However, they are indirect tests and include not only the errors of their own measurement, especially those in the formula calculation, but also the errors in the fat test.

Several states have developed systems for adapting information from field tests on protein and SNF to routine processing with IBM. Much more information is needed on the various aspects of field testing, including the standardization of procedures, development of simplified testing equipment, and preservatives for composite samples for dealers.

P92. Sorbitol metabolism by bovine spermatozoa. R. J. FLIPSE, Pennsylvania State University, University Park.

Cellular uptake and $C^{14}O_2$ production by washed ejaculated spermatozoa were measured following incubation with sorbitol-C^{14} under various conditions. Cellular uptake occurred both aerobically and under nitrogen. With a constant tracer dose of sorbitol-C^{14}, the relative yields of $C^{14}O_2$ with 10^{-2} M unlabeled sorbitol, glucose, and fructose were 100, 18, and 43, respectively. Fatty acids (acetate and octanoate) were less competitive with sorbitol; concentrations up to 10^{-2} M did not reduce $C^{14}O_2$ from sorbitol-C^{14} by more than 30%. Malonate and fluoride at 10^{-2} M caused little or no depression of uptake or $C^{14}O_2$ production. However, 10^{-3} M cyanide reduced $C^{14}O_2$ production 65% without affecting uptake and 10^{-2} M cyanide essentially eliminated $C^{14}O_2$ production while reducing uptake by 55%. Dinitrophenol, 10^{-4} M, reduced $C^{14}O_2$ production 20% and cellular uptake 55%. Sorbitol obviously is metabolized less readily than glucose or fructose, but it is catabolized rapidly enough to limit its usefulness for measuring rate of transfer across the sperm cell membrane.

P93. Carbon dioxide as a factor in glycolytic reactions of epididymal-like bovine spermatozoa. J. R. LODGE* AND C. N. GRAVES, University of Illinois, Urbana.

Previous results (J. Dairy Sci., 44:1181. 1961.) with epididymal-like spermatozoa (ELC) diluted with a citrate-bicarbonate medium containing fructose and incubated 4 hr at 37 C showed a necessity for CO_2 in the initiation of glycolysis and for the maintenance of motility. Further study shows that motility and metabolism of ELC can be recovered by regassing with 5% CO_2 95% N_2 after 1, but not after 2, 3, or 4 hr of incubation under N_2. Glycolysis was initiated by a level of CO_2 as low as 0.5%, but the optimum glycolytic rate occurred with 2% CO_2 in the N_2 atmosphere. Although 0.5% CO_2 maintained some motility, optimum motility after incubation was found in flasks which had contained levels above 0.5%. The addition of 0.02, 0.002, and 0.0002 M DPN and DPNH did not replace CO_2 in initiation of glycolysis and the highest level inhibited lactate accumulation under 5% CO_2. The same concentrations of DPN with pyruvate as substrate showed a stimulation by 0.02 M DPN in the conver-

sion of pyruvate to lactate and in CO_2 evolution under N_2, but not under 5% CO_2. With 0.02 M DPNH considerable CO_2 was taken up under 5% CO_2, in contrast to an evolution with the lower levels of DPNH and the control.

P94. Incorporation of radioactivity from glycine-C^{14} by mammalian spermatozoa. C. N. GRAVES, University of Illinois, Urbana.

A study was conducted to determine the rate of incorporation of the radioactive carbons of glycine into the different components of the bovine sperm cell. Washed spermatozoa were incubated at 37 C for 4 hr in glycine-1-C^{14} and glycine-2-C^{14}, washed free of the incubation diluent, and fractionated into the acid soluble, lipid, nucleic acid, and protein fractions. Although no stimulation of oxygen uptake occurred over that of endogenous controls during the incubation period, radioactivity was obtained in all fractions. The radioactivity of the CO_2 collected during the incubation period was higher from the sperm cells which were incubated in the C-1 labeled glycine than those from the C-2 labeled glycine. The lipid, nucleic acid, and protein fractions of the cells incubated with the C-2 labeled glycine showed greater incorporation of radioactivity than did those from spermatozoa incubated with the C-1 labeled molecule. The incorporation into the acid-soluble portion, however, was greater in the case of the C-1 labeled glycine. Chromatographic studies of the nucleic acid fraction indicate a definite incorporation of radioactivity from both carbons of glycine into the nucleic acid bases.

P95. Factors influencing the head size and shape of bovine spermatozoa. C. G. VAN DONGEN* AND G. W. SALISBURY, University of Illinois, Urbana.

The influence of bulls, duration and temperature of storage, and their interactions on the head size and shape of bovine spermatozoa were studied. Spermatozoa of four single ejaculates from each of four bulls were suspended to 320 $\times$ 10^6 cells/cm^3 in an egg yolk–citrate diluent and subsamples were stored at 5 and 27 C. Freshly ejaculated spermatozoa and those stored for six days were projected onto a screen with area amplification of 25 $\times$ 10^6 and area tracings were made from eosin-nigrosin unstained spermheads. In addition to the area tracings, measurements of total length, maximum width and base width of the spermheads from eight of the ejaculates were made. The data show no statistically significant ($P \leq 0.05$) bull differences for total length, base width for area and maximum width. Storage at 27 C significantly increased the spermhead area in contrast to no change at 5 C. The head shape changed during storage at both temperatures from an elongated to a more rounded form. Size data from a 500-cell distribution within one fresh ejaculate revealed a significant skewness from normal for maximum width and base width, and approached significance for area and length. In general, these data do not support evidence for binominal distribution of the spermhead size.

P96. Avian ova injected with bovine semen. E. V. CARUOLO, G. D. MARX, AND G. E. MILLER,* University of Minnesota, St. Paul.

Twenty-one developing avian ova were injected with 0.1 to 1.5 ml of bovine semen. Injection into immature ova resulted in slow resorption on the ovary. Most mature-appearing ova were laid within 24 hr post-injection. Eight injected ova were laid without the hard outer shell. Two of the eight ova contained sperm. One ova laid 6 hr post-injection contained motile sperm.

A segment of hen oviduct was ligated at both ends and injected with bull semen. Sperm cells were found 6 hr post-injection; though nonmotile, live-dead stain indicated some were alive. On microscopic examination of mucosal scrapings, no sperm cells were found 24 hr post-injection. Histological sections of oviduct one day post-injection showed few sperm cells and few polymorphs. Sections of oviduct four days post-injection contained no sperm cells.

Bovine sperm motility readings were made using the following extenders: citrated oviduct homogenate, egg yolk–citrate, and citrated egg yolk obtained from developing ova. Each extender was compared unbuffered and buffered with phosphate. Egg yolk–citrate without phosphate was the superior extender.

P97. Effect of a high ejaculation frequency on sperm output and semen characteristics of Holstein bulls from puberty to 2 yr of age. J. O. ALMQUIST* AND R. P. AMANN, The Pennsylvania State University, University Park.

Twenty randomly selected calves maintained on 100% T.D.N. were paired when motile sperm were first produced (35 to 46 wk). Ejaculation frequency for control bulls was biweekly ($\frac{1}{2}\times$) to 1 yr of age and weekly ($1\times$) thereafter as compared to $3\times$ and $6\times$ weekly for experimental bulls. The specified numbers of ejaculates were obtained by systematic changes of teaser and collection location. Three false mounts preceded each ejaculation. Semen volume, sperm concentration, and total sperm per ejaculate for the $\frac{1}{2}\times$ and $3\times$ bulls averaged 2.7 and 2.5 ml, 496 and 482 $\times$ 10^6/ml, and 1,436 and 1,199 $\times$ 10^6. Mean ejaculate characteristics for 53 to 56, 77 to 80, and 101 to 104 wk of age were: semen volume, 3.3, 4.3, 4.9 ml ($1\times$) and 2.3, 3.1, 3.6 ml ($6\times$); sperm concentration, 848, 1,427, 1,555 $\times$ 10^6/ml ($1\times$) and 741, 1,179, 1,145 $\times$ 10^6/ml ($6\times$); and total sperm per ejaculate, 2,749, 6,175, 7,649 $\times$ 10^6 ($1\times$) and 1,642, 3,677, 4,097 $\times$ 10^6 ($6\times$). Potential number

of inseminations per bull from puberty to 104 wk, assuming 10×10^6 motile sperm, averaged 21,160 for the controls and 74,429 for the experimental group. Body weight was unaffected. No deleterious effect of 6× was observed.

P98. Higher extension rates of semen as a means of increasing the usefulness of sires. R. H. FOOTE, Cornell University, Ithaca, New York.

Two field trials comparing 5×10^6 motile sperm vs. 10×10^6 motile sperm/ml of CUE extender were conducted in 1960 and 1961, using semen from bulls in the stud of the New York Artificial Breeders' Cooperative, Inc. Total first services and average 60- to 90-day nonreturns for the five-million and ten-million treatments in the 1960 trial were 10,613 and 74.6%, and 10,647 and 76.1%, respectively. Corresponding values for the second trial were 24,737 and 75.2%, and 18,933 and 76.1%. Nonreturn rates did not differ significantly in either trial, or in the two trials combined, $P > .1$. The higher extension rate nearly doubles the potential number of progeny per sire, thereby reducing the number of bulls required to breed a finite cow population. If this potential is exploited, considerable genetic improvement could result. For example, with Holstein sires evaluated on the basis of 50 or more tested daughters, and using herd-season-year adjusted records, it is expected that daughters of the top half of the bulls will exceed the average of the whole group by at least 390 lb of milk and 14 lb of milk fat.

P99. Effects of temperature and control feeding on thyroxine I^{131} degradation rates and serum protein fractions of dairy cattle. R. G. LUNDGREN* AND H. D. JOHNSON, Department of Dairy Husbandry, University of Missouri, Columbia.

Higher environmental temperatures and humidity depress thyroxine I^{131} degradation rates of lactating dairy cattle under ad libitum feeding conditions. To determine if this in vivo decline in thyroxine I^{131} degradation was due to the lowered (voluntarily) feed intake or the direct temperature effects, six lactation Holstein cows (two ad libitum and four control fed) were exposed for 3-wk periods alternately to 65 F, 50% R.H. and 88 F, 50% R.H. (six periods).

Blood samples were taken daily for 96 hr following injection of 100 μc/animal of thyroxine I^{131}. Samples for the serum electrophoretic analysis were also taken during the third week of the treatment periods.

Average differences (88 F less 65 F values) for all animals (ad libitum and control fed, respectively) were: thyroxine I^{131} degr. (K) −1.93 and −2.38 (.01); serum protein −.75 and −1.36 (.01); albumin +.6 and +3.6 (.01); γ globulin −2.05 and −3.41 (.01); α globulin +3.3 and +.41 (.05); and β globulin +.76 and −.16 (n.s.). Differences between ad libitum and control fed animals were significant for albumin (.01), α globulin (.01), and gamma globulin (.1).

Temperature effects on protein fractions are being related to the thyroid function. Thyroxine I^{131} degradation rates are depressed by high environmental and body temperatures rather than feed quantity differences.

P100. Variables influencing the uptake of radioactive triiodothyronine from plasma by red blood cells of dairy cattle. K. T. SZABO AND J. P. MIXNER,* New Jersey Agricultural Experiment Station, Sussex.

The in vitro uptake of tracer amounts of radioactive triiodothyronine (T_3-I^{131}) by red blood cells (RBC) has been suggested as a simple method for evaluating thyroid function. Since CO_2 tension has been reported as influencing the uptake of T_3-I^{131} by RBC, six blood samples were incubated with T_3-I^{131} in (a) open tubes, (b) stoppered tubes, and (c) tubes flushed with CO_2 and then stoppered. The mean T_3-I^{131} uptakes were 9.18, 9.40, and 12.82%, respectively. CO_2 flushing and stoppering was adopted as a standard procedure. The mean RBC uptakes of T_3-I^{131} by three blood samples stored for 0, 1, 4, 8, and 15 days at 5 C were 15.0, 13.8, 13.5, 11.1, and 10.0%, respectively. Twelve blood samples stored for zero and one day had mean T_3-I^{131} uptakes of 13.64 and 13.16%, respectively, the difference not being significant. Different lots of diluted T_3-I^{131} were stored at 5 C for 1, 3, 5.5, and 7.5 wk prior to its use. Mean uptakes with three blood samples were 14.49, 14.35, 14.35, and 12.54%, respectively, the last being a significant drop. Four blood samples were used to determine the effect of varying tracer levels of T_3 upon T_3-I^{131} uptake of RBC. Little effect could be shown.

P101. Metabolism of uniformly labeled glucose-C^{14} introduced into the rumen of a lactating dairy cow. K. K. OTAGAKI,* A. L. BLACK, J. C. BARTLEY, M. KLEIBER, AND B. O. EGGUM, University of California, Davis.

Uniformly labeled glucose-C^{14} (G-U-C^{14}) was introduced into the rumen of a lactating cow through a rumen fistula. Respired CO_2, rumen contents, jugular blood, and milk samples were collected periodically after intraruminal introduction of G-U-C^{14}.

The specific activity of the cow's respired CO_2 reached a maximum at 45 min, blood fatty acids at 15 min, while that of blood glucose was delayed for 2 hr after administration.

Rumen bacteria cell material turned over more rapidly than that of rumen protozoa. The maximum specific activity of bacteria was observed in the first sample collected (1 hr). The specific activity of protozoa increased for

several hours, reaching a maximum in the sample collected 10 hr after introducing G-U-C^{14}.

Rumen fatty acids showed highest specific activity in the first sample collected (1 hr) and the values decreased by approximately one-half each 2 hr. After 24 hr C^{14} was no longer detectable in fatty acids.

The specific activity of milk protein and the amino acids derived therefrom will be compared with that from rumen bacteria and protozoa.

P102. Effect of pelleting alfalfa on in vitro gas production, cellulose digestion, and volatile fatty acid production. S. W. Rojas, G. M. Ward, and R. G. Hinders, Colorado State University, Fort Collins.

Pelleted dehydrated alfalfa, pelleted sun-cured alfalfa, and long sun-cured alfalfa hay was fed to rumen-fistulated cows and the collected rumen fluid used for in vitro studies, with the same feeds serving as substrates.

Under in vitro conditions highly significantly less gas production and cellulose digestion and a lower acetate/propionate ratio was the result of using rumen fluid from a cow fed dehydrated alfalfa pellets as compared to rumen fluid from the cow fed hay. Significantly less gas production, slightly more cellulose digestion, and a lower acetate/propionate ratio was produced by rumen fluid from a cow fed sun-cured alfalfa pellets as compared to hay.

Dehydrated alfalfa pellet substrate resulted in highly significantly less cellulose digestion and in a lower acetate/propionate ratio than hay substrate regardless of the type of rumen fluid.

Isovaleric acid could not be detected, by gas chromatography, in the rumen fluid of cows fed dehydrated alfalfa pellets nor in the fermentation fluid when rumen fluid was used from these cows. Isovaleric acid was found to the extent of 2.5 to 7.3% when sun-cured alfalfa hay or pellets were fed or used as substrates.

P103. Effect of pH, roughage, glucose, and an added enzyme preparation on in vivo and in vitro production of volatile fatty acids in rumen fluid. W. H. Brown* and H. Tucker, University of Arizona, Tucson.

One each of four lactating cows was assigned to the following diets: A. 6 lb alfalfa and 20 lb grain. B. 6 lb alfalfa, 20 lb grain, and 1.7 g agrozyme (a product of Merck and Company exhibiting proteolytic and lipolytic action). C. 24 lb alfalfa and 10 lb grain. D. 24 lb alfalfa, 10 lb grain, and 1.7 g agrozyme. Four aliquots [control, added glucose (20 μM/ml rumen fluid), added agrozyme (0.3 mg/ml rumen fluid), and added glucose plus agrozyme] of rumen fluid from each cow were incubated at 39.5 C for 2 hr at pH levels of 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, and 7.5. HCl, $HKC_8H_4O_4$, KH_2PO_4, and/or K_2HPO_4 were used as buffers.

In vivo levels of total acids, acetate, propionate, and butyrate in the rumen were increased by the low-roughage diet and by the feeding of agrozyme. Glucose increased the in vitro production of all three acids and total acids; however, agrozyme had no effect on in vitro production. There was a general increase in production of all the acids as the pH was raised from 4.5 to 6.5. There was no significant change from 6.5 to 7.5.

P104. An in vitro artificial rumen technique, with studies on the relative value of different ration constituents. R. E. Smith, R. G. Hinders, and G. M. Ward, Colorado State University, Fort Collins.

The influence of the following variables on a manometric evaluation of feeds was studied: (1) ration of donor cow, (2) amount of substrate, (3) amount of rumen fluid inoculum, (4) volume of buffer, (5) time of reading, and (6) physical state of substrate.

Rumen fluid inoculum was obtained 4 hr after feeding from a fistulated Brown Swiss cow maintained on an average-quality alfalfa hay ration. Twenty-five milliliters of strained rumen fluid was incubated with 15 ml of .25 M phosphate buffer and 0.5 g feed. Gas production was measured by liquid displacement in 100-ml graduated burettes, readings being taken at 6, 12, and 18 hr intervals. Average 18-hr gas production (in milliliters) from 13 samples was as follows: barley, 81.4; nonfat-dried-milk-solids, 61.9; oats, 58.8; commercial grain mix, 58.0; 27% protein alfalfa hay, 53.1; beet pulp pellets, 49.1; green chop alfalfa, 46.2; low protein alfalfa, 39.0; average-quality corn, 38.5; high-fiber alfalfa, 35.6; pelleted alfalfa, 29.7; barley straw, 7.0; and sagebrush, 3.1.

This simple and rapid method shows promise as a means of in vitro evaluation of feedstuffs.

P105. Specificity of the heme requirement for growth of Bacteroides ruminicola—a ruminal saccharolytic bacterium. D. R. Caldwell,* D. C. White, and M. P. Bryant, Dairy Cattle Research Branch, Beltsville, and Rockefeller Institute, New York.

Previous studies demonstrated that the rumen fluid growth requirement of *Bacteroides ruminicola* subsp. *ruminicola* was replaced by hemin. Studies on Strain 23 show that hemoglobin, catalase, peroxidase, protoporphyrin IX, hematoporphyrin, mesoporphyrin, deuteroporphyrin, uroporphyrinogen, coproporphyrinogen, and manganese and zinc protohemes will replace hemin. Compounds unable to replace it include $FeSO_4$ in the presence or absence of citrate or ethylenediaminetetraacetic acid, ferrichrome, coprogen, terregens factor, delta-

aminolevulinic acid, porphobilinogen, uroporphyrin, cytochrome c, bilirubin, and chlorophyll. Small amounts of growth occurred in media containing coproporphyrin and copper protoheme. Media containing 7-40% of cell-free rumen fluid from cattle fed alfalfa hay-grain rations contained sufficient heme-replacing factors to support good growth of Strain 23. Phosphate-buffered suspensions of heme synthesizing Strain GA33 of *B. ruminicola* subsp. *brevis* produced porphyrins and porphobilinogen from delta-aminolevulinic acid but Strain 23 did not. The results indicate that *B. ruminicola* subsp. *ruminicola* has a specific requirement for porphyrins, but can utilize a wider range of porphyrins than other heme-requiring microorganisms so far studied. The results suggest that it lacks the enzymes delta-aminolevulinic acid dehydrase and, possibly, porphobilinogenase involved in porphyrin synthesis. The organism's capacity to synthesize delta-aminolevulinic acid was not studied.

P106. Vitamin B_{12} and the metabolism of rumen fatty acids. A. M. HARTMAN,* AND L. P. DRYDEN, Animal Husbandry Research Division, USDA, Beltsville, Maryland.

Experiments with rats were performed to study the relation of vitamin B_{12} to the metabolism of individual rumen fatty acids and related acids, particularly propionic and certain branched chain and odd-carbon acids which give rise to propionate in animal metabolism, under conditions where they are directly available to the animal as in the ruminant. The acids were added to the rations of B_{12}-deficient and B_{12}-supplemented animals and growth and survival followed.

Formic acid caused a further growth depression and some deaths in B_{12} deficiency, whereas with B_{12} supplementation no deaths occurred and growth remained normal. These results are in accord with other workers' findings, indicating, in a bacterially derived in vitro enzyme system, participation of B_{12}, along with active folic acid, in formate metabolism. Propionic, methylmalonic, n-valeric, iso-butyric, and 2-methylbutyric acids also produced further growth depression, and with some of these acids also deaths, in the absence of B_{12}; vitamin B_{12} supplementation prevented deaths and allowed normal growth. In contrast, without B_{12}, the even-carbon straight chain acids, acetic, butyric, and caproic, brought about no further lowering of the growth rate and caused no deaths. Succinic, isovaleric, lactic, and pyruvic acids also were without effect in B_{12} deficiency.

P107. In vitro studies of volatile acids (VFA) transfer through the rumen epithelium, using a new instrument. GYORGY VIDACS, GERALD M. WARD, AND GYULA NAGY, Colorado State University, Fort Collins.

Our observations on rumen epithelial changes occurring on different rations led to the conclusion that the absorption of VFA may not be uniform over the entire surface of rumen, and may be altered by lesions of the rumen epithelium (parakeratosis, destroyed papillae, etc.). Artificial rumen and diffusion chamber techniques were combined in an instrument to investigate the problem. Rumen epithelium from freshly slaughtered animals (within 20 min) were clamped between two glass elbows (1.5-inch inside diameter). One elbow was used as an artificial rumen (125 ml of rumen fluid, 2.5 g of substrate). The amount of gas produced was measured manometrically. The other elbow was filled with blood serum, and oxygen (95% O_2–5% CO_2) was bubbled continuously through it. The units were submerged in a water bath. Blood serum and rumen samples were withdrawn at 0, 1, and 2 hr, tested for pH, and analyzed by gas-chromatography for volatile fatty acid content. The results were related to the wet and dry weight of the epithelium sections, and showed differences in transference of VFA.

P108. In vitro studies on alfalfa substrate using deer, cow, sheep, and cattle as rumen fluid donor. GYULA NAGY,* GYORGY VIDACS, AND GERALD M. WARD, Colorado State University, Fort Collins.

Samples of rumen fluid were obtained from (1) mule deer from mountain range, (2) Brown Swiss cow fed alfalfa hay, (3) sheep fed a heavy grain ration, (4) Holstein steer fed on corn silage. Triplicate samples were fermented in artificial rumen on alfalfa substrate for 16-hr periods. The gas produced was measured manometrically. The volatile fatty acids (VFA) were determined by gas chromatography.

The deer rumen fluid resulted in the lowest acetate and highest propionate percentage.

The following range was found in molar percentage of acetate, propionate, and butyrate, respectively: deer, 42-49, 36-27, 17-18; cow, 60-65, 18-16, 16-14; sheep, 55-54, 23-27, 16-14; steer, 55-54, 20-21, 19-20.

When the rumen fluids of deer and cow were mixed (1:1), molar percentages of 43% acetate and 37% propionate were produced without substrate, compared to 55% to 27% on alfalfa hay substrate.

Comparisons were made of the importance of the rumen solids and supernatant from the cow and deer. The solids from cow rumen fluid were mixed with the supernatant from deer and the reverse. The higher propionate production by deer is a function of the solid fraction (probably microorganisms).

P109. Grasses and alfalfa for annual forage and pasture in South Central Alaska. A. L. BRUNDAGE,* W. J. SWEETMAN, L. J. KLEBESADEL, N. E. MICHAELSON, AND C. I. BRANTON,

Alaska Agricultural Experiment Station, Palmer.

Many grasses and legumes achieve phenomenal seedling year growth rates under the long-day, cool temperature environment of South Central Alaska, but do not survive the ensuing winter. This experiment compared four grasses; common timothy and ryegrass, Tualatin tall oatgrass and orchard grass; for annual forage and late pasture when sown singly or in combination with New Mexico common alfalfa. One-half acre plots were seeded in May 23, 1961, in a randomized block with two replications and irrigated as required. Both replicates were cut for silage on July 24. One replicate was grazed by 22-24 cows from August 28 through September 5 and the other grazed by 20-22 cows from September 6 through 17. All plots were sampled for dry matter before grazing. The number of cows grazing on each plot was recorded at 5-min intervals for the first three days on each replicate. The ryegrass plots ranked highest in production (2.8 T dry matter per acre) and lowest in palatability. Tall oatgrass ranked highest in palatability and fourth in production (2.1 T D.M/acre). Orchard grass–alfalfa, which ranked third in palatability and yield (2.4 T D.M/acre), was superior for forage and pasture.

P110. Intake, digestibility, and animal gain of three different cuttings of birdsfoot trefoil, alfalfa, bromegrass, and reed canarygrass. J. R. Ingalls,* J. W. Thomas, and M. B. Tesar, Michigan State University, East Lansing.

Pure stands of first, second, and third cuttings of birdsfoot trefoil, (BT) alfalfa, (A) bromegrass, (B) and reed canarygrass (RC) were harvested 6/7, 8/3, and 9/8, respectively, and fed ad lib. for periods of 25 days. Dry matter (DM) intake/cwt, digestibility, digestible dry matter (DDM) intake/cwt and gain were determined, using four sheep per cutting in a Latin-square design. Average DM intake/cwt was BT = 3.25 > A = 2.97 > B = 2.59 ($P < 0.05$), and RC = 2.38. Average per cent DDM of all cuttings was BT = 61.9, RC = 63.1 > B = 60.6 > A = 57.5, ($P < .05$). Average first, second, and third cutting digestibilities were 2nd = 56.6 < 1st = 62.2 ($P < .01$), 3rd = 63.0. Using 0, 24, or 48 hr lag periods, between feeding and excretion, to calculate per cent DDM did not alter this value when collections were made between Days 16 and 22. Average DDM intake/cwt was BT = 2.01 > A = 1.71, B = 1.57, RC = 1.50 ($P < .01$) A > RC ($P < .05$). Average daily gains were T = .28 > A = .13, B = .11, and RC = .09 ($P < 0.05$). Simple correlation coefficients between gain and DM/cwt, per cent DDM and DDM/cwt were .52, .34, and .63, respectively, using individual values; between DM intake/cwt and per cent DDM r was −.20. The limitation of using per cent DDM as a criterion in forage evaluation is evident.

P111. Alfalfa-orchardgrass versus alfalfa-bromegrass for lactating cows. F. G. Owen and R. G. Hinders,* University of Nebraska, Lincoln.

Alfalfa-orchardgrass and alfalfa-bromegrass pastures were strip-grazed with Holstein and Brown Swiss cows during two grazing seasons. A high and low rate of alfalfa seeding gave the following dry weight percentages of alfalfa to grass the first spring after seeding: alfalfa-orchard, high (AOH) 76% alfalfa, 24% orchard; alfalfa-orchard, low (AOL) 60% alfalfa, 40% orchard; alfalfa-brome, high (ABH) 67% alfalfa, 33% brome; alfalfa-brome, low (ABL) 40% alfalfa and 60% brome. The second season, very little brome persisted. Supplemental rations were fed on an equivalent energy basis on all pastures.

FCM and TDN production per acre was highest for AOL. Persistency of FCM for the three-month grazing season averaged for AOH, AOL, ABH, and ABL, 72, 80, 83, and 79%. Digestibility (chromogen technique) averaged about 62% and was practically the same for all pasture treatments.

P112. Effects of age at harvest and method of preservation of Coastal Bermudagrass upon milk production. C. M. Clifton,* W. J. Miller, and N. W. Cameron, University of Georgia, Athens.

Coastal Bermudagrass was harvested 30 and 42 days after previous cutting, and preserved as silage for hay. Ground corn was added to the ensiled forage at the rate of 100 lb per ton.

These forages were fed to 24 cows, in a 6-wk continuous feeding trial. Grain was fed at two levels (1:2 or 1:4) based on production of FCM during the standardization period. In the 2 wk standardization period all cows received Coastal Bermudagrass silage and hay with an intermediate level of concentrates (1:3).

The average milk production for the standardization period was 38.0 lb cow/day. Average 4-wk persistency percentages for the silage-fed cows was 90 compared to 80 for those fed hay. Persistencies for those fed early and late harvested forages were 87 and 83, respectively. Cows fed the higher level of grain had an average persistency percentage of 92 compared to 78 for those on the lower level.

P113. Dry matter intake and digestibility of a Ladino-orchardgrass mixture, nitrogen fertilized orchardgrass, and a Bermudagrass-rye forage grazed by lactating cows. J. I. Leslie,* R. W. Hemken, and N. A. Clark, University of Maryland, College Park,

Groups of four lactating cows were grazed on each of five forage treatments laid out in a

split-plot design, in two replications. Split-plots were the forage treatments consisting of a Ladino-orchardgrass mixture with no nitrogen fertilization, orchardgrass forage fertilized with 100, 200, and 300 lb of N/acre/annum, and Bermudagrass (sod-seeded with rye for early spring grazing) fertilized with 200 lb of N in 1960 and 300 lb of N in 1961. The cows were fed grain at a 1:6 ratio. Grazing seasons were from early April through September. Forage dry matter intake and digestibility were calculated from Cr_2O_3 and Chromogen(s) determinations over four seven-day periods in each season.

The average daily dry matter intakes (total) were 29.6, 24.8, 22.5, 24.9, and 22.7 lb for 1960, and 24.3, 22.8, 21.3, 20.4, and 23.6 lb for 1961, respectively, for the five forage treatments. The seasonal forage D.M. digestibilities were 55.1, 56.2, 57.4, 57.7, and 53.9% in 1960, and 53.8, 53.8, 55.5, 56.2, and 53.8% in 1961, for the respective treatments. The relative size of the intake values reflected the relationship of milk production among treatments.

P114. Nitrogen and energy utilization of orchardgrass fed at two protein levels. E. A. KANE, C. G. MELIN, AND O. M. BOWMAN, Dairy Cattle Research Branch, USDA, Beltsville, Maryland.

Two silages, containing 25.4 and 14.7% protein, were fed to six sheep as the sole ration. Digestibility trials were conducted by total collection method in a cross-over design experiment. Significant differences in digestibility coefficients were found for the high-N silage only in protein (80.1 vs. 67.7), and nitrogen-free extract (65.1 vs. 70.6). In regard to amounts of components digested, significant increases were shown by the high-N silage in dry matter, protein, and ether extract, and a significant decrease in nitrogen-free extract.

A study of the nitrogen utilization of the two silages revealed that although the high-N silage had significantly larger amounts of nitrogen consumed, digested, excreted, and retained, its over-all efficiency of nitrogen utilization, although higher, was not significantly different from that of the control silage.

The digestible and metabolizable energies of the silages were determined. The high-N silage displayed significantly higher values in both categories.

Silages will be reported in accordance with current feed evaluation systems to show unsatisfactory digergence of systems in assessing the nutritive value of forages.

P115. Nutrient value of forages. II. The influence of two stages of development of an Alfalfa–brome grass hay on consumption and milk production. J. H. BYERS AND L. E. ORMISTON, University of Illinois, Urbana.

Twenty cows were used in a 4×4 Latin-square designed study of four lots of hay. Hays fed were first-cutting bud stage (B1), second-cutting bud stage (B2), first-cutting three-quarter bloom (L1), and second-cutting three-quarter bloom (L2). Each period was of 3 wk duration with a 1-wk transition period. Hay lots were so fed as to insure at least a daily 10% refusal. A simple grain mixture approximately 15% total protein was fed at the rate of 1 lb of grain to 2.5 lb of FCM. Average pounds of hay consumed per 100 lb. of body weight per day were B1, 2.31; B2, 2.30; L1, 2.18; and L2, 1.71. Average daily body weight changes in pounds were B1, +0.05; B2, +0.7; L1, −0.05; and L2, +0.5. Average daily pounds of FCM produced were B1, 41.2; B2, 39.9; L1, 40.8; and L2, 35.1. Average solids-not-fat of the milk produced were B1, 9.31; B2, 9.28; L1, 9.36; and L2, 9.19%. The data suggest that the nutritive value of the hay accounts for about one-third and consumption of the hay for about two-thirds of the differences in performance.

P116. Effect of the size of grind and the level of intake of pelleted alfalfa hay on its nutritive value in cows and sheep. G. F. W. HAENLEIN,* C. R. RICHARDS, AND W. H. MITCHELL, University of Delaware, Newark.

Alfalfa hay was processed into five rations: ground ¾ inch (I), 1 inch (II), ⅜ inch (III) and each pelleted; chopped at 2 inches (IV); chopped at 2 inches (V) and pelleted. Ration II was also fed (VI) at the same intake levels as Ration IV to distinguish effects of intake level from size of grind.

Digestibilities of nutrients were determined from ten-day total collections with six wethers each; and four-day, grab-sample collections (Cr_2O_3 method) with three Guernsey cows in late lactation using Rations II, III, IV, and I.

Results (rations in parentheses) were: daily dry matter consumption per 100 lb body weight: Cows: 2.0 (II), 2.2 (III), 1.6 (IV), and 2.3 lb (I); wethers: 2.6 (I), 2.9 (II), 2.8 (III), 1.5 (IV), 2.7 (V), 1.4 lb (VI). Milk fat contents (pretrial: 6.6%): 6.5 (II), 6.2 (III), 7.5 (IV), and 6.5% (I). Milk production declined from 13.7 lb 4% FCM (pretrial) to 4.1 lb during the last trial.

Dry matter digestibilities and standard errors of the mean for the wethers were: 52.4 ± 0.5, 50.9 ± 2.0, 51.0 ± 1.7, 56.4 ± 1.1, 55.7 ± 1.4, and 56.2% ± 1.3. Digestibilities of energy, crude fiber, and digestion in the cows paralleled these changes, while digestibilities of protein differed insignificantly.

P117. Effect of pelleting on the utilization of Coastal Bermuda grass hay. W. A. KING,* G. D. O'DELL, AND C. C. BRANNON, Clemson College, Clemson, South Carolina.

Digestion trials have been conducted on field-cured Coastal Bermuda grass hay for two different years. Digestibility was determined on the baled, ground, and ground and pelleted

hays. The processed hay was ground in a hammer mill with ¼-inch screen. The pellets were ⅜-inch. The pellets were quite hard, the bulk density measuring 47 lb per cubic foot. Analyses showed pelleting apparently caused a 3.77 percentage point drop in crude fiber. The hays plus 20 g of trace-mineralized salt daily were fed separately to three dairy heifers each. The average TDN and digestible dry matter, protein, and fiber were as follows: baled hay —52.1, 54.0, 67.4, and 59.1%; ground—49.2, 51.3, 64.3, and 54.9%; and pelleted—43.6, 45.3, 59.8, and 42.0%. Digestible energy the second year averaged 49.3, 48.7, and 42.0%, respectively.

Dyed hay fed in the three physical states indicated that the pelleted hay was passing through the digestive tract almost twice as fast as baled hay. The reticulo-rumen dry matter content of the pellet-fed heifer was 33% of the baled hay. The ground hay apparently stayed longer in the reticulo-rumen and omasum than the baled hay.

P118. Pelleted and baled Coastal Bermudagrass and commercial alfalfa hay for dairy cows. O. L. Brooks, W. J. Miller,* E. R. Beaty, and C. M. Clifton, University of Georgia, Athens.

Beginning 2 wk after calving, 21 cows were fed a standardization diet of concentrates and Coastal Bermuda hay, ad libitum for 5 wk. They were then fed, ad libitum (a) Coastal Bermuda hay; (b) the same hay ground and pelleted, plus 2 lb of long hay daily; or (c) commercial alfalfa for 30 (replication 1) or 20 (replication 2) wk. During the treatment phase concentrates were fed initially at the rate of 1 to 5 lb of FCM produced during the standardization period with a 6% reduction every 4 wk. When changed from the standardization to the treatment diets all of the groups dropped sharply in milk production. Cows fed baled Coastal Bermuda hay consumed less forage, produced less milk and FCM, and gained less weight than the other groups. Those fed the pellets and 2 lb of hay produced milk richer in fat and protein than those fed Coastal hay and higher in SNF than that of both other groups. No other differences between those given the pellets plus 2 lb of hay and the alfalfa group were statistically significant ($P = 0.05$).

P119. Pelleted alfalfa and Coastal Bermudagrass as dairy roughages. E. G. Moody, Arizona State University, Tempe.

Field-cured roughages were studied in a double-reversal trial involving 24 cows for three 5-wk periods. The crude protein and crude fiber were for alfalfa hay and pellets 16.8 and 26.2%, and for Bermuda 11.4 and 24.4%, respectively.

In order of alfalfa hay-, alfalfa pellet-, and Bermuda pellet-fed groups results were: average daily consumption 1.50, 1.67, and 1.58 lb experimental roughage and 3.50, 3.43, and 3.45 lb silage per hundred pounds body weight; 1 lb grain was fed per 3.81, 3.78, and 3.92 lb FCM. The apparent digestion coefficients were for protein 70.6, 68.0, and 64.5%, for fat 75.7, 72.3, and 71.9%, and for fiber 94.7, 94.0, and 94.0%, respectively. Average daily milk production was 43.9, 45.4, 43.4 testing 3.80, 3.58, and 3.71% fat, 8.68, 8.69, and 8.64% SNF, and 3.46, 3.42, and 3.51% protein, respectively. Rumen volatile fatty acids averaged for acetic 53.6, 55.8, and 33.2, propionic 18.4, 20.6, and 12.6, and butyric 9.3, 10.5, and 8.2 micromoles per milliliter. Body weight increased 1.01, 1.51, and 1.69 lb daily. Treatment differences were statistically significant only in case of milk fat test, SNF, rumen acetic and propionic acids, and body weight gains.

P120. Comparative acceptability of wafered and baled alfalfa hay for dairy animals. B. I. Veltman,* J. W. Thomas, and J. Molitorisz, Michigan State University, East Lansing.

Three different wafers made by MF Hay Packer machine were compared to their companion baled hay in three different single reversal trials. The wafers were 2¼ × 2½ inch with lengths of ½ to 6 inches and a bulk density of c. 33 lb/cu/ft. Daily dry matter (D.M.) consumption of a high-quality alfalfa hay by heifers was 17.8 lb ± 2.8 as wafer vs. 17.0 ± 2.7 as bale and for ten cows it was 25.6 and 25.5 lb, respectively. Body weight gain of heifers was 1.70 lb/day when fed wafer vs. 0.7 as bale ($P = 0.05$). Daily D.M. consumption of ten cows fed an average-quality hay was 22.0 lb ± 4.8 as wafer vs. 23.6 ± 6.4 as bale. Orts amounted to 22.5 and 21.7%, respectively. Daily D.M. consumption of eight cows fed a below-average-quality hay was 19.6 ± 2.9 as wafer vs. 22.5 ± 3.8 as hay. Orts amounted to 23.9 and 21.6%, respectively. In two out of three cases, the consumption was less, the milk production and milk fat tests were slightly more (by 0.6 lb/day and .07%, respectively) and in all three cases the orts were more for wafered than for baled hay. These differences did not approach statistical significance. Handling loss and penetrometer resistance was most for wafer from low-quality hay and least for wafer from high-quality hay.

P121. Pituitary re-activation after prolonged thyroxine therapy and withdrawal. B. N. Premachandra* and C. W. Turner, University of Missouri, Columbia.

It is a matter of common experience that sudden withdrawal of thyroprotein from food in cattle results in abrupt lowering of milk production, presumably caused by a total lack of activity of the pituitary and the thyroid. Time needed for thyroid re-activation after thyroprotein withdrawal is not exactly known and to investigate this problem 100 μc of radi-

active iodine (I^{131}) were injected in eight dairy animals (the day after thyroxine was withdrawn completely) which were being maintained on a high plane of thyroxine for 21–26 wk for maximum stimulation of milk secretion. Twenty-four-hour thyroidal I^{131} uptake was determined every other day with successive injections of small amounts of I^{131} until there was definite pick-up of radioactivity by the thyroid. Between 12–14 days were required for significant thyroidal I^{131} uptake. Milk yield decreased precipitously for 10–15 days in different animals after thyroxine withdrawal, followed by a transient variable small increase in milk production before a plateau could be observed. Cessation of precipitous decline in milk yield after thyroxine withdrawal synchronized with the re-activation of the pituitary and thyroid as determined by I^{131} technique.

P122. Interrelationships of certain climatic conditions and productive responses of lactating dairy cows. J. C. JOHNSON, JR.* AND B. L. SOUTHWELL, Georgia Coastal Plain Experiment Station, Tifton; R. L. GIVENS, AERD, AND R. E. MCDOWELL, AHRD, ARS, USDA, Beltsville, Maryland.

Summertime observations of milk production, feed and water consumption, and rectal temperatures collected for 219 days during 1959, 1960, and 1961 from 16, 18, and 16 cows, respectively, were used to appraise the relative influence of climate on dairy cow response. Weather observations used included vapor pressure, dew point, dry and wet bulb temperature at 0800, 1100, 1400, 1700 and also an average of readings at these hours, plus daily solar radiation, hours above 80 F, maximum and minimum temperature, rainfall and wind velocity.

Weather measurements and cow responses were separated into 31 independent and 15 dependent variables, respectively. Correlations between all variables were computed. Climatic variables most highly correlated with cow responses were average and 1100 dry bulb and maximum temperature. The influence of the independent variables, including year and lactation decline, on cow response was estimated by multiple regression.

Of the variation observed in milk production and feed consumption 94 and 40%, respectively, could be attributed to the combined influence of the independent variables. Even so, examination of partial regression coefficients revealed that climate had a greater influence on feed consumption than on milk production.

Statistics obtained are presented and their application in herd management is discussed.

P123. Single versus frequent observations for estimating some summer climatic conditions in South Georgia. J. C. JOHNSON, JR.,* Georgia Coastal Plain Experiment Station, Tifton, and R. L. GIVENS, Agricultural Engineering Research Divison, ARS, USDA, Beltsville, Maryland.

Weather observations were recorded for 219 days during summers of 1959, 1960, and 1961. All possible correlations were computed between vapor pressure (VP), dew point (DP), wet bulb temperature (WB), and dry bulb temperature (DB), at 0800, 1100, 1400, 1700 and also an average of readings at these hours, plus daily solar radiation, hours above 80 F, and maximum temperature. Daily climate is best described by using continuous weather observations. Generally, this is not practical.

Correlations indicated that WB more satisfactorily measures humidity than DP or VP. The correlation of .87 between WB at 1100 and average WB indicated that WB at 1100 is probably the best single measure of humidity.

Maximum temperature, solar radiation, and hours above 80 F are related to daytime DB. Of the DB readings, DB at 1100 had the highest correlation (.85) with average DB. Correlations of 1100 DB with solar radiation, hours above 80 F, and maximum temperature were .53, .70, and .84, respectively. The best single measure of daily temperature conditions seemed to be 1100 DB. The best two observations for evaluating daily climatic conditions appeared to be DB and WB at 1100, whereas the best single observation was 1100 DB and next was maximum temperature.

P124. Influence of age at first breeding on growth, reproduction, and production of well-fed Holstein heifers. E. W. WICKERSHAM* AND L. H. SCHULTZ, University of Wisconsin, Madison.

Thirty-six purebred Holstein heifers were divided according to age into three groups (Group I, 14 to 18; Group II, 10 to 14; and Group III, six to ten months) and bred the first estrus after 18, 14, and ten months of age, respectively. Up to first calving all heifers received a ration of 4 lb of grain, 15 lb of corn silage, and hay ad lib.

At first breeding the percentages of heifers conceiving at first service and the average conception rates were as follows: Group I, 25% and 2.17; Group II, 42% and 2.50; and Group III, 50% and 3.25. The average body weights at conception and at first calving for those heifers conceiving within a 100-day period of the above breeding ages were 1,064 and 1,483; 907 and 1,361; and 733 and 1,204 lb, respectively. Although average body measurements did not differ markedly when compared at the same ages, Group III heifers were generally smaller at calving time and experienced more difficult calvings than the older animals. Slightly larger calves contributed to the calving difficulties of the youngest heifers (average birth weights: Group I, 82.8; Group II, 83.7; and Group III, 86.0 lb). The average first-lactation production of the 10, 11, and 7 heifers calving at an average age of 27.9, 24.2, and 20.3 months

was 10,990, 9,449, and 9,399 lb of 4% FCM, respectively, based on incomplete lactations averaging 274, 278, and 269 days in length and adjusted to 305 days.

P125. Lactation depression due to no dry period. E. W. SWANSON, University of Tennessee, Knoxville.

Five pairs of identical twins were used in a study of the effect of continuous milking through two or more lactations upon milk yields. One of each pair was given a 60-day dry period before each lactation while its mate had no dry period. In 40 wk of the first lactation the two groups averaged 5,850 and 5,836 lb milk, respectively. The complete first lactations averaged 6,546 and 6,658 lb milk, respectively. The respective average milk yields for the second lactation were 6,889 and 4,897 lb in 40 wk or 7,272 and 5,339 lb in complete lactations. The twins with no dry period produced 26% less FCM in the second lactation than their mates. Both twins of a pair were fed the same level of concentrates during lactation, but none was fed during the dry period. This caused the twins with no dry period to average 33 lb more body weight than their mates during the second lactation. The lowered production cannot be explained logically by a lack of nutrient reserves. Four pairs now in the third lactation indicate that yields from the twins with no dry period will be depressed more than in the second lactation.

P126. Milking machines and milking practices in Illinois. L. R. FRYMAN* AND J. L. ALBRIGHT, University of Illinois, Urbana.

Sixty dairy herds in the East Central, St. Louis, and Chicago milksheds with a level of production above average were visited at milking time to observe milking practices and to determine the condition of the milking machines in use.

Inadequate vacuum reserve due to small or worn vacuum pumps or small reserve tanks, sticking vacuum control valves, and faulty pulsators were the most important problems noted with the milking machines. Over one-half (55%) of the farms visited did not have an adequate vacuum reserve to operate the milking machines satisfactorily. Faulty vacuum control valves were found on 40% of the farms and 49% of the pulsators checked were not operating efficiently. There seemed to be a direct relationship between the condition of the milking machines in use and the type of field service offered in the immediate area by the manufacturer representative and the interest of the dairyman.

Trying to operate too many milker units, priming too long before the machines were put on the cows, and keeping cows in stalls too small for them were the most serious problems in milking practices and management observed.

P127. Eight to sixteen vs. 12-12 hour milking intervals. A. C. LINNERUD,* J. B. WILLIAMS, AND J. D. DONKER, University of Minnesota, St. Paul.

Nine identical and two fraternal pairs of twins completed 34 lactations. Group I—11 pairs of lactations were started on 12–12 hour intervals and Group II—six pairs of lactations were started on 8–16 hour intervals. Set members were milked at different intervals after a control period.

Records were terminated when one pair member became five months pregnant. Even-interval cows exceeded their mates by 7.0% (Group I) and 6.4% (Group II) in milk and 5.5% (Group I) and 2.5% (Group II) in milk fat during the experimental period. Even-interval cows produced 8.7% (Group I) and 6.5% (Group II) more milk and 8.2% (Group I) and 0.7% (Group II) more milk fat than their mates during the control period.

Per cent relative production was computed to adjust records for differences in control production.

$$\% \text{ RP} = \frac{P}{(CP)\ \ (CF)\ \ (A)} \times 100$$

P = experimental production; CP = control production; CF = DHIA conversion factor; A adjusts lactation length.

When corrected for differences other than interval, cows milked at even intervals produced about 1% more milk and milk fat than their mates. No correlation was found between level of production and per cent difference in yield.

P128. Comparison of the vacuum applied at the teat cup and that measured within the gland and teat cisterns of the lactating bovine mammary gland. E. V. CARUOLO* AND G. D. MARX, University of Minnesota, St. Paul.

This study was designed to measure vacuum within each quarter during and following milking. Five lactating, mastitis-free glands were obtained from cows immediately following slaughter. Each gland was supported on a metal frame. A 3-inch No. 20 needle was inserted into the gland sinus. A second needle, bent at a 90-degree angle, was inserted in the teat cistern through the cricoid ring. Each needle was attached via rubber tubing to a vacuum gauge. The teat cup was attached to one quarter at a time. Vacuums of 8 inches through 18 inches were applied. Pulsation rates of 34–120 pulsations/minute were compared.

No vacuum was reflected within the gland while milk flowed. When milk flow ceased, the intra-test cistern vacuum approached the vacuum being applied. Vacuum readings were never reflected within the gland cistern. Varying pulsation rates did not affect the intra-teat cistern vacuum reading. One live cow was used to test the applicability of the results to the

live condition. The same over-all effects were noted. Analysis of variance indicated significant ($P = .001$) variation of vacuum readings among quarters.

EXTENSION SECTION

* Designates author who presented paper.

E1. Direct mail as an effective educational medium for extension dairy specialists. E. H. ROCHE, Federal Extension Service, USDA, Washington, D. C.

Extension dairy specialists can use circular letters, newsletters, envelope stuffers, self-mailers, and other forms of direct mail to communicate with dairy farmers, county agents, industry personnel, and other specific audiences.

Effective direct mail has three ingredients: a good idea, a good approach, and a good mailing list. A good idea is one the audience wants, needs, or can use at the time the message is received. With a good approach, copy and format relate to the audience's needs or wants. Copy is in the reader's language and the mailing has an easy-on-the-eye layout. For a good mailing list, the sender must know who his prospects are, where to find them, how to set up the list, and how to keep the list up to date.

In the final analysis, success of direct mail depends on the sender, who controls all the variables which determine whether the message lands in the reader's mind or in his wastebasket. The sender determines what, how much, and how the message is written. He controls the illustrations, appearance, time of mailing, and the mailing list. If he does all these things well, he will communicate effectively.

E2. In-service training for extension agents. D. L. MURRAY, Michigan State University, East Lansing.

A major responsibility of an extension specialist is to provide subject mater and program development training of agents. Dairy specialists are continually viewing trends of the past, evaluating the present situation, and setting forth some immediate and long-time objectives that will achieve progress for dairying in the future. Results will be realized only to the extent that county workers are sufficiently well informed to recognize problem situations and solutions for solving them. Also, an extension worker with knowledge of a given subject matter area will be more aggressive in his leadership of various educational and service programs.

In-service training is accomplished in many ways. Individual farm calls by the agent and specialist are widely used and becoming more popular as we have an increasing number of large specialized dairy farm operations. District training sessions have been used for many years where specialists were the major resource people. During recent years it has been our observation in Michigan that training sessions should be held on campus where research staff members can assist and research results can be reviewed. The number in attendance, along with an evaluation survey, indicates the latter method of in-service training to be effective and favored by agents.

E3. Project M, an agricultural extension service program on milking management. H. R. AINSLIE, R. ALBRECTSEN, A. M. MEEK, W. G. MERRILL,* G. H. SCHMIDT, AND R. W. SPALDING, Cornell University, Ithaca, New York,

Project M is an extension program on milking management including information on milking machines (Department of Agricultural Engineering), managed milking (Department of Animal Husbandry), milk quality (Department of Dairy and Food Science), and mastitis control (New York State Veterinary College). The office of State Leaders of County Agricultural Agents and the Department of Extension Teaching and Information provide additional support. Project M is sponsored by the College of Agriculture Dairy Committee and will be conducted during the next few years, primarly by college specialists and county agricultural agents.

Project M activities include: 1, A three-day agricultural agent training school. 2. State industry conference. 3. Regional industry conferences (10). 4. Regional dairymen's meetings (12). 5. Meetings with different milking machine companies (7). 6. Planning meetings; a college specialist and each county agent to plan county programs. 7. County programs; the many activities with dairy farmers, aimed at achieving the educational objectives in each phase of Project M. 8. Printed materials; service letters, news articles, posters, etc.

E4. Development of 4-H judging to include type production (dam-daughter comparison) and calving intervals. W. A. DODGE, University of Vermont, Burlington.

Some years ago we became convinced that teaching 4-H dairy members how to select dairy calves, based on conformation only, was not doing the job desired. We decided the following factors should be given relative importance: (1) conformation, (2) production, (3) calving interval.

We go to a herd that has been on DHIA for a number of years and select four cows with production records, and each with a daughter in milk, with one completed lactation. The dams and daughters are rated, using an

adaptation of the New York type of appraisal system, and all animals are given relative scores compared to official classification.

Production scores are figured on each dam, using their lactations based on 2 ×, 305-day ME records. A production of 400 lb is given a rating of 100. Provision is made for computing scores on production average above or below 400 lb for the dams and daughters.

Calving interval score is figured on a dam, multiplying the fraction (of times calved over age two by 20).

4-H members assume they have the privilege of purchasing a heifer calf from their choice of four cows. They have information on conformation, production, and calving interval on each dam, and production and conformation on each daughter.

E5. Graded 4-H material in dairy production. J. N. MADDUX* AND H. K. WELCH, JR., University of Georgia, Athens.

Cloverleaf (for fifth- and sixth-grade boys and girls), Junior (for seventh- and eighth-grade boys and girls), and Senior (for senior 4-H Club boys and girls) dairy production manuals have been prepared for the use of county agricultural agents and local dairy club leaders. The Cloverleaf manual introduces the club member to dairy 4-H Club work and aids the member primarily with dairy calf projects for 2 yr.

The Junior manual is an attempt to guide the seventh- and eighth-grade members into the yearling bred heifer project. Feeding, care, and management are emphasized and the continuation of dairy demonstrations (project achievement competition), judging, showing, and fitting of the yearling heifer are encouraged.

The Senior manual is related to the breeding, feeding, milking, production testing, and management of the dairy cow. Emphasis is placed on caring for the cow at calving time.

Appropriate project record blanks are included in each manual to record the progress of the 4-H Club member.

E6. A 4-H dairy record program. D. A. HARTMAN, Cornell University, Ithaca, New York.

The dairy record-keeping and feeding and management program emphasizes the importance of keeping records and helps 4-H members determine if their calves are making satisfactory growth and if their cows are producing economically. The members learn the importance of keeping and studying records.

More than 40 counties entered their best dairy records in the State Dairy Record-Keeping Program.

The analysis of a survey showed that 75% of the dairy members fill out a record; 16% do a poor job; 34% do an acceptable job, and 25% do a good job. Thirty-seven per cent of the best records were sent by the 4-H agent to the Specialist's office for grading and recognition. The records were graded excellent, very good, good, fair or poor by the Specialist and a committee of 4-H agents. Forty-four per cent of them were graded excellent or excellent minus.

Awards were made by member companies of the National Dairy Products Corporation and the various Dairy Breed Associations to those members whose books were graded excellent or excellent minus. A total of 506 young stock and 196 cow records were graded in this group. A total of 1,589 records were submitted for grading. This was an increase of 325 over last year.

E7. New thoughts in 4-H dairy projects. RALPH W. WAYNE, University of Minnesota, St. Paul.

1. To expand enrollment with producing cows. Consider the production of the cow, the feeding and management record, and type in placing producing cows at fairs; thereby placing the project achievement and not type alone.

2. Provide more fundamental information in 4-H publications. In 4-H bulletins discuss and illustrate principles of nutrition, digestion process, and utilization of feed nutrients, modes of inheritance, physiology of milk secretion, and milk as a food. Teach the why, as well as recipes, of how to do things.

3. Dairy Herd Management Analysis Project. A bulletin and workbook for analyzing the home herd is used. Club member, working with parents, analyzes the herd for culling and special matings, takes inventory of feed supplies and needs, studies costs of different sources of feed, keeps breeding, health, and other retail records, studies level of most efficient concentrate feeding, checks milking machine operation, and studies cost of producing veal. Intended for older club members. Special interest to 4-H member becoming partner with parent. Teaches club member important management considerations in operating successful dairy farm.

E8. Electronic financial accounts for dairymen. LEWIS E. CLARK, University of Maine, Orono.

Electronic data processing machines have been directly serving dairymen for at least 20 yr. In the Northeast the Ayrshire Breeders' Association started to utilize punch-card processing machines to do some of their breed work in the early 40's. Dairymen are using the DHIA Central Process production records system.

In the middle 50's Michigan State University initiated a system of financial accounts and business analysis involving the use of punch cards. Approximately 20 states are now involved in operating or developing systems of electronic farm accounting.

On January 1, 1961, Maine and Vermont jointly initiated on a pilot basis a system of

electronic farm accounting (ELFAC). The Extension Directors of the 12 Northeastern States gave to the Northeast Farm Management Extension Committee the responsibility of developing the ELFAC system for regional use. Any state in the Northeast may now enroll farmers at the discretion of the respective State Extension Services. On January 1, 1962, dairymen from each New England State and New York were enrolled.

The ELFAC system meets the needs of dairymen for complete financial records, regardless of the size or complexity of the farm business. Participating dairymen receive monthly financial reports and year-end analyses which help them to manage their farms more effectively. The ELFAC system has several unique features of value to the dairyman.

E9. Controlled roughage, high concentrate feeding of dairy cows. GERALD H. STOTT, University of Arizona, Tucson.

By controlling roughage intake and feeding high levels of concentrate according to production, an approach is made to two major problems in feeding lactating dairy cows with high productive potential, namely, supplying sufficient net energy for production and maintenance, particularly during initial stages of lactation, and lowering heat increment, which can be detrimental to both production and reproduction during the summer season.

When roughage is fed at too low levels or in ground form the resultant lower solids and milk fat test is well known. However, it has been found by experiment, using alfalfa hay (crude fiber 28–31%), that roughage can be fed at levels of 1.5–1.6 lb per 100 lb of body weight for the best productive performance. When fed to heavily lactating cows throughout the hot summer months and for most of one lactation, the cows produced more milk with a higher solids nonfat content than their controls. Breeding efficiency was also improved, resulting in 23% less culling based on production and fertility.

E10. Relationship between size of cow and relative efficiency of milk energy production. R. E. ERB, Purdue University, Lafayette, Indiana.

Yields of 4% fat-corrected-milk (FCM) on the average increases about 225 lb per 100 lb increase in body size when stated as a partial regression independent of age of cow. However, breed differences have been noted and, therefore, generalizations are not justified from the limited data presently available.

Metabolic rate declines with increasing size and, therefore, maintenance requirement is similarly less for successive increases in unit weight. Limited studies involving inter-species comparisons indicate that milk energy production and total food consumption also declines as size increases. These average changes have been described as proportional to the 0.67 to 0.75 power of body weight.

Good cows annually produce FCM at a rate equivalent to ten times their body weight, but added increments of weight on an average result in increases approximating 2.0 to 2.5 times the additional weight. However, efficiency of energy conversion in terms of size (however described) only partially reflects the economical merit of a cow, since other cost items have a very large effect. Additional FCM to pay for increased maintenance and increased yield amounts to 125 to 250 lb annually, depending on milk value as body weight increases 100 lb. Other cost factors would tend to increase rather than decrease these estimates.

E11. Grain replacement values of hays cut at various dates. A. H. RAKES,* R. L. REID, AND I. D. PORTERFIELD, West Virginia University, Morgantown.

An alfalfa-grass mixture and a pure stand of alfalfa were subdivided, cut, and field-dried during three different periods: Plot A, May 15–19; Plot B, May 29–June 2; and Plot C, June 12–16. Weather damage occurred in Plots A and B of the second field. The hays were analyzed as a part of the West Virginia Forage Testing Program. Six groups of five cows each, allotted according to age, stage of lactation, and body weight, were used in two 20-day lactation trials to compare the feeding value of the hays. During a six-day preliminary period the voluntary intake levels of the hays were determined. Grain mixtures were used to adjust the total energy and protein intakes of the different groups. Digestible energy values for the different hays were 1.29, 1.16, 1.09, 1.23, 1.28, and 1.18 therms per pound, respectively, for Field I, Plots A, B, and C, and Field II, Plots A, B, and C. Similarly, the proportions of the total energy intakes supplied by hay for the different treatments were: 61.24, 48.27, 36.69, 48.56, 55.94, and 45.56%. Milk production was not significantly altered by the different treatments ($P < .05$).

E12. Observations of changes being made in milking equipment and their effect on CMT readings in Colorado herds. D. C. JORDAN* AND O. J. TRENARY, Colorado State University, Fort Collins.

The effects of a demonstration and educational program on milking machine installation and maintenance has been studied in 196 Colorado herds, using basic recommendations established by the University of California mastitis team. The results of recommendations made in the first 100 equipment checks have been compared to the results of recommendations made in the next 96 checks on 12 major items following demonstrations and circulation of educational material throughout the state.

To study the effects of changes in milking equipment installation and maintenance, the

California Mastitis Test (CMT) was used on DHIA supervisor composite samples in eight herds for three months before checking milking equipment and for three months after all recommended changes had been made. Improvement in these herds based on the number of negative reactors to CMT before and after ranged from none to 60.2%, with an average improvement on all eight herds of 18%.

The same type of study using the CMT on six herds checking individual quarters for three months before and after shows a range in improvement from none to 33.5%, with an average improvement of 11.3%.

E13. Problem areas in milking equipment under herd conditions. W. C. FAIRBANKS AND C. L. PELISSIER,* University of California, Davis.

During the period from March, 1959, through April, 1961, 306 analyses of milking installations were made on 262 dairies. The 672 recommendations for improvement made reflect the most common problem areas in milking equipment operating under California conditions.

	No. of improvements recommended	*Per cent of total recommendations*
Vacuum pump	41	6.1
Vacuum controller	83	12.5
Vacuum supply line	127	18.9
Milk line	91	13.5
Air bleeder holes	30	4.5
Pulsators	68	10.1
Liners	85	12.1
Vacuum level	61	9.1
No significant recommendation	86	12.8

Systematic analyses of milking systems are greatly enhanced by the proper use and interpretation of the following instruments: A vacuum recorder charts the magnitude, duration of applied vacuum, and pulsation characteristics. These charts provide a permanent record and make possible evaluation of equipment improvements. A vacuum guage is useful to measure the stability and magnitude of vacuum at any point in the system. An airflow meter is useful to measure the volume of vacuum or air delivered at any critical point. A volt meter can detect inadequate power delivery to magnetic pulsators.

E14. Vacuum stability in the pipeline milker. M. S. BECKLEY* AND F. F. SMITH, University of California, Davis.

A 2-yr study of 1921 cows, in 12 dairy herds, using pipeline milkers, shows the relationship of vacuum stability to teat irritation. An air flow meter and a dual vacuum recorder were used to determine capacity and characteristics. The degree of teat irritation was determined by CMT reactions read each month on the DHIA test sample. A strong correlation was found to exist between stable vacuum as measured at the teat cup under full load (i.e., maximum milking rate) and a low rate of reaction to the CMT. Conversely, where vacuum stability was poor, a much higher level of CMT reactions existed. Moreover, where correction of instability was made during the course of the 24-month study period, through increase in pump capacity, elimination of restrictions, replacement of faulty vacuum controllers, etc., the CMT reactions always showed marked improvement.

The vacuum pump capacity necessary to achieve stability will depend upon the type of barn, height of milk lift, characteristics of the milking machine, and general design of the pipeline installation. The suggested criterion for irritation-free milking is a stable milking vacuum accompanied by a snappy pulsated vacuum, at the teat cup, under full load conditions.

E15. Report of field check of DHIA equipment. G. W. HARPESTAD* AND R. V. JOHNSON, University of Illinois, Urbana.

Spring scales and milk meters used by Illinois DHIA supervisors are checked by an Extension Dairyman during the supervisors' conferences in the fall of each year. One hundred spring scales were checked in 1960 and 102 in 1961. A set of four weights was used to check the scales at 8-, 16-, 24-, and 32-lb levels. A tolerance of + or − 0.2 lb was established. Ten of the scales checked in 1960 and six of those checked in 1961 exceeded these tolerances.

Milk meters were checked by mounting in a level position and running water through them until the meter recorded 17 lb. The water was caught in a bucket and weighed on a tested scale. A tolerance of + or − 0.5 was established. Nine of the 45 meters checked in 1960 and 20 of the 60 checked in 1961 exceeded this tolerance.

Supervisors were instructed to have the scales which exceeded the above tolerances replaced and the meters which exceeded them returned to the manufacturer for recalibration.

E16. Dairy cattle breeding program for Extension. J. E. LEGATES, North Carolina State College, Raleigh.

Artificial insemination continues to serve an ever-increasing proportion of our commercial and purebred dairy herds. Most of the operational and supervisory responsibility for the A.I. programs rightfully has been assumed by the breeding organizations. However, effective future programs will continue to depend on the support of informed breeders and dairymen alert to the potential in continuing cooperative efforts for genetic improvement.

Three major points can serve to provide a framework for developing an extension educational program in dairy cattle breeding for herds using either artificial or natural service. First, dairymen should be directed in developing realistic goals for breeding programs in their individual herds. These goals must be developed with an appreciation of the sources of net return to their dairy enterprise, taking cognizance of future market demands and trends as well as the genetic implications. Secondly, the potential for improving specific traits in our cattle through breeding must be set forth in the light of the most reliable information available. How can breeding serve to move toward the realization of the herd goal? Thirdly, the educational program must recognize the supporting areas which can assist in the profiitable expression of the genetic potential available in the herd. The breeding program must be interpreted as a partner to the managemental, feeding, and testing phases of the total extension program.

E18. Proposed changes in the national sire-proving program. J. F. KENDRICK, Animal Husbandry Research Division, USDA, Beltsville, Maryland.

For the past 2 yr, the trend has been toward the use of daughter-herd-mate comparisons to evaluate the breeding worth of DHIA sires.

The DHIA herd coding system has now been in operation long enough to provide herd-mate data in sufficient volume to warrant the use of daughter-herd-mate comparisons to evaluate sires. This comparison summary will replace the dam-and-daughter proved-sire record.

The new DHIA sire summary record is designated USDA-DHIA Sire Summary Record. The sire summary record of sires in natural service will include, in addition to the summary of the records of the daughters of the sires and their herd-mates, a listing of daughters of the sires whose records were used in the tabulations.

The sire summary record of a sire in A.I. service will include, in addition to the summary of the records of the daughters of the sire and their herd-mates, a Regressed-Adjusted Daughter-Average which is an estimate of the production level of future daughters of the sire. In addition, records of registered daughters exceeding their herd-mate average by more than $1\frac{1}{2}$ standard deviations of milk will be listed for A.I. sires.

E19. Evaluation of adjustments used in the new sire-proving approach. R. H. MILLER, Animal Husbandry Research Division, USDA, Beltsville, Maryland.

Estimates of breeding values of sires are needed to choose the elite individuals of a group of sires available for service. Estimates of the rank according to breeding value can be improved by decreasing nongenetic variability among records of daughters. Standardization of records for age, length of lactation, and frequency of milking are universally accepted.

The herd-mate comparison also removes influence of herds, years, and seasons, regardless of their causes. Accuracy in ranking on the basis of herd-mate comparisons can be improved by appropriate attention to the following fluctuations among sire groups: (1) differences in sizes of herds in which the bulls were used, (2) differences in genetic levels of herds in which sires were used, (3) differences in number of daughters available.

Other problems which may affect our ability to identify the best sires are (1) possibility of selection bias by comparing relatively unselected daughters against herd-mates which have survived more intense culling, (2) mating unproven AI sires to inferior cows, (3) selective mating of different sires within herds supplying naturally proven bulls to AI.

Expected gain by use of statistical adjustment should be balanced against ability to estimate the relevant relationships.

PRICE SCHEDULE FOR REPRINTS OF PAPERS THAT APPEAR IN THE JOURNAL OF DAIRY SCIENCE

H. F. JUDKINS, Secretary-Treasurer
32 Ridgeway Circle, White Plains, New York

The Executive Board, at the time of the Annual Meeting of the American Dairy Science Association at the University of Wisconsin, increased the price of reprints 25%, effective July 1, 1961. The new reprint schedule follows:

No. of reprints	Number of pages								
	2	4	8	12	16	20	24	28	32
	(Cost in dollars)								
50	17.50	20.00	36.25	51.25	67.50	78.75	97.50	115.00	125.00
100	20.00	22.50	41.25	61.25	77.50	92.50	112.50	132.50	145.00
200	22.50	28.75	51.25	76.25	97.50	117.50	143.75	162.75	185.00
300	28.00	33.75	62.50	91.25	117.50	143.75	173.75	205.00	226.25
400	30.00	40.00	72.50	107.50	137.50	170.00	205.00	241.25	266.25
500	33.75	45.00	83.75	122.50	157.50	195.00	236.25	277.25	306.25
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

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