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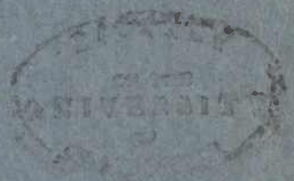
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NUMBER 2

PAPERS ON BACTERIOLOGY AND
ALLIED SUBJECTS

BY FORMER STUDENTS OF
HARRY LUMAN RUSSELL



MADISON
1921

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DEDICATED TO
BACTERIOLOGIST DEAN HARRY LUMAN RUSSELL
BY HIS FORMER STUDENTS ON THE TWENTY-FIFTH
ANNIVERSARY OF HIS
DOCTORATE

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FOREWORD

In June 1917 the twenty-fifth anniversary of Dean H. L. Russell's doctorate was celebrated by a complimentary breakfast. As a part of the exercises, it was announced that a quarter century volume by certain of his students would be published in his honor. Most of the manuscripts were received June 1, 1918, but war-time conditions have delayed the completion of this task until now. It is hoped, however, that the unavoidable delay will not lessen the pleasure which the volume will bring to either the teacher or his students.

H. A. HARDING,
E. G. HASTINGS,
W. D. FROST.
Committee.

A REVIEW OF THE SCIENTIFIC WORK OF H. L. RUSSELL

E. G. HASTINGS

It is impossible to present in a few pages an adequate statement of the work of him in honor of whom this volume is issued. In most of the fields that engaged his attention, as a member of the Wisconsin Experiment Station staff, the work was of a pioneer nature and has served as a foundation for the work of others who were to follow, making a more detailed study of the varied fields.

The University of Wisconsin has been fortunate in the loyalty of its staff and in the cooperation that has existed between its various departments. The work presented by Dr. Russell in many of his papers was done in association with colleagues in other departments of the College of Agriculture. Chief among these collaborators may be mentioned Dr. S. M. Babcock whose knowledge of the chemistry of the dairy supplemented and indeed made possible much of the work in dairy bacteriology, a subject that is as much chemical as biological in nature.

Men are creatures of circumstances. Their careers are governed largely by their associations. One of the great factors in directing the future of the subject of this sketch was his father, a medical man by education, a scholar by nature, who realized the value of a university education and who made possible the years of graduate study. H. L. Russell was born on March 12, 1866, son of E. Fred Russell and Lucinda Estella Russell. He entered the University of Wisconsin in 1884, receiving his bachelor's degree in 1888.

Another important factor in the determination of the future career of the student was the presence in the University of Wisconsin of William Trelease, now in charge of the department of botany in the University of Illinois. Dr. Trelease

had been attracted by the new subject, the new science of bacteriology, that was developing so rapidly under the tutelage of Pasteur and Koch. The thesis submitted to Harvard University in 1884 by Professor Trelease for his doctorate was entitled *Observations on Several Zooglaea and Related Forms*. It involved a study of the growth of certain bacteria on potato. The interest of Professor Trelease in bacteriology led him to introduce it into his courses in botany. Dr. Trelease left the University in 1885 and his work fell to the then professor of zoology, President E. A. Birge, under whose guidance the student was to receive his introduction to bacteriology. Two years were spent in graduate study at the University of Wisconsin. During this period the first paper was prepared. It was entitled *Preliminary Observations on the Bacteria of Ice from Lake Mendota*, and was published in 1889.

In those days two laboratories were attracting all students who wished to become bacteriologists, that of Robert Koch at the University of Berlin and that of Louis Pasteur in the Institute which bears his name. The years of 1890-1891 and 1891-1892 were spent in these laboratories and at the Zoological Station at Naples where the data were collected for the first extensive paper which was published in the *Zeitschrift für Hygiene* in 1892 under the title *Untersuchungen über im Golf von Neapel lebende Bakterien*. On returning to this country a year was spent under Dr. William H. Welch of Johns Hopkins University. By this university the doctor's degree was granted in 1893. The thesis submitted for the degree was entitled *Bacteria in their Relation to Vegetable Tissue*. It was published in the *Johns Hopkins Hospital Reports* in 1893. A year was then spent at the University of Chicago as fellow in biology.

Under the influence of Weigmann in Germany, Storch in Denmark, and Conn in this country, dairy bacteriology was rapidly attracting the attention of those interested in this phase of animal industry. It had long been evident to the director of the Wisconsin Experiment Station, Professor Henry, that Wisconsin was destined to become a great dairy

state. He had brought to Wisconsin the most prominent and the most honored dairy investigator of this country, Dr. S. M. Babcock, and had sought in every way to favor the development of the dairy industry. It was but natural that these men, wide awake to the developments that were to come in dairying, should at once recognize the importance of this new phase of bacteriology to the state of Wisconsin. It was again but natural that in seeking a man to develop this subject, both from the research and instructional points of view, they should turn for aid to the first student of the University who had prepared himself for work in this field. In 1893 H. L. Russell was appointed assistant professor of bacteriology in the University of Wisconsin. Advancement to full professor was made in 1897.

The training received in general biology led to the development of a course in general bacteriology, in which the general relations of the bacteria were considered, apart from their practical relations. This course was given most successfully for many years. The constant knocking at the door of the list of required studies by the new phases of agricultural work that had been introduced from time to time into the college led to the abandonment of the course and to the development of a course in which the practical aspects of bacteriology as related to farming were especially emphasized. From the laboratory of the University have gone many men who have had much to do with the development of agricultural bacteriology in this country.

The Wisconsin College of Agriculture in 1893 was expending a large portion of its energy in the development of the Dairy School and in the training of young farmers in the Short Course in Agriculture. Dean Henry furnished to these groups of students the best instruction the College could provide. Thus the instructional work of the bacteriologist was not limited to the regular university students, but was extended to both the students in the Dairy School and in the Short Course in Agriculture. Many hundreds of young men have returned to their homes with not only some idea of the

great importance of the bacteria in the life and work of the dairy and farm, but also with the inspiration for their work.

The first annual report of the Agricultural Experiment Station of the University of Wisconsin, published after the appointment of the bacteriologist, contained four papers by him; one on the relation of bacteria to milk and three on bovine tuberculosis, two lines which were to be followed actively for many years. In each subsequent report of the Station and in a number of bulletins were presented the results of the research work of the department.

It is impossible to review in any detail the things accomplished. A few of the chief lines of endeavor will be mentioned. In 1892 Robert Koch prepared tuberculin, a product that was not to fulfill the hopes of him and his friends as a therapeutic agent, but whose value as a diagnostic agent in the case of bovine tuberculosis was soon recognized. The college herd was tested in February, 1894. This test was one of the first made in this country and the first made west of the Allegheny Mountains. Twenty-five of the thirty animals comprising the herd were found to be diseased. The whole herd was destroyed and a new herd formed which for twenty years has served as an example to the farmers of the state of what can be done in the control of this disease. The destruction of the college herd was a serious step and one that would have been authorized only by a far-seeing administrator as the history of the Experiment Station and College of Agriculture has and is showing Professor Henry to have been. The tendency then as now was to temporize with the disease. No question remains as to the wisdom of the decision made in 1894 with reference to the fate of the college herd. Twenty years later sister institutions were still hesitating about doing with their herds what they were advising the farmer to do with his.

The pioneer in any line of human endeavor is not likely to see the fruition of his work. To no one is this more likely to happen than to the investigator in agricultural fields. Facts are discovered, the importance of which is most evident to those whose background of knowledge enables them to appre-

ciate their bearing. Those without such a background must accept them on faith. The natural and proper conservatism of the farmer leads him to accept new facts only after their value has been demonstrated. Yet delay in their acceptance may often mean great loss and irreparable damage. This fact is well illustrated by bovine tuberculosis.

The lack of recognition of the importance of bovine tuberculosis by the farmer has enabled the disease to gain greater and greater headway. In 1894 great areas of our country were free from the disease which now exists in every locality and is still spreading, due to the fact that the importance of the truths pointed out twenty-five years ago is not yet realized by the great mass of farmers.

If the breeders and the farmers of the state and of the country had accepted and followed the advice given in the first papers published on bovine tuberculosis by the Wisconsin College of Agriculture, the conditions today would be far different. It was recognized that the pure bred herds were the chief centers from which the disease was being disseminated. It was recognized that a healthy herd would be a great asset to its owner. But few of the breeders of the state saw fit to follow the advice. Those who did have never regretted it.

The knowledge that was obtained concerning the conditions that existed in the state with reference to bovine tuberculosis made possible the formulation of a campaign against it. Briefly, this consisted of an educational propaganda which should bring to the farmer evidence of the economic importance of the disease, which should acquaint him with the tuberculin test and its value in keeping tuberculosis out of his herd or in eliminating it therefrom.

It was recognized that by far the greater part of the dairy herds of the state were free from the disease, especially in the more newly settled regions of the state, that the important and immediate thing was not the elimination of the disease from the infected herds, but the prevention of its introduction into healthy herds. In furtherance of the plan of action, animals that had reacted to the tuberculin test were slaugh-

tered at various gatherings of farmers and thus there was presented to them evidence of the ravages which the disease may occasion in an apparently healthy animal.

If progress was to be made, the tuberculin test must be widely used. The limited number of veterinarians available for the work made evident the necessity of extending its application to the laity. With this idea in mind, special instruction in the use of the test was given to the students of the agricultural college, both in the regular and in the short course.

It was suggested that the prevention of the sale of tubercular animals would be the most effective way of stopping the spread of the disease. The passage of a law was urged that would require the testing of all cattle sold for breeding and dairy purposes. Such a law was enacted.

The first of these steps was opposed by the veterinarians who felt that it was an encroachment on their field; the second was opposed by the farmers who had not been or who would not be convinced of the importance of the disease. The plan was too early to meet with the approval of those most concerned, and by the time they had become ready to give to such a plan their sympathy and approval, it was too late for such or any plan to have much effect.

Looking backward from the vantage point of present knowledge, one can see how great a factor the plan would have been in preventing the spread of the disease which is rapidly becoming the most important as far as the economic and sanitary aspects of the dairy are concerned.

Dean Russell served as a member of the State Live Stock Sanitary Board for a number of years. The work in bovine tuberculosis led to an interest in human tuberculosis. The services rendered in this field were in connection with the establishment of the State Tuberculosis Sanatorium at Wales. Dean Russell served as president of the advisory board of this institution.

For a number of years, work was done for the State Board of Health in the sanitary examination of water and in the diagnosis of tuberculosis, diphtheria, and typhoid. In 1903

the State Hygienic Laboratory was created by the legislature. Dean Russell was made director, a position he held until 1908.

Another field in which pioneer work was done was in the pasteurization of milk and cream for direct consumption. Bulletin 44 of the Experiment Station was issued in 1895. The method suggested therein conforms with one exception to that which is accepted by the health officials as the best today. The milk was treated in an apparatus that insured the heating of all of the milk to the desired temperature and for the desired time. This is nothing more or less than the "holding" method of pasteurization so widely used today. The temperature recommended was 155° F. for 20 minutes. At this time the tubercle bacillus was considered to be much more resistant to heat than was later found to be the case.

For many years pasteurization of milk for direct consumption did not meet with the approval of health officials. They considered it in the class with preservatives. The process was gradually introduced into the milk trade by the distributors whose interest lay in imparting to their product a better keeping quality. They adopted the machines that had been found satisfactory in the pasteurization of cream for butter making. These machines subjected the milk to a high temperature for a short period, the "flash" method of pasteurization. This process did not insure the freedom of the milk from pathogenic organisms since a portion was subjected to the maximum temperature for such a short period that the organisms therein might not be destroyed. The recognition of this fact by health officials did not lead them to favor pasteurization of milk for direct consumption. Indeed, for many years, the whole weight of influence of the medical profession was against the pasteurization of milk. They failed to recognize the trend of affairs in the provisioning of our cities with milk, that it was becoming increasingly difficult to obtain a sufficient supply of milk close to the point of consumption, that as supplies must be sought farther away, the period between the moment of production and of consumption lengthened. It became increasingly difficult to retard the process of souring of the milk by the methods then used

without increasing the price of the product to an extent that would limit its use. It became more and more evident that freedom of the municipal supplies of milk from pathogenic organisms could never be insured by inspection at the point of production. These two facts indicated to every opened-minded person the necessity of pasteurization of milk. The medical profession is on the one hand the most radical group and on the other the most conservative. As a group the medical men could see no use, no advantage, in the pasteurization of milk and until the process was favored by the physicians, its extension was slow.

The work done from 1894 to 1900 laid the foundation for the rational pasteurization of milk and cream. It involved a study of the effect of heat on the physical properties of milk and a study of the resistance of the tubercle organism to heat. It was shown that milk could be so treated as to insure its freedom from pathogenic organisms and yet retain its original chemical and physical properties to such an extent as not to make it undesirable from the standpoint of the distributor or the consumer. The development of the pasteurizing process has been along the lines that were laid down in the first publication on this subject from the Wisconsin Experiment Station. Many of the facts discovered and the points emphasized did not exert the influence they deserved to exert. They were rediscovered by later workers at a time when the world was ready to accept them. This is the fate of the pioneer in science.

At the time Dean Russell came to the Experiment Station, research work on the factors concerned in the production of cheddar cheese was being carried on under the leadership of Dr. S. M. Babcock. The first bacteriological paper in this field, *Gas-Producing Bacteria and the Relation of the Same to Cheese*, was published in the report of the Experiment Station for 1895. This was quickly followed by other papers dealing with the bacterial flora of cheese and their rôle in the ripening of cheese and the importance of the quality of milk in the cheese industry. The development of the Wisconsin Curd Test gave to the cheese maker a means of testing the milk delivered

by the patrons for harmful types of bacteria. This method furnished the cheese maker a reasonable way of overcoming the most frequently occurring and most important trouble that confronted him.

The field of enzymology was a relatively unexplored one in 1897, when a report was made by Dr. Babcock and Dean Russell on the presence in milk of a proteolytic enzyme to which the term galactase was applied. A study of the occurrence of this enzyme showed it to be present in the milk of various species of animals. The study of the properties of this enzyme and its rôle in the ripening of cheddar cheese led to a modification in the curing of cheese that has become of the greatest importance to the cheese industry. The ripening of cheese at low temperatures was held by men of long practical experience to be impossible. The results of the experiments made at Wisconsin showed that at much lower temperatures than had been used in the curing rooms, the occurrence of abnormal flavors was much less frequent than at the higher temperatures. The experiments in the cold curing of cheese were extended in 1901 and 1902 and later were carried on in cooperation with the Dairy Division of the U. S. Department of Agriculture and the New York Experiment Station at Geneva. This work has served to revolutionize the entire business of curing cheese. It has saved and is saving every year large sums of money to the industry through the prevention of abnormal cheese. It is an excellent example of the practical results that may follow from work that apparently can promise no practical results.

In the early years of this century the presence of cellular elements in milk was attracting the attention of health officials. Rules were being formulated that meant the rejection of a large part of the milk produced. The rules had been formulated out of a clear sky. Their authors thought that milk should contain not to exceed a certain arbitrary number of cells. They had not taken the trouble to determine by a study of milks anything concerning the normal content in these elements and some of the factors that influence their number. Work done on the University herd showed that the standards

that had been proposed were far too exacting, that what were being considered abnormal conditions in milk were really normal. The modern views of the significance of the cellular elements in milk were forecast in the papers published in 1906 and 1907.

Another field in which pioneer work was done was that of the relation of bacteria to diseases of plants. The views held by those highest in the field of plant pathology were that bacteria could not be of importance in the causation of disease in plants due to the acidity of the plant juice. It was known that bacteria as a group grow best in nutrient solutions that are alkaline to litmus. Plant juices are usually acid to litmus, therefore the inference was drawn that bacteria could not grow in the plant tissues. Again the structure of the plant did not seem to favor the entrance of bacteria or their spread in the tissues of the plant. The German investigators of 1895 in bacteriology were not accustomed to pay much attention to what had been done in America. One of the first bacteriological investigations in this country was that of Dr. T. J. Burrell of the University of Illinois on pear blight. This should at least have indicated to the bacteriologists the possibility of bacterial diseases of plants.

The training which Dean Russell had received in botany and in connection with the preparation of his doctor's thesis had given him an admirable preparation to undertake an investigation of a disease that was causing great havoc in the cabbage-growing sections of Wisconsin. In 1898 Bulletin 65 of the Experiment Station was published. It was entitled *A Bacterial Rot of Cabbage and Allied Plants*. This work is to be classed as one of the first extensive pieces of research in a field that has assumed so great an importance.

The period from 1893 to 1907 was crowded with work of instruction and research; the period from 1907 to 1917, with executive duties whose manifold demands have made impossible any active continuance in the former lines of work. This condition is to be regretted, on the one hand, for it has robbed the students of the college of an inspiring instructor, of whom there are few, and the world of an able research man,

but it has given to the state a successful administrator of its College of Agriculture and its Experiment Station. It is fortunate for the state that energies have not been dissipated by attempting to follow many lines, but that they have been confined to one line, that of administration.

As earlier stated, the University of Wisconsin has been fortunate in the loyalty of its staff. This has been noteworthy in every division of the University. For thirty-four years the destiny of the agricultural work of the University has been in the hands of but two men. It is to be hoped that the same may be said when the half-century is reached.

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THE DEVELOPMENT OF CITY MILK SUPPLY PROBLEMS

H. A. HARDING

While records of fermentative changes in milk and its products extend back practically to the dawn of history, little significance can be attached to observations of the details of germ life in the dairy prior to the beginning of modern bacteriological technique in 1881.¹

About 1890 there was a widespread development of interest regarding germ life in the dairy. Adametz² in Austria and de Freudenreich³ in Switzerland were then at work on cheese problems, Storch⁴ in Denmark and Weigmann⁵ in Germany were busy with the relations between germ life and flavors in butter, while in America Conn⁶ was examining the bacteria in cream and Sedgwick⁷ had made some observations on the bacteria in city milk.

In connection with the World's Fair in Chicago in 1893, Conn displayed a collection of dairy bacteria illustrating the changes which these germs were able to produce in milk and cream. Whatever else this exhibit may have accomplished, it was an important factor in convincing Dean Henry that

¹ R. Koch, *Zur Untersuchung von pathogenen Organismen in Mitt. aus dem Kaiserl. Gesundheitsamte*, 1, pp. 1-18, 1881.

² L. Adametz, *Bakteriologische Untersuchungen über den Reifungsprozess der Käse in Land. Jahr.*, 18, pp. 227-270, 1889.

³ Ed. v. Freudenreich, *Bakteriologische Untersuchungen über den Reifungsprozess des Emmenthalerkäses in Milch. Zeit.*, 21, pp. 368-369, 1892.

⁴ V. Storch, *18 Beretning kgl. Veter. og Landbohøjskoles Laborat. f. landökon. Forsög. Kjobenhavn.*, 1890.

⁵ H. Weigmann, *Landw. Wochenbl. f. Schleswig-Holstein*, 40, p. 549, 1890. Also *Milch Zeit.*, 19, p. 593, 1890.

⁶ H. W. Conn, *Bacteria in Milk, Cream and Butter in Storrs Agr. Exp. Station Ann. Rept.*, 2 (1889), 52-67, 1890.

⁷ W. T. Sedgwick and Batchelder, *A Bacteriological Examination of the Boston Milk Supply in Boston Medical and Surgical Journal*, 126, pp. 25 ff., 1892.

here was a field of study which should be further cultivated in behalf of the dairy industry. The result was the appointment of Dr. H. L. Russell as head of the department of bacteriology in the University and Bacteriologist to the Agricultural Experiment Station. The uniqueness of this action may be judged from the fact that at that time there was no similar official position in any American agricultural experiment station. The nearest analogy was Dr. H. W. Conn, professor of biology at Wesleyan University, Middleton, Conn., who was doing some cooperative work with the Storrs Agricultural Experiment Station and the Federal Department of Agriculture.

To those coming recently into the field of dairy bacteriology it is difficult to picture the meagreness of the facts available to Dr. Russell when he was inducted into this field of work in September, 1893. These available facts may be fairly summarized as follows:

1. Bacteria were known to be widely distributed in nature and to find their way accidentally into milk.
2. They multiplied in milk and in connection with their growth produced various compounds among which acid was the most evident. The work of Storch, Weigmann, and Conn suggested that the flavors in cream and butter resulted in part from such growths. The relation of bacteria to cheese was less evident.
3. It was known that tuberculosis was caused by a definite germ and a number of other diseases of cows and people were more or less definitely ascribed to germ causes.

During the succeeding twenty-five years Dr. Russell, in person and through his students, has contributed largely to increasing our knowledge along all lines of dairy bacteriology. One of the first problems which engaged his attention was that of bovine tuberculosis⁸ and the influence he exerted has helped to bring Wisconsin to the front in the struggle against

⁸ H. L. Russell, *On the Efficiency of Tuberculin as a Diagnostic Agent in Tuberculosis* in *Annual Report, Wis. Agr. Exp. Sta.*, 11 (1894), pp. 166-195, 1895.

this disease; his clear analysis of B41⁹ and its relation to butter flavors saved the creamery industry large sums of money; his work in connection with cheese ripening has not only thrown much light upon the intricacies of this problem but out of these studies has come the cold curing of cheese¹⁰ which has been of untold advantage to the cheese industry; and his influence in the field of city milk supplies will be presented in some detail in this paper.

Because his influence through his own investigations and those of his students has touched every problem in connection with city milk supplies during the past twenty-five years this presentation will be simplified by treating these problems in the order of their historical development.

City milk supply problems have developed with the growth of cities. The small city where the necessary milk was produced within the municipality or upon adjoining farms and delivered within a few hours was slow to recognize that it had city milk problems.

The last quarter of the nineteenth century was marked by a rapid increase in urban population, particularly in the eastern portion of the United States. Along the coast from Boston to Washington there developed a succession of rapidly growing cities with a correspondingly increasing demand for milk. The ocean prevented production on one side and the expanding zone of milk producing territory tributary to each city soon met, compelling an even more rapid development of the source of supply to the West and North. As late as 1890 the source of supply for the majority of these coast cities was essentially local while at the present time practically all of the accessible territory east of Buffalo and up to and even across the Canadian border is engaged in supplying them.

The tardy awakening of a consciousness of their milk problems on the part of the American cities is quite evident from

⁹ E. H. Farrington and H. L. Russell, *The Use of Bacterial Culture Starters, with Especial Reference to the Conn Culture (B41)*, in *Annual Report*, Wis. Agr. Exp. Sta., 12 (1895), pp. 174-226, 1896.

¹⁰ S. M. Babcock and H. L. Russell, *Influence of Temperature on the Ripening of Cheese in Annual Report*, Wis. Agr. Exp. Sta., 14 (1897), pp. 194-210, 1897.

another angle. The city can acquire little exact information regarding the quality of its milk supply and can do even less in the matter of correcting abuses until it provides a system of inspection and collection of milk samples. As late as 1890 only about a dozen American cities had made specific provisions for such inspection and examination.¹¹

In their attempts at handling the milk situation the cities have given their attention successively to various aspects of the milk question and for the time being each of these aspects has been considered as essentially the milk problem. While the chronological order in which these subdivisions of the question have been emphasized in different cities has varied, the common sequence has been food value, healthfulness, cleanliness, and keeping quality.

The necessity of determining existing facts with regard to these problems has quite naturally created the problem of milk inspection and the complicated results of more recent milk inspection have emphasized the need of milk grading as a way of clearly summarizing the situation for the consuming public.

THE PROBLEM OF FOOD VALUE

Since milk is uniformly retailed by volume, there is a temptation to increase the volume through the addition of water. Moreover, the ready market for cream offers an additional temptation to partially skim the milk before sale. Many of the early examinations of milk supplies showed that both practices were fairly common. The earliest recognized problem of city milk supplies was safeguarding the composition or food value of the milk.

Massachusetts passed an act forbidding the watering and skimming of milk in 1856 as did New York City in 1873.¹² However, little could be accomplished in the absence of simple and accurate means for detecting these practices. The lactometer was of some service in this connection but it was

¹¹ H. N. Parker, *City Milk Supply*, p. 371.

¹² *Ibid.*, p. 370.

not until after the discovery of the Babcock test in 1890¹³ that municipal control of skimming and watering became reasonably efficient. In the decade following 1890 state and municipal regulations against skimming and watering became practically universal. As an aid in securing court convictions minimum legal standards were commonly included in these regulations. While these minimum legal standards have undoubtedly served a useful purpose in combating skimming and watering they have exerted an unexpected and unfortunate influence because they have tended to standardize the entire supply at a low level.

The growing appreciation of the wide variation in the food value of the milk now upon the general market is giving the problem of food value a new form. There is a desire on the part of the public for a milk considerably richer in fat than required by the minimum legal standards. Milk distributors desire to enter to this trade and wish to distribute a milk with a uniform fat content distinctly above that of the ordinary supply. The fat content of the normal milk supply is subject to variations. A supply which on the average gives the desired fat content will fall below this desired composition during a portion of the year and be above the desired point during another portion. This difficulty could be readily met by adding cream during one period and skim milk at another were it not that such additions are a violation of some of the laws regarding milk adulteration. The proper regulation of milk standardization is now receiving careful consideration, and standardization seems destined to be an early development in the retail milk business.

Until somewhat recently public consideration of food value of milk concerned itself almost exclusively with the amount of fat present. Later some attention began to be given to the accompanying solids, and the total energy value or the calorific value of the milk came under discussion. The newest angle of this old question of the food value of milk

¹³ S. M. Babcock, *A New Method for the Estimation of Fat in Milk, Especially Adapted to Creameries and Cheese Factories in Annual Report*, Wis. Agr. Exp. Sta., 7 (1890), pp. 98-113, 1890.

is the recognition of the fact that milk is the most available source of certain substances indispensable to the growth of the young and the well being of the adult. Much of the study of these important compounds designated as "fat soluble a" and "water soluble b" has been conducted at Wisconsin.¹⁴ The nutritive importance of these compounds is emphasized by the fact that recent studies of pellagra make it evident that milk is practically a specific for this disease.¹⁵ Pellagra in this country in a single year is variously estimated at between 150,000 and 200,000 cases, with a mortality of approximately 5 per cent. These figures suggest that there is more disease and death in this country every year from the failure to use sufficient milk than results from the use of bad milk. While this fact should not militate against all reasonable efforts to improve the quality of the present milk supply, it should lead to vigorous efforts to increase the consumption of fluid milk.

THE PROBLEM OF HEALTHFULNESS

Healthfulness as here applied to milk refers to the absence of germs capable of transmitting specific diseases.

The early objections to watering and skimming of milk were based in part upon the supposition that such practices rendered the milk unhealthful. When carried to such lengths as to reduce the amount of food received by the child below a maintenance ration this conception is in a sense correct. However, in modern thinking these practices are objected to rather as a fraud in that they reduce the food value which the purchaser receives.

Tuberculin as a means of diagnosing bovine tuberculosis was just beginning to be tried in 1893. A test of the Wisconsin Experiment Station herd showed it to be largely tuberculous. Dr. Russell immediately took an important part in the strug-

¹⁴ E. V. McCollum, *The Newer Knowledge of Nutrition*, MacMillan, 1918.

¹⁵ J. Goldberger, G. A. Wheeler and E. Sydenstricker, *A Study of the Diet of Nonpellagrous and of Pellagrous Households in Textile Mill Communities in South Carolina* in *Jour. Amer. Med. Asso.*, 71, pp. 944-949, 1918.

gle with this disease, a struggle which has continued unabated for twenty-five years and the end is not yet. He promptly concluded that the tuberculin test properly conducted was the best available means of diagnosis.¹⁶ He was among the first, if not the first, in America to recognize the value of the Bang Method in treating valuable herds.¹⁷

He was quick to recognize the need of some method of rendering the public milk supply safe from the danger of carrying tuberculosis to the consumers. He turned at once to pasteurization¹⁸ as a safeguard and in the succeeding years no more practical method of accomplishing this purpose has been discovered. Pasteurization then labored under the disadvantage that the available data on the thermal death point of the tubercle bacillus called for a time and temperature of pasteurization which seriously impaired the commercial qualities of the milk. As a result pasteurization as it was done commercially up to 1900 was rarely satisfactory.

In 1898 Theobald Smith¹⁹ published his studies on the thermal death point of the tubercle bacillus showing that in laboratory tests the germ of tuberculosis was killed in milk by a heating at 140° F. for 15 minutes. Studies²⁰ were immediately begun at Wisconsin which showed that under commercial conditions the germs in question were killed by exposure to 140° F. for 10 to 15 minutes thus substantiating the findings of Smith. It was recommended that in commercial plants the heating be continued for 30 minutes at 140° F. in order to provide a satisfactory margin of safety. This method of pasteurization has been found satisfactory from the commercial point of view and has become the standard procedure in protecting the milk supplies of the country.

¹⁶ See footnote 8.

¹⁷ H. L. Russell, *The History of a Tuberculous Herd of Cows*, Bulletin 78, Wis. Agr. Exp. Sta., 1899.

¹⁸ H. L. Russell, *Pasteurization of Milk and Cream for Direct Consumption*, Bulletin 44, Wis. Agr. Exp. Sta., 1895.

¹⁹ Theobald Smith, *The Thermal Death-point of Tubercle Bacilli in Milk and Other Fluids* in *Jour. Exp. Med.*, 4, pp. 217-233, 1899.

²⁰ H. L. Russell and E. G. Hastings, *Thermal Death-point of Tubercle Bacilli under Commercial Conditions* in *Annual Report*, Wis. Agr. Exp. Sta., 17 (1900), pp. 147-170, 1900.

The process of pasteurization not only frees milk from the danger of transmitting tuberculosis but at the same time it frees it from the danger of transmitting the other diseases which have been observed to have been occasionally so transferred. Accordingly pasteurization is coming to be recognized as the simplest and most efficient protection against the spread of disease germs through milk supplies.

THE PROBLEM OF CLEANLINESS

Even under most favorable conditions it is difficult to prevent limited amounts of foreign matter from getting into milk. On the other hand, the consumer insists that the milk shall be as clean as practicable. Fortunately the amount of soluble foreign matter which finds its way into milk is very slight and the white color of the milk forms a background against which any insoluble matter present stands out distinctly.

The amount of insoluble dirt actually present in milk as it is delivered to the consumer naturally varies considerably.²¹ The present available data suggest that it rarely amounts to more than five milligrams per quart or roughly five parts per million while the average is nearer one part per million.

The Wisconsin Sediment Tester²² is a convenient device for collecting upon a cotton disk the insoluble dirt from a pint of milk and has proven a satisfactory instrument for this purpose. At the Chicago Department of Health this method has been made roughly quantitative by preparing test disks from samples of milk containing weighed amounts of dirt.²³ At the University of Illinois the standards for quantitative examinations of sediment in milk have been still further perfected until it is believed that the weight of the insoluble dirt in milk may be quickly determined with an accuracy rivaling that obtained by any other available means.

The standards for this purpose are prepared by powdering

²¹ H. N. Parker, *City Milk Supply*, p. 256.

²² S. M. Babcock and E. H. Farrington, *New and Improved Tests of Dairy Products*, Bulletin 195, Wis. Agr. Exp. Sta., 1910.

²³ F. O. Tonney, *Report of the Municipal Laboratory*, Department of Health, City of Chicago, 1907-1910, p. 25.

carbon and passing it through a 100-mesh to the inch sieve. The desired amount of this carbon is then weighed in a ground glass slide and transferred to a small beaker containing 50 c. c. of milk. After stirring until uniformly distributed, the contents of the beaker, while rotating, are poured quickly into the sediment tester. The carbon is deposited upon the cotton disk in the tester and this disk may be used as a standard for comparison with disks resulting from the testing of milk samples.²⁴

Standards prepared in this way are quite uniform and in the lower dilutions clearly show differences of 0.25 milligram. A series of these standards may be conveniently mounted on a card and protected from dirt by a strip of glass or celluloid. With such a series of standards at hand, the making of a sediment test from a sample of milk and the estimation of the amount of dirt present by comparison with these standards occupies but two or three minutes.

THE PROBLEM OF KEEPING QUALITY

The first article prepared by Dr. Russell for his first annual report was entitled *The Source of Bacterial Infection and the Relation of the Same to the Keeping Qualities of Milk*.²⁵ At that time the idea was almost universal that the overshadowing importance of bacteria in milk lay in their relation to the health of the consumer. A portion of this fear of germ life arose from the feeling that since certain germs cause definite diseases, all germs must be dangerous. The faulty logic in this reasoning is too evident to need discussion. The nightshade is truly a poisonous plant while its near relative, the potato, is one of our most important sources of food.

The fact that the death rate of babies is highest during the hot months when the germ content of the milk supply is also highest early led to the belief that the high germ content

²⁴ The details of the preparation of these standards were worked out by James D. Brew under the general direction of the author.

²⁵ H. L. Russell, *The Source of Bacterial Infection and the Relation of the Same to the Keeping Qualities of Milk in Annual Report*, Wis. Agr. Exp. Sta., 11 (1894), pp. 150-165, 1895.

of milk was responsible for the death of the babies. However, the results from the most careful studies of this subject fail to substantiate this view. Park,²⁶ in studying conditions in New York, found that while under tenement house conditions high germ content milk and fatalities among the babies were commonly associated there were likewise present many other factors inimical to the health of the baby. On the other hand in baby hospitals where the germ content of the milk supply was uniformly high but good general care was given the babies, their health was satisfactory. Williams²⁷ later made a careful study of all baby deaths during one year in Rochester, N. Y. He concluded that the milk supply could be directly connected with only a small portion of such deaths. Price,²⁸ by instructing the mothers, reduced the baby death rate in Detroit to a low level without changing the character of the milk supply.

On the other hand, it is generally agreed that bottle-fed babies are prone to digestive disturbances which are usually attributed to something connected with the milk since that is practically their sole source of nourishment.

It has often been asserted that these digestive troubles were due to the presence of large numbers of germs in the milk. On the other hand, when these same ailing infants are placed upon a diet of milk containing immense numbers of germs, their digestive disturbances usually promptly vanish. This would seem to dispose of the argument that their original trouble was due to the mere presence in milk of large numbers of germs.

Logically, the next suggestion is that the difficulty with the babies is due, not to the germs themselves, but to the changes which the germs bring about in the milk. The

²⁶ W. H. Park and L. E. Holt, *Report upon the Results with Different Kinds of Pure and Impure Milk in Infant Feeding in Tenement Houses and Institutions in New York City*, Dept. of Health, City of N. Y., *Ann. Rept.* (1902), pp. 275-299, 1904.

²⁷ J. R. Williams, *A Study of Infant Mortality in Rochester. The Relation of Market Milk Thereto* in *N. Y. Med. Jour.*, July 13, 1912.

²⁸ W. H. Price, *Some Statistics Regarding Infant Mortality* in *Ann. Rept.*, Int. Asso. Dairy and Milk Inspectors, 3, pp. 95-103, 1915.

marked improvement in the health of ailing infants when fed upon sour milk is commonly attributed to a difference in the kinds of germs present in the milk consumed before and after the improvement with a corresponding difference in the character of the changes produced in the milk by these germs.

So far as studies have been made, there is shown surprisingly little qualitative difference in the bacterial flora of the ordinary public milk supply when carrying a high germ content and the flora of the milk which has acknowledged therapeutic value in the treatment of sick babies. There are, of course, quantitative differences in that the total number of germs in the therapeutic milk is much higher and this increase in numbers is largely in germs with marked acid-forming tendencies.

Too little is yet known of the chemical products of bacterial action in milk to justify conclusions, but the known ability of milk compounds to absorb or otherwise neutralize small amounts of bacterial by-products suggests that many of them may be cared for in this way. Acid is the most evident by-product of this bacterial action but this is most abundant in the therapeutic milks.

Failing to find a satisfactory explanation for the illness of children in the number of germs involved or in any of their recognizable by-products, it seems logical to seek for other explanations of the constantly observed coincidence of high germ content milk supplies and baby sickness.

A part of the diarrheal diseases of children is undoubtedly due to the action of specific dysentery organisms. No technique is available by which the presence of these organisms in milk can be readily determined but pasteurization offers the same protection against them as against other pathogenic organisms.

Experience in the feeding of domestic animals has abundantly demonstrated that an animal accustomed to a uniform diet is markedly disturbed by sudden changes in its ration. It is an axiom in the feeding of dairy cows that changes in the amount or character of their ration should be made gradually. The gratifying improvements which have followed

the district nurse system of instructing mothers in uniform and regular feeding of babies suggests that babies respond similarly to like physiological stimuli. In the feeding of calves it is observed that calves like the human infant thrive when fed uniformly upon sweet milk or when fed uniformly upon sour milk but that diarrhea appears promptly when a system of feeding sweet milk is varied by an occasional feeding of sour milk. While frankly admitting the danger of reasoning by analogy, the similarity of the factors observed and of the results obtained in the feeding of calves and of babies strongly suggests that in both cases diarrheal conditions result from sudden changes in the acid reaction of the diet. If this reasoning is correct, a satisfactory supply of baby milk is characterized among other things by a constant acid reaction. Under ordinary conditions the reaction which will be most satisfactory is approximate neutrality.

If the above explanation of the evil results attending the use of occasional samples of partially sour milk is accepted, it offers an excellent reason for insisting that the public milk supply shall have such a keeping quality as to maintain an essentially unchanged reaction during the time which would ordinarily elapse before its consumption.

Since the increase in acid reaction of milk is due to the growth of bacteria, the problems of keeping quality are problems of limiting the entrance and activity of germ life and of measuring the activity of the germs which enter.

The work of Dr. Russell, above referred to,²⁹ is one of the earliest inquiries as to the source of the bacteria which enter the milk and this type of inquiry has been continued by a number of his students. In connection with this series of studies it has developed that the barn and barn conditions exert surprisingly little influence upon the germ content of milk.³⁰ While in a few instances the udder flora heavily

²⁹ See footnote 25.

³⁰ H. A. Harding, G. L. Ruehle, J. K. Wilson and G. A. Smith, *The Effect of Certain Dairy Operations upon the Germ Content of the Milk*, Bulletin 365, N. Y. Agr. Exp. Sta., 1913.

M. J. Prucha and H. M. Weeter, *Germ Content of Milk as Influenced by the Factors at the Barn*, Bulletin 199, Ill. Agr. Exp. Sta., 1917.

infect the milk,³¹ under ordinary conditions the contamination of the milk arises almost exclusively from the utensils in which it is handled.³² Among the utensils the milking machine,³³ where it is used, the shipping can, and the cooling and bottling machinery at the milk plant are ordinarily the principal sources of this contamination.

The problem of a suitable test for the keeping quality of milk is not as simple as might at first appear.

Since the keeping quality of milk depends upon germ life, the number of germs present has been suggested as the logical measure of keeping quality, and this idea has been accepted by a considerable number of municipalities. However, there seems to be no basis for an agreement as to the maximum number of bacteria to be used as a standard of proper keeping quality. Actual municipal standards vary from as low as 50,000 per c. c.³⁴ to as high as 2,000,000 per c. c.³⁵

A very practical difficulty with bacterial standards is the extreme variations which occur in determining the germ content of milk even under the most favorable conditions. This was most strikingly illustrated in a comparative test made under the direction of Dr. H. W. Conn and conducted cooperatively in four laboratories in New York City. These studies showed that occasionally plate determinations made from milk approximately sour indicated a lower germ content than other plate determinations made from certified milk,

³¹ E. G. Hastings and C. Hoffman, *Bacterial Content of the Milk of Individual Animals*, *Research Bulletin* 6, Wis. Agr. Exp. Sta., 1909.

H. A. Harding and J. K. Wilson, *A Study of the Udder Flora of Cows*, *Tech. Bul.* 27, N. Y. Agr. Exp. Sta., 1913.

³² M. J. Prucha, H. M. Weeter and W. H. Chambers, *Germ Content of Milk as Influenced by the Utensils*, *Bulletin* 204, Ill. Agr. Exp. Sta., 1918.

³³ F. C. Harrison, *Machine-drawn Milk Versus Hand-Drawn Milk* in *Cent. F. Bak.*, II. Abt. 5, 183-189, 1899.

E. G. Hastings and C. Hoffman, *Bacterial Control of Machine-drawn and Hand-drawn Milk* in *Annual Report*, Wis. Agr. Exp. Sta., 24 (1907), 214-222. See also *Cent. f. Bak.* II. Abt. 22, 222-231, 1908.

H. A. Harding, J. K. Wilson and G. A. Smith, *Milking Machines: Effect of Method of Handling on the Germ Content of Milk*. *Bulletin* 317, N. Y. Agr. Exp. Sta., 1909.

³⁴ *Bacteriological Standards for Milk*, U. S. Public Health Reports, 29, pp. 1218-1221, 1914.

³⁵ Limit for potable milk set by ordinance at St. Louis, Mo.

although the real germ content was vastly higher in the sour milk. It should be noted that this is a comparison of the results from individual plates and that the average from a considerable number of simultaneous plate determinations gave more logical results. A study of the above data by Dr. Reitz showed that the results of approximately twenty-five simultaneous plate determinations should be averaged to give results which are satisfactorily accurate.³⁶ Ordinary laboratory studies are made with four to six simultaneous plates³⁷ but routine municipal determinations are frequently based upon the count from single plates. In the face of the demonstrated variability of bacterial plate counts, the use of the results from single plates in municipal laboratories is extremely unsatisfactory.

Another stumbling block in the way of bacterial plate standards is the fact that there are so few municipalities equipped with men and facilities for making as accordant plate counts as those discussed above. The number of cities in the United States thus properly equipped is considerably less than the number which have already adopted such municipal standards. Any standard of keeping quality which is to be a satisfactory aid in measuring the keeping quality of city milk must be much more widely applicable than standard bacterial plate counts are at present. The "little plate" method suggested by Frost³⁸ meets some of these objections but as yet has not been commonly employed.

The direct microscopic estimation of the bacteria in milk has a number of advantages over the plate count among which are the quickness with which it can be made and the relatively

³⁶ Data presented by H. L. Reitz and H. A. Harding to the Laboratory Section of the Amer. Public Health Association at the Rochester meeting, 1914.

³⁷ M. J. Prucha and H. M. Weeter, *Bul.* 199, Ill. Agr. Exp. Sta., p. 31, 1917.

³⁸ W. D. Frost, *Rapid Method of Counting Bacteria in Milk* in *Sci.* 42, pp. 255-256, 1915; *Comparison of a Rapid Method of Counting Bacteria in Milk with the Standard Plate Method* in *J. of Inf. Dis.*, 19, pp. 273-287, 1916; *A Rapid Method of Counting Bacteria in Milk and Other Richly Seeded Materials* in *J. Amer. Med. Asso.* 66, pp. 889-890, 1916; *Counting the Living Bacteria in Milk—A Practical Test* in *J. of Bact.*, 2, pp. 567-583, 1917; *Improved Technique for the Micro or Little Plate Method of Counting Bacteria in Milk* in *J. of Inf. Dis.*, 28, pp. 176-184, 1921.

small expense for laboratory equipment.³⁹ Among its limitations the fact that it is not readily applicable to milk of low germ content nor to pasteurized milk are the most important.

As acid formation is the most prominent objectionable feature in milk of poor keeping quality it has been suggested as a measure of keeping quality. Studies of acid development in milk show that at the temperatures to which milk is ordinarily exposed during delivery and in the household, changes in acidity occur at first so slowly as to appear of little significance but that later acidity increases very rapidly.⁴⁰ As a result milk at the time of delivery may be so little changed as to show on titration a normal acidity and yet be sour before the lapse of twenty-four hours.

A simple and on the whole fairly satisfactory test of keeping quality may be made by holding a sample of milk at a definite temperature for a definite time and then examining for acid and flavors. This test has the merit of not requiring apparatus more complicated than a good ice chest and facilities for the titration of acidity. It is a test which is within the financial possibilities of any municipality which has sufficient funds to provide an inspector to collect and observe milk samples.

THE PROBLEM OF INSPECTION

Attention has already been drawn to the fact that a city can learn little about the actual condition of its milk supply until it provides some form of inspection, but much of the value of such information depends upon the form of inspection provided.

Until about 1900 city milk inspection consisted almost exclusively of the collection and examination of samples within the municipality. At succeeding periods attention was focused upon skimming and watering as affecting the food

³⁹ R. S. Reed and J. D. Brew, *Counting Bacteria by Means of the Microscope*, Tech. Bul. 49, N. Y. Agr. Exp. Sta., 1916.

⁴⁰ E. G. Hastings and A. C. Evans, *A Comparison of the Acid Test and the Rennet Test for Determining the Condition of Milk for the Cheddar Type of Cheese*, Circ. 210, U. S. D. A., Bureau of A. I., 1913.

value, upon cleanliness and upon the germ content as affecting keeping quality and also as an index of healthfulness.

After 1900 in milk inspection work attention was shifted from the city to the country. This shift was made possible by the development of the dairy score card.⁴¹ The dairy score card was originally an attempt to assign such values to the equipment and methods employed in the dairy that the resulting score would give a correct index of the general desirability of the dairy. A dairy scoring 100 would be one in which all items were ideal. The score of actual dairies varied greatly but ordinary producing dairies had an average score of less than 50⁴² which at once suggested that they were open to considerable improvement.

There is little question but that the quality of milk is determined by two general factors, heredity and environment; the former determines the food value of the milk and the latter controls healthfulness, cleanliness, and keeping quality. The score cards paid no attention to the food value of the milk but took account of the environmental factors.

While dairy score cards were originally designed as measures of the general desirability of dairies, as such it is easy to see how in the absence of better standards the dairy score was taken by health officials as an index of the quality of the milk produced from the dairy. The application of this new means of rating and improving milk supplies seemed so fascinatingly simple that the New York City Department of Health was given an initial appropriation of \$100,000 with which to begin the system of farm inspection.

The keen interest in this form of farm inspection lasted about a decade. During this time it was shown that when the dairy score became of financial importance to the producer, either because he was offered a bonus for high scores or because he would be excluded from the market if his score was

⁴¹ Wm. C. Woodward, *Ann. Rept. of the Health Officer*, Dist. of Columbia, 25 (1903-4), p. 27, 1904. Another card independently proposed by Dr. R. A. Pearson, Feb. 25, 1905.

⁴² Geo. M. Whitaker, *The Extra Cost of Producing Clean Milk*, *Ann. Rept.*, of Bureau, 26 (1909), pp. 119-131, 1911.

too low, he changed the conditions of production as profoundly as financial conditions seemed to warrant.⁴³

The decline in popularity of the score card as the basis for farm inspection may be ascribed to two general factors. The first was connected with the matter of germ life in milk. The period during which interest in the germ content of milk practically overshadowed other phases of milk quality in the minds of health officials was practically coincident with the period of interest in dairy score cards. At the time the score cards were formulated there was little available information as to just how bacteria most commonly found their way into milk. This question soon thereafter received careful study and unfortunately for the score cards these studies showed that the avenues through which the great bulk of the germ life enters the milk⁴⁴ had been given slight emphasis on the score cards. The correctness of this conclusion was shown by a separate line of inquiry through which it was demonstrated that there was no observable relation between the germ life present in the milk of dairies and the score of these dairies on any of the score cards in general use.⁴⁵ It is entirely conceivable that in the light of added knowledge regarding the sources of bacteria in milk, new and better score cards might be formulated.

A second set of facts has militated even more strongly against farm inspection. Mention has been made of the large appropriation with which New York City initiated its farm inspection. However, it soon became evident that with this appropriation it would not be possible to inspect all the farms furnishing milk to that city more than once per year. A survey of the general situation showed that only under exceptional conditions could funds be made available to provide

⁴³ H. A. Harding and J. D. Brew, *The Financial Stimulus in City Milk Production*, Bul. 363, N. Y. Agr. Exp. Sta., 1913.

⁴⁴ M. J. Prucha, H. M. Weeter and W. H. Chambers, *Germ Content of Milk. II. As Influenced by the Utensils*, Bul. 204, Ill. Agr. Exp. Sta., 1918.

⁴⁵ J. D. Brew, *Milk Quality as Determined by Present Dairy Score Cards*, Bul. 398, N. Y. Agr. Exp. Sta., 1915.

for more than two farm inspections per year.⁴⁶ On the other hand the studies of germ life referred to above have shown that the factors controlling the germ content of the milk change twice per day on each farm. Under such circumstances little can be accomplished toward controlling the germ content of milk by the amount of farm inspection which the health officials find to be financially possible.

The need of effective supervision of milk supplies is unquestioned. Farm inspection failing to provide this supervision, attention is returning to an inspection of the milk itself. As a matter of fact, if the consumer can assure himself that the milk as delivered to him is in satisfactory condition, he has very little interest in the preceding details. The four questions which he is accustomed to ask regarding a milk are the following:⁴⁷

Is it rich? (The problem of food value)

Is it safe? (The problem of healthfulness)

Is it clean? (The problem of cleanliness)

Is it sweet? (The problem of keeping quality)

The housewife commonly estimates the richness of the milk by the depth of the cream in the neck of the bottle. The food value of milk cannot be adequately expressed by any single measurement because in addition to its value as a source of energy, it has an important relation to growth and health. However, in comparing the relative food values of two samples of normal milk, the net calories of energy contained are perhaps the best basis for comparison. There are at present available only a few analyses of milk of known purity and these analyses grouped according to their fat content are given in Table I.

⁴⁶ H. Lloyd, *Report of Committee on Cost of Dairy and Milk Inspection, Ann. Rept., Int. Asso. of Dairy and Milk Insp., 7, 42-47, 1919.*

⁴⁷ H. A. Harding, R. S. Reed, W. A. Stocking and E. G. Hastings, *What is Meant by Quality in Milk, Circ. 205, Ill. Agr. Exp. Sta., 1919.* Also published by the N. Y. and Cornell Agr. Exp. Stations.

TABLE I.—ENERGY VALUES OF MILKS ⁴⁸

Protein	Fat	Carbo- hydrates	Food substance per quart*	Calories per gram	Calories per quart	Total calories per quart
1	2	3	4	5	6	7
per cent	per cent	per cent	grams	calories	calories	calories
2.648			25.87	4	103.48	
	3.00		29.31	9	263.79	
		4.596	44.90	4	179.60	546.87
3.068			29.96	4	119.84	
	3.498		34.18	9	307.62	
		4.903	47.90	4	191.60	619.06
3.044			29.74	4	118.96	
	3.994		39.02	9	351.18	
		4.875	47.63	4	190.52	660.66
2.082			30.11	4	120.44	
	4.516		44.12	9	397.08	
		4.958	48.44	4	193.76	711.28
3.62			35.37	4	141.48	
	5.048		49.32	9	443.88	
		4.922	48.09	4	192.36	777.72
3.743			36.57	4	145.28	
	5.534		54.07	9	486.63	
		4.93	48.17	4	192.68	825.59
3.992			39.00	4	156.00	
	5.94		58.03	9	522.27	
		4.878	47.66	4	190.64	868.91
4.12			40.25	4	161.00	
	6.50		63.52	9	571.68	
		4.90	47.87	4	191.48	924.16
4.22			41.23	4	164.92	
	7.00		68.39	9	615.51	
		4.84	47.29	4	189.16	969.59

* In computing these values, 977 grams have been used as the weight of one quart of milk. This is the weight of a quart of milk of specific gravity 1.0323, or nearly the average specific gravity of milk. The use of the minimum or maximum limits of specific gravity of normal milk (1.029-1.035) would change the values so slightly as to be negligible in so far as the purpose of this circular is concerned.

The figures given in Table I show that accompanying an increase of fat from 3 per cent to 7 per cent, there is a corresponding increase in net calorific value from 546.87 to

⁴⁸ O. R. Overman, *Food Values and Dairy Products*, Circ. 235, Ill. Agr. Exp. Sta., 1919.

969.59 calories or an increase of approximately 105 calories with each increase of 1 per cent fat. The use of the calorimeter, however, is too technical even for inspection work. From the above data it is evident that the use by the housekeeper of the fat content as an index of food value is correct in principle though the method of estimation is necessarily crude. The Babcock test for fat determination is both simple and accurate with the added advantage that the test itself is well understood and the necessary apparatus is ordinarily at hand. Accordingly, the fat content is in all particulars well adapted to serve as an index of food value of milk as it is delivered to the consumer.

The safety of the milk as delivered may be about equally well guaranteed in either of two ways; by frequent inspection of the health of the cows and people concerned in its production and handling, or by proper pasteurization and protection from reinfection. Safeguarding the milk by means of health inspection of cattle and men adds from 3 to 5 cents per quart to the cost of the milk while the expense of pasteurization varies from about 1 cent per quart in small quantities to about $\frac{1}{8}$ cent per quart in large quantities. Under such conditions it is not strange that practically all the actual advance in safeguarding municipal milk supplies has been made through the adoption of pasteurization. Where pasteurization is relied upon to make milk safe from germs of infectious diseases, there should be a requirement of temperature recording devices in connection with the process and a frequent inspection to ascertain that the apparatus is in proper working condition and that the milk is properly protected after pasteurization.

The housewife is aware that when a bottle of milk is undisturbed for some time the dirt in the milk settles to the bottom and she accordingly looks at the bottom of the bottle to determine the cleanliness of the milk. In inspection work the amount of insoluble dirt in milk as delivered to the consumer may be estimated very accurately by means of the sediment test.

There can be no question regarding the relative ease and accuracy with which the above outlined examinations will give the facts regarding the food value, freedom from germs of infectious diseases, and cleanliness of a sample of milk. However, there is not the same agreement regarding the possibility of determining the keeping quality. There are at present no means available for quickly measuring the keeping quality of milk except in samples which are rapidly approaching an undesirable condition. In such samples titration of the acidity may indicate their condition and in the case of unpasteurized milk a microscopic examination is even more useful. However, the results from both such examinations of some milk samples may seem favorable and still the milk may not remain sweet for twenty-four hours under household conditions.

In the absence of any test which will immediately yield the desired information in the case of pasteurized milk, recourse may be had either to the bacterial count or to the observation of a sample of the milk itself. Since the question at issue is the ability of the milk to remain sweet and in satisfactory condition for twenty-four or at most forty-eight hours, this fact can be determined from the sample of the milk itself quite as quickly as the bacterial count can be determined from the standard plates. The examination of the sample has the added advantage that it does not require as extensive equipment nor as delicate manipulation. Most important of all, it gives precisely the information desired while the bacterial count even when accurately determined must first be translated before it can be of service.

Following the plan above outlined it is practicable to collect samples of the milk as delivered to the consumer and from the examination of such samples, supplemented by some inspection of pasteurization plants, determine the food value, healthfulness, cleanliness, and keeping quality of the milk supply.

GRADING MILK

An accurate answer regarding the richness, safety, cleanliness, and sweetness of a sample of milk is the real beginning of successful milk inspection. However, inspection presupposes the existence of standards of quality, and in every other line of merchandise these standards are arranged so as to recognize classes or grades corresponding to market demands.

The idea of grading milk is not a new one, as in 1866 in England⁴⁹ regulations were promulgated recognizing two grades of milk, one adapted to city trade and one to manufacturing purposes. Suggestions for grading the city milk supply began to be made in New York City before 1907 but the first official grading of a municipal milk supply in this country was probably that at Geneva, N. Y. in 1907.⁵⁰ A plan of grading the milk supply of New York City went into effect in January, 1912.⁵¹ This plan established a number of commercial grades and required that each bottle of milk bear the designation of its grade. The Milk Commission appointed by a philanthropic organization called the New York Milk Committee in 1912⁵² recommended a plan for milk grading which was essentially an amplification of the previous New York City plan. The grading plans of the city of New York and of the Milk Commission have been based mainly upon the results obtained from dairy scores and the bacterial count of the milk. It should also be noted that both of these plans for grading recognize the importance of pasteurization. As already explained no relation has been demonstrated between the dairy scores as actually obtained and the quality of the milk while bacterial counts are at best only an indirect way of measuring the keeping quality of the milk. The city of New York has continued with its

⁴⁹ H. N. Parker, *City Milk Supply*, p. 370.

⁵⁰ H. A. Harding, *Publicity and Payment Bases on Quality as Factors in Improving a City Milk Supply*, Bul. 337, N. Y. Agr. Exp. Sta., 1911.

⁵¹ W. H. Park, *The Future Milk Supply of New York City*, Ann. Proc., Amer. Asso. Med. Milk Com., 5, pp. 99-103, 1911.

⁵² *First Report of the Commission on Milk Standards Appointed by the New York Milk Committee*, Public Health Reports 27, pp. 673-700, 1912.

experiments in milk grading, and in 1913 milk grading was extended to include the state of New York. Encouraged by these attempts milk grading has been undertaken by municipal and private enterprise in various cities in a number of states.

In 1912 the Official Dairy Instructors Association or, as it is now called, the American Dairy Science Association, through a committee, undertook to determine the essential facts in milk quality. In its first formal report in 1917,⁵³ this committee defined quality in milk and indicated how grades might be so constituted as to conform to market needs.

The markets in the large cities and in the smaller towns present somewhat different problems. In both, the main need is for a moderately rich, safe, clean, sweet milk which will be satisfactory for adults and for children. This may well be characterized as "table milk." In both there is a limited demand for an extra attractive grade of milk which is usually somewhat richer, perhaps a little cleaner, and has an increased keeping quality. This may be referred to as "special" or "baby milk."

On the other hand there is on both markets a considerable amount of milk which does not come up to the standard for "table milk." In the large cities its most common deficiency is keeping quality induced by age or careless handling. In the small town healthfulness is usually the point to be criticised since in such places pasteurization is the exception rather than the rule. Such milk is either undesirable or unsafe, depending upon the nature of its deficiency, and still in either case it is ordinarily well adapted to cooking purposes. Such milk might be characterized as "cooking milk."

It is common for milk reformers to insist that any system of classification which contemplates the sale of unsafe milk is unsound but any workable system of classification must take account of market conditions as they exist. Again, cooking milk as here discussed is safe for cooking purposes. Any plan which contemplates accurate labeling is at least a step

⁵³ See footnote 47.

in the right direction, and as promptly as the local situation makes this possible the objection can be met by an ordinance requiring the proper pasteurization of all of the local milk supply. Until this point in the development is reached, the situation is not made any safer where unsafe milk is sold without a label than it would be if the unsafe milk were expressly labeled "cooking milk." On the other hand, in such communities the proper use of the "table milk" label would guide at least the most intelligent to the use of a safe supply and permit the authorities to indicate to the public that "cooking milk" was not safe for general use.

As an illustration of the way the qualities of food value, healthfulness, cleanliness, and keeping quality may be used in establishing grades of milk the following is quoted from an ordinance adopted by the city of Champaign, Illinois, May 1918.

GRADING OF MILK

Section 16. On and after June 1st, 1918, all milk sold in bottles in the City of Champaign must state on its label its commercial grade and must be true to grade according to the following definition of grade, provided that nothing in this ordinance shall be construed as applying to certified milk as supervised by the Champaign County Medical Milk Commission.

Two grades of milk shall be recognized, viz.: table milk and cooking milk.

TABLE MILK

Table milk as used in this ordinance is defined as milk having the following qualities:

Food Value: Butter fat content at least 3 per cent. A higher butter fat content may be stated upon the container. When so stated it must be as indicated upon the container. (A reasonable variation or tolerance from the statement on the container will be permitted, provided the fat content is at least 3 per cent.)

Healthfulness: As resulting from pasteurization at 140 to 145 degrees Fahrenheit for thirty minutes or from a physical and tuberculin test of the cattle, and a medical examination of those who come in contact with the milk during its production. The tuberculin test and the medical examinations must be made by a person or persons approved by the Health Officer. Complete

records of all tuberculin tests and medical examinations must be promptly filed with the Health Officer. Cows must not be admitted to the herd until after they have passed the physical examination and tuberculin test. Tuberculin tests of the herd must be repeated annually and wherever any animals react they must be promptly removed from the herd and in such cases the tuberculin test be repeated within an interval of six (6) months.

Cleanliness: As indicated by a sediment test showing not more than 3 milligrams per pint. The amount of sediment to be determined by comparison with a scale formed by passing through a cotton filter, milk in which the designated amount of powdered carbon has been suspended.

Keeping Qualities: Sufficient to remain sweet and in a satisfactory condition for 24 hours at 60 degrees Fahrenheit, as delivered to the consumer.

COOKING MILK

Cooking milk as used in this ordinance is defined as milk having the following qualities:

Food Value: Butter fat content at least 3 per cent. A higher butter fat content may be stated upon the container. When so stated it must be as indicated upon the container. (A reasonable variation or tolerance from the statement on the container will be permitted, provided the fat content is at least 3 per cent.)

Cleanliness: As indicated by a sediment test of not more than 6 milligrams to the pint. The content to be determined by a comparison with a scale formed by passing through a cotton filter, milk in which the designated amount of powdered carbon has been suspended.

Keeping Qualities: Sufficient to remain sweet and in a satisfactory condition for twenty-four (24) hours at 60 degrees Fahrenheit, as delivered to the consumer.

This ordinance is not suggested as a model but rather as an illustration of the adaptation of milk grading to a city where the authorities are desirous of indicating the safe milk upon the general market and at the same time are unwilling to require that all the milk be made safe.

Today city milk supplies utilize approximately one-half of the milk produced in the United States. The problems connected with city milk include a large part of those connected with the dairy and its products.

When Dr. Russell entered upon his duties as bacteriologist to the Wisconsin Agricultural Experiment Station in 1893 practically all of these problems were unsolved. During the succeeding quarter of a century, the rapid growth of the cities has demanded a growing milk supply and the problems have arisen in rapid succession. This Pioneer Bacteriologist in person and through his students has taken an honorable part in the solution of these problems.

THE RESISTANCE OF MOLD SPORES TO THE ACTION OF SUNLIGHT

JOHN WEINZIRL

The present paper is one of a series on the action of light on microorganisms, and grew directly out of the work on the action of light on bacterial spores.¹ In that paper it was shown that bacterial spores resist the action of direct sunlight from two to eight hours, i. e., they show about sixty times the resistance of non-spore-bearing bacteria. Having found bacterial spores to be highly resistant, it was quite natural to inquire how this resistance compares with that of mold spores. The simple method devised for making the exposures has not been tried on mold spores so far as the writer is aware and it seemed worth while to extend it into this field. The method has previously been described at length;² briefly stated, it consists in first making a suspension of the spores in water or physiological salt solution; from this suspension a droplet is spread upon a small slip of sterile paper (1 x 3 cm.) contained in a sterile petri dish; for this purpose the standard platinum loop used by bacteriologists works very well; the infected paper slips are first dried, then exposed to the sun in a petri dish without the presence of any medium. This avoids all disturbing factors such as germination, formation of disinfectants, and the excessive absorption of the chemical rays by the medium. The plates are exposed on wire trays raised about 16 inches above the floor, and are held so that the sun's rays fall as nearly vertically as practicable. After being exposed a given length of time the inoculated slips are transferred by means of a sterile forceps to a suitable medium, nutrient agar being very satisfactory. If the surface of the medium is dry, then it is well to add a few drops of bouillon.

¹ *American Journal of Public Health*, 4, 11, p. 969, 1914.

² *Journal Infectious Diseases*, Sup. No. 3, p. 128, 1907.

For the work with molds an agar containing the nutrient salts, potato broth, and cane sugar gives excellent growths, although ordinary nutrient agar serves nearly as well.

For obtaining the spores it is well to use a culture a month or two old. Controls were always employed to determine whether the inoculations were successful. Two controls, one made at the beginning and the other one at the end of the experiment, were sometimes made but this is scarcely necessary for mold spores possess a remarkable resistance against desiccation.

CULTURES USED

Representatives of the more common molds were employed in the trials. A black mold of as yet undetermined species represented the *Mucoraceae*; *Aspergillus niger*, *Aspergillus fumigatus*, *Aspergillus nidulans*, *Oidium lactis* and *Penicillium glaucum* were the other forms used. All the forms are exceedingly common and were obtained from the air or from decayed fruits, excepting the *Oidium* which was isolated from milk. *Aspergillus fumigatus* was included because of its pathogenic powers for animals.

The following tables present the results when the spores of these molds were exposed to direct sunlight.

TABLE I.—MUCOR SP.

Date	Age of Culture	Time Exposed (hours)	
		Growth	No growth
October 30, 1913.....	7 days.....	$\frac{1}{4}$, $\frac{1}{2}$, 1.....
March 27, 1915.....	17 ".....	$1\frac{1}{4}$, $2\frac{1}{4}$, $3\frac{3}{4}$
June 28, 1915.....	107 ".....	2, 4, 6, 10.....

TABLE II.—ASPERGILLUS NIGER

Date	Age of Culture	Time Exposed (hours)	
		Growth	No growth
June 21, 1915.....	60 days.....	1, 3, 8.....	
June 28, 1915.....	67 ".....	2, 4, 6, 10.....	
June 29, 1915.....	68 ".....	9, 11, 12, 13.....	
July 4, 1915.....	73 ".....	5, 13, 18, 27.....	
July 10, 1915.....	79 ".....	13, 23, 36, 47, 58.....	
July 18, 1915.....	87 ".....	7, 20, 31, 42.....	

TABLE III.—ASPERGILLUS FUMIGATUS

Date	Age of Culture	Time Exposed (hours)	
		Growth	No growth
June 21, 1915.....	60 days.....	1, 3, 8.....	
June 28, 1915.....	67 ".....	2, 4, 6, 10.....	
June 29, 1915.....	68 ".....	9, 11, 12, 13.....	
July 4, 1915.....	73 ".....	5, 13.....	18, 27
July 10, 1915.....	79 ".....	13.....	23, 36, 47, 58
July 18, 1915.....	87 ".....	7, 20, 31, 42.....	

TABLE IV.—ASPERGILLUS NIDULANS

Date	Age of Culture	Time Exposed (hours)	
		Growth	No growth
March 27, 1915.....	17 days.....	1¼, 2½, 3¾.....	
June 28, 1915.....	107 ".....	2, 4, 6, 10.....	
June 29, 1915.....	68 ".....	9, 11, 12, 13.....	
July 4, 1915.....	73 ".....	5, 13, 18, 27.....	
July 10, 1915.....	79 ".....	13, 23, 36, 47, 58.....	
July 18, 1915.....	87 ".....	7, 20, 21, 42.....	

TABLE V.—OIDIUM

Date	Age of Culture	Time Exposed (hours)	
		Growth	No growth
June 21, 1915.....	60 days.....	1, 3, 8.....
July 10, 1915.....	79 ".....	23, 36, 58.....	47
July 18, 1915.....	87 ".....	20, 42.....	7, 31

TABLE VI—PENICILLIUM GLAUCUM

Date	Age of Culture	Time Exposed (hours)	
		Growth	No growth
September 30, 1913.....	?	¼, ½.....
October 25, 1913.....	25 days.....	1, 2, 3.....
October 30, 1913.....	30 ".....	2½, 5.....
July 10, 1915.....	18 ".....	36, 58.....

An examination of the above tables shows that the mold spores possess an extraordinary resistance to direct sunlight. This resistance was so unexpected that in the earlier trials the cultures were exposed for only a few hours; this time was gradually lengthened until in the last trials the exposure was continuous for four or five days during the long days of July, and yet *Aspergillus niger* and *nidulans* never failed to grow. The other species showed some irregularities, but possessed remarkable endurance. The irregularities may be due to a failure to infect the paper strips, for in no other way can we explain the discrepancy in the case of *Aspergillus fumigatus* which withstood 42 hours of exposure one time and only 13 hours in an earlier trial.

It is difficult to explain this extraordinary resistance of mold spores as compared with bacterial spores which rarely survive more than five hours. The fact that these spores are colored suggests that this pigment exerts a protective influence;

doubtless this is true in a measure, but there still remains the fact that *Oidium* with colorless spores also shows remarkable resistance.

The fact that mold spores are relatively lighter than other spores would suggest that they have succeeded better in eliminating water from their protoplasm and hence are better able to resist adverse agents. How far this may really enter into the explanation it is difficult to say.

The results help to explain the prevalence of mold spores in air where they greatly preponderate over bacterial spores. While bacterial spores can resist the action of sunlight but a few hours, mold spores can resist this action for days. Indeed, it is doubtful whether sunlight is able to kill mold spores.

SUMMARY

Spores of the following molds were exposed to direct sunlight on paper slips in glass petri dishes: *Mucor Aspergillus niger*, *Aspergillus fumigatus*, *Aspergillus nidulans*, *Oidium*, *Penicillium glaucum*. The results show that mold spores are able to withstand 58 hours or five days of continual exposure to the intense rays of a summer sun.

Apparently, sunlight possesses slight, if any, germicidal powers upon mold spores. This fact helps to explain the greater prevalence of mold spores than bacterial spores in air, for the latter are killed usually within five hours.

THE INFLUENCE OF THE PLANE OF NUTRITION UPON THE PERCENTAGE OF FAT IN MILK AND THE PROPERTIES OF THE FAT

C. H. ECKLES

It is a well-known fact that the percentage of fat present in milk is subject to wide variations. Among the most important causes known to be responsible for these variations are breed of animal, stage of lactation and the individuality of the animal dependent upon heredity. In addition the author has found certain other factors not previously recognized to be of considerable importance. Among these are seasonal variations,¹ fatness of the cow at parturition,² and plane of nutrition.³ Although the second and third factors are not identical, they are closely related and some material concerning both is included in the present paper.

The fat percentage of milk may be increased decidedly above the normal for the animal concerned for a short time after parturition, and increased to a less extent for a longer period, provided the animal has been fed on a high plane of nutrition for a sufficient time previous to parturition to make it possible for a large amount of fat to be deposited in the body tissues and that the plane of nutrition following parturition is reduced to a point below the actual requirements of the body. The underfeeding following parturition does not necessarily result from feed being purposely withheld by the feeder but because the animal when in high flesh at the

¹ C. H. Eckles, *Jahreszeitliche Schwankungen des prozentischen Fettgehaltes in Kuhmilch in Milchwirtschaftliches Zentralblatt*, Heft 11, pp. 488-502, 1909.

² *Ibid.*, *Influence of Fatness of Cow at Parturition on Per Cent of Fat in Milk*, Bulletin 100, Mo. Agr. Exp. Station, 1912.

³ C. H. Eckles and L. S. Palmer, *Influence of the Plane of Nutrition of the Cow Upon the Composition and Properties of Milk and Butter Fat, Overfeeding*, Research Bulletin 24, p. 35; *Underfeeding*, Bulletin 25, p. 106, Mo. Agri. Exp. Station, 1916.

beginning of lactation does not appear to be able to consume sufficient feed to supply the heavy demands upon her body.

Our attention was first attracted to this factor by the results of an experiment carried out for another purpose. A Jersey cow was fed very liberally with grain for several months before calving in order to have her excessively fat. Immediately after parturition she was put upon a ration, estimated from feeding standards to be sufficient to maintain her body weight allowing nothing for milk production. The surprising part of the result was that the cow continued to give practically the same amount of milk, 18-24 lbs. a day for the thirty days she was kept on this ration. The continued production of milk on a ration sufficient only for maintaining the body strongly supports the theory now generally accepted that milk secretion, at least in the early stages of lactation, is the result of the action of a hormone which stimulates the udder into activity. The nutrients necessary for the milk production unquestionably were taken from her body as evidenced by a decline in weight of nearly four pounds daily.

The point that especially attracted our attention, however, was the fact that the fat percentage in the milk during this period of underfeeding was abnormally high and that it declined within twenty-four hours after the ration of the cow was increased to a point where sufficient nutrients were supplied for both maintenance and milk production. These data are shown in Table I.

It will be noted that the average fat content for the thirty days of underfeeding was 6.01 per cent while the average for the entire year, which was normal for the animal, was 4.8 per cent. The experiment was repeated with similar results with a Holstein cow. The average fat percentage for the year with this animal was 2.99 and during a period of underfeeding seven days after calving the fat content was 4.47 per cent.

In order to secure further data on this subject an experiment was conducted with another Holstein cow. This animal was fed a very heavy grain ration for over a year resulting

TABLE I.—EFFECT OF UNDERFEEDING UPON THE PER CENT OF FAT
IN MILK
Jersey Cow No. 20

Days after calving	Per cent fat	Yield of milk Pounds	Grain fed Pounds	Alfalfa hay fed Pounds	Weight Pounds
2	4.37	22.2	3.5	7	830
4	5.80	20.9	3.5	7	807
6	6.89	22.3	3.5	7	790
8	7.21	23.1	3.5	7	787
10	6.60	22.6	3.5	7	785
12	5.86	21.5	3.5	7	780
14	6.82	20.0	3.5	7	765
16	6.00	19.1	3.5	7	755
18	5.07	21.8	3.5	7	755
20	4.94	17.2	3.5	7	730
22	6.37	19.0	3.5	7	730
24	6.82	19.6	3.5	7	725
26	5.70	19.1	3.5	7	720
28	6.26	17.8	3.5	7	720
30	5.60	19.9	3.5	7	710
32	5.53	20.4	5.5	11	690
34	4.74	24.4	7	11	710
36	4.28	25.4	7	14	710
38	4.49	25.0	7	14	720
40	4.45	25.0	7	14	710
42	3.98	27.2	7	14	715
44	4.83	26.7	7	14	740
46	4.27	26.1	8	16	700
48	3.80	25.9	8	16	735
50	4.23	27.0	8	16	747
52	25.6	8	16	725
54	4.01	25.7	8	16	715
56	4.47	23.7	8	16	720
58	4.37	24.3	8	16	740

in her being excessively fat at parturition. Beginning ten days after the birth of the calf, this cow in seven days produced 291 pounds of milk containing 5.1 per cent of fat. Her average for the entire milking period of eleven months was 3.2. During the year she was fed a normal ration for a dairy cow, resulting in her being in a moderate condition only at the time of next parturition. Beginning the same interval after calving as before she produced 224 pounds of milk in seven days with an average fat percentage of 3.63. The average for the entire milking period was 3.00 per cent.

These results are corroborated by a large number of data from other experimental animals which will not be given here.

Other evidence on this point is found in the reports of official tests of dairy cattle as reported by the various breed associations. All interested in the practical phases of dairy husbandry are familiar with the immediate application which followed the publication of the facts⁴ regarding the possibility of increasing the richness of the milk by these means and the astonishing results that have been obtained in seven day tests of dairy cows in recent years as a result. It is well known that the great increase in butterfat yield in these tests is due largely to a decidedly higher fat percentage. For example in 1903 the cow, Sadie Vale Concordia, held the Holstein butter fat record for seven days with a yield of 694.3 lbs. of milk containing 3.52 per cent of fat.⁵ The record up to 1917 is 730 lbs. milk containing 5.54 per cent of fat.⁶ At the same time there is no evidence of any appreciable raise in the average fat percentage for the breed. The average fat percentage reported by the Holstein Association for the highest twenty-five cows in seven day tests is 5.1. The average for the highest twenty-five yearly records is 3.96.

The practical use of this means of greatly increasing the butterfat production temporarily has resulted in an entirely new basis of fixing value for breeding stock among certain of the dairy breeds.

The question may be raised whether it is not normal regardless of the state of flesh of the animal for the fat percentage to be high during the first four weeks after parturition followed by a decline to a lower level. Data are also at hand showing this is not the case. In Table II are found data giving the fat content by weeks in the milk of cows that were in a moderate state of flesh at parturition. It will be noted that there is a constant rise in the fat percentage from the beginning and that the average for the year is decidedly higher

⁴ C. H. Eckles, *A New Factor Influencing the Per Cent of Fat in Cows' Milk* in *Hoard's Dairyman*, 40, 23, p. 696, Aug., 1909.

⁵ *Holstein Friesian Yearbook*, 3, p. 93, 1903.

⁶ *Hoard's Dairyman*, 43, 1, p. 14, Jan., 1917.

than in the beginning of the lactation period which is exactly the reverse of that found when the animals are in a fat condition.

TABLE II.—SHOWING FAT CONTENT NORMAL TO LOW IN BEGINNING OF LACTATION PERIOD

Cows Moderate to Thin in Flesh

Time after calving	No. 57	No. 215	No. 304	No. 59	No. 55	No. 307
Weeks						
1	3.2	3.5	3.6	3.2
2	3.0	3.4	4.6	3.9
3	3.2	3.8	4.1
4	3.2	3.15	3.4	4.0	4.5
5	4.0	3.1	3.4	3.8	3.12
6	4.4	3.2	3.7	3.8
7	4.7	3.2	4.1	4.0	3.9
8	4.5	3.0	3.5	4.2
9	4.2	3.2	3.2	4.7	4.20
10	5.6	3.2	3.9	5.0	4.3
11	4.5	3.2	3.9	4.5
12	4.6	3.3	4.0	3.53
Months						
4	5.9	3.2	4.0	5.6	4.7	3.30
5	5.8	2.9	3.9	5.2	5.8	3.67
6	5.8	3.5	3.6	5.3	5.8	3.80
7	5.2	3.4	4.0	5.3	5.6	3.85
8	5.3	3.6	4.7	5.0	6.3	4.19
9	5.5	2.8	5.0	5.1	6.4
10	5.1	3.2	5.2	5.6	5.9
11	5.1	2.9	5.0	6.1
12	3.4	5.9
Year	5.33	3.29	4.01	5.11	5.18

We are also able to present data showing the effects of underfeeding upon the fat percentage are not confined to the interval immediately following calf birth. Table III gives the results for Jersey Cow 62 beginning at a point 96 days in milk. It will be seen that there was almost an immediate rise in the fat percentage when the ration was reduced below the requirements and that the fat declined again as soon as the ration was raised.

TABLE III
EFFECT OF UNDERFEEDING—ADVANCED LACTATION

Jersey Cow No. 62

Date	Per cent of fat	Pounds of milk	Live weight	Energy supplied	Energy required
				Therms	Therms
March 7.....	4.6	16.9	930	10.00	11.80
8.....	4.5	16.3	930	10.00	11.58
9.....	4.9	15.6	915	7.00	11.24
10.....	4.8	14.6	910	7.00	10.85
11.....	5.5	14.8	900	7.00	10.86
12.....	5.7	15.1	890	7.00	10.91
13.....	5.5	14.6	870	4.66	10.60
14.....	5.9	14.3	865	4.66	10.43
15.....	6.1	13.6	865	4.66	10.21
16.....	5.6	12.9	855	4.66	9.88
17.....	6.0	13.5	855	4.66	10.11
18.....	6.5	13.1	845	4.66	9.91
19.....	5.7	12.9	835	4.66	9.71
20.....	6.2	12.9	835	4.66	9.77
21.....	5.6	12.9	840	4.66	9.81
22.....	5.8	13.3	830	4.66	9.89
23.....	5.2	12.8	850	4.66	9.83
24.....	5.4	12.0	830	4.66	9.42
25.....	5.7	12.8	815	10.00	9.62
26.....	4.8	13.1	845	10.00	9.91
27.....	4.8	14.5	855	10.00	10.47
28.....	4.3	15.2	855	10.00	10.73
29.....	4.1	15.3	830	10.00	10.92
30.....	4.5	16.1	880	10.00	11.21

Table IV gives similar data for Jersey Cow 2, beginning 100 days after parturition. It will be seen that the results are the same as for Cow 62. When the ration was dropped suddenly on the fifth day there was an immediate rise in the fat content followed by a decline when the ration was again raised.

Outside the publications of the author, and his co-worker, Dr. L. S. Palmer, the only reference to be found in the literature regarding the relation of underfeeding to the fat content of milk is by Lusk who conducted a brief experiment with a goat. He reports that the fat content of the milk was increased during two days when the goat received no feed. In explanation, Lusk advances the theory that when suf-

ficient sugar is not oxidized in the cells the sugar hungry cells attract fat. Dextrose is converted into milk sugar in the mammary gland and cannot then be burned in the organism; the mammary cell becoming a sugar hungry cell attracts large amounts of fat which is present in the blood.

TABLE IV
EFFECT OF UNDERFEEDING—ADVANCED LACTATION

Jersey Cow No. 2

Date	Per cent of fat	Pounds of milk	Live weight	Energy supplied	Energy required
				Therms	Therms
April 22.....	4.8	11.9	870	15.76	9.69
23.....	4.4	11.3	870	15.76	9.47
24.....	4.5	10.9	870	13.53	9.32
25.....	5.8	11.1	865	10.58	9.36
26.....	5.4	10.6	842	9.07	9.05
27.....	5.5	10.7	840	8.32	9.07
28.....	5.4	10.8	855	9.47	9.20
29.....	5.0	10.9	860	11.27	9.26
30.....	4.7	10.7	852	11.98	9.14
May 1.....	5.0	13.5	852	15.78	9.79
2.....	5.2	11.1	850	15.78	9.27

The author in connection with Dr. L. S. Palmer has given some attention to the question of the cause of this increase in fat content which accompanies underfeeding. A series of experiments was conducted to test a theory that the increase in the fat in the milk is due to an increase in the fat in the blood of the animal during underfeeding, a condition known to occur with smaller animals, at least, under conditions of starvation.

Two cows, a Shorthorn and an Ayrshire, were selected for the test. These animals were fattened to an excessive point before parturition in order to make certain that high fat content of the milk would result after calving. Ten days before the animals were due to calve, blood was drawn from the jugular vein and analyzed for percentage of fat. Blood samples were again taken after parturition when the conditions of underfeeding were strongest and again later when the animals were normal. The method of analysis used was the Kama-

gaira-Luto as modified by Rosenthal and Trowbridge.⁷ Table V gives the results.

TABLE V
INFLUENCE OF UNDERFEEDING ON PERCENTAGE OF FAT IN BLOOD AND PERCENTAGE OF FAT IN MILK

Date	Lactation	Daily milk yield	*Fat in milk	Fat in Blood		
				Test 1	Test 2	Average
	Days	Pounds	Per cent	Per cent	Per cent	Per cent
Cow 407						
1914						
Dec. 24	-10	0.484	0.396	0.440
1915						
Jan. 5	3	46.1	6.80	.318	.328	.323
Jan. 6	4	33.7	4.80	.281	.315	.298
Feb. 16	45	49.5	3.65	.323	.324	.324
Cow 305						
1915						
Feb. 4	-4257	Lost	.257
Feb. 9	2	24.3	6.46	.241	.223	.232
Feb. 10	3	27.0	6.68	.237	.221	.229
Feb. 11	4	29.8	6.10	.210	Lost	.210
Feb. 18	11	33.1	6.10	.233	Lost	.233
Mar. 3	20	38.0	5.00

* The average of Cow 407 for the year was 3.36 and of Cow 305, 4.03 per cent.

The negative results obtained on the blood fat seem to indicate that in the case of the cow, starvation does not result as is the case with small animals in an increase in the fat in the blood. It would seem therefore that the increase in the fat in the milk could hardly be looked upon as the result of a direct transfer from the body.

It is believed the explanation may be sought along other lines and the following is suggested. When the body is forced to draw on its reserves for needed energy, the fat is liberated by an increased lipase activity. The hormone or chemical messenger that stimulates this lipase activity must be carried by the blood, and it is reasonable to believe that it will affect the lipase activity in all parts of the body through which

⁷H. Rosenthal and P. F. Trowbridge, *The Estimation of Fat in Blood in J. Biol. Chem.*, 20, pp. 711-717, 1915.

the blood passes, particularly those parts in which lipases are already normally at work. Other enzymes are also involved in this activity, for the synthesis of fatty acids and glycerol from which the lipases build the neutral fat is fundamentally reactions of oxidation and reduction. The general hypothesis, however, is at once seen to offer a plausible explanation for the increase in the fat of the milk during underfeeding. It explains how the lipases and other enzymes normally accelerating the formation of milk fat in the mammary gland may bring about a greater formation of milk fat at the same time the lipases in the tissue fat of the body are liberating more fat from the cells for energy required by the body. It also explains why the highest fat tests in underfeeding were secured in the cases of the fattest animals, and why animals in thin or only moderate flesh showed the least effects on the percentage of fat in the milk.

The relation of the lipase activity of the mammary gland to the lipase activity in other parts of the body also explains the low fat percentage of milk often secured during very hot weather. It is a well-known fact that an abnormally low percentage of fat in the milk will accompany a period of hot, humid weather in summer as has been shown by the author, while a period of very cold, dry weather in winter will cause an increase in the percentage of fat in the milk.

INFLUENCE OF PLANE OF NUTRITION UPON COMPOSITION OF THE FAT

A striking result of underfeeding a lactating cow is the effect upon the composition of the milk fat. This change becomes noticeable almost as soon as the plane of nutrition drops below the requirements of the animal. In the experiment with the Jersey cow mentioned in connection with the relation of the plane of nutrition to the fat percentage of the milk, the fat constants exhibited the typical effect of underfeeding during the period when the ration was below the requirements of the animal. When the ration was increased to a normal amount the fat constants quickly returned to normal for the animal in question.

At the end of thirty days' underfeeding the saponification number was 222.1; after fourteen days on a normal ration it was 225.7. During the same interval the iodine number decreased from 42.62 to 34.15; the Reichert-Meissl increased from 21.43 to 24.2, and the melting point declined from 36.35 to 33.60.

A summary of the results of twenty-three experiments is given in Table VI. The plane of nutrition as indicated in percentage is calculated on the basis of the requirements of the animal in question calculated according to the Armsby Feeding Standard.

The data presented in Table VI have several striking features. The most striking is the fact that without exception all

TABLE VI

VARIATION IN FAT CONSTANTS FROM NORMAL TO ABNORMAL DUE TO PLANE OF NUTRITION

Exp.	Cow	Plane of nutrition	Variations from Normal of			
			Saponification value	Reichert-Meissl number	Iodine value	Melting point
		Per cent				° C.
1.....	20	-62	- 7.2	-5.5	+12.4	+3.5
2.....	206	-60	-9.9	+ 8.0	+7.0
3.....	301	-39	-15.2	-1.5	+17.5	+2.2
4.....	300	-38	- 3.3	-2.6	+ 1.3	+2.2
5.....	2	-23	- 5.4	-2.9	+ 4.2	+0.3
6.....	301	-16	- 4.4	-4.5	+ 1.0	+0.8
7.....	2	-33	-12.2	-6.9	+14.9	+0.7
8.....	301	-25	- 7.9	-4.1	+10.8	-0.8
9.....	20	-23	- 2.0	-2.0	+ 4.5	+0.0
10.....	206	-20	- 2.3	-1.9	+ 2.7	-0.1
11.....	62	-55	- 5.8	-1.3	+13.3	-5.0
12.....	2	-30	- 6.7	-3.7	+ 7.3	+1.5
13.....	300	-21	-10.5	-4.1	+11.6	-2.7
14.....	300	-18	- 4.7	-4.0	+ 6.9	+0.1
15.....	301	-17	- 4.1	-3.3	+ 5.0	-2.4
16.....	300	-14	- 8.2	-3.2	+10.8	-1.2
20.....	301	+18 to -1.5	- 6.9	-5.0	+ 7.5	-1.0
21.....	300	+25 to -1.1	- 9.4	-3.2	+ 4.1	-0.2
22.....	205(*).....	-4.9	+11.0	+3.5
23.....	209(*).....	-14.0	-7.0	+ 6.3	+6.0
25.....	211(*).....	-13.8	-8.2	+ 5.8	+8.5
26.....	407(*).....	- 3.7	-3.3	+ 4.5	+2.7
28.....	305	-59	- 5.2	-2.0	+ 5.2	-1.2

* Subnormal.

of the experiments show a decline in the saponification value and Reichert-Meissl number of the butter fat, and an increase in the iodine-absorption value. The melting point, however, in some cases was increased, in some cases was not affected, and in other cases declined. Another striking feature of the results is the extreme variations in the chemical fat constants obtained in a great many of the experiments. In fifteen of the twenty-three experiments given in the tables the maximum iodine-absorption value rose to a figure of 40.0 or above and in twenty-one of the twenty-three experiments to a figure of 36.0 or above. The extent of these variations is emphasized when they are compared with the average normal iodine value of 32.7 for all the experiments. Similarly, Reichert-Meissl numbers below 24.0, the legal standard for butter in the United States, were obtained in ten of the twenty-three experiments. Reichert-Meissl numbers below 28.0, the Belgian legal standard for butter, were obtained in twenty of the twenty-three experiments. Again, the extent of these variations is emphasized by comparison with the average normal value of 29.1 for the twenty-three experiments.

Application of the results of underfeeding studies. The marked variations in the chemical and physical constants of the milk fat which are found to accompany a subnormal plane of nutrition, even in cases of mild underfeeding, or when the plane of nutrition is merely reduced from super-normal to normal, at once indicate the importance of taking into account this important factor in both human nutrition and in experimental work involving the influence of specific factors on the composition of milk fat.

It is certain that at times milk comes to the market from herds in such a condition of underfeeding that the fat will have the somewhat abnormal composition noted in the experimental work reported. It is of interest in this connection to raise the question as to the possible relation between the character of the fat and human nutrition, especially of infants. Unfortunately up to the present no information is at hand concerning this possible relation although it seems entirely possible it may be a matter of some importance. The im-

portance of controlling the plane of nutrition of the cow becomes especially apparent in studying the influence of specific feeds on the composition of the milk fat, in which the changes in the ration which are necessary for studies of this character may bring about a temporary underfeeding of the cow. The great care which it has been found necessary to exert in studies of this character at this Experiment Station to avoid involving the effects of underfeeding with the effects of specific feeds throws great doubt on the results of many of the studies of this kind that have been published in the past. Not only is it necessary to control this factor in feeding studies, but it is also necessary to take it into account in the interpretation of the results. The data secured in the underfeeding studies are also useful in explaining some of the heretofore unexplained cases of abnormal butter that are occasionally reported in the agricultural literature.

Application to feeding experiments. The danger of allowing the effects of underfeeding to interfere with the correct interpretation of the effects of specific feeds on the composition of the milk fat is no more strikingly shown than in experiments to determine the effects of fresh pasture grass on the composition and properties of butter fat. Not only is there danger of a cow in good flow of milk being underfed if suddenly turned from dry feed to pasture, but more or less underfeeding is practically certain to follow such a procedure. This is due to the relatively low nutritive value of fresh pasture grass together with the fact that the animal is not accustomed to depend upon her own activity for the feed which she requires.

An example of underfeeding accompanying a sudden change from dry feed to pasture is shown in Table VII. The data were taken at this Station. The animal used was a pure-bred Jersey weighing about 850 pounds. Her energy requirement for maintenance and milk production was about eight therms, which was just supplied by the dry ration. The animal was turned to pasture for the first time on the morning of May 20 and thereafter received no additional feed. The certainty that the animal would be underfed for a few

days at least is apparent when it is considered that she suddenly was required to gather by her own activity about seventy pounds of pasture grass to supply the eight therms of energy which she required. Even this figure may be too low, for it is based on the energy value of green alfalfa, which is in all probability higher than that of the fresh blue grass pasture to which the animal was turned and for which no data are available. The underfeeding results, which were temporary, are seen in the marked drop in the saponification and Reichert-Meissl values of the milk fat, and in the great increase in the iodine value. The fact that underfeeding accompanied a change to pasture in the case of this cow with a low milk production emphasizes the danger as well as the probability of a similar but more pronounced result accompanying the turning to pasture of cows with much greater milk production.

TABLE VII

CHANGING THE RATION FROM DRY FEED TO FRESH PASTURE, SHOWING THE EFFECT OF UNDERFEEDING *

Milk yield	Date	Fat in milk	Saponification value	Reichert-Meissl number	Iodine value	Melting point
Pounds		Per cent				° C.
18.8	May 18-19	4.55	224.0	25.92	37.02	32.83
4.5	May 20 a. m.	4.35	225.7	24.05	39.11	33.40
4.8	May 21 a. m.	4.47	221.1	23.99	42.68	32.10
4.0	May 21 p. m.	5.76	219.5	22.69	45.34	31.40
5.0	May 22 a. m.	4.04	223.2	25.10	38.28	33.37
5.7	May 22 p. m.	5.29	223.6	24.68	41.67	32.67
9.8	May 23	5.00	223.6	25.87	43.19	31.87
19.6	May 24-25	4.92	223.7	27.15	42.15	32.27

* Fed on May 18-19, 4.5 pounds of grain, 4.5 pounds of alfalfa hay, and 18 pounds of silage. The other data are based on pasture only.

Equally striking results were obtained in several other experiments when the ration was grain and dry roughage. In all these cases the striking change in the properties of the fat might have been attributed to the change in ration while the real cause was an insufficient amount of nutrients.

It is clear special care must be taken in studying factors influencing the properties of butter fat that the results are not vitiated by this factor of under-nutrition. A survey of the literature of the subject makes it clear that certain investigators have made this error and the results they attributed in some cases to certain feeds are really the results of under-nutrition.

Application to cases of abnormal butter.—Cases of abnormal butter are not infrequently reported in the agricultural and chemical literature, for which no adequate explanation can be offered. Most of these cases are reported by food officials whose duty it is to detect adulterated butter, and accordingly have to do largely with the Reichert-Meissl number of the butter fat. Such cases are much more frequently reported by officials in European countries than in the United States. It will not be possible to take up all of the cases that have been reported. Several typical ones have been selected, however, and the applications of the results of the underfeeding studies pointed out in explanation of the abnormalities found.

Van der Zande⁸ has reported a feeding experiment in which a decrease in volatile acids occurred while the cows were on pasture. The change in the composition of the butter fat was attributed by the author to a period of severe weather during the experiment. In view of the experiments pointed out above showing the ease with which cows may be underfed while on pasture it seems evident that this factor was responsible for the change in the composition of butter fat found by Van der Zande.

Reinsch⁹ has reported a sample of abnormal butter fat from five Holstein cows, which showed a Reichert-Meissl number of 19.7 and a saponification value of 213.9. The cows were in good health, and had been in milk but a few months. Their ration consisted of pasture grass *ad libitum* with the addition of six

⁸ K. H. M. Van der Zande, *Verlag. Ver. Exploit. Proef. Hoorn*, pp. 45-50, 1902.

⁹ A. Reinsch, *Ein Fall von sogenannter "anormaler" Butter* in *Z. Nahr, Genussm.*, 8, pp. 505-508, 1904.

to eight pounds of potato pulp and three-fourths to one pound of rice polishings a head daily. The explanation of the abnormally low saponification and Reichert-Meissl values unquestionably lies in this fact. The conclusion that would naturally be drawn in the light of the studies of the influence of underfeeding is that the animals were underfed on account of the short pasture.

Ziegfeld¹⁰ reported a sudden drop in the Reichert-Meissl value of the butter fat during a period of feeding cows on beet-tops, which is attributed to a sudden fall in the temperature, causing the leaves to become to some extent frozen. Inasmuch as the cows were fed the beet-tops *ad libitum* with no additional feed, it would hardly be expected that they would consume the required amount to support maintenance and milk flow if they were suddenly required to eat the partly frozen beets. A number of other equally clear cases have been gathered from the literature.

CONCLUSIONS

The percentage of fat in milk can be influenced to a marked extent for the first twenty to thirty days by the fatness of the animal at parturition. This influence appears to extend in some cases in a less degree for at least three months.

Underfeeding of the animal after parturition seems to be a necessary condition to bring about this abnormal percentage of fat in the milk.

Tests of dairy cows made for short intervals in the beginning of the lactation period cannot be depended upon to indicate the normal percentage of fat produced by the cows tested.

Underfeeding of the lactating cow results in a decline in the Reichert-Meissl number and the saponification value and an increase in the iodine value of the milk fat.

The increase in the percentage of milk fat and the changes in the character of the fat which accompany underfeeding

¹⁰ M. Ziegfeld, *Die Zusammensetzung des Butterfettes bei Rübenblattfütterung* in *Z. Nahr. Genussm.*, 17, 179-86, 1909.

are not satisfactorily explained by the hypothesis that underfeeding causes a transfer of tissue fat to the mammary glands. Blood fat analyses made during extreme underfeeding in the case of two cows failed to show any increase in the amount of fat carried by the blood stream in comparison with normal conditions in the same animals.

The effects of underfeeding on the composition and properties of milk and butter fat show the importance of controlling this factor in feeding experiments involving the effects of specific feeds on the composition of milk and butter.

The effects of underfeeding must be taken into account in the interpretation of all data involving variations in the composition of milk and butter fat due to specific conditions of the cow, specific environments of the cow, changes in the feed of the cow, or to feeds of specific character.

CHARACTERISTICS AND DISTRIBUTION OF THE COLON-AEROGENES GROUP *

L. A. ROGERS

INTRODUCTION

The results of a series of investigations carried on in this laboratory on the gas-forming bacteria, commonly known as the colon-aerogenes group, have appeared in various journals.¹ This paper has offered an opportunity to bring these results together and present them in a briefer and more concise form than has been possible in the earlier papers. The results which will be discussed in this paper represent the accumulative work of a number of persons for which the writer has no desire to take undue credit. Particular credit is due my colleague, Dr. Clark, who has been responsible for the greater

* Published by permission of the Secretary of Agriculture.

¹L. A. Rogers, W. M. Clark and B. J. Davis, *The Colon Group of Bacteria in Jour. Inf. Dis.*, 14, 3, pp. 411-475, 1914.

L. A. Rogers, W. M. Clark and A. C. Evans, *The Characteristics of Bacteria of the Colon Type Found in Bovine Feces in Jour. Inf. Dis.*, 15, 1, pp. 99-123, 1914.

Wm. Mansfield Clark, *The Final Hydrogen Ion Concentrations of Cultures of Bacillus Coli in Jour. Biol. Chem.*, 22, 1, pp. 87-98, 1915.

L. A. Rogers, W. M. Clark and A. C. Evans, *The Characteristics of Bacteria of the Colon Type Occurring on Grains in Jour. Inf. Dis.*, 17, 1, pp. 137-159, 1915.

W. M. Clark and H. A. Lubs, *The Differentiation of Bacteria of the Colon-Aerogenes Family by the Use of Indicators in Jour. Inf. Dis.*, 17, 1, pp. 160-173, 1915.

L. A. Rogers, W. M. Clark and H. A. Lubs, *The Characteristics of Bacteria of the Colon-Aerogenes Type Occurring in Human Feces.* (Unpublished.)

S. H. Ayers and P. Rupp, *Simultaneous Acid and Alkaline Bacterial Fermentations from Dextrose and the Salts of Organic Acids Respectively.* (Unpublished.)

L. A. Rogers, *The Occurrence of Different Types of the Colon-Aerogenes Group in Water.* (Unpublished.)

W. M. Clark and H. A. Lubs, *The Colorimetric Determination of Hydrogen Ion Concentration and its Application in Bacteriology in Jour. Bact.*, 2, 1, pp. 1-34; 2, pp. 109-136; 3, pp. 191-236, 1917.

part of the exceptionally fundamental and searching chemical investigations on which the bacteriological work has been based. While intended originally as a study of some of the gas-forming bacteria of milk, it has developed into an investigation of the characteristics of the colon-aerogenes group in general and of the more intimate processes by which some of the familiar end products are produced. In this way it has been possible to bring about some semblance of an orderly arrangement of the large number of cultures studied and to correlate the types so produced with certain definite habitats in nature.

There has been a tendency to arrange bacteria according to the effect that they have on our bodies or on the food we eat. This has certain advantages, but is as illogical from a taxonomic standpoint as a classification of men on the basis of their occupation, an arrangement which would be useful for industrial purposes, but which would separate members of the same family, and bring together distinct ethnological groups. In the same way bacterial classifications of convenience frequently separate closely related bacteria and bring together types agreeing only in superficial characters. On the other hand, there has developed in some quarters a tendency to follow the logical method of classifying bacteria by determining actual relationships and lines of descent. This raises the difficult question of the method of ascertaining how the evolution of a bacterial species or genus may be traced; in other words, which characters will be considered as fundamental and showing broad relationships, and which are superficial and thus of secondary importance. The record of the struggle to secure food and opportunity for reproduction is found especially in morphology in animals and the higher plants and in physiological characters in the bacteria and similar microorganisms.

The colon-aerogenes family is particularly distinguished by its ability to bring about a gaseous fermentation of carbohydrates, alcohols and glycerines, and in attempting to separate this extensive family into species, bacteriologists have usually relied on this fermentative activity as a basis of

classification. The hopelessness of establishing a stable classification by using only the fermentations of various substances is shown by the large number of proposed schemes, some of which permit over one hundred possible varieties. The one which has been most generally followed was proposed by MacConkey and is merely the possible combinations of the plus and minus signs under two test substances. The four species permitted by this arrangement may, by applying similar methods, be further divided into varieties, limited only by the number of test substances used. A classification of this kind may be convenient in that it adapts itself readily to a workable key but there is no evidence to show that it has any relation to the evolution of the group or that it serves in any way to indicate the origin of the culture.

It should be possible to discover some combination of characters which separates the group into natural species. Since the evolution of a bacterial species is largely a question of environment, the species so established should be coincident with more or less well-defined habitats. For taxonomic purposes it may safely be assumed that the nature of the fermentation is of more significance than the particular material fermented.

THE CHARACTERS STUDIED

The characters of a group of bacteria can be properly established only by studying a considerable number of cultures. The value of this method of study has been so completely demonstrated by some of the recent work on systematic bacteriology, particularly by the work of the Winslows on the Coccaceae, that its use should need no further justification. In attacking this problem we have so far as possible used exact chemical methods in determining the reactions produced by the bacteria in various culture media. This has limited the number of cultures which could be studied but has permitted distinctions on the basis of quantitative measurements which have been of the greatest value. The details of the methods followed have been given in the papers cited and for the sake of brevity will not be repeated here. The cul-

tures used consisted of a collection of 689 cultures fermenting dextrose with evolution of gas, without spore formation, with abundant growth on agar and diffused growth in broth. One hundred and fifty-two of these came from 27 samples of human feces, 148 from 17 samples of bovine feces, 132 from 31 samples of water, 143 from 33 samples of grains, and 114 from milk. They were obtained for the most part by direct plating on asparagin lactose litmus agar followed by replating and a test for fermentation in dextrose or lactose broth.

THE FORMATION OF GASES

The active evolution of gases, indicating a more profound fermentation of the carbohydrates, is the striking character which distinguishes the colon-aerogenes group from most of the carbohydrate-fermenting bacteria. The nature and the relative proportion of gases evolved has come to be regarded as of little significance and the fermentation tube of Theobald Smith is now used merely to determine the fact of gas production. The gas ratio, proposed by Smith² as a diagnostic character, has fallen into disrepute through its failure to correlate with habitat or with other physiological characters. This attitude is well illustrated by the paper by Longley and Baton.³ The probable cause of this failure is found in the work of Keyes⁴ who pointed out the great variation in the gas ratio as ordinarily determined and its constancy when determined by accurate methods. Our results have confirmed the conclusions of Keyes in every way and show that the nature and amount of the gaseous by-products formed under uniform conditions are remarkably constant.

The same culture, or even different cultures of the same variety, will repeat the ratio of carbon dioxide to hydrogen with almost mathematical accuracy. This is illustrated by

²Theobald Smith, *The Fermentation Tube with Special Reference to Anaerobiosis and Gas Production among Bacteria in Wilder Quarter-Century Book*, pp. 187-232, 1893.

³F. F. Longley and W. U. C. Baton, *Notes on the Determination of B. Coli in Water in Jour. Inf. Dis.*, 4, 3, pp. 397-416, 1907.

⁴Frederick G. Keyes, *The Gas Production of Bacillus Coli in Jour. Med. Res.*, 21, 1, pp. 69-82, 1909.

the fact that under the conditions used in our work 264 cultures from a great variety of sources gave CO_2/H_2 ratios varying only from 1.0 to 1.1. The amount of gas, as would be expected, was subject to greater variation, but for a certain type of culture under definite conditions the volume of gas could be predicted with considerable accuracy. The kind of gases formed in the anaerobic fermentation of sugars was limited to carbon dioxide and hydrogen with a very small amount of a residual gas which was undoubtedly nitrogen. The origin of this residual gas is not certain but it obviously has no part in the main fermentation.

A total of 615 cultures has been subjected to an exact determination of the gas produced in the anaerobic fermentation of dextrose. The results of these determinations are assembled in Table I and Fig. 1, and include all of the cultures with the exception of a few from water.

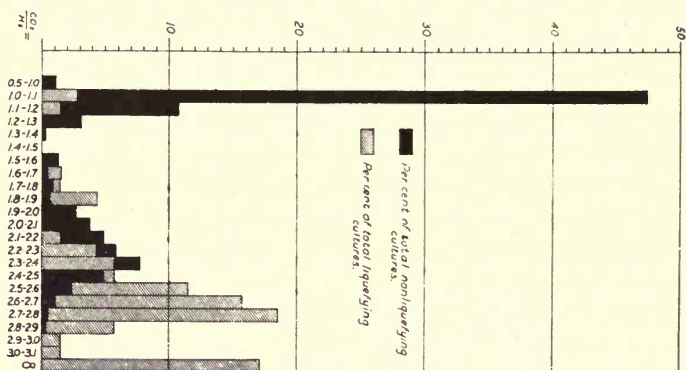


Fig. 1

In making the frequency polygons shown in Fig. 1, the liquefying and non-liquefying cultures have been separated and the percentages calculated for the two groups separately. The proportion of liquefying cultures was so small that it was necessary to make the calculations in this way. While this distinction may seem to ascribe an undue importance to the liquefaction of gelatin it follows the usual custom and is probably justified.

TABLE I
DISTRIBUTION OF CULTURES BY CO₂ : H₂ RATIO

CO ₂ / H ₂	.5-1	1.0-1.1	1.1-1.2	1.2-1.3	1.3-1.4	1.4-1.5	1.5-1.6	1.6-1.7	1.7-1.8	1.8-1.9	1.9-2.0	2.0-2.1	2.1-2.2	2.2-2.3	2.3-2.4	2.4-2.5	2.5-2.6	2.6-2.7	2.7-2.8	2.8-2.9	2.9-3.0	3.0-	Infinity
Total	6 .98	262 41.92	60 9.60	17 2.72	2 .32	7 1.12	4 .64	6 .96	7 1.12	15 2.40	21 3.36	27 4.32	32 5.12	46 7.36	31 4.96	20 3.20	17 2.72	16 2.56	5 .80	1 .16	1 .16	12 1.92
Liq.	2 2.85	1 1.42	1 1.42	1 1.42	3 4.28	1 1.42	3 4.28	4 5.71	4 5.71	8 11.42	11 15.71	13 18.57	4 5.71	1 1.42	1 1.42	12 17.14
Non-Liq.	6 1.09	260 47.35	59 10.74	17 3.09	2 .36	7 1.27	3 54	5 .91	4 72	15 2.73	21 3.82	27 4.91	32 5.82	42 7.65	27 4.91	12 2.13	6 1.09	3 .54	1 .18	0

This figure shows these 615 cultures in three distinct groups based on the relation of the volume of carbon dioxide and hydrogen produced. By far the larger number produced carbon dioxide and hydrogen in almost equal amounts, thus giving a ratio of approximately 1:1. The average was $\text{CO}_2/\text{H}_2=1.06$, with only an occasional culture giving less CO_2 than H_2 and comparatively few varying appreciably on the other side of the mean. It was observed that as the technique of the gas determinations became more refined, the variation in the gas ratio became less, and it is probable that the greater part of the variation shown for this group is in the determinations rather than in the fermentation. The volume of gas produced by this group is relatively small. From 10 c. c. of 1 per cent dextrose broth about 14 c. c. of gas was produced. Of the 350 cultures giving the 1.06 ratio only 3 liquefied gelatin. These will be considered later with the liquefying cultures.

Distinctly separated from this group so far as the gas ratio is concerned is a second type characterized by the production of considerably more CO_2 than H_2 , by a wider range of ratio, and by a greater variation in the gas ratio of individual cultures. Nearly all of the liquefying cultures belong in this group. A study of the tables and figures in some of our previous papers will show that the actual amount of hydrogen produced is nearly constant throughout these two groups and that the increase in the ratio is brought about almost entirely by a larger production of carbon dioxide.

In our first paper⁵ we suggested, as a possible explanation of the remarkable constancy of the *B. coli* ratio of $\text{CO}_2/\text{H}_2=1.06$ and the variation found in the aerogenes ratio, that the equal volumes of carbon dioxide and hydrogen were produced by a fermentation common to both groups while in the aerogenes group there occurs an additional and independent fermentation producing carbon dioxide only.

Clark has shown that while an increase of sugar in the medium has no appreciable effect on the relation of CO_2 to H_2

⁵ *Loc. cit.*

in the gas produced by the low ratio group, a similar change in the composition of the medium materially affected the gas ratio of the high ratio group by increasing the CO_2 . The possibility of distinct fermentations, one producing CO_2 and H_2 in equal parts and one producing CO_2 independently of the hydrogen, is strengthened by the fermentation observed in the third group which gives carbon dioxide only and for which the ratio may, for convenience, be designated by the α sign. Only twelve cultures of this type were found, but the unusual end product of their fermentation entitles them to consideration as a separate type.

The statement by Burton and Rettger⁶ that we believe that the two reactions are mutually dependent does not express the opinion we have held on this subject. On the contrary we have found considerable evidence to lead us to believe that these fermentations were independent. Burton and Rettger have taken a similar view and have elaborated an hypothesis to account for the two fermentations. They assume that in one fermentation nearly equal volumes of carbon dioxide and hydrogen are produced with sufficient acidity to suppress the second fermentation when the former predominates. In the second fermentation butylene glycol is fermented with the formation of considerable quantities of gas and acetyl-methyl-carbinol, the substance giving the Voges-Proskauer reaction. Since the end products of the reaction are not acid in their character, a proteolysis is permitted and sufficient ammonia to account for the alkaline reaction sometimes observed is produced.

CORRELATION OF OTHER CHARACTERS WITH THE GAS RATIO

The "Methyl red test."—It has been shown by Michaelis and Marcora⁷ that the growth of a colon culture ceases when a certain hydrogen ion concentration is reached. This re-

⁶ L. V. Burton and L. F. Rettger, *Correlations in the Colon-Aerogenes Group in Jour. Inf. Dis.*, 21, 2, pp. 162-195, 1917.

⁷ L. Michaelis and F. Marcora, *Die Säuerproduktivität des Bacterium Coli in Ztschr. f. Immunitätsforsch. u. exper. Therap.*, Orig., 14, 2, pp. 170-173, 1912.

lation of hydrogen ion concentration to growth has been worked out in considerable detail for this group by Clark and Lubs⁸ and was made the basis of the "methyl red test." The exact point at which growth stops is dependent in some measure on the buffer effect of the medium and, therefore, is constant only under fixed conditions. If the buffer and fermentable constituents of the medium are properly proportioned, the limiting hydrogen ion concentration is reached and the fermentation stops. If, on the other hand, the sugar content is low and the buffer action high, the sugar may be entirely fermented without raising the hydrogen ion concentration to the point at which fermentation stops. In this case the reaction "reverts" to the alkaline side. This action is very well illustrated by chart I of Clark and Lubs' paper. It is pointed out, in this paper, that this "reversion" cannot be due to the production of ammonia as has been commonly assumed.

Unpublished work of Ayers and Rupp of this laboratory has shown that this phenomenon is brought about by two simultaneous, but independent, fermentations. While the sugar is being fermented, a secondary fermentation is converting the salts of the acids, which are the end products of the first fermentation, to gases, carbonates and other products.

The reaction at any given time is dependent on the relative rates at which these two fermentations are progressing.

The change from an acid reaction toward the alkaline side is not due, as has been assumed, to a neutralization by ammonia from a protein decomposition but to an actual decrease in the acids through a secondary fermentation and in some measure to a neutralization by carbonates which appear as end products of the decomposition of the acid salts.

The work of Harden,⁹ Harden and Walpole,¹⁰ and Thomp-

⁸ *Loc cit.*

⁹ Arthur Harden, *The Chemical Action of Bacillus Coli Communis and Similar Organisms on Carbohydrates and Allied Compounds* in *Jour. Chem. Soc.* (London, 1901), 79, pt. 1, pp. 610-628.

¹⁰ A. Harden and G. S. Walpole, *Chemical Action of Bacillus Lactis Aero-genes (Escherich) on Glucose and Mannitol; Production of 2: 3-Butylene-glycol and Acetylmethylcarbinol* in *Proc. Roy. Soc.* (London, 1906), Ser. B, 77, pp. 399-405.

son¹¹ shows that the acids formed by the accepted types of *B. coli*, and *B. aerogenes* are identical but that the amounts of these acids produced by *B. coli* are less than those produced by *B. aerogenes*. The results obtained by Ayers and Rupp show that the final difference is due primarily to differences in the rates of the various fermentations. In the low ratio type the acid formation proceeds faster than the fermentation of the acid salts and a hydrogen ion concentration which inhibits growth may be reached. In the high ratio group the fermentation of the acids is more rapid and the initial acid reaction may be converted into an alkaline one. With these facts before us it is easier to explain the more complicated and more variable gas evolution of the high ratio group.

By carefully adjusting the amount of sugar and the buffer action, Clark and Lubs¹² devised a medium in which the low ratio cultures reached the limiting hydrogen ion concentration while the high ratio cultures fermented the greater part of the sugar without reaching this point, thus permitting a reversion of the reaction toward the alkaline side. The P_H value of 5.0 - 4.8 (Sorensen's scale), reached very uniformly by the low ratio group, gives a red color with methyl red while the high ratio cultures are uniformly yellow. Nearly all of our cultures have been subjected to this test and it has been found to agree with the gas determination in every case. With only a few cultures was there any question as to the reaction and in these cases it was found that there was some abnormality in the gas ratio which would justify classing these cultures as atypical. Other workers who have used this test have also found it a reliable method of distinguishing between the *B. coli* and *B. aerogenes* type, but some, who have considered it less reliable than the Voges-Proskauer test, have apparently failed to realize that it was designed to in-

¹¹ James Thompson, *The Chemical Action of Bacillus Cloacae (Jordan) on Glucose and Mannitol* in *Proc. Roy. Soc.* (London, 1912), Ser. B, 84, pp. 500-504.

¹² W. M. Clark and H. A. Lubs, *Improved Chemical Methods for Differentiating Bacteria of the Coli-Aerogenes Family* in *Jour. Biol. Chem.*, 30, 2, pp. 209-234, 1917.

dicate the nature of the fermentation which would take place under certain very definite conditions. A variation in the medium, even in the kind of pepton, may change the buffer action so much that the test no longer serves the purpose for which it was designed. For this reason, Clark and Lubs,¹³ in a second paper, have proposed a synthetic medium which will obviate this difficulty.

Properly speaking the methyl red test cannot be considered as a character correlated with the gas ratio. By indicating the group to which the culture belongs, the necessity for determining the gas ratio is obviated. The chemical changes producing the reactions on which the test depends are the changes which produce the distinct gas ratio and the two are thus merely indicators of a fundamental difference in the course of the fermentation. The methyl red test is so designed that if it is properly manipulated it must be correlated with the gas ratio.

The Voges-Proskauer test.—MacConkey and Clemesha considered a positive Voges-Proskauer test as one of the characters which distinguished *B. aerogenes* from *B. coli*. This view was confirmed by Levine,¹⁴ and Johnson and Levine,¹⁵ observed that there was a very high negative correlation between the methyl red test and the Voges-Proskauer test. In our own work we did not use the Voges-Proskauer test at first but after the appearance of Levine's paper, 374 cultures which were then available were subjected to this test. The results, which have been reported by Clark and Lubs,¹⁶ show a perfect correlation between the gas ratio, the methyl red test and the Voges-Proskauer test. In some cases, however, the reaction was so faint and disappeared so quickly that without careful observation it would have been overlooked.

The formation of acetyl-methyl-carbinol is not directly de-

¹³ *Loc. cit.*

¹⁴ Max. Levine, *Preliminary Note on the Classification of Some Lactose Fermenting Bacteria* in *Jour. Bact.*, 1, 6, pp. 619-621, 1916.

¹⁵ B. R. Johnson and Max. Levine, *Characteristics of Coli-like Microorganisms from the Soil* in *Jour. Bact.*, 2, 4, pp. 379-401, 1917.

¹⁶ W. M. Clark and H. A. Lubs, *Improved Chemical Methods for Differentiating Bacteria of the Coli-Aerogenes Family* in *Journal Biol. Chem.*, 30, 2, pp. 209-234, 1917.

pendent on the fermentation which produces the gas and it is, therefore, additional and important evidence of the validity of the distinction between the high and low ratio groups.

Indol.—The formation of indol has always been associated more closely with the fecal or *B. coli* type than with *B. aerogenes*. The correctness of this assumption is indicated by the results shown in Table II and Fig. 2. Over 90 per cent of the low ratio cultures formed indol against 26 per cent for the high ratio cultures. This characteristic is evidently subject to some variation and the percentage of positive results obtained depends in some measure on the technique adopted.

TABLE II
PHYSIOLOGICAL REACTION OF NON-LIQUEFYING CULTURES,
ALL SOURCES

C O ₂ H ₂	Low Ratio		High Ratio	
	Number	Per cent	Number	Per cent
Indol +	372	92.30	72	26.66
..... -	31	7.69	198	73.33
Saccharose +	157	39.05	248	91.85
..... -	245	60.94	22	8.14
Raffinose +	173	43.14	246	90.77
..... -	228	56.85	25	9.22
Starch +	24	6.16	133	50.57
..... -	365	93.83	130	49.42
Inulin +	1	.29	16	7.80
..... -	342	99.70	189	92.19
Mannitol +	397	99.00	171	64.77
..... -	4	.99	93	35.22
Glycerol +	321	93.58	140	68.62
..... -	22	6.41	64	31.37
Salicin +	197	74.06	255	95.86
..... -	69	25.93	11	4.13
Dulcitol +	205	51.25	86	31.73
..... -	195	48.75	185	68.26
Adonitol +	53	15.45	137	49.28
..... -	290	84.54	141	50.71

A perfect correlation with the more basic characters could not be expected but so far as the evidence goes it forms another distinction between the low and high ratio group.

The fermentation of carbohydrates and alcohols.—In considering the ability of these cultures to utilize different sources of carbon some consideration must be given to the difficulty sometimes encountered in determining if a fermentation has actually taken place. With the ordinary culture and most of the sugars and alcohols there is no difficulty in determining fermentation either by the gas formation, the change in titratable acidity, or in hydrogen ion concentration. With some cultures the reaction is obscured by a weak evolution of gas or a rapid reversion of the reaction.

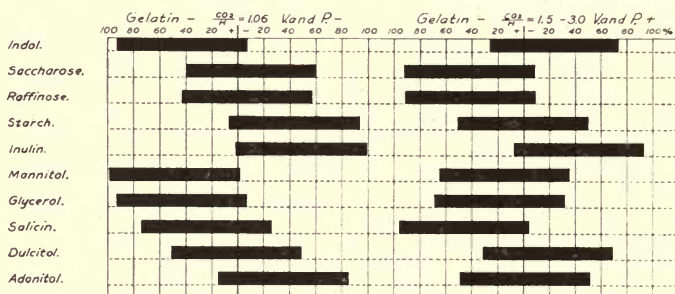


Fig. 2

In most of the sugars the change in reaction is rapid and distinct but in some of the alcohols, notably glycerol, the fermentation is so slow that it may be in doubt for many days. We have considered an evolution of gas in the absence of any apparent change in acidity or an appreciable change in acidity without evident gas formation as fermentations. Figure 2 shows that while there is no perfect correlation, there is a distinct difference in the fermentative ability of the two groups. The cultures of the high ratio group almost without exception ferment saccharose and raffinose while only about 40 per cent of the low ratio group utilize these sugars. Starch is also utilized much more commonly by the high ratio cultures. On the other hand, more of the low ratio cultures ferment the alcohols mannitol, glycerol and dulcitol than is the case with the high ratio group. This does not hold for adonitol which seems to be more available for the high ratio type. The percentage of cultures fermenting the glucoside

salicin is slightly higher for the high ratio group. Only a very few cultures of either group are able to utilize inulin.

These results agree in a general way with those reported by Burton and Rettger¹⁷ and Johnson and Levine,¹⁸ except that the latter found a higher percentage of glycerol fermenters among the Voges-Proskauer plus cultures. They give no data for mannitol. In summarizing these observations we may say that the greater fermentative ability of the high ratio group is shown in the volume of the gas formed, the extent of the decomposition usually effected, and in the range of material available for fermentation. While the correlations are not perfect, they indicate an additional real difference between the two groups.

Subdivision of the low ratio or *B. coli* group.—It has been customary to divide *B. coli* into two varieties designated as *communis* and *communior* on the basis of the fermentation of saccharose. This classification has been partially followed by Kligler,¹⁹ but Levine²⁰ has made of this V and P- group six species, including one which is still further subdivided into two varieties. A major division is made on the ability to ferment saccharose, and the specific and varietal distinctions on motility and the fermentation of salicin correlated with dulcitol and glycerol. Burton and Rettger²¹ suggest three species of which one liquefies gelatin and two, designated as *communior* and *communis*, are distinguished by the saccharose fermentation.

The data on which Levine's separation is based are not given and, as we did not include motility in our observations, we are unable to apply his classification to our cultures. Without admitting the validity of basing specific differences on the fermentation of a single sugar we have separated the low ratio cultures in the usual way as shown in Table III and Fig. 3. The low ratio liquefiers, which probably agreed with

¹⁷ *Loc. cit.*

¹⁸ *Loc. cit.*

¹⁹ I. J. Kligler, *Studies on the Classification of the Colon Group in Jour. Inf. Dis.*, 15, 1, pp. 187-204, 1914.

²⁰ *Loc. cit.*

²¹ *Loc. cit.*

Burton and Rettger's group IV included only three cultures and are omitted. There is no difference in the proportion of cultures forming indol in the two groups. Raffinose is fermented by nearly all of the cultures fermenting saccharose and is fermented by only a very few of the cultures which do not ferment saccharose, but considering the chemical relation of these two sugars, this high correlation becomes of minor significance. The percentage of cultures fermenting glycerol and salicin is slightly higher in the saccharose group but the difference is so slight that it has no significance. On the whole, these data furnish no sound basis for separating the low ratio group into species.

TABLE III

CHARACTERISTICS OF LOW RATIO, GELATIN NEGATIVE CULTURES

	Saccharose (positive)		Saccharose (negative)	
	Number	Per cent	Number	Per cent
Indol	145	91.19	197	90.78
	14	8.80	20	9.21
Raffinose	153	96.22	29	13.30
	6	3.77	189	86.69
Starch	18	12.08	20	9.21
	131	87.91	197	90.78
Inulin	1	0.75	0	0.00
	131	99.25	186	100.00
Mannitol	158	99.37	214	98.16
	1	0.62	4	1.83
Glycerol	120	90.90	176	94.62
	12	9.09	10	5.37
Salicin	62	80.51	109	66.87
	15	19.48	54	33.12
Dulcitol	118	74.21	91	42.12
	41	25.78	125	57.87
Adonitol	8	6.10	49	26.48
	123	93.89	136	73.51

The idea of making species or even varieties on a single fermentation seems to us a very questionable one. A fermented sugar is merely a source of carbon and, while the fermentation may be a constant reaction, it does not represent

a fundamental difference, especially if it is not correlated with other reactions. So far as data are available, the low ratio or *B. coli* group appears to be a very definite and circumscribed entity and there is no apparent reason for separating it into species. Varieties on the basis of saccharose fermentation might be recognized but the need for these is not very evident.

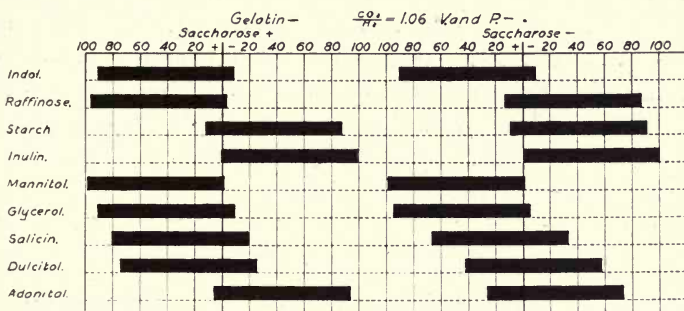


Fig. 3

Subdivisions of the high ratio group.—The high ratio or aerogenes type shows more evidence of being a heterogeneous group capable of subdivision into species than the group just considered. Johnson and Levine,²² following the earlier suggestions of Levine,²³ have two species, in one of which all cultures are motile and nearly all liquefy gelatin while in the other all are immotile and nearly all fail to liquefy gelatin. They have also a V and P— group which otherwise resembles the aerogenes type and which they consider as intermediate between coli and aerogenes. Burton and Rettger²⁴ have three groups, one of which is a liquefying spore former, one a non-spore forming gelatin liquefier of the cloacae type and one a non-liquefier of the aerogenes type. In our collection from grains we found evidences of four high ratio groups including one of 40 liquefying cultures and one of 90 non-liquefiers with

²² *Loc. cit.*²³ *Loc. cit.*²⁴ *Loc. cit.*

nearly identical characters. The other two included only a few cultures and were of doubtful validity.

The value of divisions based on the liquefaction of gelatin may be very properly questioned. It is well known that the ability to excrete a proteolytic enzyme may be lost and Burton and Rettger think that there may be an error in determinations which do not permit an incubation period longer than 20 days. If the fact of gelatin liquefaction can be established, there is no doubt that it indicates a distinct evolution in methods of obtaining food supply and consequently has taxonomic significance. The indications are that the liquefiers of our high ratio group have other characters in common which separate them from the non-liquefiers and we have accordingly considered them separately.

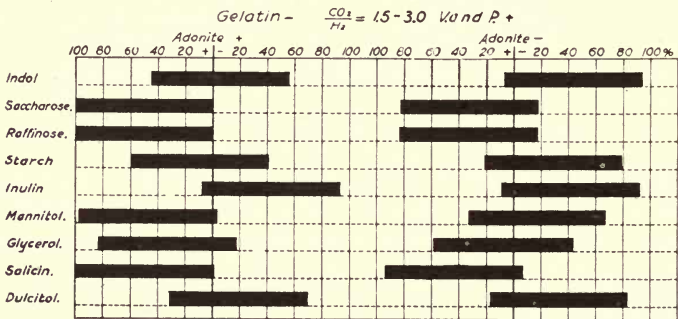


Fig. 4

While there is a considerable range in the gas ratio of the high ratio group, the variation under different conditions and between cultures otherwise identical is so great that this character cannot be safely used for further subdivision. Many groupings on the basis of substances fermented are possible. The correlation of adonitol fermentation with a definite source has suggested the use of this character as a possible indicator of a line of demarcation.

The differences in the two groups formed in this way are shown by Fig. 4, which is based on data given in Table IV. The adonite + cultures are more active in every way than the adonite - cultures, and are especially so in fermenting

starch and mannitol. It is entirely possible that this difference is due to the fact that nearly all of the adonite + cultures were freshly isolated from feces while the adonite — cultures came for the most part from dried grains. To account for these differences in this way, it is necessary to assume that the fermentative abilities of aerogenes become attenuated by exposure to unfavorable conditions. While there is some circumstantial evidence to support this view, it is by no means established. On the evidence of the fermentative reactions these varieties have only a little more substantial basis than the *communis* and *communior* of the *B. coli* group, but, as will be pointed out later, they may serve a useful purpose in indicating the probable source of the organism.

TABLE IV

CORRELATION OF ADONITOL FERMENTATION WITH OTHER PHYSIOLOGICAL CHARACTERS

	Adonitol (positive)		Adonitol (negative)	
	Number	Per cent	Number	Per cent
Indol +	61	44.20	9	7.20
..... —	77	55.79	116	92.80
Saccharose +	139	100.00	104	82.53
..... —	0	0.00	22	17.46
Raffinose +	139	100.00	105	83.33
..... —	0	0.00	21	16.66
Starch +	78	59.09	26	21.13
..... —	54	40.89	97	78.86
Inulin +	7	7.52	9	8.41
..... —	86	92.47	98	91.58
Mannitol +	132	97.05	41	32.80
..... —	4	2.94	84	67.21
Glycerol +	76	82.60	62	58.48
..... —	16	17.39	44	41.50
Salicin +	131	99.24	117	93.60
..... —	1	.75	8	6.40
Dulcitol +	42	30.43	22	17.59
..... —	96	69.56	103	82.40

THE LIQUEFYING CULTURES

Figure 1 shows that while there are liquefying cultures in all of the gas ratio groups, a large percentage of them form a mode which is quite distinct from that of the non-liquefying high ratio cultures. In other words, the cultures with a very high ratio are nearly all liquefiers. On account of the method of calculating percentages, the relative numbers of liquefying cultures are exaggerated. The ratio of approximately 1.06 was given by only three liquefying cultures, a number so small that they have not been considered at this time.

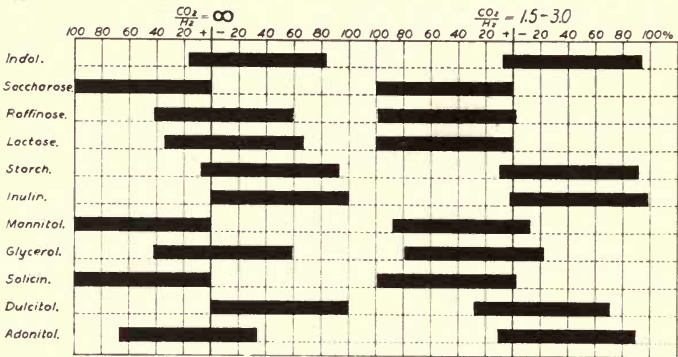


Fig. 5

An additional mode over the infinity ratio marks a physiologically distinct group. A comparison of Fig. 5 with Fig. 2 shows that in fermentation reactions, the high ratio liquefying cultures do not differ in a very marked degree from the high ratio non-liquefiers. Indol, saccharose, raffinose, inulin, glycerol, salicin and dulcitol are fermented in about the same way by the two groups. Starch is fermented more actively by the non-liquefiers, and mannitol by the liquefiers. If, however, we accept the arrangement shown in Fig. 4 we find that the liquefiers do not agree with either the adonitol + or adonitol - types. The liquefiers differ decidedly from the adonitol + type in indol formation, and in the fermentation of adonitol and starch, and from the adonitol- type in the fermenta-

tion of mannitol. The lactose fermenting E. coli have always been grouped together as *B. cloacae*, and we are inclined to favor the retention of this arrangement until more conclusive evidence can be produced to support a revision.

Although we have only a small number of cultures which produce only CO₂ in the anaerobic fermentation of dextrose, this character indicates such a fundamental physiological departure from the type that we have had no hesitation in putting them in a class by themselves.

Active liquefaction of gelatin and the fact that 8 of the 12 cultures included in this group failed to ferment lactose suggests the identity of this type with the proteus group. The three striking characteristics of the proteus group are the failure to ferment lactose, the liquefaction of gelatin and the formation of characteristic swarming colonies in gelatin. Two-thirds of our cultures agree with the first character and all with the second. The typical colony of proteus on gelatin, according to the original description by Hauser,²⁵ has secondary colonies which appear in the medium around the original colony. Evidently many cultures are classed as proteus which are not known to form this peculiar type of colony. Herter and Ten Broeck²⁶ mention one culture which did not form swarming colonies until it had been passed rapidly through a series of milk tubes and then plated on dextrose gelatin.

We studied the colony fermentation of twenty-five liquefying cultures on 5 per cent gelatin at 20°C. There was a great variation in the type of colony, and even in different colonies by the same culture on a single plate. Only a few of these twenty-five cultures formed what could be described as typical proteus colonies. In most cases the colony was round, with a smooth margin and without out-growths of any kind. Liquefaction appeared slowly, and the margin of the colony would be unliquefied. There were many exceptions to this, some of which are shown in Fig. 6. At *a* is shown a single strand

²⁵ Gustav. Hauser, *Über Fäulnisbakterien u. deren Beziehungen zur Sepsitæmie*, Leipzig, 1885.

²⁶ C. A. Herter and C. Ten Broeck, *A Biochemical Study of Proteus Vulgaris* Hauser in *Jour. Biol. Chem.*, 9, 6, pp. 491-511, 1911.

more highly magnified than the other figures in the cut. The plate became filled with secondary colonies of this kind connected by almost microscopic filaments. At *d* is another type in which the colony is almost entirely filament with an occasional bud-like colony. The filament formation is frequently limited to fringe-like outgrowths on the margin, as

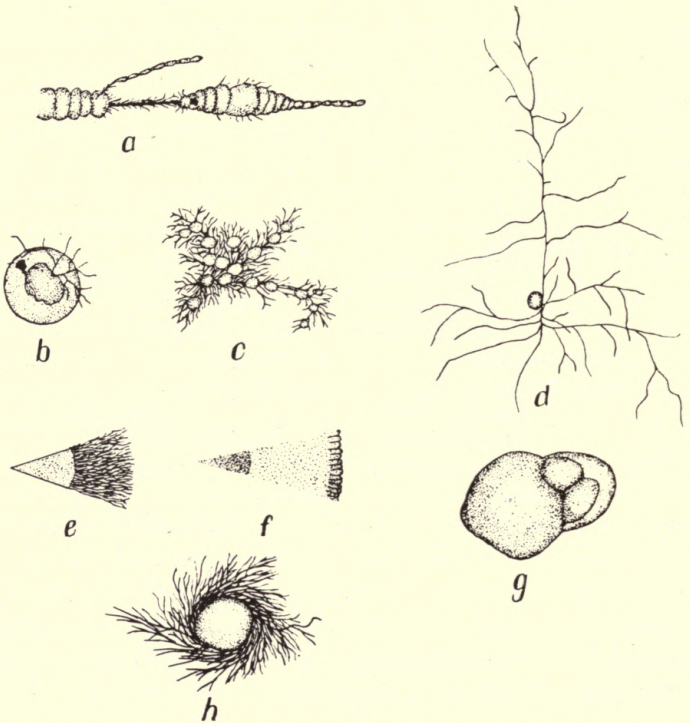


Fig. 6

shown by the sections of liquefying colonies at *e* and *f*. Sometimes on a plate filled with smooth, round, solid colonies, an occasional colony like *b* or *g* may develop. The type of colony from an individual culture may be variable and, on a single plate, may be found solid smooth colonies, large colonies liquefying in the center, and almost any one of the colonies shown in Fig. 6. Sub-cultures have been made from the different types of colonies of a single culture, but these sub-

cultures were identical in all their reactions. So far as the evidence obtained from these twenty-five cultures goes, there is no relation between the type of colony and the fermentation of lactose or the gas ratio. It seems that the tendency to form outgrowths is an expression of an attempt to push out into the medium where conditions are more favorable. Some strains have developed this tendency to a greater extent than others, but it is very evident that it could not be placed on a par with physiological characters for taxonomic purposes.

The infinity group liquefies gelatin more actively than the cloacae group, but its fermentative ability, as shown in Fig. 5 is less. A comparison based on only twelve cultures must be tentative, but some of the differences are of special interest. This is the only group in which the correlation between saccharose and raffinose is not nearly perfect.

The amount of gas formed from dextrose by the infinity group is usually much less even than that obtained from the low ratio cultures. Traces of gas and a slight change in the hydrogen ion concentration sometimes observed in lactose broth indicate that there may be a feeble fermentation of this sugar by those cultures classed as lactose negative.

The general characters of the cultures of the infinity group identify them with the proteus type. While a few of them differ from the usual conception of proteus by fermenting lactose, it seems to us that the nature of the fermentation as indicated by the end products is so much more basic than the nature of the material fermented that the separation into species should be made on the gas ratio rather than on the failure to ferment lactose. A number of cultures sent to us from other laboratories as proteus failed to ferment lactose but gave the carbon dioxide-hydrogen ratio of the aerogenes group. It has been customary to include in the colon-aerogenes group only cultures that ferment lactose. This is largely a matter of convenience and there is no good reason why dextrose + lactose — cultures should not be included.

THE DISTRIBUTION OF THE COLON-AEROGENES GROUP

When physiological or morphological characters are correlated with a definite habitat, their taxonomic significance is greatly increased. The restriction of species of the higher animals to a more or less definite habitat is too well known to need discussion. Among the plants this relation is even more marked and the fungi are sometimes limited in their habitat to a single species of host plants. The source of the various tentative groups into which we have divided our 689 cultures should be in a way a test of the validity of this grouping. The distribution of these groups according to their origin is shown in Table V and Figure 7.

TABLE V
SHOWING THE DISTRIBUTION OF CULTURES ACCORDING TO ORIGIN

Type	Human Feces		Bovine Feces		Water		Milk		Grains	
	No.	Per cent	No.	Per cent	No.	Per cent	No.	Per cent	No.	Per cent
$\frac{\text{CO}_2}{\text{H}_2}$ 1.06 Sacch. +	23	14.45	86	54.08	27	16.98	21	13.20	2	1.25
$\frac{\text{CO}_2}{\text{H}_2}$ 1.06 Sacch. -	83	38.07	61	27.98	32	14.67	37	16.97	5	2.26
$\frac{\text{CO}_2}{\text{H}_2}$ 1.5-3.0 Adonite +	46	33.09	1	.71	46	33.09	32	23.02	14	10.07
$\frac{\text{CO}_2}{\text{CO}_2}$ 1.5-3.0 Adonite -	0	0	14	13.59	12	11.65	77	74.75
Gel. liquef. $\frac{\text{CO}_2}{\text{H}_2}$ = ... ∞	0	0	4	33.33	1	8.33	7	58.33
Gel. liquef. $\frac{\text{CO}_2}{\text{H}_2}$ 1.5-3.0	0	0	9	15.51	11	18.96	38	65.51

This collection cannot be taken as accurately representing the relative distribution, because in some cases the isolation was selective, as, for instance, when a special attempt was made to get high ratio cultures from feces. No attempt was made to isolate liquefiers and the results may be misleading in that

they show no liquefiers in feces where they no doubt occur in small numbers. Water and milk can hardly be considered as the habitat of bacteria of this kind. They occur and multiply there, but these fluids must be looked upon as carriers into which the bacteria have been introduced from some other source. This statement will probably hold also for grains which may be merely carrying soil or fecal organisms mechanically.

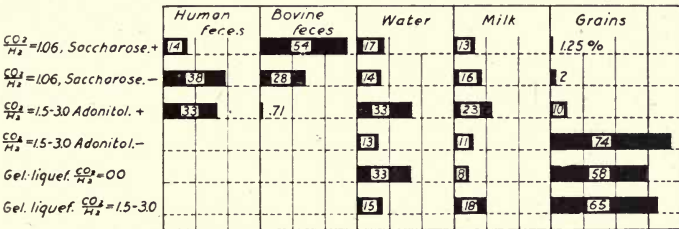


Fig. 7

The most striking feature brought out by Fig. 7 is the very great preponderance of the low ratio groups in feces, and especially in bovine feces from which, in spite of the fact that a special effort was made to isolate high ratio cultures, only a single one was obtained in 150 cultures isolated. They were more frequent in the human feces, although *B. coli* was in great preponderance there also. There was great variation in individuals and it is difficult to say what the proportion of *B. coli* to *B. aerogenes* would be, but it would probably vary from 10 to 1 to 100 to 1.

This seems to agree with the earlier work which touches on this point. MacConkey,²⁷ in 241 cultures from human feces, found none that were V and P+. Ferreira, Horta and Parades²⁸ found 8 V and P+ cultures in 117 from human feces. Clemesha²⁹ says that aerogenes (V and P+) cultures occur very rarely in feces. This all confirms our conclusions

²⁷ Alfred MacConkey, *Lactose-fermenting Bacteria in Feces* in *Jour. Hyg.*, 5, 3, pp. 333-379, 1905.

²⁸ A. Ferreira, A. Horta and C. Parades, *Recherches sur le B. coli communis de l'intestin de l'homme* in *Archiv. du Real Inst. Bact. Camara Pestana*, 2, fac. 2, pp. 153-197, 1908.

²⁹ W. W. Clemesha, *The Bacteriology of Surface Waters in the Tropics*, London, 1912.

that the predominant organism of the intestinal tract is the low ratio or *B. coli* type.

Figure 7 indicates that the saccharose fermenting type is more common in bovine than in human feces. It is not safe, however, to put too much weight on this deduction. If we were to assume that the failure to ferment saccharose indicated human origin, we would be obliged to conclude that a majority of the *B. coli* cultures from milk came from human feces. Unequal rates of multiplication by the different types may completely rearrange the original relations.

The origin of the cultures of the two groups into which non-liquefying high ratio cultures have been divided is perhaps of some significance. Of the 139 adonitol+ cultures, 33 per cent were from human feces, 33 per cent from water, 23 per cent from milk and only 10 per cent from grains. On the other hand, 74 per cent of the adonitol— cultures were from grains and none at all from feces. In other words, all of the high ratio non-liquefying cultures from feces fermented adonitol while nearly all of those from grains failed to ferment this alcohol. If we assume that the 14 adonitol + cultures found on grains came originally from feces, we have a sharp distinction between the fecal and non-fecal types. In water and milk the fecal type predominates. It is not surprising that this should be so since the greater part of the colon-aerogenes cultures isolated from waters would naturally come from samples more or less infected with fecal matter. It may seem peculiar that so large a percentage of the fecal type occurred in milk while they were very rare in bovine feces, but this anomaly may perhaps be accounted for by the difference in rates of growth of different types. Mr. Ayers has observed that while high ratio cultures will not be found in bovine feces by the usual methods of plating, milk infected with this material and incubated at a low temperature for twenty-four hours may contain large numbers of this type.

It will, no doubt, be suggested that the adonitol— cultures are merely fecal cultures which have become attenuated through existence under the unfavorable conditions found in the soil, on the surface of grains, or in water. We have no

evidence supporting or controverting this theory. If the loss of ability to ferment adonitol is effected only after many generations under the new conditions, it is only another way of saying that a variety has been created by a change of environment. If the change takes place in the original cells, it may be considered as an indication of the remoteness of the contamination.

While none of the liquefying cultures in this collection were isolated from feces there is no doubt that they occur there under certain circumstances. Ford³⁰ reports the occurrence of *B. cloacae* in feces. Clemesha³¹ says that *B. cloacae* may be present in human feces in numbers as high as 15 or 20 per cent of the total. MacConkey³² found occasional gas forming liquefiers in feces. On the other hand, nearly one half of the coli-like organisms isolated from soil by Johnson and Levine³³ were liquefiers. Unless we assume that the liquefiers of this group are more resistant to unfavorable conditions than the other types and therefore persist longer in water and in the soil it is evident that the principal source of *B. cloacae* and *B. proteus* is outside of the intestinal tract.

CONCLUSIONS

It may be considered as established by the results reported here, supported as they are by the observations of many other investigators, that *B. coli* (low ratio) and *B. aerogenes* (high ratio) are very distinct types. This is based on: (1) a fundamental difference in the course of the fermentation. Carbohydrates are fermented more readily by *B. aerogenes* and *B. cloacae* with a secondary rapid fermentation of the by-product, resulting in a greater production of CO₂ and a decrease in acidity. The fermentation brought about by

³⁰ W. W. Ford, *Classification of Intestinal Bacteria in Jour. Med. Res.*, 6, 1, pp. 211-219, 1901.

³¹ W. W. Clemesha, *The Bacteriology of Surface Waters in the Tropics*, London, 1912.

³² Alfred MacConkey, *Further Observations on the Differentiation of Lactose-fermenting Bacilli with Special Reference to those of Intestinal Origin in Jour. Hyg.*, 9, 1, pp. 86-103, 1909.

³³ *Loc. cit.*

B. coli is of a similar nature, but the primary and secondary fermentations proceed at different rates and the end products are different from those produced by *B. aerogenes*. (2) With this are correlated various other characters, particularly a higher percentage of indol formers in *B. coli* and the formation of acetyl-methyl-carbinol by *B. aerogenes*. (3) There is also correlated with these physiological differences a difference in habitat. *B. coli* is the characteristic gas-forming organism of animal feces and is commonly found elsewhere only in localities recently contaminated with fecal matter. *B. aerogenes* and *B. cloacae*, on the other hand, occur rarely in bovine feces and in relatively small numbers in human feces, but are common in soil and materials contaminated with soil.

B. coli is probably of specific rank and the subdivisions usually made on the basis of saccharose fermentation should not be looked upon as more than varieties of doubtful validity. It is possible to make two similar varieties of *B. aerogenes* on the basis of the fermentation of adonitol. This character is given an added significance by a high correlation with habitat. All of the aerogenes cultures from feces fermented adonitol while a large percentage of those from grains were adonitol negative.

The distinction between *B. aerogenes* and *B. cloacae*, beyond the liquefaction of gelatin, is not very sharp, but everything considered *B. cloacae* should be a separate species.

A small group of cultures distinguished by the liquefaction of gelatin, the fermentation of dextrose with the formation of CO₂ only, and the fermentation of saccharose but usual failure to ferment lactose is evidently identical with *B. proteus*. The failure to ferment lactose, usually considered the distinguishing character of *B. proteus*, does not coincide perfectly with the more fundamental character indicated by the single gaseous end product of the fermentation. The unusual colony formation commonly looked upon as peculiar to proteus is probably shared with some *B. cloacae* cultures. A more logical separation between *B. proteus* and *B. cloacae* would be on the end products of the fermentation rather than on the nature of the material fermented.

B. cloacae may be considered as the stem from which the other members of the group have sprung. By its vigorous habits of growth, the wide range of substances from which it is able to obtain its supply of carbon and its ability to excrete proteolytic enzymes, it is able to thrive under the saprophytic conditions in which it is found. *B. aerogenes* has lost the proteolytic ability and acquired semi-parasitic habits. *B. coli* is still more parasitic in its habits, is more restricted in its range of carbon supply, and is further removed from *B. cloacae* and *B. aerogenes* by the loss of the carbinol reaction and by the development of the ability to form indol. Still more parasitic in their habits and correspondingly more removed from *B. cloacae* by their physiological characters are *B. enteritides*, *B. typhosus* and *B. dysenteriae*. Removed from *B. cloacae* in the opposite direction is *B. proteus* which has developed the proteolytic ability but lost in the utilization of carbohydrates both in the range of materials fermented and in the extent of the fermentation.

THE IDENTITY OF AMERICAN AND FRENCH SPOROTRICHOSIS*

DAVID JOHN DAVIS

INTRODUCTION

The reason for presenting this subject at this time will no doubt be deemed adequate by those who have followed the literature on sporotrichosis during the past several years. This disease is known to be relatively common in France, the number of cases observed now running into the hundreds. In America the disease is being commonly reported in both man and horses; the number of human cases now closely approximates a hundred, and several extensive outbreaks in horses in different localities have been observed.

The disease is known under the name of Sporotrichosis in both countries. In France and generally on the continent, also in certain other parts of the world, the cause is given as the *Sporotrichum beurmanni*. In the United States the causal organism is generally but not uniformly recognized as *Sporothrix schenckii*. Certain writers here, now and then, refer to the organism from American cases as *Sporotrichum beurmanni* or as *Sporotrichum schenckii-beurmanni*. The impression is general on the continent and especially in France that the American and French organisms are distinct and that we have to do with two different though closely related diseases. It is my purpose in this paper to analyse the existing data and to present certain new data bearing upon this matter of the identity or non-identity of these two infections.

This discussion does not concern other distinct varieties of Sporotricha either pathogenic or non-pathogenic. The existence of these is recognized. Many saprophytic sporotricha

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grow in the soil, in water, and under other conditions; certain varieties are pathogenic for plants.

Infection with varieties pathogenic for man, excepting the two here under consideration, are apparently very rare. The following are mentioned in this connection.

Sporotrichum dori, an organism isolated from a human case and very imperfectly described by Dor in 1906, was evidently a different organism morphologically, culturally, and in its pathogenicity for animals. The culture has been lost and no similar organism has since been found.

Sporotrichum indicum, an organism described by Castellani in 1908, was isolated from two cases of sporotrichosis in Ceylon. It is impossible now to compare them with other varieties since these cultures have also been lost. From the original description given by Castellani it is clear that the organisms are very similar to, if not identical with, the French and American varieties. He says that "it closely resembles *Sporotrichum beurmanni*; the mycelial threads are somewhat larger, between 2 and 3 microns wide; spores roundish (3 to 5 microns in diameter) or oval (4 to 5 microns long and 3 to 4 microns in breadth). Colonies on maltose agar may be of various colors,—greyish, light brownish, dark brownish, black".¹ There are no differential characteristics here that are important and I am inclined to believe these strains are identical with the American variety of Schenck. De Beurmann and Gougerot provisionally classify it as *Sporotrichum beurmanni* var. *indicum*.

Sporotrichum gougeroti is an organism isolated from a case in France by Gougerot who thought it different from the Beurmann type in several respects, chiefly in macroscopic growth and pigment production. De Beurmann and Gougerot observed one case only.

The *Sporotrichum jeanselmei* was isolated by Jeanselme and Chevallier in 1910 from a human case and a second case appeared as an experimental infection accidentally obtained in the laboratory from the culture of the first case. According to de Beurmann and Gougerot this organism is very similar

¹ Castellani & Chalmers, *Manual of Tropical Medicine*, London, 1910.

to *Sporotrichum beurmanni*, indeed identical with certain pleomorphic forms of the latter. They make the significant statement in this connection: "Plusieurs de ses pléomorphismes s'identifient au *Sporotrichum Schenki* et au *Sporotrichum Beurmanni*, voire même au *Sporotrichum Gougeroti*. Cette unification dans les pléomorphismes est la meilleure preuve d'une commune origine de ces germes." Their position seems to be that this organism, the American organism *Sporotrichum schenckii*, and the *Sporotrichum beurmanni* were originally one and the same organism, and certain strains later developed pleomorphic characteristics leading to new varieties.

A new pathogenic sporotrichum, *Sporotrichum councilmani*, described very recently by Wolbach, Sisson and Meier² of Boston was found in a case of acute arthritis of the knee following injury. From their description it appears quite different from all other strains of Sporotricha. They summarize the distinguishing features as follows: "(1) its pleomorphic growth, characterized by a free aerial growth of hyphae; (2) the abundant spore formation, large size of the spores and absence of lateral spore clusters, and (3) the occurrence in lesions as septate, branching filaments." The last character is especially significant. The clinical history and the character of the lesion in the patient are also of interest and are possibly of importance for differential purposes.

For the purpose of this paper I think we may eliminate *Sporotrichum dori* as being quite different from the other sporotricha; also *Sporotrichum councilmani*. The very rare cases of so called *Sporotrichum indicum*, *Sporotrichum jeanselmei* and *Sporotrichum gougeroti* are much more closely related to *Sporotrichum beurmanni*, the first two indeed being probably identical with it. The existence of these very rare varieties is here recognized but this fact is only indirectly related to the main question at issue here, namely, the possible identity of the organisms causing sporotrichosis as commonly observed in America and in France.

In the further analysis of this question it will be desirable

² *Journal of Medical Research*, 36, p. 337, 1917.

to discuss briefly the history of sporotrichosis in America and France and also in the other countries where it has been observed.

SPOROTRICHOSIS IN NORTH AMERICA

The recorded history of this disease in America is brief and simple. In 1898 Schenck³ reported a case of chronic subcutaneous abscesses from which he isolated in pure culture a fungus which grew readily on artificial media and which was identified by Dr. Erwin F. Smith of the U. S. Dept. of Agriculture as belonging to the genus *Sporotricha*. The organism was found to be distinctly pathogenic for mice and dogs and from the characteristic lesions the same fungus was recovered pure. Thus, in the first case observed, all of Koch's laws were fulfilled. Illustrations of the human lesions, cultures, and microscopic appearance of the fungus accompany the paper of Schenck.

In 1900 Hektoen and Perkins⁴ observed and very carefully described a second case in which the fungus was isolated in pure culture and its pathogenicity for various animals determined. They were able to confirm the results of Schenck and after a careful comparison of the fungi from both cases concluded that they were identical. Schenck also examined their strain and pronounced it identical with his organism. They definitely named this organism *Sporothrix schenckii* at this time. Therefore it is to be noted that in two of the most prominent medical publications of the time, an accurate clinical, pathological, bacteriological, and experimental description of this disease appeared.

A case reported in 1899 by Brayton⁵ agreed clinically with the case of Schenck and of Hektoen. The organism was not detected however and cultures were not made. Definite statements as to the nature of the infection cannot be made though it was probably a case of sporotrichosis.

³ Johns Hopkins Hospital Bulletin, 9, p. 286, 1898.

⁴ Jour. of Exp. Med., 5, p. 77, 1900.

⁵ Indianapolis Med. Jour., 18, p. 272, 1899.

The next report from America was that of Duque⁶ in Cuba in 1908.⁷ Duque reported three cases that clinically were disseminated, *gummatous sporotrichosis*. They were treated surgically and in two the amputation of an extremity was resorted to without result. Under treatment of iodide of potassium they all responded promptly and made complete recoveries in periods of from one to two months. Details concerning the diagnosis are not given. Duque states that the diagnosis was made by a careful examination of the pus but does not say whether or not cultures were made. From the clinical and therapeutic data we should judge that these cases were sporotrichosis, but the lack of more complete pathological data prevents further analysis.

Burlew⁸ in 1909 observed a case of sporotrichosis of the disseminated gummatous type in a farm laborer in Santa Anna, California. Both legs and the face were involved. The organism was cultured and identified as the *Sporothrix schenckii*.

From this year (1909) to the present time the number of cases recognized and reported has increased rapidly so that

⁶ *American Jour. Dermat. and Gen. Urin. Dis.*, 12, p. 240, 1908.

⁷ The period of eight years lapsing between the reports of Hektoen and Perkins and those of Duque, Burlew, Hyde and Davis, etc., in which no cases appeared has been commented upon by de Beurmann and Gougerot. *Bull. et Mem. de la Soc. Med. des Hospit. de Paris*, 35, p. 798, 1910. They infer that sporotrichosis in North America had practically been forgotten and that only after attention had been called to this disease through the later work of the French did Americans begin again to recognize this disease. There is little truth in this statement. Naturally the large amount of work that was being done by the French between 1906 and 1908 did attract attention in America. However, it is surely not correct to state that the disease had been forgotten here when men like Hektoen, Welch, Smith and others, who had recognized or seen the disease and the fungus, continued to engage in active work in pathology. The real reason no doubt was the fact that the disease in the human is restricted as we shall presently see, almost entirely to the valley of the Missouri River. This locality in the central and western portion of the country, especially at that time, was not developed medically and naturally only the cases which drifted out of the region to medical centers would be apt to be detected. Such apparently was true of the cases of Schenck and of Hektoen and Perkins. In later years when men like Sutton and others worked in that locality cases were recognized in much larger numbers.

⁸ *South. Calif. Pract.*, 24, p. 1, 1909.

now they can no longer be considered rare. Ruediger⁹ gathered together and analysed all the cases in the United States in 1912. He found 57 in which the diagnosis had been made with reasonable certainty. Since then the literature each year has furnished a considerable number of additional reports. Many cases have no doubt not been recorded in the literature. The writer is aware of several in which cultures were obtained and identified as *Sporothrix schenckii* but never reported.

Ruediger called attention to the interesting fact that the disease occurred chiefly in the Missouri River Valley. Five-sixths of the 57 cases were from this locality,—the others being scattered more or less diffusely over the country. North Dakota, which furnished 22 authentic cases, seems to be the chief focus of human infection in this country. Kansas has also furnished a large number of cases. A map showing the location of the cases in the United States accompanies Ruediger's paper and brings out strikingly the geographical distribution. However, K. F. Meyer¹⁰ has more recently analysed the data, especially those dealing with the relation of animal and human sporotrichosis in this country. He shows that as new cases appear it becomes increasingly evident that the disease is widely distributed, though certain localities like the Missouri River valley furnish the great majority of the cases. It has been reported from the following states: Missouri, Kansas, Iowa, Nebraska, Texas, Virginia, West Virginia, Ohio, New Jersey, District of Columbia, South Dakota, North Dakota, California, Illinois, Pennsylvania, New York, Minnesota, Wisconsin, Indiana, Montana and Michigan. Two cases have been reported from Canada.

Sporotrichosis has appeared in horses in several localities in the United States. The disease was recognized clinically in horses some time before it was accurately studied bacteriologically in this animal. Horses were found in 1908 or before in North Dakota suffering from what was then taken to be mycotic lymphangitis. From the description and the

⁹ *Jour. of Inf. Dis.*, 12, p. 193, 1912.

¹⁰ *J. A. M. A.*, 65, p. 579, 1915.

illustrations given in the *Second Annual Report* (1908) of the Live Stock Sanitary Board to the Governor of North Dakota, it is evident that clinically these horses were afflicted with sporotrichosis. Furthermore, organisms obtained from these Dakota horses were declared to be identical by the Bureau of Animal Industry in Washington with the organisms from certain horses afflicted with a similar disease in Pennsylvania, from which an organism was isolated and clearly shown to be the *Sporothrix schenckii*. The Dakota organism was at first taken to be *Saccharomyces farciminosus*, the cause of lymphangitis in horses as described by Tokishike and Pallin. Through the comparative studies of Paige, Frothingham and Paige, and also of the writer, of the organisms isolated from the Pennsylvania horses and an organism isolated from a human case from North Dakota by the writer, it was shown that the organisms were without question identical. This established the first clear identity of the organism from lesions in horses and in the human and showed too that it was apparently identical with the *Sporotrichum schenckii* as described by Schenck, Hektoen and others.

K. F. Meyer¹¹ has also recently studied this disease in horses. He concludes that spontaneous sporotrichosis in this animal is very common, especially in two localities, Pennsylvania and North Dakota. He cites a case of accidental laboratory infection in man as proof of the pathogenicity of equine strains for the human. The evidence collected, however, does not support the theory that sporotrichosis is very frequently transmitted from horse to man in the United States. His opinion is that the *Sporotrichum schenckii*, *Sporotrichum beurmanni*, the organisms from mules and horses in Madagascar, and the South American strains are all identical. He proposes the use of the term *Sporotrichum schenckii-beurmanni* for all.

¹¹ *Loc. cit.*

SPOROTRICHOSIS IN FRANCE

In France the history of this disease begins with the report of a case by de Beurmann and Gougerot in 1903. Apparently they completely overlooked the work of the American investigators published several years earlier and thinking they had discovered a new organism they submitted it to Matruchot and Ramond who identified it as a sporothrix and in 1905, in a note to the Biological Society of Paris, named it *Sporotrichum beurmanni*. A second case was observed in France in 1906 by de Beurmann and Gougerot and later they identified other cases and made numerous extensive and admirable studies on all phases of the disease.¹² New cases rapidly accumulated in the French literature and it was soon evident that the disease in that country was not rare. It was observed also in the dog and the horse, the organisms being identical with that from the human. It is to be noted that not until 1906 did the French learn of the American cases and of the *Sporothrix schenckii*. French workers generally contend that the *Sporotrichum beurmanni* is different from the *Sporothrix schenckii*.

SPOROTRICHOSIS IN SOUTH AMERICA

In 1907 Lutz and Splendore¹³ in San Paulo in Brazil were the first workers to recognize spontaneous sporotrichosis in lower animals. They observed the disease in both gray and white rats. They also reported five human cases from the same locality. They noted that the disease in rats was transmitted through bite wounds usually on the extremities or tail and following an initial lesion a generalized infection would result. Transmission from the rat to man, while probable, was not demonstrated. These studies, it should be noted, were made independently, the work of American and French investigators not being known to them until some time later. The organism as described by them cor-

¹² De Beurmann et Gougerot, *Les Sporotrichoses*, Paris, 1912.

¹³ *Cent. für Bact.*, 45, p. 631, 1907.

responds in detail with the North American variety and de Beurmann and Gougerot have examined it and pronounced it identical with the French strains. This identity has been conceded by Lutz and Splendore.

Balino and Marco del Pont¹⁴ in 1907 discovered a case of this disease in Buenos Ayres, and Greco,¹⁵ also in 1907, one from Uruguay. Greco suggested calling his organism *Sporotrichum schenckii-beurmanni*. According to him it agrees with the organisms of both Schenck and de Beurmann. Other cases have since appeared in South America.

SPOROTRICHOSIS IN MADAGASCAR

On the island of Madagascar Carougeau¹⁶ in 1908 found this infection in mules and in horses. It is a common disease there. Clinically and pathologically it agrees in every way with the disease as it appears in man. It is either a disseminating or an ascending gummatous sporotrichosis and responds promptly to potassium iodide. Carougeau reproduced the disease experimentally in the mule by intravenous injection. He reports a human infection in a veterinarian who punctured himself while operating on a sick mule. He clearly differentiates this infection from the closely related but more serious one of *Saccharomyces farciminosus*. The sporotrichum from the mules was carefully described by Carougeau and agrees with *Sporotrichum schenckii*. De Beurmann and Gougerot have identified it with the French organism and this identity has been acknowledged by Carougeau.

As to distribution, then, sporotrichosis is practically a world-wide disease having now been noted in North America, South America, Europe, Madagascar and probably India. The chief focus in Europe is France but cases have been observed also in Germany, Austria, Switzerland, Italy, England, Belgium and Spain. In North America, as already stated, it is largely confined to the Missouri River Valley.

¹⁴ *Argentina Med.*, 2, p. 23, 1908.

¹⁵ *Argentina Med.*, 45, p. 699, 1907.

¹⁶ *Bull. et. Mem. de la Soc. Med. de Hopit. de Paris*, 34, p. 507, 1909.

Animal susceptibility is rather general, the spontaneous disease having appeared in man, horse, mule, dog and rat. It has been observed as an accidental infection in man. Experimentally it has been produced in a large number of the lower animals, the rat being probably the most susceptible and useful animal for this purpose.

A STATEMENT OF THE QUESTION

At their request, Hektoen in 1906 sent to de Beurmann and Gougerot at Paris a culture of the American organism which he had isolated seven years before. After studying and comparing this organism with their strains they declared that the American and French strains were different and they continued to retain the name of *Sporotrichum beurmanni* for the French fungus and to use the term *Sporotrichum schenckii* for the North American strains. It is to be noted, too, that de Beurmann and Gougerot and their French colleagues considered the South American strains, the Madagascar strains, the German, Austrian and other strains, all of which were described after their work, as identical with the French organism.

In 1910 the writer took to Gougerot in Paris a strain isolated by himself from a typical human case from North Dakota and reported later by Hyde and Davis.¹⁷ I received from him and also from Sabouraud at that time strains isolated from cases in France and called by them *Sporotrichum beurmanni*. I also obtained from Hektoen a culture of his sporotrichum which he had preserved from his case of 1899, a culture of which, as stated above, he had sent to de Beurmann and Gougerot in 1906. De Beurmann, Gougerot and I, therefore, have French strains, American strains and the original Schenck-Hektoen strain for comparison. In order to simplify and limit the discussion as far as possible I will make the following statement: first, excluding for the time being the Schenck-Hektoen strains, we may consider all the later strains, except *Sporotrichum councilmani*, isolated in

¹⁷ *Jour. of Cut. Dis.*, 28, p. 321, 1910.

the United States from man and horses by numerous workers including the writer identical with each other. My own work as well as the work of K. F. Meyer, Sutton, Ruediger, Page, Frothingham, Paige, and others, all tend to confirm this point. The strains have usually been designated as *Sporotrichum schenckii*. Again excluding *Sp. dori*, *Sp. jeauselmei* and *Sp. gougeroti* in France, all the French strains of sporothricha are admitted by all, both French and Americans, to be alike. They have been designated *Sporotrichum beurmanni* by the French. Gougerot in a publication¹⁸ made after examining the American cultures which I gave him in 1910 admits that they are identical with the French strains but different from the old Schenck-Hektoen cultures. In another article on this subject he writes as follows: "Hyde & Davis bezeichnen also *Sp. Schencki* einen Parasiten welchen uns Davis ubermittelt hat und der zweifellos identisch mit dem *sp. Beurmanni* ist."¹⁹ A further statement in the same paper on this point is as follows:

Die Beantwortung der aufgeworfenen Frage lässt drei Möglichkeiten zu:

(1) Der Stamm Hektoen-Gougerot des *Sp. Schencki* stellt den Typus des *Sp. Schencki* dar; das *Sp. Beurmanni* muss von ihm unterschieden werden, steht ihm jedoch nahe und stammt von derselben Urstamme ab wie er.

(2) *Sp. Schencki* und *Sp. Beurmanni* stammen beide von ein und demselben Urstamme ab; sie zeigen zwar gegenwärtig einige Verschiedenheiten, sind aber durch Zwischenglieder, wie z. B. den Stamm von Hyde & Davis, miteinander verbunden.

(3) Der Stamm Hektoen-Gougerot des *Sp. Schencki* ist ein fest gewordener pleomorpher Vertreter des *Sp. Schencki* und von den Stämmen "Schencki initial" und "Hektoen initial" unterschieden. Es ist jedoch unmöglich, auf Grund unserer Kenntnisse von diesem Stamm das echte *Sp. Schencki* zu beurteilen, weil letzteres sich vollkommen vom Stamm Hektoen-Gougerot unterscheidet und beispielsweise durch den Stamm Hyde-Davis vertreten ist. Da aber der Stamm Hyde-Davis mit dem *Sp. Beurmanni* identisch ist, so müssen das *Sp. Schencki* und das *Sp. Beurmanni* ebenfalls identisch sein.

¹⁸ *Sporotrichosis Nord Americanus: Bull. et Mem. de la Soc. Med. de Hop. de Paris*, 35, p. 798, 1910.

¹⁹ Gougerot, Kolle & Wasserman, *Handbuch der Pathogenen Microorganismen*, 2 Auflage, 5, 225, 1913.

Die Frage kann nur entschieden werden durch Vergleich der Original-kulturen von Schenck und Hektoen. Entschliesst man sich, diese dritte Hypothese einer Unität des Sp. Schencki und des Sp. Beurmanni anzunehmen, so wurde trotzdem die Tatsache bestehen bleiben, dass dieser Parasit in zwei Typen sich darstellen kann; als Sp. Schencki, der seltener Typus, welcher einzig von den amerikanischen Forschern im Jahre 1898 and 1900 beschrieben worden und von welchem der Stamm Hektoen-Gougerot eine Abart bildet (Sp. Schencki-Beurmanni var. Schencki), und schliesslich als Sp. Beurmanni, ein sehr verbreiteter Typus, der zum ersten Mal von Matruchot & Ramond im Jahre 1903-1905 und später im Jahre 1906 von Beurmann & Gougerot studiert worden ist (Sp. Schencki-Beurmanni var. Beurmanni.)

From the above statements it is evident that Gougerot himself concedes the identity of the French and the later American strains and I entirely agree with him on this point. I have examined several French strains very carefully and compared them with later American strains from man and horses without noting any essential points of difference. Admitting then that the French strains and the later American strains are identical, the question resolves itself into a comparison of the disease and organisms as observed by Schenck and by Hektoen on the one hand and the French and later American disease and organisms on the other. These latter, it is to be noted, are conceded to be identical with the strains from South America, Madagascar, and the other foci mentioned above. If it is shown that they are identical with the Schenck-Hektoen strain, they should all be called *Sporotrichum schenckii*. If they are different, they would be called *Sporotrichum beurmanni* and the strains of Schenck and Hektoen would remain as isolated and unique organisms differing from all other described sporotricha.

COMPARISON OF THE TWO CASES OF SPOROTRICHOSIS OBSERVED
BY SCHENCK AND BY HEKTOEN AND PERKINS WITH
THE LATER AMERICAN CASES

Clinically these two cases were typical ascending gummatous sporotrichosis identical in every way with the many cases observed later by the French and by many observers

here in the United States. No one, so far as I know, has attempted to differentiate them on clinical grounds.

Therapeutically they responded promptly to potassium iodide as did the later French and American cases.

The morbid anatomy and histology in the human were, so far as studied, the same in the two types, the lesions being those of a chronic abscess. In experimental animals the lesions produced by the two types are identical as admitted by Gougerot.

Bacteriologically there is greater opportunity to detect minute or subtle differences should they be present and these we shall consider in detail. In the cases of Schenck and of Hektoen and Perkins, the organisms were not seen in the human tissues or in the pus. In experimental animals the organisms appeared as oval and elongated forms with occasional round forms. They stained with Gram. In no way did they differ from the forms seen in tissues infected with organisms of the French strains. I have made a special study²⁰ of the tissue forms in experimental animals using strains from France and from America as well as the Schenck-Hektoen strain. No differences could be detected between them. When these various strains are grown in animal fluids, blood etc., in the test tube, elongated forms similar to those seen in the tissues are produced and here again no differences between the various strains were noted.

The question of the virulence of the various strains may not be of any importance in differentiation since this property is such a variable one. However, it may be stated that the Schenck-Hektoen strain even after years of artificial culture is still about as virulent for rats as are the freshly isolated strains of the French type. According to the paper of Schenck, these organisms were virulent for mice and dogs. Hektoen and Perkins produced lesions in mice, dogs, rats and guinea pigs (slight). As far as these results are comparable with those obtained with the French and later American strains, they agree in all essential points.

We now come to a discussion of the morphological and cultural characteristics, both microscopic and macroscopic, of

²⁰ Davis, *Jour. Infect. Diseases*, 12, p. 453, 1913.

the organisms and in doing this it becomes necessary to state in detail the differences which the French workers have pointed out between *Sporotrichum schenckii* and *Sporotrichum beurmanni* since they very largely center around these properties. They have been stated succinctly by de Beurmann and Gougerot in their article on North American sporotrichosis²¹ and in order to avoid misstatements I quote as follows:

Sporotrichum Beurmanni

Cultures difficiles, mais possibles à 38 degrés. Optimum 22 à 30 degrés: donc développement plus lent.

Aspect macroscopique des cultures sur gelose glycosée peptoneé de Sabouraud (milieu d'épreuve).

Pigmentation rapide et complète. Colonies toujours très colorées, de teinte chocolat ou noire.

Circonvolution à la façon des circonvolutions cerebrales.

Aspect microscopique des cultures sur lames sèches et en gouttes pendantes.

Filaments myceliens de 2 u de large plus rectilignes, quelquefois agrégés, mais surtout enchevêtrés, non parallèles.

Spores de 3 sur 5 à 6 u, très nombreuses, insérées sur de longs filaments ou à l'extrémité de filaments lateraux courts ou longs.

Sporotrichum Schenckii

Cultures facile à 38 degrés. Optimum 30 à 38 degrés: donc développement plus rapide.

Pigmentation très lent, le plus souvent inconstante ou absente. Colonies peu colorées ou blanches le plus souvent.

Sillons presque rectilignes divergeants à partir d'un centre comme des vallées du cone d'un volcan.

Chlamydospores.

Filaments myceliens de 2 u de large plutôt curvilignes, onduleux, presque toujours agrégés et parallèles en faisceaux, sans enchevêtrement habituel.

Spores très rares, souvent même absentes, insérées le long et surtout à l'extrémité de longs filaments. Peu ou pas de conidiophores courts lateraux.

Pas de chlamydospores connues.

²¹ *Loc. cit.*

Caractères biologiques (Blanchetiere et Gougerot).

Fait fermenter la saccharose.

Fait fermenter la lactose.

Ne semble pas faire fermenter la lactose, etc.

Ne semble pas faire fermenter la saccharose, etc.

Matruchot has stated the differences more in detail but since all the essential points are covered by the above outline it will not be necessary to state them again. I shall take up these points in order and attempt to analyze them in the light of data from both American and French sources.

First, as to the optimum temperature for growth, Schenck states that for his organism it was between 20° C. and 37° C. Hektoen says it would seem to be about 37° C. Growth is much slower at 20° C. My own experiments have not convinced me that there is any appreciable or constant difference in optimum growth temperature between *Sp. schenckii* and *Sp. beurmanni*. Slight differences are often observable between various strains of sporotricha. In growing many cultures side by side, including the original *Sp. schenckii*, growth was most rapid and most abundant at temperatures from 28° C. to 32° C. Variation in optimum growth temperatures is common in fungus organisms of this type. They are not delicate in this respect and small differences should not be unduly emphasized as differentiating features.

In the outline quoted above de Beurmann and Gougerot have next emphasized certain points concerning the macroscopic characters on special media of the cultures which in their opinion are important in differentiation. These points center chiefly round the fact that sporotricha generally are especially prone to change and modify their cultural properties on artificial media, a character referred to as pleomorphism. This is so important and so much has been made of cultural differences in distinguishing *Sp. schenckii* and *Sp. beurmanni* that I must discuss it somewhat in detail.

First, the colonies may in a great variety of ways alter their pigmentation, the tints changing through various shades of brown and black; portions or all of the culture may be pure white. These changes may or may not be permanent. I

have for some time made a study of chromogenesis²² in these cultures and have now some pure white strains which sprang from deeply pigmented cultures. Indeed, from the culture I received from Gougerot of Paris, a white colony appeared which has remained pure white and smooth, and though tested on numerous media of the most favorable sort (carrot, potato, Sabouraud medium), remains pure white. Passage through a rat for six weeks did not alter it. It has now passed through twenty-four generations without change. The black colonies continue to produce pigment as usual. Similar alterations have been observed in other strains. Distinct and similar changes have been noted in the Schenck-Hektoen strain but they are less marked. It is interesting to note Gougerot's statement²³ in this connection: "Par exception nous avons eu des pléomorphismes blancs qui sont trestis irréductibles; ils étaient associés à des pléomorphismes de surface et ces pléomorphismes complexes donnaient un *Sporotrichum Beurmanni*, identique d'aspect au *Sporotrichum Schenckii*."

A second pleomorphism relates to form of growth, smoothness, wrinkling, etc. Colonies tend to lose their irregular and corrugated surface and become smooth and leathery in appearance. This is a common change in strains of sporotricha which can be brought about, at least to some extent, in all strains by suitable culture, especially on ill adapted media.

A third is the tendency to form on the surface growth hair-like processes or finely pointed spines. This is seen quite commonly and is a striking feature of many cultures of the original Schenck-Hektoen strain.

A fourth pleomorphic change is the appearance of a powdery growth covering part or all of the media. The color is variable and may range from black through brown to pure white. This alteration is largely dependent on surface deposits of spores.

It is to be emphasized that these pleomorphic changes above noted are common. In some strains they are far more frequent than in others, but probably occur in all strains at

²² Davis, *J. Inf. Dis.*, 17, p. 174, 1915.

²³ *Les Sporotrichoses*, Paris, p. 91, 1912.

times. They are variable but often fixed and permanent. They are so manifold in character that cultures show a great variety of appearances and two strains identical at first may later through these changes become quite different in appearance. De Beurmann and Gougerot state in their monograph on page 133 (*Les Sporotrichoses*) that they have observed strains of *Sporotrichum beurmanni* (notably of the race *alpha*), through pleomorphic change, become identical with *Sporotrichum schenckii*. Others have become identical with *Sporotrichum jeanselmei* or have even simulated *Sporotrichum gougeroti*. Again certain strains have reverted to short forms comparable to yeast or blastomyces. My own work also confirms in general the above observations of de Beurmann and Gougerot. I have noted yeast-like forms in certain strains and a great many changes in pigmentation and other morphological appearances, some of which are fluctuating, others are apparently permanent.

In the light, then, of the above facts it seems to me that distinctions of these sporotricha based on pigmentation become valueless because of these easy and striking fluctuations. So, too, surface convolutions and forms simulating the cone of a volcano are factors which change under conditions favoring pleomorphism.

Under microscopic aspect of cultures on slides and in hanging drop in their outline de Beurmann and Gougerot consider especially spore formation. They have repeatedly stated that in cultures of *Sporotrichum schenckii* the spores are rare or even at times absent on the filaments. This is true, at least, of certain cultures that develop little or no pigment. As a differentiating feature, however, this point is not necessarily significant. I have noted other strains, especially the non-pigmented ones, which show this same characteristic. This dearth of spores in the strain of *Sporotrichum schenckii* which they examined was, I think, no doubt due to a pleomorphic change. It is important to note the fact, which they have not referred to in their publications, that in the original articles of both Schenck and Hektoen and Perkins several photographs of unstained organisms show the mycelium with spores attached to the sides and ends in great abundance. I think

these photographs are conclusive on this point and show that no doubt a change occurred later in these cultures resulting in a strain bearing fewer spores. Spore formation is extremely variable as de Beurmann and Gougerot admit. Furthermore in their monograph they state that the white pleomorphic forms—those approaching the type of *Sporotrichum schenckii*—are very poor in spores, certain ones becoming entirely devoid of them. I wish again to emphasize this point as practical proof that changes did result in this strain from the time it was first described and the time, some seven years later, when it was sent to de Beurmann by Hektoen. Furthermore, de Beurmann and Gougerot write²⁴ that Hektoen stated in his letter when transmitting the culture that it seemed to have lost its power of producing spores as compared with the preceding generations. This, I believe, is definite proof of a change which no doubt occurred on artificial media and which these writers have used to differentiate the *Sporotrichum beurmanni* from *Sporotrichum schenckii*. Original organisms and original descriptions should be compared for this purpose, not organisms changed through growth on artificial media.

As to chlamydo spores, de Beurmann and Gougerot state that *Sporotrichum beurmanni* forms them while the *Sporotrichum schenckii* does not. Matruhot also makes this statement. I discussed this matter in a special paper some time ago²⁵ and showed that, at least under certain conditions, the *Sporotrichum schenckii* readily forms typical chlamydo spores. This was especially true on media poor in nutrient material. K. F. Meyer²⁶ confirmed my results in this respect noting chlamydo spore formation not only in the original *Sporotrichum schenckii* but also in the many strains of sporotricha which he isolated from horses in the United States.

With reference to the arrangement of mycelial filaments, it may be stated that this is a property decidedly pleomorphic, and with the appearance of the pleomorphic alterations noted

²⁴ Bull. et. Mem. de la Soc. Med. des Hop., Paris, 35, p. 745, 1910.

²⁵ Jour. Inf. Dis., 15, p. 483, 1914.

²⁶ Jour. of Inf. Dis., 16, p. 399, 1915.

above on artificial media the mycelial filaments may run in straight or curved bundles with little entangling.

As to biological characters the fermentation reactions of these organisms are apparently not very uniform or definite. Gougerot says that *Sporotrichum beurmanni* ferments saccharose but not lactose, whereas *Sporotrichum schenckii* ferments lactose, but not saccharose. He states, however, that he does not know whether these properties are fixed for all strains and for the pleomorphic forms. Greco noted that his strain from South America failed to ferment lactose, saccharose and mannite. This does not agree with the fermentation reactions for *Sporotrichum beurmanni* as given by de Beurmann and Gougerot but they nevertheless classify the South American strains as *Sporotrichum beurmanni*. At the same time they use this difference in the fermentation of lactose and saccharose to differentiate *Sporotrichum beurmanni* from *Sporotrichum schenckii*.

Meyer and Aird²⁷ have made a careful study of the fermentation of American strains and of *Sporotrichum beurmanni*. They were not able to confirm the finding of Blanchetière and Gougerot that *Sporotrichum schenckii* fermented lactose. Furthermore, they found the fermentation of saccharose irregular. They state that "in considering these results purely from a differential diagnostic viewpoint it is quite evident that it cannot be used for this purpose and the fermentation of carbohydrates is just as little a criterion of the type of sporotrichum as is the absence of pleomorphism and the chlamydospore formation". Their conclusions are so definite and so relevant that I quote them:

The differentiation of pathogenic sporotricha into two distinct species by means of the fermentation of carbohydrates, is impossible. The reactions are not fixed and are as inconstant as the many variations noted in the formation of chlamydospores and, frequently, in pleomorphism. There does exist however an apparent relation between the pigmentation of the sporotrichum strains and the ability of these strains to ferment saccharose. The alpha and beta types are the most active fermenters.

²⁷ *Jour. of Inf. Dis.*, 16, p. 399, 1915.

This and other evidence, which will be presented elsewhere, make it apparent that the American sporotricha—of which we studied thirty-five strains—have, in many respects, type characters in common with *Sporothrix beurmanni*. In the light of de Beurmann's and Gougerot's work, some of the American strains are doubtless *Sporothrix beurmanni*, and it is not permissible to call such strains "*Sporothrix schenckii*" merely for the sake of simplicity. The discussion of de Beurmann and Gougerot (28) on this subject can now also in our opinion, be satisfactorily closed, namely: that *Sporothrix schenckii*, Hektoen-Gougerot strain, is an absolutely fixed type. The true *Sporothrix schenckii* is represented however by all of the recently isolated strains. Inasmuch as most of these strains are undoubtedly identical with *Sporothrix beurmanni*, the *Sporothrix schenckii* is identical with the *Sporothrix beurmanni*.

The American strains of pathogenic sporotricha are therefore best classified as one species. *Sporothrix schenckii-beurmanni* (as suggested by Greco.)

Having now completed the discussion of the several points of differentiation quoted above from de Beurmann and Gougerot, I shall next briefly consider certain other similarities of the French and American strains of sporotricha that deserve mention, it seems to me, in a discussion of their possible identity. Slight and otherwise insignificant differences between organisms may be determined often by differences in serum reactions in varying concentrations. In the study of this group of organisms, Widal and Abrami showed that positive agglutination occurred in patient's serum in dilutions often of 1/400 or 1/500 or even higher. This has been confirmed by several workers. For differential purposes they noted that patients suffering with other mycelial infections like actinomycosis, nocardiosis, etc., give a positive but much lower agglutination. Gougerot and Caraven noted that the serum of a case of hemisporosis agglutinated in dilution of 1/400. This was evidently exceptional.

The writer immunized rabbits with several strains of sporotricha for a period of about 8 months. The strains included *Sporotrichum schenckii* obtained from Hektoen, *Sporo-*

²⁸ Bull. et Mem. de la Soc. Med. de Hop. de Paris, 26, p. 9, 1908.

trichum beurmanni from Gougerot of Paris, a sporotrichum isolated by K. F. Meyer from a horse in the United States, and a strain isolated in 1909 by the writer from typical sporotrichosis in a man from North Dakota. The sera of the various rabbits were tested with the homologous organisms and also with other strains. It was found that agglutination appeared quite uniformly in the dilutions of serum varying from about 1/320 to 1/640. In most instances a slightly higher agglutination appeared in the homologous sera but this was not always the case. The several strains tested could not be differentiated by these interagglutination tests in animals.

Similar tests were made with serum from a human case in which agglutination with the homologous organism occurred at 1/160. Here again the original *Sp. schenckii* and *Sp. beurmanni* were agglutinated at approximately the same dilution, namely, 1/160. The controls were negative.

Wilder and McCullough²⁹ studied the serum from a case of sporotrichosis of the eye. Tests for agglutinins and opsonins in the serum of the patient against several strains of sporotricha, including the infecting strain, the original Schenck-Hektoen strain, a French strain, an equine strain from Meyer, and two other American strains from typical cases, revealed no specific differences in the antibody content of the serum.

The reaction of complement fixation is positive in cases of sporotrichosis but it seems less reliable than that of agglutination. The studies of Widal and Abrami and other French workers have shown that the results are very definite but that an infection with many other mycoses (actinomycosis, hemisporosis, discomycosis, etc.) will also give a positive test. *A priori*, then, one would not expect this test to be useful in differentiating strains of sporotricha. J. J. Moore³⁰ in our laboratory has made such studies, finding a definite fixation in human serum from a case of sporotrichosis using the homologous organism. He obtained similar results when antigens made from the various other strains, including *Sporotrichum schenckii*, *Sporotrichum beurmanni* and an equine strain, were

²⁹ *J. A. M. A.*, 62, p. 1156, 1914.

³⁰ *Jour. Inf. Dis.*, 23, p. 252, 1918.

used. He concluded that these organisms are identical so far as this test is concerned.

Bloch³¹ in 1909 was the first to obtain a positive skin reaction in a case of sporotrichosis though some work had been done along this line by de Beurmann and Gougerot in 1906 without definite results. Bloch used a "sporotrichosine" extracted from a broth culture. The reaction was very definite. French workers, especially de Beurmann, and Gougerot and Chopin, about the same time took up this work, using intracutaneous injections of extracts of the killed organisms in salt solution and obtained positive results. The reaction, however, was shown by them not to be absolutely specific. Other mycoses (actinomycosis, oosporosis, saccharomycosis, exascosis, at times tuberculosis) responded so that the method was useful according to them only for the differentiation of a rather large group. From their data, one would conclude that this method could have no value for differentiating closely related organisms belonging to the genus *sporotricha*.

Recently Moore and the writer tested a human case of typical sporotrichosis with a sporotrichosine consisting of killed sporothrix in salt solution. The patient was tested both when receiving and not receiving potassium iodide. A very distinct skin reaction was obtained with the sporotrichosine made from the original *Sporotrichum schenckii* and also from a strain of *Sporotrichum beurmanni* obtained from Gougerot. No differences were noted in these reactions which were very definite and measured 5 to 7 centimeters across. Controls with "blastomycine" made in exactly the same way from a blastomycete isolated from a typical case of blastomycosis did not give a positive reaction in this patient. Sporotrichosine injected into the skin of the patient with blastomycosis gave no reaction; nor did he react to his own blastomycine. Agar alone in $\frac{1}{2}$ per cent suspension injected into persons when taking potassium iodide (t. i. d. 10 grains) gave a definite reaction but was not nearly as pronounced as that given by the "sporotrichosine". Normal persons receiving potassium iodide (t. i. d. 10 grains) reacted no stronger to

³¹ *Beihefte zur Med. Klinik*, Basel, 8, p. 179, 1909.

sporotrichosine than before the iodide had been administered to them. In either case the dermal reaction measured from 5 to 15 mm. whereas the sporotrichosine reaction in the patient measured 5 to 7 cm.

While, therefore, a striking reaction may be elicited in these cases with sporotrichosine, it probably cannot be considered sufficiently specific to be of value for differentiating these organisms. But it is to be noted that patients taking or not taking potassium iodide may react intradermally to these various strains of sporotricha and not to strains of blastomyces prepared in exactly the same way. Whether or not all cases will so react we do not know.

SUMMARY AND DISCUSSION

It appears that the first case of sporotrichosis was reported by Schenck in 1898. The second case was reported in 1900 by Hektoen and Perkins and the organism definitely identified by comparison with that isolated by Schenck. Hektoen named the organism *Sporothrix schenckii*.

In 1903 de Beurmann and Gougerot reported the first case in France and the organism was named by Matruchot and Ramond *Sporotrichum beurmanni*. The work of the American investigators published several years previously was not known to the French workers.

On comparing the American strain sent to them by Hektoen in 1906, seven years after its isolation, de Beurmann and Gougerot pointed out certain differences between this strain and their recently isolated strain. They noted certain differences also between their own strains (strains a, b, c) but contended that these differences were not sufficient to justify creating a new species. But the differences between their strains and *Sporotrichum schenckii* were sufficient, they contended, to justify a new species. The organisms, isolated later in North America and those found in South America and in Madagascar, they claim are the same as their organism, *Sporotrichum beurmanni*. These were all isolated after they discovered and named the organism in France.

It is pointed out that sporotricha, French, American and other strains, are especially subject to undergo pleomorphic changes, some of which are transient while others are fixed and permanent. De Beurmann and Gougerot themselves have called especial attention to this and admit that some of the pleomorphic alterations in the macroscopic growth of certain strains render them identical with the strain of *Sp. schenckii* as it exists today. Furthermore, Hektoen has stated that the culture as sent to them had changed in the seven year interval on artificial media especially in its ability to produce spores. This change is not an uncommon one in both American and French strains and no doubt was associated with other pleomorphic changes. Yet de Beurmann and Gougerot and also Matruchot used this loss of ability on artificial media to form spores as a differentiating characteristic from their own *Sporotrichum beurmanni* though they observed this same change in strains of the latter. There can be no doubt that pleomorphic changes took place in *Sporotrichum schenckii*; and this is borne out also by the photographs in both Schenck's and Hektoen's papers which clearly show that at first both strains produced spores in large numbers.

Changes in pigmentation are common in all strains of sporotricha; poorly or non-pigmented strains may arise from deeply pigmented strains and remain fixed. One would scarcely classify such an organism as sporotrichum on the basis of such a fluctuating character as pigmentation, though this property is mentioned by them as an important differentiating one.

The statements of de Beurmann, Gougerot and Matruchot that the *Sporotrichum schenckii* (original) does not form chlamydo-spores must be considered erroneous. Under suitable conditions these structures have been observed by the writer, and these results were confirmed by Meyer, not only in the original Schenck-Hektoen strain but in many other American strains from both man and horses. The attempt to differentiate *Sporotrichum schenckii* and *Sporotrichum beurmanni* on this basis must therefore be given up.

The fermentation of sugar is quite inconstant. It is difficult to understand why de Beurmann and Gougerot would use

the differences in the fermentation of lactose and saccharose as a distinguishing feature between the American and French organisms since they say that not all their strains fermented saccharose. Meyer and Aird have shown conclusively the inconstancy in fermentative powers of not only many American strains but also of different French strains. They conclude it is impossible to differentiate sporotricha into two distinct species by means of the fermentation of carbohydrates. These results have been confirmed by the writer. Greco's observation on a South American strain does not agree with those of de Beurmann and Gougerot on French strains.

Specific serum and dermal tests are probably of limited value in differentiating these closely related organisms though they may furnish important data for the basis of a group relationship. So far as the results indicate they show no differences between the French and American strains.

From the above analysis it would seem that the basis upon which French investigators differentiate the *Sporotrichum schenckii* and *Sporotrichum beurmanni* is, to say the least, very inadequate. On account of the pleomorphism of this organism there is an excellent opportunity to take advantage of slight and unimportant differences in order to create new species. It is of course true that no two strains are *exactly* identical. De Beurmann and Gougerot noted that certain of their strains manifested fixed pleomorphic changes that made them appear identical with the original *Sporotrichum schenckii*. Yet they did not suggest that these strains be called *Sporotrichum schenckii*. Concerning these slight differences, especially in organisms of this type, it would seem that it would be wise to assume a conservative attitude and to refrain as much as possible from the use of new and unnecessary terms.

The question arises in connection with an organism of this kind, as it arises so frequently in biology, what differences are sufficient to warrant the creation of a new variety or species? Where shall the line be drawn between varieties since no two cultures of sporotricha are absolutely identical in every detail and strains are ever prone to these striking

changes? To this the answer must be made that this is largely a conventional matter and often it is impossible to state clearly where the line of demarcation should lie. But this I wish to point out, that it is evidently not proper or scientific to use pleomorphism, or any other character for that matter, as a basis for the classification of an early American strain, and not use it in the classification of French strains or later American strains.

In résumé, I believe we are justified in stating that the differences between the American strains, including the original cultures of Schenck and of Hektoen, and the French strains of de Beurmann, Gougerot and others, are easily explained as pleomorphic variations and therefore are insignificant. Furthermore, the disease, clinically, pathologically, experimentally, and therapeutically, is admitted by all to be identical in France and in America.

The above statements being true, according to the rules of botanical nomenclature the organisms in both countries should be called by the name first given to them in 1900 by Hektoen, namely, *Sporotrichum schenckii*. The fact that de Beurmann rediscovered the organism several years later deserves no consideration so far as determining nomenclature is concerned. As regards the use of the compromise term *Sporotrichum schenckii-beurmanni*, suggested first by Greco of South America and more recently concurred in by Meyer in this country, it may be said that this is objectionable because it not only introduces a long cumbersome term but it is not in accord with the rules of botanical nomenclature. There is obviously therefore but one legitimate term for this organism, namely *Sporotrichum schenckii*.

It should be pointed out that even though one maintains that the small differences noted between the pleomorphic forms of the Schenck-Hektoen strain and the other sporotricha are sufficient to justify a species distinction, the important fact remains that the hundreds of strains of sporotricha found in France and in North America are alike. This is admitted by both sides of the controversy. Therefore, whichever view of the original Schenck-Hektoen strain is taken by

the French, the identity of sporotrichosis, excluding the very rare strains mentioned earlier in the paper, in France and America must be admitted. One is as justified in making this statement as in saying tuberculosis in France and in America is identical.

THE SIGNIFICANCE OF YEASTS AND OIDIA IN PASTEURIZED BUTTER

F. W. BOUSKA AND J. C. BROWN

The original object of pasteurization in buttermaking was to produce a better immediate flavor by controlling fermentations. The aim was to destroy undesirable microorganisms as well as microorganisms whose effect is unknown. By means of a pure culture of selected lactic acid bacteria (starters), regulated temperatures, and the acid test, the desired flavor was to be developed. In more recent times it was discovered that pasteurization greatly improved the keeping quality of creamery butter made from cream which had soured spontaneously on the farm. A considerable part of American butter is now made from such cream. The succession of sweet milk by sour cream as a source of creamery butter is a result of economic conditions on the farm. Some farmers think that the expense of hauling milk to the creamery every day is too great. So they skim the milk at home and deliver the cream twice a week in the winter and three times a week in the summer. The skim milk thus derived has the best feeding value because it is warm and sweet. But the cream obtained by this farm method is usually sour. It became our lot to design methods of making butter from spontaneously soured cream and to overcome butter defects that sometimes occur here. While our experience, investigations, and results apply principally to sour cream, it will be seen that in some respects a general application can be made.

Butter which has a good flavor when churned but develops a bad flavor at low temperatures within one month has poor keeping quality. Properly made pasteurized butter scoring 90 will, in current commerce, remain eatable to the last morsel. In cold storage it scores 89 to 90 at the end of seven to ten months. We have known churnings of butter to score 90 at the end of eighteen months.

A quicker test is made by storing small samples at 60° to 70° F. A very poor keeper develops a bad flavor within three days. A good keeper, such as is usually made, scores 89 to 90 at the end of two weeks.

It became our problem to ascertain the cause of poor keeping and to find a remedy for it. Bacteriological philosophy of the keeping quality of butter has exerted an exaggerated influence in every sphere of butter-making. For a time we accepted the impression from Sayre, Rahn and Farrand¹ that only yeasts can tolerate the well high saturated solution of salt which is the watery part (brine) of butter. To our disappointment, we did not find any connection between yeasts and a very long and serious epidemic of fishy butter. Never present in great numbers, sometimes absent, they practically did not multiply in the course of time.

At the beginning of 1913 we thought that by ascertaining the number of yeasts and *Oidia* (*lactis*) in butter we could foretell its keeping quality. We made a study of the cold storage of 177 lots of butter from a number of states. As the butter went into cold storage, the commercial judge made his predictions according to the quality of the butter and previous experience with the creamery. We predicted from the number of yeasts and *Oidia*. At the end of the storage season the predictions were compared with the final quality of the butter. We predicted that 39 lots would keep well. Only five (13 per cent) of these kept poorly. The judge predicted that 83 lots would keep well. Twenty-four (29 per cent) of these came out poor. But our way selected so few good keepers that a cold storage could not have made a living by our method.

We now know that under American creamery conditions keeping quality is due to acidity, elimination of buttermilk, pasteurization, and proper working. The deterioration of butter is mainly the result of physical or biochemical causes. An indirect part may be played by micro-organisms.

These bacteriological attempts taught us how to make counts of yeasts and *Oidia*. We found that vatfuls and churnfuls

¹ *Technical Bulletin*, No. 1, Michigan Experiment Station.

of raw spontaneously soured cream always contained from 10,000 to 100,000 yeasts (per c. c.) and not quite so many Oidia. In the winter the numbers are smaller with the Oidia often preponderating. Properly pasteurized cream, right from the pasteurizer, never contains yeasts or Oidia. Here, then, is the test for pasteurized cream and pasteurized butter! But cream off the cooler, in the cream vat, in the churn, and the resulting butter, showed many yeasts and Oidia. The first and last cream issuing out of flash pasteurizers was not pasteurized and had to be diverted by a by-pass till a pasteurizing temperature had been reached and maintained for several minutes. The last cream had to be heated in a can or held over till the next pasteurization. The outlet of retarding and pasteurizing vats formed a long pocket in which cream escaped pasteurization. This pipe was shortened. Raw cream dripped into the pasteurized cream from pipes and conductors that had conveyed the raw cream. These were removed. Raw cream splashed upon the sides and cover of a vat escapes pasteurization and is usually washed into the churn with the hose. This is partly overcome by rinsing with hot water or hot cream during pasteurization.

No amount of scrubbing of utensils or pipes will produce good bacteriological results. This must be completed by sterilizing by means of hot water or live steam. Permanent steam connections on cream pipes give the best results. The packing glands of vats, pumps, and churns and the wood of churns, as they are today, are difficult to sterilize and furnish some of the yeasts and Oidia. Sometimes the starter becomes contaminated and introduces yeasts and Oidia. It is easy to see how laymen handling both raw cream and pasteurized products may contaminate the latter with their hands, thermometer, dipper, and measuring stick.

In December, 1912, in one plant we began yeast and Oidia counts in a cubic centimeter of butter. The totals ranged from two to fifty. In August, 1913, the counts declined to ten or less. We continued improving methods and equipment and educating the men, and by July, 1916, we reduced the counts to zero to five. This efficiency is now main-

tained by 10 per cent of the plants; 20 per cent of the fifty plants on which we have records are below 10. The majority of them have less than six months' education. As they learn, we hope for better results. By means of written directions and photographs, creamery workers are instructed how to take samples of butter. (see Fig. 1.) They have proved quite dependable. For transporting we use small metal capped



Fig. 1.—Sampling butter for yeast and mold count.

vaseline jars sterilized in parchment wrappers. Butter is nearly always sampled in the churn by means of a scalded teaspoon. Prints and tubs are sampled by removing inner portions by means of scalded knife, spatula, or trier. Enamelled or copper syrup pitchers are used for collecting a composite sample of a day's churnings. The lid on the pitcher automatically drops down and prevents possible contamination.

Samples of salted butter sent by mail or stored for a week do not show any multiplication. Unfortunately, yeasts and *Oidia* multiply rapidly in unsalted butter. Counts of sweet butter over 24 hours old are unreliable.

Whey agar is prepared as follows: Skimmilk is warmed to about 100° F., acidified with lactic or hydrochloric acid, coagulated with rennet or pepsin, the curd is cut, allowed to settle and then heated to 115° C. in the autoclave, the whey is filtered off through cotton, neutralized, made up with 1.5 per cent agar, 1 per cent peptone, and filtered. In plating, 1 c. c. of sterile 1 per cent tartaric acid solution (by weight) is placed in the petri dish. Then 1 c. c. or 1 gram of the material to be analyzed is introduced. With this is mixed 10 c. c. of the agar. In two or three days at room temperature the yeast colonies are about 3 mm. in diameter,



Fig. 2.—Yeasts and Oidia (Left) 1 c. c. of inefficiently pasteurized cream plated in whey tartaric acid agar. (Right) In 1 c. c. of butter plated in whey tartaric acid agar.

raised, moist, and glistening. The Oidium colonies are about 1 to 3 cm. in diameter, dry and velvety. One soon learns to recognize them at once with the unaided eye (see Fig 2). Mixed colonies occur, but they do not affect the practical interpretation of the count. Colonies of bacteria seldom reach such a size as to interfere. Occasional air molds are regarded as an accidental contamination unless they recur in considerable numbers in the subject from the same source. Professor Lund has successfully used beerwort and lactic acid instead of whey and tartaric acid. Where obtainable, the wort is more convenient than whey.

We have not made any study of the species or races of yeasts and Oidia that occur here. We are unable to give any

interpretation of a large proportion of yeasts and a small proportion of *Oidia* and vice versa. Only the sum total has a meaning. The yeasts are more persistent. To simplify the language for laymen we call *Oidium lactis* "mold".

On the basis of the record made by a number of good plants we have adopted an arbitrary commercial standard of ten or less. This is as good work as the best men are able to do. A count of thirty or more means one or more of the following defects: Failure of pasteurization, i. e., in temperature, in time, or by contamination; lack of cleanliness or of sterility of utensils and conduits; or contaminated starter. In every case where we made a personal survey where the count exceeded 30 we demonstrated that one of these defects existed to such a degree that the laymen could easily see it when it was pointed out to them. By mail and without making personal trips we have corrected many defects. That we detected dirty cream pipes 500 miles beyond our eyesight and caused them to be cleaned is one of the wonders of our uninitiated.

When testing pasteurization or when searching for defects, we present a survey of the plant by means of the graphic report shown here (see Fig. 3). This maps out the course of the material through the plant and shows where defects occur. In the case exhibited here yeasts and molds in the pasteurized products made their first appearance in the churn, thereby proving it to be the source of contamination. If the cream were the original source of yeasts and molds, then the butter would contain much fewer than the cream because the drainage of the buttermilk and washing of the butter eliminate the larger part of them. But since the churn was the source of the yeasts, their number increased the longer the materials remained in the churn. The working of the butter expelled more yeasts than the churning because the fixtures of the worker are the main refuge of yeasts. These churns were old and difficult to sterilize. Nevertheless this exhibit is excellent work and far above the average.

Other conditions being favorable, a butter having only a few yeasts and molds is a safer hazard for long distance shipments and for storage. Indeed, our records show that the

creameries that have the best commercial reputation for their butter also have the lowest yeast and mold counts. We must acknowledge that in this case good all around methods contribute to both results. At the plant where we made our original study we have observations on a large part of its stored make of 1912. Twenty-five per cent of this kept poorly.

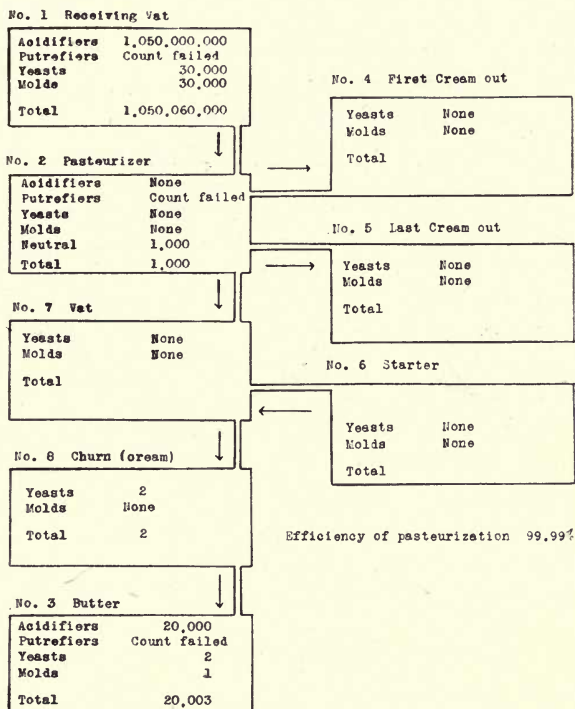


Fig. 3.—Bacteriological Survey of the — Creamery, May 15, 1917. By F. W. Bouska.

The commercial storage referred to above is another case and there 43 per cent of the butter did not keep. In 1915 at the original plant 1.75 per cent of the storage spoiled. But in 1916 only 1/15 of one per cent did not keep. During this time big improvements in every facility that affects keeping quality were made. There is no way of ascertaining how much credit for keeping quality should be awarded to each facility. All men in any way associated with the making of this butter

concur in giving a fair share of credit to the yeast and mold counts.

The conditions of pasteurization that yield good results by our test are considerably above the usual hygienic and board of health requirements. Moreover, plating a whole c. c. of butter gives a test one hundred times as exacting as plating 1/100 of a c. c. Pasteurized butter yielding a low yeast and mold count therefore has a clean bill of health. If butter that purports to be pasteurized contains hundreds of yeasts or molds, we would hesitate to say that the pasteurization that produced it fulfilled hygienic or commercial requirements. Nor could we positively say that it is lawfully entitled to be branded "Pasteurized butter".

We cannot form even a conjecture of the meaning of large or small numbers of yeasts and molds in raw butter. It is not uncommon to find hundreds of yeasts and molds in raw butter that enjoys the very best commercial reputation.

Our investigations and observations cover approximately 153,000,000 pounds of creamery butter.

THE ACTION OF CERTAIN BACTERIA ON THE NITROGENOUS MATERIAL OF SEWAGE

E. G. BIRGE

Edward Grant Birge was born April 24, 1881, at Leipzig, Germany, at which time his father was studying at the University of Leipzig.

Educated in the public schools at Madison, Wisconsin, graduating from the high school in 1899.

Entered the University of Wisconsin in the fall of that year where he took the premedical course, graduating in 1903.

Entered Johns Hopkins, graduating with the M. D. degree in 1907.

For three years was bacteriologist with the Sewerage Commission of the city of Baltimore, going from there to Altoona, Pennsylvania, where he became bacteriologist for the Pennsylvania railroad.

In the fall of 1912 he went to Harvard Medical School as assistant to Dr. Milton J. Rosenau in the department of Hygiene and Preventive Medicine.

In 1914 he went to Florida as State Bacteriologist where he remained until the United States went into the war.

In April, 1917, he volunteered with the Medical Reserve Corps and was given the commission of Captain.

He was sent to Fort Oglethorpe in August of that year where he remained for nearly a year. From there he was sent to Camp Beauregard, Louisiana, and then to Camp Wadsworth, Spartanburg, North Carolina. He received his discharge in August, 1919, going immediately to the University of Iowa as Professor of Bacteriology and State Epidemiologist, which position he filled until his death from influenza on February 4, 1920.

The bio-chemical work which has been done on sewage in the past has been confined almost entirely to the changes taking place in the various forms of filter beds. We have then considerable information concerning bacterial action in the filter beds, but our knowledge of this action in the septic tank is in a more chaotic state. What little we know is the "mass action" of all organisms, bacterial and otherwise, which play a rôle in the preparation of sewage for further treatment.

The primary object of this paper, which must be considered as preliminary, is to determine what certain individual species

of bacteria do chemically when allowed to act on sewage in pure cultures singly or mixed. It was hoped that something could be shown which would justify us in trying to control the bacterial flora in large masses of sewage under treatment. It was also expected that the results would enable us to say what we might expect if certain groups or species of bacteria proved to be present in predominant numbers.

It is reasonable to assume that mere numbers of micro-organisms do not guarantee any definite chemical action. It is only when certain species or groups are predominant that a definite chemical change may be predicted or expected. At the present time we can make no prediction of that sort, because it is not known what bacteria or groups of bacteria are responsible for those changes which are considered desirable.

In the past, work on putrefaction and decomposition has been confined largely to the study of the nitrogenous material, although there are certain other cycles which, as Fuller¹ points out, must be quite as carefully studied. In conformity with past work along this line, it was decided to study the nitrogen cycle.

Clark,² in his report on a somewhat similar investigation done at the Lawrence Experiment Station in Massachusetts, points out that there are five lines of action, i. e., putrefaction, nitrification, de-nitrification, nitrogen liberation, and nitrogen fixation. While undoubtedly all five of the processes go on simultaneously, the process which predominates depends entirely upon conditions present. His work was done in connection with the nitrification in filter beds, but it is reasonable to suppose that much the same thing would hold true for septic tanks, except that the conditions are such, usually, that putrefactive action is more likely to predominate, especially in the one story type of tank.

At the beginning of this work it was assumed that the most likely change in composition would be an increase in the free ammonia content, and a corresponding decrease in those decomposition products represented by the organic nitrogen,

¹ Fuller, *Sewage Disposal*, p. 47.

² Clark, *Report of the Mass. State Board of Health*, p. 244, 1904.

with little or no change in either the nitrites or nitrates. In order to bring about conservation of the higher oxidation products of nitrogen, it is essential that sufficient oxygen be present so that the micro-organism is not forced to reduce these substances to support life. I believe that at the present time it is considered wise to have as much residual oxygen as possible in the septic tank. I found, as I will point out later, that the action of certain bacteria, in fact of nearly all of the particular species studied, was much more dependable under aerobic than anaerobic conditions.

METHODS

The methods used simulated natural conditions as much as possible in laboratory experiments on a small scale. In order to use sewage as a culture medium it is necessary to sterilize it, and any method of sterilization changes the chemical composition of the sample. It is therefore necessary to select that method which produces a minimum change. Chemical methods are naturally out of the question, because of their ineffectiveness in bringing about complete sterility without a great excess of the chemical, which would have an inhibiting action on the bacteria subsequently added. The incompleteness of sterilization by chemical means has been shown by Lederer and Hommon³ in their paper before the American Public Health Association, at the meeting of 1910.

If the gross solid material is filtered out, the sewage can be sterilized by heat, without greatly affecting the chemical composition.

While there is a small difference in composition between the unsterilized and sterilized sewage, the differences in the flasks of sterilized sewage made up from the same large sample are so slight as to be negligible.

The sewage used was filtered through cotton to remove the gross solid material, put into liter flasks and sterilized at 120° C. (15 pounds pressure) in the autoclave for 45 minutes. Two sets of flasks were made up at a time for each series. To

³Lederer and Hommon, *Journal American Public Health Association*, I (New Series), p. 267, 1911.

one set, paraffin oil was added to give anaerobic conditions and one was left without the oil to favor aerobic conditions. In each set there was one flask which acted as a control. The changes in those flasks which were inoculated with bacteria were compared with this control, after making allowances for difference in composition and errors in methods.

In connection with the dissolved oxygen content, the preliminary work brought out several interesting facts. It was found that the heat of sterilization would in itself drive off the greater amount of the dissolved oxygen, but that the flasks which were not treated with paraffin oil would pick up oxygen rapidly, even at the temperature of the incubator, 37.5° C. On the other hand those which had been treated with paraffin oil continually lost what little dissolved oxygen there was left. There is no doubt but that the unsaturated hydrocarbons of the oil extracted the dissolved oxygen, and did it so rapidly that at the end of twenty-four hours there was not, for all practical purposes, any left.

The flasks were inoculated twenty-four hours after sterilization with 1 c. c. of a twenty-four hour bouillon culture of the bacteria to be studied and incubated at 37.5° C. for twenty-four hours. They were then analysed for free ammonia, organic nitrogen, nitrites and nitrates according to the Standard Methods for Sewage Analysis.

Sewage.—The sewage was obtained from the Ward Street Pumping Station of the Metropolitan System of Boston, and was a typical domestic sewage. The district served by this pumping station was mainly residential. There was probably a small amount of manufacturing wastes and some storm water. Care was taken to obtain the samples at the same time each day, so that the sewage would have a similar composition throughout the work.

Bacteria.—The bacteria chosen for the work were not isolated from the sewage itself. It was thought better to use bacteria of known stock, typical of those groups which are normally found in sewage. For this reason *B. coli*, *B. cloacae*, *B. pyocyaneus*, *B. proteus*, *B. mesentericus var. ruber* and *B. subtilis* were selected. All of these have been reported as

having been isolated from or are representative of groups normally occurring in sewage.

The bio-chemistry of these bacteria, when grown on artificial media, shows that they possess characteristics which, theoretically at least, should be of value in breaking down a complex organic substance such as sewage. The only exception to this statement is possibly the power of *B. coli* to reduce nitrates. It will also be recalled that all of them are facultative anaerobes.

The action of the bacteria when grown in mixed cultures brought out two very interesting points. One was the behavior of *B. coli*. Invariably in the anaerobic flasks it was completely overgrown in the twenty-four hour period. Under aerobic conditions it overgrew *B. pyocyaneus*, but was overgrown by other bacteria.

The other fact was that both *B. proteus* and *B. subtilis* overgrew the other bacteria in the proportion of three to one under all conditions. When these two were grown together they about balanced each other at the end of the twenty-four hour period.

THE ACTION OF PURE CULTURES

The action of pure cultures was studied under two conditions of temperature, at 37.5° C. and at room temperature. A summary of the results follows:

B. coli.—There was a marked tendency to decrease the nitrites and nitrates, with a very decided decrease in the organic nitrogen. This was more marked in the anaerobic flasks than the aerobic flasks, and was much more vigorous at 37.5° C. There was very little action on the free ammonia, although the general tendency was to decrease it.

It was noted that the correction for the amount of organic nitrogen added to the sample in the bouillon tended to give, in all of the experiments, a reduction of the organic nitrogen. This was studied in the hope that some intermediate decomposition products might be found to account for it. At the present time I am unable to explain it in any other way than

that the standard methods may tend to give results too low, and that possibly some of the nitrogen is liberated.

B. cloacae.—In general it may be said that this micro-organism did not materially differ from *B. coli*. The greatest action was on the nitrites and nitrates, which were decreased markedly in both the anaerobic and aerobic flasks. There was also a decided tendency to decrease the amount of free ammonia, although this was not constant.

B. pyocyaneus.—At 37.5° C. the action was irregular. At times the nitrites and nitrates were increased and under apparently the same conditions they were decreased. The same holds true for the free ammonia and organic nitrogen. There was nothing to indicate that the oxygen content of the samples had any effect on the action.

At room temperature the action on the free ammonia and organic nitrogen had the same irregular tendencies that were noted above.

This irregularity was also noted in the aerobic flasks in connection with the nitrites and nitrates. In the anaerobic flasks the nitrates were decreased with an increase in the nitrites. This increase and decrease nearly balanced.

B. proteus.—This also showed a marked irregularity in its action at 37.5° C. At room temperature the action was more constant, the free ammonia being increased with a corresponding decrease in the organic nitrogen. The nitrates were increased with a corresponding decrease in the nitrites. This activity was more marked in the anaerobic than in the aerobic flasks, and I believe that with this micro-organism, better results can be obtained under anaerobic, or nearly anaerobic conditions, than where the oxygen content is relatively high.

B. mesentericus var. ruber.—This has nearly the same characteristics as the previous micro-organism, the action being irregular in the experiments conducted at 37.5° C. while those at room temperature were more constant, especially under anaerobic conditions. At the lower temperature the free ammonia was increased and the organic nitrogen was decreased. The nitrites were, in the majority of the experiments, increased, with no changes noted in the nitrates. Under

aerobic conditions at room temperature, both the nitrites and nitrates were increased, while the organic nitrogen was decreased. These changes did not balance, leaving a large deficit in the organic nitrogen which has not been satisfactorily accounted for.

B. subtilis.—This micro-organism gave really consistent results. They were obtained under aerobic conditions and confined themselves to the free ammonia and the organic nitrogen. There was in all of the experiments an increase in the free ammonia with a decrease in the organic nitrogen that practically balanced it. This activity was relatively very great, amounting to as much as eight parts per million at 37.5° C. and two parts per million at room temperature. In regard to the nitrites and nitrates, the tendency was more often to decrease than to increase them, but this action was not constant.

Under anaerobic conditions the action of the bacteria was regular at 37.5° C., both the free ammonia and the organic nitrogen being decidedly decreased with an increase in the nitrites and nitrates, the latter being very decided. At room temperature the action was not so marked and in general followed that of the aerobic conditions, showing an increase in the free ammonia and a decrease in the organic nitrogen.

From the results which were obtained, I believe that this micro-organism and the entire group of allied micro-organisms will bear much careful study in their relation to the bacterial processes in the purification of sewage.

The action of mixed cultures.—I have already pointed out that in mixed cultures *B. coli* was completely overgrown in the anaerobic flasks, and was overgrown by all of the bacteria in the aerobic flasks, with the exception of *B. pyocyaneus*. Also that *B. subtilis* and *B. proteus (vulgarus)* overgrew the other bacteria in both sets of flasks. When grown together they about balanced each other.

The fact that the chemical action very closely followed the predominating bacteria was extremely interesting. The action was in certain cases, as I will point out later, very different in the aerobic and anaerobic flasks with the same

bacteria. This difference was particularly noticeable when *B. subtilis* and *B. coli* were grown with other bacteria.

It was to be expected that the chemical results of these experiments would follow in a general way the influence of the predominating bacteria, although the action of the other organisms was at times apparent. The time of incubation was in some cases evidently too short for the predominating bacterium to manifest itself chemically. This was plainly evident where more than two bacteria were inoculated into one flask. In these cases also it is to be noted that *B. coli* dropped out of sight in twenty-four hours in both sets of flasks. In those flasks where all of the bacteria were grown together the overgrowth of *B. proteus* and *B. subtilis* was apparent, but it was not sufficient to markedly affect the chemical results. A summary of the results follows:

B. coli and B. cloacae.—I have pointed out previously that these two organisms have almost the same action on sewage. The results when grown together are quite different from what might be expected, especially in the aerobic flasks. Here the free ammonia was increased with a corresponding decrease in the organic nitrogen. The nitrites were also decreased. No changes were noted in the nitrate content. Apparently the presence of *B. coli* was a decided advantage in the early part of the incubation period, since it was greatly overgrown in twenty-four hours.

The overgrowth in the anaerobic flasks was probably much quicker than in the aerobic flasks, and was much more complete. The chemical changes noted in this set of flasks are those of a pure culture of *B. cloacae*, the only exception being that neither the nitrites or nitrates were attacked.

B. coli and B. mesentericus var. ruber.—The changes here are those due almost entirely to *B. mesentericus var. ruber*, especially in the anaerobic flasks.

In the aerobic flasks the small number of *B. coli* present affected the results so that they were very irregular.

B. coli and B. pyocyaneus.—In spite of the fact that *B. coli* overgrew *B. pyocyaneus*, there were no changes which could be attributed to either micro-organism. Evidently *B.*

coli established itself so late in the incubation period that the little chemical effect it might have had was not apparent.

B. coli and B. proteus.—In pure culture both of these bacteria had a slight and irregular chemical action. However, when grown together, the changes in composition were regular and consistent. In both the aerobic and anaerobic flasks there was an increase in the free ammonia and a decrease in the organic nitrogen. No changes were noted in either the nitrites or nitrates.

B. coli and B. subtilis.—In the aerobic flasks the changes were those of a pure culture of *B. subtilis*, i. e., an increase in the free ammonia and a corresponding decrease in the organic nitrogen. Except in one case where there was a slight increase in the nitrites, neither the nitrites or nitrates showed any change.

As I have noted, neither of the bacteria has a regular chemical action at 37.5° C. under aerobic conditions. However, when grown together there is an increase in the free ammonia and a decided decrease in the organic nitrogen. This increase and decrease balanced each other.

Neither the nitrites nor nitrates were affected in any way.

Just what the explanation for this condition is, I am unable to say at the present time, as *B. coli* was completely overgrown.

B. subtilis and B. cloacae.—In the aerobic flasks, in which *B. subtilis* markedly overgrew *B. cloacae*, the chemical changes were typical of *B. subtilis*. The free ammonia was increased with corresponding decrease in the organic nitrogen. The nitrates were decreased with an increase in the nitrites.

In the anaerobic flasks where the overgrowth of *B. subtilis* was not apparent, the chemical changes were in the main those of *B. cloacae*, differing from those of the pure culture in the tendency towards increasing the nitrites and nitrates.

B. subtilis and B. mesentericus var. ruber.—In the aerobic flasks the action was irregular, resembling that of *B. mesentericus var. ruber*. Both the nitrites and nitrates were increased.

In the anaerobic flasks the free ammonia was increased with a corresponding decrease in the organic nitrogen and

nitrites. The nitrates were unaffected. This, except for the decrease in the nitrites, follows very closely what was found under similar conditions for *B. mesentericus var. ruber* in pure cultures. The cultures, however, showed a considerable overgrowth of *B. subtilis*.

B. subtilis and B. pyocyaneus.—In both sets of flasks the bacteria grew practically equally well.

In the aerobic flasks the free ammonia and the organic nitrogen were decreased, while the nitrites and nitrates were both increased.

In the anaerobic flasks the free ammonia was increased with a decrease in the organic nitrogen and nitrites. The nitrates were unaffected.

B. cloacae and B. mesentericus var. ruber.—*B. mesentericus var. ruber* overgrew *B. cloacae* in both sets of flasks.

In the aerobic flasks the chemical action was more irregular than was found for *B. mesentericus var. ruber* in pure culture, but in general coincided very closely to it. The most marked effect was on the nitrites and nitrates. The nitrates were decreased with an increase in the nitrites.

In the anaerobic flasks the action was much more typical of *B. mesentericus var. ruber*, the free ammonia being increased with a decrease in the organic nitrogen. The nitrates were decreased and the nitrites were increased.

B. cloacae and B. proteus.—The action of these bacteria was very irregular and typical of neither. This was true for both the anaerobic and aerobic flasks.

The nitrites were generally increased. In one experiment all of the nitrogen was reduced in amount.

B. proteus and B. mesentericus var. ruber.—In the anaerobic flasks the tendency was to decrease both the free ammonia and the organic nitrogen, while the nitrites and nitrates were increased. These results follow those obtained from *B. proteus* more closely than *B. mesentericus var. ruber*.

The cultures showed a slight overgrowth of *B. proteus* in the anaerobic flasks.

B. pyocyaneus, B. proteus, B. pyocyaneus and B. mesentericus var. ruber.—Neither of these gave results in either set of flasks.

The chemical results in those experiments in which four of the bacteria were inoculated into one flask were very irregular and disappointing. The time of incubation was apparently insufficient for any one or any combination of the bacteria to establish themselves chemically.

In the experiments in which all of the bacteria were inoculated into one flask there was but one change worthy of note. That was the increase in the nitrates in all of the experiments. This was found under both aerobic and anaerobic conditions,—amounting in one instance to six parts per million. There was a decided decrease in the rest of the nitrogen present.

SUMMARY AND CONCLUSIONS

Certain bacteria have been studied to determine their effect upon sewage. Those selected were *B. coli*, *B. cloacae*, *B. pyocyaneus*, *B. vulgaris*, *B. mesentericus var. ruber*, and *B. subtilis*. They were selected because they represent aerobic types which are found frequently or constantly in sewage. The effects of these bacteria were studied in fresh sewage, filtered and sterilized by heat at 120° C. (15 pounds pressure) for forty-five minutes. They were studied under aerobic and anaerobic conditions, also in pure and in mixed cultures. Particular attention was paid to the changes in free ammonia, organic nitrogen, nitrites and nitrates.

Bacteriologically it was shown that *B. coli* was completely overgrown under anaerobic conditions in the twenty-four hour period. Under aerobic conditions it was able to overgrow *B. pyocyaneus*, but was overgrown by the rest of the bacteria studied.

When grown in pure cultures, with the exception of *B. proteus*, the bacteria gave more constant results under aerobic than anaerobic conditions.

B. subtilis showed a marked ammonifying power throughout the work under aerobic conditions. Under anaerobic conditions it regularly decreased the free ammonia and organic nitrogen content, increasing the nitrites and nitrates, especially the latter.

B. coli and *B. cloacae* had a decided reducing action on both the nitrites and nitrates. Under some conditions, as yet undetermined, they reduced the free ammonia content also.

B. proteus had a considerable ammonifying power under anaerobic conditions. This was very slight under aerobic conditions, and was more constant at room temperature than at 37.5° C.

The action of *B. pyocyaneus* and *B. mesentericus var. ruber* was irregular under both aerobic and anaerobic conditions.

The experiments with mixed cultures showed that the chemical changes followed very closely those of the predominant bacteria in pure culture. Those experiments which did not follow this rule, and in which there was a decided predominance of one micro-organism, showed that the bacteria had become predominant too late in the incubation period to effect a chemical change.

B. coli and *B. cloacae*, *B. coli* and *B. proteus* gave more constant results when grown in mixed than when grown in pure cultures. These were the only instances of an apparently true symbiotic relationship.

The results of the experiments in which more than two bacteria were grown in mixed culture showed that the incubation period, twenty-four hours, was too short to allow any one micro-organism of any group of bacteria to establish itself chemically.

The work has shown that we will be able to predict what the changes in the chemical composition of sewage are going to be if a group or species of bacteria are predominant.

The results of this work would certainly not justify us in attempting to control the bacterial flora of the septic tanks. However, I am strongly convinced that in the future this course will be attempted.

I again wish to emphasize the results obtained from *B. subtilis*. Everything indicates that this organism may be made to play a most important rôle in the treatment of sewage.

THE DETECTION OF PASTEURIZED MILK

W. D. FROST

The necessity for pasteurizing all milk destined to be used as human food is becoming more and more firmly established as the dangers from the use of raw milk are more generally recognized. But in order to further safeguard the public health it is necessary to control the methods of pasteurization.

The range of temperatures permissible in pasteurization is very narrow. In order to render a milk safe it must be heated above the thermal death point of *Bacillus tuberculosis*. Only a few degrees above this necessary temperature the physical properties of milk are altered. The cream line is changed and a "cooked taste" may be acquired. Both of these changes lessen the commercial value of the milk. Hence there is constant temptation for the milk dealer to underheat his milk. Public health authorities must therefore be constantly on the alert to prevent the sale of underheated or improperly pasteurized milk.

How determine whether or not milk has been properly pasteurized? So far as I know the milk analyst is helpless unless he makes use of the method discussed here.

It is true, of course, that various methods have been suggested. One of these proposes to regulate the temperature and time of holding in pasteurization. To this end certain municipalities require the use of automatic thermoregulators and recorders on all pasteurizing apparatus. A bacteriological test tells whether or not a milk is high or low in bacteria, but cannot always, by any means, indicate the thoroughness of the process of pasteurization. Other tests have been advocated which depend upon the changes which the protein undergoes in heated milk. These have not proved applicable in practice. Still others have been suggested which depend on the presence of oxidizing enzymes in milk. Of these,

Storch's test is generally regarded as the most satisfactory. This test, however, as is well known, can be used only on milk heated from 78° to 80° C. (172.4° to 176° F.). It cannot, therefore, be applied to milk heated to the temperature employed for pasteurization in this country now. A microscopic test was devised by me and described in a paper by Frost and Ravenel in 1911.¹ Two features of this method interfered with its usefulness. One was the difficult technic and the other was the fact that the stain safranin clotted the milk unless added with the greatest care. So far it has been possible to avoid this danger only by diluting the stain, and in this case the action of the dye is largely neutralized.

In 1915 I proposed the method under discussion in this article in a preliminary paper.² This was described somewhat more in detail in a paper read before the International Milk Dealers' Association in October, 1916.³ The practical results that may be obtained by its use are described in a paper by Miss Moore and me.⁴

It is here proposed to discuss this method in a somewhat more adequate way.

BODY CELLS IN MILK

There occur in cow's milk under all conditions a variable number of body cells or leucocytes. There are at least two kinds: the mononuclear and the polymorphonuclear varieties. The latter ones are of chief interest here. Whether or not these cells are leucocytes from the blood which have escaped through the gland walls or are cells from the walls of the mammary gland, given off at the time of milking, is a question on which histologists are not agreed.

¹ Frost and Ravenel, *A Microscopic Test for Heated Milk* in *Proc. Am. Assn. Med. Milk Commissions*, p. 127, 1911.

² W. D. Frost, *A Microscopic Test for Pasteurized Milk* in *Jour. Am. Med. Assn.*, 44, pp. 821-822, March 6, 1915.

³ W. D. Frost, *Milk Dealer*, p. 84, Dec., 1916.

⁴ W. D. Frost and Genevieve D. Moore, *The Cellular Test for Pasteurized Milk* in *Dairy Science*.

Lacking definite evidence to the contrary and in keeping with common practice, these cells will be referred to as leucocytes. The variety which is of service in this study is histologically described as follows:

Polymorphonuclear or polynuclear leucocytes constitute the form more frequently encountered (in normal blood), and probably represent the fully developed condition of the white blood-cell. These elements are distinguished by the variously distorted nucleus, which, being fairly rich in chromatin, presents a striking figure in stained preparations. The nuclei appear imperfectly separated into variously disposed segments, so that they recall the letters O, S, U, V, Z, etc. The segments usually retain connection by delicate threads of chromatin; exceptionally these bridges become broken, in which case the term polynuclear is appropriate. Occasionally cells may be observed containing granules which stain deeply with eosin. Such eosinophilic leucocytes probably represent the final phase of development.⁵

PRINCIPLES INVOLVED

Wet process.—In attempting to differentiate between raw and pasteurized milk, I have⁶ shown that when certain stains are put into milk and allowed to act *wet*, there is a distinct difference in the way in which the stain acts on the cells in the pasteurized milk and on the cells in the raw milk.

When the proper amount of stain is used, the cells in the raw milk are not stained at all, while the nuclei of the cells in the pasteurized milk are well stained. If the stain should be too concentrated, the nuclei of the cells in the raw milk may be stained, but only slightly, while those in the pasteurized milk are always deeply stained.

The staining of the pasteurized cells and the failure to stain the cells in the raw milk is to be explained on the theory that the heat of pasteurization is sufficient to “fix” (in the histological sense) the protoplasm of the leucocytes and thus make staining possible.

Brief description.—The method may be briefly described as follows: The milk to be tested is mixed with an equal

⁵ Pierson, *Textbook of Normal Histology*, sixth edition, p. 106.

⁶ *Loc. cit.*

quantity of a special methylene blue stain in a specially prepared centrifuge tube. The stain and milk are thoroughly mixed and allowed to stand for at least ten minutes. The sediment is thrown onto the cork by whirling the tube in a centrifuge. The sediment is carefully spread on a microscopical glass slide. This smear is allowed to air dry without coming in contact with water at any time. When dry it is examined under the microscope with a low power and later with the oil immersion objective.

DETAILS OF METHOD

Preparation of stain.—Various stains have been tested out: safranin, methylene green, dahlia, thionine and methylene blue. On the whole methylene blue seems to be the stain best adapted to the test. Practically all of the work has been done with the German dye (Gruebler), but several American made dyes have been tested recently and have proved to be entirely satisfactory.

At first quite concentrated solutions were used. Small quantities of these were added to larger volumes of milk. While this method produced satisfactory results, there was a tendency for these dyes to curdle the milk, and when this happened the test was worthless.

I have found gradually that this danger can be overcome by using a more dilute stain and adding larger proportions of it to the milk. It is quite essential to the proper working of the test that the right amount of the dye be added to the milk. Fortunately this is indicated by the color of the milk. When methylene blue in any concentration is gradually added to milk the color becomes a deeper and deeper blue, until quite suddenly it becomes a "cadet blue". This deep blue color seems necessary to the proper working of the test. It may be arrived at in any way, but there is less danger of causing a precipitation if the dye is added to the milk in a weak solution.

To make the solution needed, 1.5 grams of the methylene blue powder is added to a liter of distilled water. I have

usually allowed this to stand in the 37° C. incubator for several hours (over night) and then filtered it through paper. Such a stain when added to milk, part for part, should produce a "cadet blue". Some powdered dyes may need to be used in greater concentration, others, especially the medicinal methylene blue, in smaller quantities.

This solution seems to be quite permanent and most of my evidence indicates that it keeps indefinitely.

Mixing stain and milk.—The addition of the dye to the milk can be done in any convenient way. At first I put the milk in a flask or beaker and added concentrated stain slowly while the milk was kept moving by a rotary motion of the vessel. When a weaker solution as recommended above is used, the method of combining them is quite immaterial except that it is never safe to drop the milk into the dye. It is better to add the dye to the milk. The mixing should be thoroughly done.

Time of action of stain.—The action of the stain requires several minutes and it has been my practice to mix the stain with the milk and then allow it to act for ten or fifteen minutes before it is centrifuged. A longer action up to one hour is not injurious, but if the stain is allowed to stand too long in contact with the milk the cells of the raw milk may take on the stain, but even under these conditions a careful study of such cells would enable one to recognize the difference between them and the cells of properly pasteurized milk.

Centrifugation.—Any form of centrifuge tube can be used for collecting the sediment. The most convenient are those which have a rubber stopper at the lower end because of the ease with which the sediment can be removed. At first I used the Stewart-Slack tubes, but lately I have made a larger tube of the same style. In this way I always get an abundance of cells for examination, and avoid the use of a special head on the centrifuge. The tubes are made from three-eighth inch glass tubing and are four inches long. One end is plugged with a rubber stopper (No. 00), the other is left open. These tubes can be put into the ordinary head of any centrifuge. The centrifuge should be run long enough

and at sufficiently high speed to throw down most of the leucocytes without injuring them, e. g., 2,000 r. p. m. for ten minutes.

Spreading of sediment.—When the tubes are taken out of the centrifuge, the fat layer at the top of the tube is loosened, the milk and stain mixture poured out, the cork removed, and the sediment on it carefully spread on a glass slide, as one would make a blood smear. It must always be borne in mind, however, that water cannot be used to spread the smear, as this violates the principles involved and invariably causes the cell nuclei to take up the stain, even in raw milk.

Microscopical examination of smears.—The smears are first examined with the low power (16 mm. lens). In the raw milks the background, or the entire microscopic field, is stained blue, the depth of the stain depending upon the thickness of the film: in this blue background appear numerous clear areas. The smaller of these are fat globules. The larger ones may be large fat globules or clusters of the same, but usually they are the leucocytes of the milk. Occasionally there are the deeply stained mononuclear leucocytes. The general impression one gets from these preparations is a blue field, which tends to be uniform, and in which there are a number of holes or clear places.

The smears of the pasteurized milk, on the other hand, do not have the background as deeply stained as do the smears from the raw milk, although in thick portions it may be quite blue. The nuclei of the leucocytes here are always stained and their color is deeper than that of the background. The area immediately surrounding the cells usually takes the stain deeply and shades off into the color of the background, forming what I have called a "dark halo".

Because of the lighter color of the field, the fat globules do not stand out as they do in the raw milk. The most prominent objects here are the leucocytes with their darkly stained nuclei.

The cells are noticeably smaller in the pasteurized than they are in the raw milk.

Even a cursory examination of the two smears shows a distinct and easily recognizable difference between the smear from the heated milk and that from the raw milk.

To examine the cells more closely, the oil immersion objective is used. The oil may be put directly onto the dried smear, or the preparation may be mounted in Canada balsam under a cover glass.

The polymorphonuclear leucocytes in the raw milk, under the high powers of the microscope, are practically all colorless, but with some experience it is not difficult to recognize them. Usually they are quite regular in outline and large, i. e., about twelve microns in diameter. The nuclear material, if differentiated at all, is poorly defined and if stained it is of a light greenish-blue color. Occasionally there are leucocytes in raw milks, and in some more than others, which take the stain. Whether or not these are dead cells has not been determined. Even if quite deeply stained the leucocytes in raw milk are distinctly different from those in pasteurized milk, in that the nuclear material is more spread out and less densely stained. The nuclei of the raw cells are usually integral with a definite isthmus connecting the lobes.

The leucocytes from properly pasteurized milks have their nuclei deeply stained, and the different portions are frequently rounded up into definite fragments so that the cells appear to be polynuclear rather than polymorphonuclear. They average about seven microns in size. The depth of the stain and the amount of shrinking vary somewhat with the degree of heat applied.

By way of summary it may be repeated that :

The effect of heat on the leucocytes, so far as this test is concerned, is twofold. It alters the shape and size of the cells, and changes their staining reactions. The shape of the cell is probably gradually changed as the degree of heat increases, and the shrinking begins to appear at a lower temperature than that used for pasteurization; but the "fixing" of the nuclear material, which makes possible the absorption of the stain, seems to take place definitely at practically the same temperature as that nec-

essary for the pasteurization of milk, namely, at from 60 to 63°C. (140-145°F.)⁷

INTERPRETATION OF RESULTS

Illustrations: The results of this method of staining are shown in Plates I and II, Figures 1 to 26. These drawings are all made under an oil immersion lens and by means of the camera lucida.

Figures 1 to 4 show cells in the raw milk. Here the background is stained, while the cells remain clear or unstained. In Fig. 5 the milk was heated to 58° C. (137° F.) for 20 minutes and in Fig. 6 to 60° C. (140° F.). Here the polymorphonuclear leucocytes have their nuclei lightly stained.

Figures 7, 8, and 9 show the cells in milk that has been pasteurized at 63° C. (145° F.) for 20 minutes. The nuclei are all deeply stained and the segments well rounded off and the fragments discrete. Fig. 10 shows cells from milk heated to 65° C. (150° F.), and Fig. 11 shows cells from milk heated to 70° C. (158° F.). Figures 10 and 12 show the bacteria well stained.

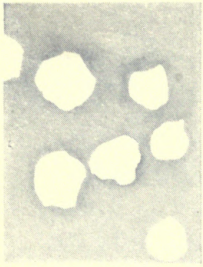
Figures 13 to 18 represent groups of leucocytes selected from various fields to show variations in form, size, and staining reaction at the various temperatures indicated. Except for the raw milk, no attempt has been made to represent the background.

Figures 19 and 20 are the results obtained by applying a counter stain to the preparations obtained by the staining with methylene blue in the usual way. The advantage of the counter stain is that it brings out or differentiates the leucocytes in the raw milk. (The preparations are made in the usual way and when the smears are dry, they are immersed for a few seconds in a 1 per cent solution of orange G in alcohol (95 per cent) and examined after drying without washing.)

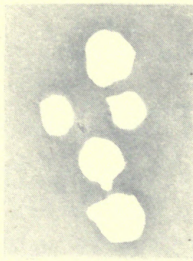
Figures 21 and 22 are from preparations made by a method suggested by Traum.⁸ In this method the cells are separated

⁷ *Loc. cit.*

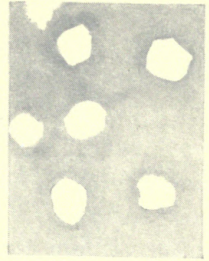
⁸ J. Traum, *Laboratory Methods to Improve Milk in Amer. Jour. Vet. Med.* 10, 717, 1915.



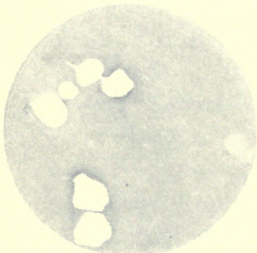
1. Raw.



2. Raw.



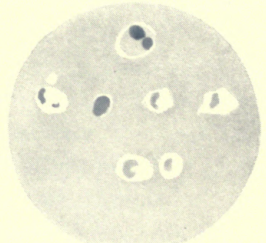
3. Raw.



4. Raw.



5. 58° C.



6. 60° C.



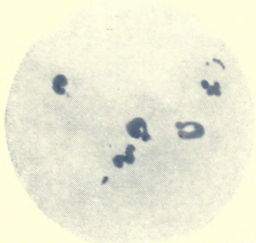
7. 63° C.



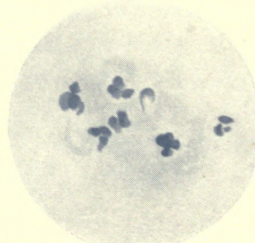
8. 63° C.



9. 63° C.



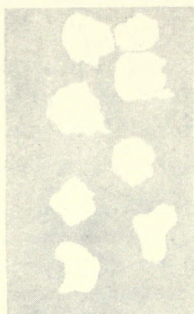
10. 65° C.



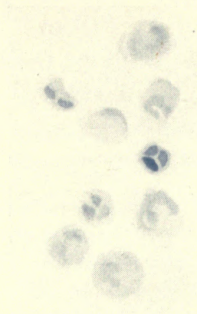
11. 70° C.



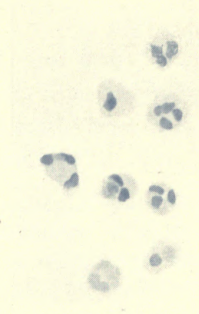
12. 63° C.



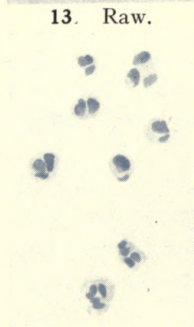
13. Raw.



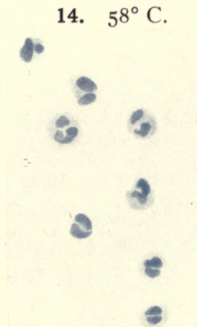
14. 58° C.



15. 60° C.



16. 63° C.



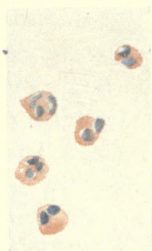
17. 65° C.



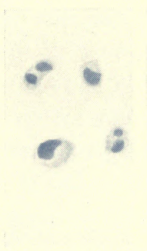
18. 70° C.



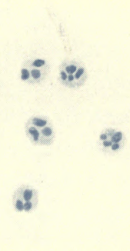
19. Raw.



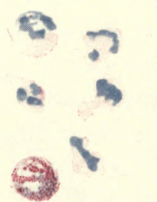
20. 63° C.



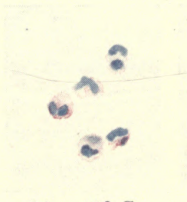
21. Raw.



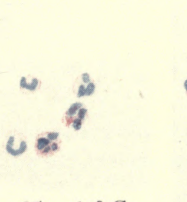
22. 63° C.



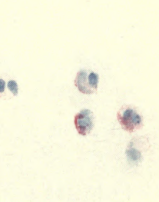
23. Raw.



24. 58° C.



25. 60° C.



26. 63° C.

by centrifugal force, smeared on slides, dried, and fixed with formalin. The staining of this dried, fixed film is done with a one-half saturated aqueous solution of methylene blue. The nuclei of the cells in the raw milk stain well (Fig. 21).

They appear as if drawn out and there is frequently no indication of cell body, the area around the nuclei fusing into the background. If a cell body is observed it has a reticulated appearance. In pasteurized milk, on the other hand, the nuclei of these cells are smaller, more compact, stain deeper, and the cells which are considerably smaller, are outlined by a clear, circular zone around the nucleus. The difference is noticeable when comparing raw milk with milk heated to 60° for 30 minutes and is very marked if the milk has been heated to 63° for 30 minutes.

Figures 23 to 26 represent preparations made from raw milk (23), pasteurized at 58° C. (24), 60° C. (25), and 63° C. (26) and stained with Wright's blood stain. The milk sediment was spread on slides, dried, and then stained in the usual way.

DETAILS OF PROCESS

Stain of background.—The body of the smear, probably the casein or milk serum, takes the stain in a varying degree. In raw milk the background is usually found to be dark and continuous. By this is meant that the entire smear is stained deeper than the nuclei of the leucocytes which are imbedded in it. Usually these are not stained at all. Whether it is actually dark or light depends very evidently upon the thickness of the film and this unfortunately cannot be controlled. In well pasteurized samples the stain of the background is found to be light and variable. By this is meant that the stain in the background is lighter than the nuclei of the contained leucocytes. It is variable in that it is usually darker about the leucocytes, forming a more or less distinct "dark halo" about them.

Stain in cells.—In raw milk the leucocytes are unstained and in the typical preparations appear as clear areas in a dark blue field. They are easily differentiated from the fat globules by their larger size and more or less irregular outline.

Frequently there is a tinge of blue to them so that there is just a suggestion of the nuclear structure of the cell visible. A few cells even in perfectly fresh milk may have their nuclei stained but less densely and compactly than in pasteurized milk. In the pasteurized milk, on the other hand, the nuclei are definitely stained, usually dark blue, while the background is lighter. The difference is striking and perfectly characteristic.

Outline of leucocytes.—The outline of the cells in raw milk is irregular. This is probably due to protrusions or pseudopodia although certain authors have hesitated to accept these cells as leucocytes because they do not show evidence of amœboid movement. The irregular outlines, as shown in the figures of raw milk, are noticeable, however, especially in Figures 4 and 13.

In the heated milk the outline of the cells usually appears more regular. Under the influence of heat the cells have rounded up. There is no constant difference between these cells under the two conditions, however, and some observers have even regarded the heated cells as the more irregular. The irregularity of the heated cells may, however, be due to quite a different cause, namely, shrinkage.

Nuclear fragments.—Only the polymorphonucleated cells are of value in this test. In a raw cell it is usual for the nucleus to be single but variously shaped (or polymorpho), while in the heated milks there is a pronounced tendency for the nuclei to become separated and thus the cells become polynucleated. The nucleus or its fragments are much more compact and demarkated in the heated than in the raw samples.

Size of the leucocytes.—As already pointed out, the heat of pasteurization not only affects the staining reaction of the leucocytes but it produces a profound change in their size. In raw milk these cells are frequently 10 or 12 microns in diameter and nearly always above 7.5 microns. In properly pasteurized milks they are very much smaller, usually less than 7.5 microns.

This marked reduction in size is no doubt due to the heat of pasteurization which shrinks the cells. The decrease in size is a progressive one, depending upon the amount of heat

used. When a temperature of 63° C. (145° F.) is maintained, the average diameter of the cells is only slightly greater than half the diameter of the cells in raw milk. It is evident from Figure 14 that the shrinking is less at 60° C. (140° F.) than it is at 63° C. The shrinking continues with the increase of heat. See Figures 15 to 18.

The table below gives the comparative size of the milk cells in raw and pasteurized samples. All of the figures given are the average of at least twenty-five cells.

SIZE OF MILK CELLS

Raw	Pasteurized
11.2	5.6
9.8	6.0
10.4	7.5
10.9	7.0
12.9	6.4
11.9	7.2
11.8	6.8
11.0	6.2
13.0	5.7
12.0	6.0
—	—
Grand average 11.4	6.4

Temperature at which change takes place.—The staining reaction typical of properly pasteurized milk occurs only when milk has been subjected to a particular temperature for a definite length of time. With the time constant, say thirty minutes, no change takes place in the leucocytes until the temperature nears 60° C. It is true that the nuclei begin to absorb the dye at temperatures from 56 to 60° C. At first the nuclei stain only lightly and there is an almost invariable tendency for them to be greenish in color even at 60° C. It is usually possible to differentiate milk pasteurized at 60° C. from that pasteurized at 63° C. and above, by the way the nuclei take up the stain. It is only when a temperature of 63° C. is reached that the nuclei become deeply stained. Beyond this temperature there is no change in the character of the stain although the nuclear fragments may be more compact and discrete and the size of the cell reduced. The definite

change in staining reaction between the leucocytes of raw and pasteurized milk takes place at the temperature of 63° C.

When milk is subjected to this temperature for varying lengths of time it is found that the definite staining reaction takes place when the temperature is maintained for about twenty minutes. When the time of exposure is eighteen minutes or less, some of the leucocytes do not take the stain in the characteristic way. So it may be stated that the characteristic staining reaction occurs when the milk is subjected to a temperature of 60° to 63° C. for upwards of twenty minutes.

Effect of stain on bacteria.—The heat which fixes the nuclei of the leucocytes for staining curiously renders the bacteria less likely to take the stain. It is only in raw milks then that the bacteria stain well. In well pasteurized milks they stain poorly. Frequently the bacteria take the stain with a varying degree of intensity, but whether this is due to inherent differences in the bacteria or to the effect of the heat on them is undetermined. The reaction of the bacteria to the stain has up to now shown illogical variations, but in general the following conclusions seem warranted. The bacteria in raw milk are always stained, usually well and sometimes very beautifully. In the sediment from properly pasteurized milk examined within a few hours after pasteurization the bacteria are usually invisible. Old pasteurized milk may show the nuclei of the cells well stained and the bacteria also. In this case we assume that the bacteria which stain have been introduced or grown subsequent to the process of pasteurization.

THE MICROSCOPICAL PICTURE OF A PROPERLY PASTEURIZED MILK

When a milk has been stained by the Wet Process described in this paper and the sediment mounted without the addition of water is examined under the microscope, the following picture will be obtained if it has been pasteurized at a temperature of 63° C. (145° F.) or above, and held at that temperature for twenty minutes or more. The nuclei of practically all of the leucocytes are in compact masses. They are well stained. The background is lighter than the nuclei

except in the thickest places. The leucocytes are usually surrounded by a "dark halo". They are small,—not more than 7.5 microns in diameter. If the sample is examined soon after pasteurization or has been kept under conditions which prevent the growth of bacteria, the bacteria will not stain at all or at least variably or indistinctly.

In making this examination the slide is ordinarily first examined under the low power, and the light, uneven distribution of the background and the deeper stained leucocytes are noted if the milk has been properly pasteurized. In a raw milk the background is quite blue in the thicker places and the unstained leucocytes appear as clear areas in it.

The slide would ordinarily next be examined under an oil immersion objective. A few leucocytes would be studied to determine their staining reaction and the condition of the nuclear fragments. The eyepiece should be provided with a micrometer scale and some of the leucocytes measured. The condition of the bacteria will be noted without effort at the same time.

THE USE OF OTHER STAINS

A number of the anilin dyes have been tried out in this test, as for example safranin, thionine, methylene green, dahlia, gentian violet and methylene blue, but none of them appear to possess advantages over methylene blue.

THE INVESTIGATION OF DRINKING WATER SUPPLIES

H. A. WHITTAKER

The question of what constitutes a safe water supply for drinking purposes, and the proper method of determining this fact are subjects that have given sanitarians much concern ever since the discovery of water-borne diseases. Investigators have worked for many years developing and perfecting field and laboratory methods for the detection of unsafe supplies, devising corrective measures to eliminate the dangers found to exist, and preventing the repetition of these errors in new installations. This work has resulted in the discovery of certain general principles concerning the protection of water supplies, and in establishing analytical methods and standards for their control. The application of these principles, methods, and standards to water supply investigations by different health organizations has differed widely throughout the country. The water supply service has often been adapted to the existing health organization rather than the organization being adjusted and equipped properly to handle the work. Some organizations have followed the dangerous practice of depending largely upon an analysis of the water without requiring a detailed field survey by a trained observer to secure accurate information regarding the location, construction, and the management of the supply. A common practice is to leave the field survey of the supply to untrained individuals in the local communities. Data sheets and sampling equipment are often furnished to local authorities and private citizens who collect the field data and water samples on which the safety of the supply is determined. There are several points in connection with this practice that are dangerous to public safety. The field survey, which is one of the most essential parts of the investigation, is placed

in the hands of an untrained observer who is often incompetent to undertake the work. This same unskilled individual is entrusted with the duty of securing samples of water that must be properly collected if satisfactory results are to be obtained. These samples are then shipped to the laboratory and subjected to a most careful examination by a skilled technician when there is little assurance of the accuracy of their collection. This method makes it necessary for the skilled worker to accept facts from an untrained person on the fundamental features of an investigation on which the safety of a water supply is to be judged. The fallacy and danger of such practice is very evident when these points are considered. The importance of thorough investigation work on water supplies is illustrated in the following tables. These tables include six years of investigation work on existing water supplies by the Minnesota State Board of Health.

TABLE I.—WATER SUPPLY INVESTIGATIONS, 1912-1918

Water Supplies Investigated	Results	
	Satisfactory	Unsatisfactory
Number	1,119	
Per cent	100	
	389	730
	34	66

The investigations recorded in Table I represent both surface and underground water supplies from a variety of sources including wells (dug, bored, drilled, driven), springs, lakes, rivers, creeks, etc. This table shows that 1,119 existing water supplies were investigated during the period indicated. Three hundred eighty-nine, or 34 per cent, were shown to be safe and 730, or 66 per cent, were found to be unsafe sources in their existing condition.

TABLE II.—UNSATISFACTORY WATER SUPPLIES, 1912-1918

Unsatisfactory Water Supplies	Shown Unsatisfactory by		
	Field survey and analytical results	Field survey	Analytical results
Number	730		
Per cent	100		
	354	338	38
	49	46	5

Table II includes the unsatisfactory supplies found during the same period. An analysis of this table covering the unsatisfactory supplies shows that 354, or 49 per cent were found to be unsafe by both the field survey and the analytical results; 338, or 46 per cent, by the field survey alone, while the analytical results on the date of the investigation were satisfactory; and 38, or 5 per cent, by the analytical results alone where the field survey did not show the possibilities for pollution and further investigation was required to find the avenues through which pollution could enter the supply. These results show that the field survey was corroborated by the analytical results in 49 per cent of the cases; that the field survey was the only index of danger in 46 per cent; and the analytical results the only indication in 5 per cent. These results demonstrate the importance of thorough field survey work, for had the analytical results been accepted as the only index, 49 per cent of the unsatisfactory supplies would have been approved. It is also true that if the analytical work had been omitted, 5 per cent of the unsatisfactory supplies would have been overlooked.

It is evident from these results that both field and analytical investigations should be made in order to determine the safety of a water supply from a sanitary point of view. It should be appreciated that the use of any method which permits the collection of haphazard information by untrained individuals is exceedingly dangerous.

The field survey should give an accurate idea of the possibilities of present and future pollution. The analytical results should provide information on the sanitary condition of the water at the particular time the investigation is made and it may supply some information of its past history. The field survey and analytical results combined should afford information on which recommendations can be made for the protection or the abandonment of an unsafe supply. The corrective recommendations are frequently very simple and a water supply can be made safe at a relatively small cost.

In order to illustrate the importance of thorough investigation work, especially in regard to the field survey, it may be

of interest to enumerate some of the most common errors that are found associated with water supplies. The errors referred to are briefly summarized as follows:

1) The use of surface waters without treatment, as all such waters must be considered open to contamination. The sanitary policing of such sources is usually impossible or impractical.

2) The installation of water purification plants in localities where underground supplies were available and would have provided a safer source.

3) The installation of water purification plants by local authorities who have little or no understanding of the theoretical and practical side of the treatment nor the difficulties attending the operation of these plants. Many such plants have been installed and the authorities believed them to be equipped with automatic devices which would insure an effluent of good sanitary quality. This impression is erroneous since these plants require the most careful attention by trained operators at all times during the operation.

4) The installation of hypochlorite or liquid chlorine plants to purify water that cannot be properly purified by these chemicals because filtration is required to render it suitable for such treatment.

5) The addition of hypochlorite or liquid chlorine to raw water as it enters a filtration plant rather than to the effluent. This practice often makes very difficult the administration of these chemicals in proper amounts.

6) The failure to keep on hand duplicate parts of important equipment, especially parts of chlorine apparatus that are likely to get out of repair. The lack of such parts may make it necessary to allow untreated water to be discharged into the distribution system.

7) The installation of by-passes around water purification plants by which untreated water can be admitted into the system without passing through the plants.

8) The improper operation of water purification plants by unskilled or inefficient operators.

9) The location of wells, pumping apparatus, exposed suction mains, reservoirs and filters where they will be subject to flooding with surface water.

10) The improper construction of well casings and covers, and the lack of adequate provision for surface drainage to prevent pollution of the well with surface water.

11) The construction of pits around wells at the surface in which all or a part of the pumping equipment is located. This applies especially to drilled wells. It is difficult to prevent surface and waste water from collecting in such pits.

12) The connection of any part of the system with sewers which makes it possible for sewage to back up into wells, well pits, storage reservoirs, etc.

13) The improper construction of underground and surface reservoirs. This includes structures that are not watertight, those where the covers admit surface water and others where the clean-outs are connected with sewers or bodies of polluted water, the surface of which may be raised to an elevation higher than the bottom of the reservoir.

14) The installation of emergency connections which may permit untreated surface water, or water of unknown or questionable sanitary quality, to be admitted to the system. There should be a complete physical separation between water supplies that are safe and those that are known to be unsafe from a sanitary point of view.

Many of these defects have been the cause of epidemics of water-borne diseases, consequently, their sanitary significance is at once apparent. These errors can be prevented in new supplies and corrected in existing supplies by proper health administration carried out by trained workers who have the proper facilities to put such supervision into effect.

CONCLUSION

The purpose of this article is to draw the attention of health authorities and others to the importance of thorough investigation work when the safety of a water supply is to be determined from a sanitary point of view. The investigation should be undertaken by trained workers who understand the

proper application of methods, the interpretation of results, and by those who are competent to make definite recommendations for correcting defects or for the abandonment of a water supply. The use of haphazard methods leads to erroneous opinions or false security which may result in loss of life among the consumers.

THE MILK SUPPLY OF CHICAGO

A. L. AMOTT

SOURCE OF SUPPLY

The city of Chicago is very fortunately situated in that it is located in the heart of one of the most fertile agricultural sections in the United States. No other large city is so favorably situated, with the exception, perhaps, of Milwaukee, where almost identical conditions prevail.

As the city grows and expands, we find the dairy district gradually moving farther from the city limits, to make room for new subdivisions, suburban towns, and to a great extent, truck gardens.

During the years 1907 to 1911, 90% of the milk reaching Chicago was produced within 56 miles of the city. Since that time the milk zone proper has moved out about 25 miles. This has reference to the regular supply in normal times. In case of a shortage in the Chicago milk zone, due to unusual or abnormal conditions, it quite often becomes necessary to go considerably farther, as is the case at the present time, when a small percentage of milk and cream is brought in a distance of 275 miles.

The accompanying map is designed to show the growth and expansion of the milk territory within recent years. Line No. 1 shows the Chicago milk district up to 1915, as authorized and inspected by the Chicago Health Department. Line No. 2 represents the district from 1915 to 1917, which was also inspected by the Health Department. The district included between Lines 2 and 3 is not inspected by the Health Department, although considerable milk is shipped into the city from this territory.

In spite of the abnormal conditions prevailing at the present time, brought on by the war, Chicago is the only large city

getting the bulk of its milk supply within 100 miles. This is all the more remarkable when it is borne in mind that no supply can come from the east on account of Lake Michigan.



Map Showing the Expansion of Milk Territory within Recent Years.

In the district, which from its geographical position, naturally should supply Chicago, there are 42 condenseries, with a combined capacity of 2,447,000 lbs. of milk per day, which divert the flow from its regular channels toward the city. This is one of the contributing causes, if not the only reason, why it has become necessary for the distributors of market milk to seek other sources of supply in Wisconsin, Indiana, Michigan, and to a less degree in Iowa.

AMOUNT OF SUPPLY

There is a total of approximately 17,000 farms producing milk for the city market. According to the most recent milk census taken by the Chicago Health Department in August and September, 1917, there are approximately 800,000 quarts reaching Chicago daily, which includes that used for retail, wholesale and industrial purposes. According to the population of Chicago, this means an average per capita consumption of .6 pint.

The Monthly Crop Report issued at Washington, D. C. credits the entire state of Illinois with 1,057,000 cows in 1917, an increase from 1,047,000 in 1916. It is very evident that Illinois produces many times the amount of milk consumed by Chicago.

Sixty per cent of the total milk of Illinois', ninety per cent of Wisconsin's and seventy-six per cent of Indiana's are made into butter, cheese and condensed milk.

McHenry County, Illinois, is the third largest market milk producing county in the United States, being exceeded by St. Lawrence and Orange Counties, N. Y. Kane County, Illinois, is the second milk producing county in Illinois and fourth in the United States. Kane County borders on Cook County, in which Chicago is located, and McHenry County borders on Kane County, which gives an idea of the proximity of the milk center to the city of Chicago.

It has been determined by the Chicago Health Department that McHenry County, Illinois, and Walworth County, Wisconsin, produce sufficient milk to supply Chicago with its needs. According to the same authority the average number of cows per farm in 1916 was $17\frac{1}{2}$ and in 1917, $14\frac{1}{2}$; also the average production per cow in 1917 was 16 lbs. per day.

The consumption of 800,000 qts. per day by the city of Chicago is a subnormal figure, brought about to a certain extent by the propaganda of economy, and the increased price. Decreased consumption in the city means a surplus in the country which must be conserved. The Chicago zone is practically devoid of butter and cheese factories, making it

necessary for the condenseries to absorb this surplus, which sometimes works a hardship on that industry.

PRODUCTION

At the present time 98 per cent of the milk consumed in Chicago is pasteurized, according to the Chicago Health Department. The other 2 per cent is certified milk. A clause in the city ordinance provides for the sale of raw inspected milk, but none is sold, unless certified milk is included under this head.

All pasteurized milk has been heated in a "holding system" since 1914. A minimum temperature of 140° F. with a minimum time of 20 minutes is required. Previous to 1914, the "flash" system was used almost exclusively.

The largest dealers in the past have advertised the fact that their milk was bottled in the country, and at the present time, this practice is maintained by them, although one of the largest companies has erected and operated a modern plant within the city limits for the past four years.

The plants located in the country are known as "bottling plants". They are situated as a rule near a railroad track. The milk is delivered at these plants, by the farmers themselves, between 6:30 and 11 A. M., depending on the season of the year. In summer deliveries are made early, and in winter late. It is a ruling of the milk distributors that the milk cans be covered with canvas during transit from farm to plant.

The milk is cooled by the farmer to 60° F. or below, usually by placing the cans in a tank of cold running water. After delivery at the plant, the cans are thoroughly washed and steamed before they are returned to the farmers. Immediately after delivery, the milk is pasteurized and cooled to 45° F. The milk is then filled into glass bottles; no single service packages are used to any extent. All milk for the retail trade has been bottled since 1891, and that for the wholesale trade is put up in 8 or 10 gallon cans.

Some of the bottling plants are equipped with separators and separate their own cream for the trade. The skim milk

obtained is utilized for the manufacture of buttermilk,—any surplus is sold back to the farmers or made into casein.

The milk is put up in quarts and pints; cream in $\frac{1}{2}$ pints, and buttermilk in quarts. After bottling, the bottles are placed in crates, covered with cracked ice, and transferred to refrigerator cars for transportation to the city.

The milk cars reach the city not later than midnight of the same day that they are loaded, under normal conditions. They are switched to side-tracks controlled by the milk companies, where they can be unloaded directly into delivery wagons for distribution. There are 47 such stations owned by the larger companies.

The big dealers order their milk from the bottling plants daily, estimating very closely the amounts needed from day to day.

The small dealers receive their milk from farmers designated as "shippers". Their milk is shipped in 8 or 10 gallon cans, in ordinary baggage cars, the shipper paying the freight. These cars are switched to sidings and platforms owned by the railroad companies. From there the cans are hauled by trucks and teams to the various pasteurizing establishments of which there are 305 in the city.

TRANSPORTATION

The city milk supply comes in over 25 different railroads and electric lines. By far the greater portion is hauled by the Chicago & Northwestern R. R. and Chicago, Milwaukee & St. Paul R. R.

The freight tariff now in force is practically the same one in effect during the last 20 years. The rates as between different railroads are practically the same for equal distances.

The milk put up at the bottling plants, or in cans at receiving stations, is brought to the city by what is known as "milk trains". These trains carry nothing but milk, and operate under normal conditions within a radius of 80 or 85 miles of the city. At the present time trains of this character are operating as far as 150 miles from the city, although

milk in small quantities is brought from points 275 miles away. The milk sent in by the "shippers" to the small dealers is handled in baggage cars, attached to the passenger trains under expedited movement.

The railroad companies are not compelled by law to ice the cars, consequently this is done by the milk dealers. The "shipper" is also obliged to furnish his own ice.

The freight rate varies, of course, with the distance, size of can and material in the can, which the following table will illustrate:

	Milk		Milk or Cream	Cream and Condensed Milk	
	8 gal. cans	10 gal. cans	Crate of 12 qts., 24 pts., 48½ pts. or less	8 gal. cans	10 gal. cans
Lake Geneva, Wis.....	18.5c	22.5c	10.5c	23c	26c
Sycamore, Ill.	17.5c	21.5c	10.5c	23c	26c
Woodstock, Ill.	17.0c	21.0c	10c	23c	26c
West Chicago, Ill.....	15.5c	19.5c	9c	19c	21c

All empty bottles, cans, crates and boxes are returned to original point of shipment free of charge. This is also true with sour milk provided it is in the unskimmed condition.

CITY DISTRIBUTION

The milk in Chicago is delivered almost exclusively by means of one horse wagons of which there are about 3,286 in number. Comparatively few, if any, motor driven vehicles deliver milk to the consumer. One reason perhaps is the short haul between houses, which would make the starting and stopping of a motor vehicle a hindrance rather than a help in efficient distribution.

According to information furnished by the Chicago Health Department, there are in the city at the present time, 70 establishments which deliver milk, but buy it already bottled, 205 bottled milk establishments, 2 wholesale only establish-

ments, 5 who deal in bulk milk only, and 21 wholesale bulk establishments.

Of late there has been considerable discussion as to the efficiency of the present system of distribution. Owing to the fact that several dealers may have become firmly established in practically the same section of the city there necessarily must result considerable duplication and criss-crossing of milk routes of these several distributors. Remedies have been suggested, such as the division of the city into zones, allotting certain sections to each dealer, or by the establishment of municipal distributing stations under the direct supervision of the city authorities. Neither proposed system has been practically applied. The establishment of milk depots has been suggested, which would permit the customer calling for his own milk, and eliminate the expense of delivery. The latter scheme in all probability would not meet with success, except perhaps in the poorer districts, due principally to the fact that the public has been educated to the present system and is especially loath to make early morning calls for breakfast milk, if it is possible to have it delivered at the door.

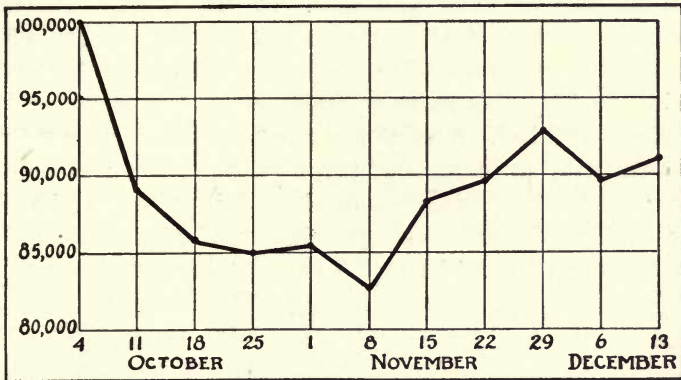
This was one phase of the subject under discussion during a recent milk inquiry conducted by the Food Administration during December, 1917, and January and February, 1918. From testimony brought out by experts on accounting and cost figures, it would appear that any attempt to change the present system of delivery would involve a considerable lapse of time and expenditure of money.

PRICES

During the past two years, there has been considerable agitation and discussion relative to the price of milk to the consumer. During April, 1916, the first raise in price for several years, from 8¢ to 9¢, caused considerable comment among the public and press. When, however, the price was raised by subsequent steps to 13¢ per quart, the consumer in consequence cut down on consumption to a considerable degree.

An effort has been made to determine the relation between increase in price and consumption of milk. The accompany-

ing chart shows a composite graph of several companies' experience during the period of raise in price. The price of milk was raised from 10¢ to 13¢ the first of October, 1917, and lowered to 12¢ per quart on November 1st, 1917, and remained stationary throughout November and December.



The figures on the left of the chart illustrate the number of points sold. A basis of 100,000 points was chosen as an arbitrary figure and the graph shows the decrease on that basis. The figures at the bottom represent the end of weeks. It may be necessary to explain the meaning of the word "point". A point is considered a quart of milk, or two pints of milk, one-half pint of cream or a quart of buttermilk. We may, therefore, assume that the figures on the left represent quarts of milk.

There was a sharp decrease in consumption when the milk was raised from 10¢ to 13¢ per quart. After November 1st consumption gradually began to rise, although it did not approach normal.

From an investigation conducted by several milk companies, it was found that the greater percentage in reduction of consumption of milk due to increase in price took place in the poorer sections of the city. Similar investigations disclosed that a very small percentage resorted to the use of canned milk.

The general decrease in consumption has been estimated at about 20 per cent, although the price of milk has not kept pace with the price of a majority of other important articles of food, during the present time of stress.

Previous to the year 1912, milk was bought from the farmer at a flat rate per hundred weight. In 1912 a bonus was paid for milk averaging 3.8 per cent fat for the month, which marked the beginning of payment for milk on the butter-fat basis. In 1915 this more equitable method of payment was finally established. A price was fixed for milk testing 3.5 per cent fat with an increase of three cents per hundred pounds for every tenth of one per cent above 3.5 per cent and a similar decrease of three cents per hundred pounds for every tenth of one per cent below 3.5 per cent down to 3 per cent.

The following prices have prevailed in the Chicago milk district for the last ten years. From 1915 on the prices are based on 3.5 per cent milk with increase and decrease as noted above. Previous to 1915 a flat rate per hundred pounds was paid, regardless of butterfat tests.

Month	1907-1908	1908-1909	1909-1910	1910-1911	1911-1912	1912-1913	1913-1914	1914-1915	1915-1916	1916-1917	1917-1918
April	1.30	1.35	1.40	1.45	1.30	1.30	1.60	1.60	1.45	1.65	2.40
May	1.00	1.05	1.10	1.20	1.10	1.05	1.40	1.40	1.20	1.45	2.00
June90	.95	.95	1.05	1.00	1.00	1.30	1.25	1.10	1.25	1.60
July	1.00	1.05	1.05	1.20	1.30	1.30	1.50	1.50	1.30	1.55	2.12
August	1.20	1.25	1.25	1.30	1.45	1.45	1.60	1.60	1.55	1.70	2.30
September	1.30	1.35	1.35	1.40	1.45	1.50	1.60	1.60	1.55	1.70	2.30
October	1.55	1.45	1.55	1.70	1.70	1.60	1.85	1.90	1.53	1.90	3.42
November	1.65	1.55	1.70	1.90	1.85	1.75	1.95	2.00	1.65	2.10	3.22
December	1.65	1.55	1.75	2.00	1.90	1.80	2.00	2.00	1.65	2.10	3.22
January	1.65	1.55	1.75	2.00	1.85	1.75	2.00	1.95	1.64	2.05	3.22
February	1.65	1.55	1.70	1.90	1.80	1.70	1.85	1.85	1.54	2.00	3.07
March	1.55	1.45	1.55	1.70	1.65	1.60	1.75	1.75	1.43	1.85	2.90

The prices beginning with November, 1917, were set by the Food Administration during their hearing on milk prices which will be referred to later. It has been customary to

sign a contract for six months with each individual farmer for his supply during these months.

ORGANIZATION

The farmers in the Chicago district are members of the Milk Producers Association. This Association came into prominence about two years ago, although previous to that time local organizations existed of very little importance to the industry as a whole. Since the organization of this Association, numerous strikes or boycotts have been instituted on the part of the farmers over the price question, resulting in partial milk famines in the city. During the winter of 1917-1918, this matter became so serious that the United States Food Administration started an inquiry to determine the cost of production and distribution, in an effort to establish a just and equitable price to the farmer and likewise to the consumer.

The producers refused to abide by the findings of the Commission and instituted a boycott or "strike". The State Food Administrator called another conference establishing a price satisfactory to the producers.

The drivers of the milk delivery wagons are well organized in the Milk Wagon Drivers Union. Their organization regulates the hours of labor and wages of its members. The wages are fixed at \$21.00 and \$22.00 per week, in addition to a commission of six-tenths cents per point over 1,333- $\frac{1}{3}$ points sold per week or about 191 points per day. They are also entitled to two weeks' vacation every year with pay.

There exists also an organization known as the Milk Dealers Association, its purpose being to promote the welfare of the milk industry, especially as regards distribution. Local conditions have made it possible for a firm of milk brokers to develop. This firm serves to establish an equilibrium between supply and demand. If a farmer or group of farmers wish to dispose of their milk at some outlying point, the broker endeavors to find a market and also if a dealer in the city has an over-supply the broker will also make an attempt to remedy this condition.

CERTIFIED MILK

Certified Milk was first introduced in Newark, N. J., originating with Dr. Coit. Gradually other communities adopted this system of producing safe raw milk, and it was finally introduced into Chicago in 1909. At this time the Chicago Medical Society appointed a Medical Milk Commission to certify the milk from certain farms. At the present time eight different farms are sending Certified Milk to Chicago, four located in the northern part of Illinois and four in the southern part of Wisconsin.

MILK INSPECTION

From 1890 to 1900 great stress was laid on the fat side of milk. From 1900 to the present time, the pressure which has been brought to bear by the health authorities has been largely from a sanitary standpoint. About 1908-1909 a milk ordinance was drawn up, which required the pasteurization of milk or the tuberculin testing of all cows producing the milk. The tuberculin test was not or could not be enforced, which made pasteurization obligatory, inasmuch as this part of the ordinance could be enforced. Chicago was the first city of any size in America to demand pasteurization of market milk.

No systematic sanitary inspection of farms was established until 1910. In 1909 the state legislature passed a law providing that in determining the quality of milk nothing should be taken into consideration except the result of the analysis of the milk in the can, which no doubt was a blow to farm inspection. This law was repealed in 1915. The Commissioner of Health has general supervision of the milk inspection. There is also a Chief Food Inspector working under the direction of the Commissioner of Health, and he in turn has supervisors to carry out his orders.

The entire dairy district is divided into fifteen inspection districts, each with one inspector. Three inspections per year per farm are the maximum requirements. If a farmer is found below standard on the first inspection, a re-inspection

is made after a reasonable lapse of time. If no improvement has been made, the milk is excluded until such time as the rules and regulations are complied with.

In addition there are four "field platers", whose duties consist in making bacterial tests on pasteurizing equipments as to efficiency. These men travel from plant to plant continually.

One of the larger companies maintains its own system of inspection. One inspector is located at each plant, and visits each farm delivering milk to that plant at least once every month. Cooperating with these men, this company has on its staff a corps of veterinarians, who have direct charge of the elaborate inspection system of this concern.

The city is also divided into inspection districts, fifteen in number, with one inspector in each district. These men inspect the various milk establishments throughout the city and take chemical and bacterial samples of the various milk products for analysis at the city laboratories.

The city of Chicago maintains a laboratory for the examination of milk and cream samples. During the year 1916, this laboratory examined 44,000 samples of milk and cream chemically, and 10,000 samples bacteriologically.

On the whole the milk situation has improved in Chicago in late years from certain points of view. The Health Department is continually making strenuous efforts to insure a safe supply by eliminating as rapidly as possible establishments that are substandard from a sanitary standpoint.

During the last 15 years, according to Dr. W. A. Evans, former Health Commissioner, the baby death rate has been cut in two. The typhoid death rate in 1917 was 1.6 per thousand, the lowest of any large city in the world.

THE BACTERIOLOGY OF ICE CREAM

B. W. HAMMER

Within the last few years, the growth of the ice cream industry has been phenomenally rapid. Instead of being a product associated with holidays and similar occasions, ice cream has become a common confection and standard dessert that is readily available in even the smallest towns. Its manufacture has grown from a home, or at least a local affair, to an industry of immense magnitude with regular shipments over steam and electric roads to large numbers of dispensing establishments.

The rapid development of the ice cream business has introduced numerous problems, many of which are essentially bacteriological in nature. The enormous demands that are made by the manufacturers on the sweet cream supply, especially during the warm months when shipping is more difficult and when the amount of milk produced is ordinarily decreasing, have necessitated the institution of various shipping procedures. Suitable methods of holding cream have been required, not only to provide for possible heavy demands but also to take care of over-stocks during cool weather. Sharp competition, with the consequent tendency to more efficient methods and a better product, has likewise necessitated procedures that inevitably involve bacteriological considerations, and the public health side has been considered because with the increase in the consumption, the importance of ice cream as a means of spreading disease has been realized and an effort made to decrease the danger from this source.

THE NUMBERS OF BACTERIA IN ICE CREAM

The numbers of bacteria present in ice cream have been studied by many laboratories, particularly those associated with health departments. The cubic centimeter has been

much more frequently used as the basis for determinations than the gram, since it offers a big advantage in the saving of time that is possible in the securing of the portion to be examined. Samples can be conveniently collected in sterile petri dishes or sterile flasks and can then be slowly and carefully melted down and mixed. The thorough mixing of the sample is very essential as it results in the working out of the incorporated air that otherwise interferes with the accurate measuring of the material. Ice cream can be collected from freezers with a sterile spoon or can be allowed to fall directly into a petri dish and can be removed from containers of hardened ice cream by the use of a sterile spoon or a sterile butter trier. Under ordinary conditions it seems desirable to discard the ice cream at the surface of containers, and where a trier is used this can be conveniently done by cutting the drawn core over the edge of the bottom half of a petri dish in such a way as to allow as much of the core as is desired to fall into the dish. A wide range of media has been used in the plating of ice cream but, since milk derivatives are the greatest source of the bacteria in ice cream, there seems to be a very good reason for using the same medium for this product that is used for milk. The incubation conditions most commonly used are 37° C. for 48 hours; Ayers & Johnson¹ found however, that 30° C. for 5 days gave them practically double the count they secured at 37° C. for 48 hours.

The bacterial counts that have been reported in the literature vary widely and are in general surprisingly high. Counts of only a few thousand per c. c. have been occasionally recorded but those running into the hundreds of thousands or millions are much more common. A count of 8,000,000,000² per c. c. was secured in Milwaukee. The average of a series of samples examined in our American cities at the present time is almost certain to run into the millions per c. c. unless unusual conditions prevail. Seasonal differences of consider-

¹ U. S. Dept. of Agr., *Bulletin* 563, p. 12.

² *Bulletin* of the Milwaukee Bureau of Economy and Efficiency, No. 13, p. 35.

able importance have been observed. Ayers and Johnson³ working in Washington, D. C. found that 94 samples of ice cream examined during the summer months showed counts from 120,000 to 510,000,000 with an average of 37,859,907 per c. c., while 91 samples examined during the winter months ranged from 13,000 to 114,000,000 and averaged 10,388,222 per c. c.

The great bulk of the ice cream manufactured in the U. S. is vanilla ice cream and accordingly this is the type that has been dealt with in most of the bacteriological examinations. The Iowa Agricultural Experiment Station⁴ has found that the bacterial content of ice cream other than vanilla is essentially the same as that of vanilla and this finding is substantiated by data published by various laboratories. Water sherbets, on the contrary, contain comparatively small numbers of bacteria; the Iowa Agricultural Experiment Station⁴ found in 17 samples that the counts ranged from 6 to 7,800 per c. c. with no evident relationship between the bacterial count and the flavor. The small numbers of bacteria in water sherbets are undoubtedly due to the fact that cream is not employed in the manufacture, although the acid that is ordinarily present in these products may also play a part in keeping down the count by destroying certain organisms.

Because of the viscosity, the high percentage of solids, and the large part of the solids made up of fat, the distribution of the organisms in melted ice cream is perhaps never as uniform as in a sample of milk. Duplicate determinations made on unbroken containers of ice cream agree very well however. In a comparison of 17 duplicates the Iowa Agricultural Experiment Station⁵ found variations from .79 per cent to 22.73 per cent with an average of 6.75 per cent. On broken containers from which ice cream was being dipped, however, much higher variations were found, one of 137.19 per cent being recorded. Ayers & Johnson⁶ have more re-

³ U. S. Dept. of Agriculture, *Bulletin* 303.

⁴ Unpublished data.

⁵ *Bulletin* 134, p. 298.

⁶ U. S. Dept. of Agriculture, *Bulletin* 563.

cently studied the variation between results secured on the same lot of ice cream and found "the general variation among the samples from each gallon of ice cream was from 20 to 30 per cent". These investigators consider that the method used by them gives results "which indicate that bacteria in commercial ice cream are distributed quite evenly and that an analysis of one sample from a gallon of ice cream gives results which will hold for any other similar sample from the same gallon". The variation in results secured on the same lot of ice cream is one of the objections to a bacterial standard that is advanced by commercial men and it seems that it is entitled to much study although there is ample evidence that a bacterial count is a very good index of the sanitary quality of the material employed and the care used in the manufacture.

TYPES OF BACTERIA IN ICE CREAM

As would be expected from the wide range of sources of contamination, practically all of the common types of bacteria can be found in ice cream, while the prevailing types are determined by various factors. If cream which has been held under conditions favorable for the development of *Bact. lactis acidi* is used, the finished product is practically certain to contain this organism as the predominating type. In the same way, other organisms may be present in excessive numbers if conditions are favorable for their development in any of the materials used or in the utensils employed. Ice cream with a low bacterial count is very likely to have the most of the bacteria of the spore-forming types due to the efficient pasteurization and holding.

Ayers and Johnson⁷ studied the types of bacteria that were present in the ice cream sold at retail in Washington. The group percentages for 71 summer samples were acid-coagulating 49.82 per cent, acid-forming 20.72 per cent, inert 13.98 per cent, alkali-forming 1.86 per cent, and peptonizing 13.62 per cent, and for 28 winter samples acid-coagulating 30.84 per

⁷ U. S. Dept. of Agriculture, *Bulletin* 303.

cent, acid-forming 38.03 per cent, inert 4.81 per cent, alkali-forming 5.42 per cent and peptonizing 20.90 per cent. In general these were high count ice creams and would be expected to show a considerable percentage of acid producing organisms.

The occasional presence of pathogens in ice cream is evident from the cases of disease that have been traced to this product and is to be expected, due to the presence of these types of organisms in cream.

It is evident that ordinarily the types of bacteria found in ice cream are determined practically entirely by the sanitary quality of the materials used and by the handling given and that the range in flora is limited only by the extent of variation that is possible with these.

THE SOURCES OF THE BACTERIA IN ICE CREAM

The bacteria that are present in ice cream come from two main sources: (1) the materials used and (2) outside contamination.

(1) The Materials Used

Cream. The cream used in the manufacture of ice cream is quite likely to carry many bacteria unless pasteurization is used, since ice cream manufacturers ordinarily buy cream under conditions such that they have little, if any, control over the methods of production. Commonly also considerable difficulty is experienced in securing sufficient sweet cream; this is particularly true during the hottest months when the demands on the manufacturer are heavy. Accordingly, the ice cream factories in certain sections are sometimes virtually forced to accept cream with practically no other requirement than that it be sweet and, in certain cases, the ice cream sold is ample evidence that this requirement is not rigidly adhered to. A portion of this cream is shipped without refrigeration facilities and, although pasteurization before shipment is quite common, it inevitably arrives at the ice cream factories with enormous numbers of bacteria.

Under the condition usually prevailing, the modern ice cream manufacturer finds it very necessary to pasteurize the cream which he receives. This is necessary because he has no control over the production and desires to put out a product containing no living pathogens and also because he wishes it to have satisfactory keeping qualities, as it is ordinarily necessary for him to hold it at least a short time. The pasteurization of cream is usually followed by aging in order that the ice cream maker may secure the most desirable yield and texture. The length of the aging depends, under commercial conditions, on the demand for ice cream and on various other factors over which the manufacturer has little or no control. It is usually considered that 48 hours is a sufficiently long aging period, but this may be reduced due to a heavy demand or lengthened as a result of cooler weather. The holding of the cream can be accomplished without an increase in the bacterial content by the use of proper methods. If the pasteurization is followed by prompt cooling to 32° F. or slightly below (e. g. until a half inch layer of cream freezes to the wall of the storage tank) there will be no multiplication of the bacteria for a considerable period of time. From the results secured on the storage of cream at 32° F. by the Iowa Agricultural Experiment Station⁸ and from data secured by various investigators on milk held at this temperature, it seems that even under very favorable conditions cream should not be held for more than a week, since there are certain bacteria that will grow in cream at this comparatively low temperature. For the storage of cream, the use of temperatures which will freeze the cream has been suggested in connection with agitation during the cooling process. Such a procedure would be expected to prevent the separation of the solids that occurs when milk or cream is frozen without agitation and may eventually prove widely practical, particularly when the cream or the ice cream mix is to be homogenized. The use of temperatures below the freezing point would make possible the storage of cream for extended periods of time without an increase in the bacteria contained.

⁸ *Bull.* 134, p. 285.

Ordinarily cream is one of the very important sources of the bacteria in ice cream. It is possible that with a very high grade cream and with some other ingredient of a poor quality the cream may be over-shadowed as a source of the bacteria, but this is rarely, if ever, the case under practical conditions. The extent to which cream may contaminate ice cream varies from the enormous number of bacteria contributed by a raw cream on the verge of souring to the small number added by a high grade cream, properly pasteurized and stored. By the employment of proper pasteurization and storage, the contamination from the cream can be controlled and when the added safety to the consumer is considered there is no justifiable objection to ordinances requiring the pasteurization of cream used in the manufacture of ice cream.

The homogenization of cream is becoming more and more common and is playing a very important part in well regulated ice cream manufacture. Ordinarily homogenization increases the bacterial content of cream, as determined by the plate method, and this is presumably due to a breaking up of the clumps of bacteria just as is the increase due to the clarification of milk. The extent of the increase with homogenization is very variable and undoubtedly depends on the numbers and types of the clumps present. When a homogenizer is used it should be given careful attention or it may become an important source of contamination because of the difficulty of cleaning.

Gelatin. Gelatin may or may not be an important source of the bacteria in ice cream. Gordon⁹ has published the bacterial contents of 20 samples of gelatin where the results ran from 200 to 30,000,000 per c. c. and the Iowa Agricultural Experiment Station¹⁰ studied 5 samples and found the counts varying from 35 to 113,000,000 per gram; from these data it is evident that the bacterial content of gelatin is extremely variable. The heating to which gelatin is subjected in order to get it into solution before its addition to an ice cream mix may destroy some of the bacteria, but it does not seem that

⁹ *Ice Cream Trade Jr.*, p. 33, Jan., 1912.

¹⁰ *Bulletin* 134, p. 286.

this can be very important as the best practice prohibits the use of excessive heat on account of its effect on the solidifying property of the gelatin and the bacteria present in dry gelatin must have considerable resistance or the desiccation to which they have been exposed would have destroyed them. The bacterial flora of gelatin is, to all appearances, made up largely of spore formers, and the number of organisms per gram is presumably closely related to the sanitary quality of the material from which the gelatin was made. High grade gelatin can be secured which has a negligible influence on the bacterial count while certain gelatins may add large numbers of bacteria to the ice cream mix and are to be avoided in the manufacture of the highest quality product.

Sugar. If sugar is properly protected its bacterial content is practically certain to be very low, but it is entirely probable that where sugar is exposed to dust and dirt it may be of some little consequence as a means of contaminating an ice cream mix. The results secured on plates indicate that the great majority of the bacteria are spore-forming types.

Vanilla. The Iowa Agricultural Experiment Station¹¹ has examined five samples of vanilla and found the bacterial content ranging from 200 to 2,300 per c. c. The alcohol present in vanilla extract is undoubtedly responsible, in part at least, for the low bacterial content and moreover this makes it reasonably certain that vanilla is never an important source of the bacteria present in ice cream.

Condensed Milk. Condensed milk, usually the bulk condensed, is used in enormous quantities in many ice cream plants. The product at the time of its use has been found by the Iowa Agricultural Experiment Station¹² to contain large numbers of organisms in certain cases. Excessive numbers of organisms are in part caused by defective shipping and holding methods and in some cases perhaps by a poor quality of material from which the condensed milk is made. Bulk condensed milk has been found in at least one instance to have

¹¹ *Bull.* 134, p. 287.

¹² Unpublished data.

been the cause of large numbers of bacteria in the product of a certain ice cream plant.

(2) Outside Contamination

Air. Air plays a part in the contamination of practically all dairy products although apparently this contamination is less serious than it was at one time supposed to be. In an ice cream plant, as in most dairy establishments, the moisture on the floors, walls and ceiling must tend to materially reduce contamination from this source. With wide-open windows and a dust laden air blowing in, it is conceivable that air contamination might be of considerable consequence but under ordinary conditions this is certainly not true.

Utensils. Utensils undoubtedly are as important a source of the contamination of ice cream as they are of other dairy products, and always should be given the best of care. The Iowa Agricultural Experiment Station¹³ determined the contamination from a freezer by adding sterile water to the machine and then operating the freezer for about the freezing period, after which the bacterial content of the water was determined. Although the freezer was one that was given more than ordinary care, the counts in 5 tests were 3,700; 141,500; 8,050; 1.195; and 300 per c. c. respectively. The increases per c. c. of an ice cream mix would have been considerably more than in the case of the water because it was necessary to use an excessive amount of water to fill the machine since the water would not hold the air beaten into it and thus failed to increase in volume. From these results, it seems evident that the careful ice cream manufacturer should study his process with the idea of reducing the contamination from the various utensils used. Because of its construction the freezer should be given particular attention; small amounts of water should not be allowed to stand in it from one washing until the next freezing because of the probability of the rapid multiplication of bacteria in this. Steam should be used with great care on a freezer, and if the freezing chamber is steamed,

¹³ Unpublished data.

water should be circulated or the freezer slowly cooled in some other way before the cold brine is circulated.

Employees. The direct contamination of dairy products from persons is especially undesirable because of the possibility of the introduction of pathogenic bacteria in this way. The real danger from employees is shown by several typhoid epidemics in which the ice cream causing the infection was contaminated by carriers coming in contact with the material during the manufacturing process. The handling of the cream after pasteurization should be done under the most careful conditions, as should also the handling of the materials that are not heated at all.

CHANGES IN THE NUMBERS OF BACTERIA DURING FREEZING, HARDENING, AND HOLDING

The changes in the numbers of bacteria during the freezing, hardening and holding of the ice cream are of considerable importance if bacterial standards for this product are to be employed.

In the modern ice cream freezers, the ice cream mix is subjected to rather violent agitation in addition to having its temperature brought down below the freezing point of water and it would be expected that significant changes in the numbers of bacteria, as determined by the plate method, might occur. The lowering of the temperature would be expected to destroy certain of the bacteria while the agitation would be expected to break up the clumps of bacteria that might be present. The Iowa Agricultural Experiment Station¹⁴ compared the bacterial content, as determined by the plate method, of the ice cream mix and the frozen ice cream in 51 cases. In 2 cases (4 per cent) there was no change; in 6 cases (11.8 per cent) the freezing lowered the count, the decrease varying from 2 to 31 per cent (Av. 13.0 per cent) while in 43 cases (84.3 per cent) it increased the count from 2 to 227 per cent (Av. 46.3 per cent). A few representative comparisons follow:

¹⁴ Unpublished data.

Trial No.	Bacteria in mix	Bacteria in frozen ice cream later	Per cent change in numbers
	Per c. c.	Per c. c.	
1	19,100,000	24,750,000	+30
2	2,220,000	2,270,000	+ 2
3	30,000	33,500	+12
4	485,000	660,000	+36
5	27,500	45,500	+65
6	5,600,000	4,700,000	-16
7	270,000	285,000	+ 6

From the results obtained it is evident that freezing commonly increases the apparent bacterial content of ice cream and it seems reasonable to assume that this increase is due to the breaking up of the clumps of bacteria as a result of the agitation in the freezer. The lowering of the temperature in all probability causes the destruction of some of the organisms, and in those mixes in which there are few or no clumps the lowering of the temperature may be of greater significance in determining the change in numbers, as a result of the freezing, than is the agitation.

The hardening of ice cream that has just come from the freezer involves a further decrease in temperature and this would ordinarily be expected to have an influence on the bacteria contained. Ice cream usually leaves the freezer at a temperature of 26° to 28° F. and is then reduced to a temperature usually below 10° F. by using either ice and salt or a refrigerated room. The effect of this lowering in temperature has been studied by the Iowa Agricultural Experiment Station¹⁵ in 52 comparisons of the bacterial content of the frozen ice cream before and after hardening. In 1 case (2 per cent) there was no change in numbers during the hardening; in 45 cases (86.5 per cent) there was a decrease, varying from 2 to 75 per cent (Av. 39.1 per cent), while in 6 cases (11.5 per cent) there was an increase varying from 7 to 22 per cent (Av. 13.8 per cent). Illustrative results follow:

¹⁵ Unpublished data.

Trial No.	Bacteria in frozen ice cream	Bacteria in hardened ice cream	Per cent change in numbers
	Per c. e.	Per c. e.	
1	2,270,000	1,660,000	-27
2	2,120,000	1,105,000	-48
3	4,700,000	5,200,000	+11
4	33,500	21,000	-37
5	30,500	30,000	- 2
6	21,000,000	5,320,000	-75
7	268,500	246,000	- 8

In general the hardening of frozen ice cream caused a lowering in the bacterial count, as determined by the plate method, due presumably to the destructive action of the lowered temperature. The slight increase in a small percentage of the cases was in all probability due to experimental error, as a multiplication of the organisms was very improbable and care was taken to prevent contamination.

The influence of holding on the bacterial content of ice cream is of a great deal of importance from the standpoint of bacterial standards. Unless definite information regarding what can be expected to happen to the bacterial content of ice cream during storage is available, bacterial standards cannot justifiably be instituted because holding ice cream undoubtedly has some influence on the numbers of bacteria contained. In 1912, the Iowa Agricultural Experiment Station¹⁶ reported data on a number of samples which showed that in all probability there is a decrease, or else very little change, in the bacterial content of ice cream during storage and pointed out certain objections to results that had been previously presented with the idea of showing an increase in the numbers of bacteria in ice cream during holding. Esten and Mason¹⁷ concluded from their study of 12 samples that when ice cream is kept frozen for periods of at least a month, there is no marked increase or decrease in the bacterial count, as shown by litmus lactose gelatin plate cultures. More recently

¹⁶ Bull. 134.

¹⁷ Bull. 83, Storrs Agricultural Experiment Station.

the Iowa Agricultural Experiment Station¹⁸ has studied 39 samples of ice cream held with ice and salt and 12 samples held in a commercial hardening room and the results secured make it still more evident that as long as ice cream is kept suitably hardened, there is no increase in the number of bacteria, but that on the other hand, in many cases, there is a tendency towards a decrease. The rate and extent of this decrease is in all probability determined by the types of organisms present and is due directly to the low temperature to which the organisms are exposed.

INFLUENCE OF SOFTENING AND REHARDENING ICE CREAM

The softening and rehardening of ice cream occurs frequently in retail establishments when the product is not properly cared for, and accordingly the influence of this procedure on the bacterial content is of considerable importance. The Iowa Agricultural Experiment Station¹⁹ has studied the influence of softening and rehardening in a number of instances and in general the results seem to be quite variable. There are evidently two influences operative in the softening and rehardening of ice cream and these act in opposite ways. The increase in the temperature of the product probably allows of a certain amount of growth, while, on the other hand, the subsequent hardening will have the usual destructive effect on the organisms; whether there will finally be an increase or a decrease will be determined by whether the multiplication of the bacteria or the destructive action of the hardening is the more important.

MANUFACTURE OF ICE CREAM WITH A LOW BACTERIAL COUNT

The use of methods that tend to keep down the bacterial content of ice cream is now widespread among the best commercial ice cream men. They practice pasteurization, the steaming of containers and equipment, careful protection of ingredients and various other procedures with the idea of

¹⁸ Unpublished data.

¹⁹ Unpublished data.

putting a clean and safe product on the market. The Iowa Agricultural Experiment Station²⁰ in 1912 attempted the manufacture of ice cream with a low bacterial count and succeeded, by using cream that had been properly pasteurized and well cared for, other ingredients of high quality, and thoroughly steamed utensils, containers, and freezer, in producing ice cream containing only a comparatively few bacteria. The methods that were employed were selected with particular attention to their practicability and can be used in any properly equipped factory. The bacterial content of some of the lots of ice cream is given below.

Trial No.	Bacterial content of ice cream
1	6,300 per c. c.
2	10,000 "
3	4,200 "

A procedure that has recently been tried out from the standpoint of its influence on the bacterial count is the pasteurization and immediate homogenization of the mix either shortly before or 24 hours before freezing. Some exceptionally low count ice cream has been secured in this way, while in other cases the count has not been quite so satisfactory; the variation is, in all probability, due to the variation in the resistance of the bacteria contained in the cream to the pasteurization temperatures. The procedure involves the pasteurization of all the ingredients that go into the ice cream and not the cream alone. Moreover, it calls for less handling after the pasteurization than the pasteurization of the cream and the subsequent preparation of the mix. The procedure is claimed by commercial men to possess certain advantages from the standpoint of factory methods, and it seems that its advisability from the standpoint of the wholesomeness of the product cannot be questioned. The changes secured in the numbers of bacteria during the procedure are shown in three trials below.

²⁰ *Bull.* 134, p. 288.

Trial I

Material	Bacteria per c. c.
Cream	720,000
Mix heated but not held.....	156,500
Mix pasteurized 142 degrees F., 20 minutes.....	1,545
Pasteurized mix through homogenizer without pressure.....	2,025
Pasteurized mix through homogenizer, 170 Kg. pressure.....	4,250
Frozen ice cream	5,900
Hardened ice cream.....	2,250

Trial II

Material	Bacteria per c. c.
Cream	5,200,000
Mix before heating.....	1,300,000
Mix pasteurized	170
Pasteurized mix through homogenizer with pressure.....	375
Frozen ice cream.....	7,950

Trial III

Material	Bacteria per c. c.
Cream	5,600,000
Mix before heating.....	2,600,000
Mix pasteurized 145 degrees F., 20 minutes.....	6,150
Pasteurized mix through homogenizer with pressure.....	6,150
Frozen ice cream.....	6,700

One of the peculiar changes shown in the data is the considerably smaller number of bacteria in the mix than in the cream. This relationship has been consistently obtained, and is likely due, in part at least, to the plasmolyzing action of the sugar. The volume change may play a part, but the very great reduction encountered in most instances cannot be explained in that way alone.

RELATION OF ICE CREAM TO PUBLIC HEALTH

The real reason for the concern regarding the bacteriology of ice cream is that this product is more or less frequently responsible for human disease. The diseases caused by ice cream can be divided into two groups: poisoning and infectious disease.

The cases of poisoning from ice cream are well advertised by the newspapers and it is very probable that ice cream gets more than its just share of the blame in this connection. It seems, moreover, that the most of the ice cream that is the cause of cases of poisoning is ice cream manufactured in homes or in small poorly equipped factories. Ice cream mixes made

up and allowed to stand at a fairly high temperature before being frozen have been shown to give trouble in certain instances; this is to be expected especially when the material has been heated and the non-spore-forming acid producers destroyed, thus leaving the field free to the spore-bearing bacteria. The long storage of cream at temperatures around 32° F. should be prevented because of the well-known chemical changes that occur in milk and undoubtedly in cream at such temperatures. The pronounced increase in the amount of soluble nitrogen is much more likely to result in the development of poisonous products than the decomposition of the lactose which takes place at higher temperatures and which by the development of acid keeps down the spore-forming peptonizing bacteria. The ice cream manufacturers have a real problem in their cream supply, particularly at times of fairs, holidays, etc., when the storage of cream for considerable periods of time is a practical necessity except in those exceptional districts where there is an abundant sweet cream supply. It seems, though, that safety demands a reasonable limit on the length of time that cream should be stored, even at such favorable storage temperatures as 32° F.

Epidemics due to ice cream have been noted by various investigators and have become common enough to no longer excite any unusual comment. The Iowa Agricultural Experiment Station²¹ cited some of the references previous to 1912 which give an idea of the extent of the outbreaks up to that time. Since then, literature on the subject has continued to appear. The disease most commonly spread through ice cream is very evidently typhoid fever and some fairly large epidemics of this disease have been traced to this source. There is no reason to doubt the claim that all diseases spread through milk can be spread through ice cream unless precautions are taken to prevent it.

Undoubtedly, the most common method of the contamination of ice cream is by bacillus carriers. Such individuals have frequently been found to be the cause of epidemics and

²¹ *Bull.* 134.

should certainly be kept out of ice cream plants by every possible method. It now seems evident also that a negative Widal is by no means definite proof that a certain individual is not a carrier. An epidemic in Brooklyn in 1914²² was not traced to its source, and in 1915 another epidemic in the same section led to stool examinations of the employees of an ice cream plant supplying most of the ice cream, with the result that a man who had been working in the factory in 1914 was found to be a carrier. A typhoid epidemic in California²³ was found to have been due to ice cream made by a woman who had had typhoid 17 years before and who gave only a partial Widal. This same ice cream also caused poisoning in the persons consuming it and all but two of the persons poisoned developed typhoid. A portion of the ingredients was heated, then cooled and added to whipped cream, after which the mix was allowed to stand from 6:30 until 1:00 o'clock without ice, before being made into ice cream.

The presence of organisms producing disease in non-epidemic form is very likely to occur unless proper care and handling are followed.

If pathogenic organisms are present in ice cream, the low temperatures to which they are subjected during the hardening and holding process cannot be depended on to destroy them. While there will likely be a falling off in the numbers of living cells, low temperature cannot be counted on to make the product safe. At the Iowa Agricultural Experiment Station,²⁴ ice cream artificially infected with the tubercle bacillus was found capable of producing tuberculosis in guinea pigs after one month, which is as long as the tests were made, this period being considerably longer than ice cream is likely to be held under practical conditions.

²² Letter from New York City Health Dept.

²³ *Jr. Am. Med. Assn.*, April 21, 1917.

²⁴ Unpublished data.

BACTERIAL STANDARDS FOR ICE CREAM

Bacterial standards for ice cream have been set by various cities but it seems that as yet our information regarding the bacteriology of ice cream is too meager to admit of the establishment of fair and safe standards. In at least one instance, however, the institution of a tentative standard was followed by a marked decrease in the average bacterial content of the ice cream offered for sale. Standards which prohibit the sale of ice cream with "excessive numbers" of bacteria undoubtedly are of value and force the manufacturers to give at least some attention to the quality of the ingredients used and the care employed in the manufacture. Until better methods of detecting pathogens in ice cream are available, it does little good to prohibit the sale of ice cream "containing disease-producing bacteria"; and it seems more advisable to use numerical standards as the bacterial content gives at least some index of the quality of the ingredients and the care used in production.

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