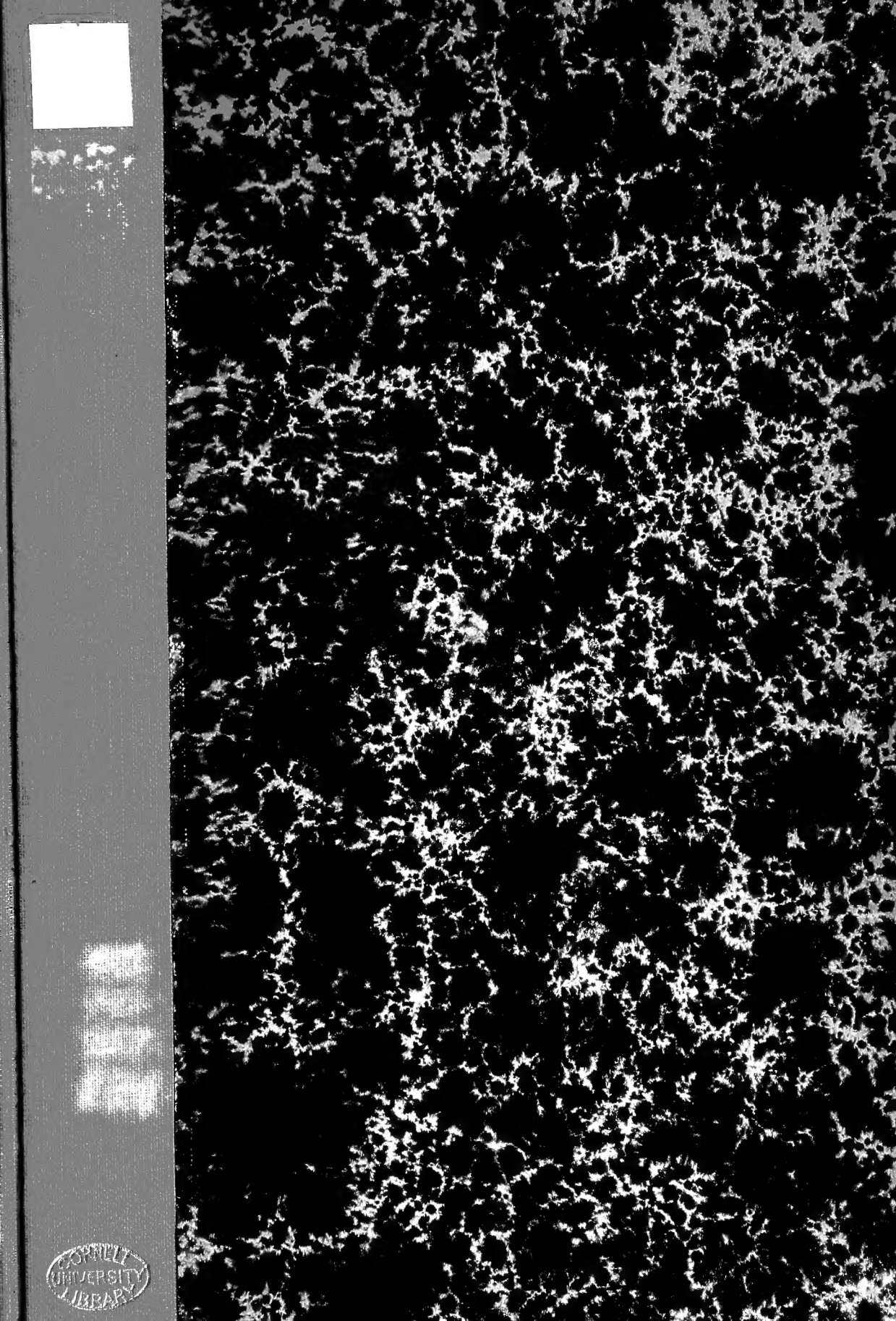
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PASTEURIZATION OF MILK

Report of Committee on Milk Supply

OF THE

Sanitary Engineering Section

American Public Health Association

Committee

H. A. Whittaker, Chairman
H. D. Pease
S. M. Heulings
A. F. Stevenson
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PRICE 35c

BOSTON
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AMERICAN PUBLIC HEALTH ASSOCIATION SANITARY ENGINEERING SECTION

REPORT OF COMMITTEE ON MILK SUPPLY

I. Introduction.

The Committee has limited its activities to certain subjects on the pasteurization of milk which have a bearing on public health, and has attempted to bring together certain information on milk pasteurization which will be of service to health officials and others interested in the subject. The members of the Committee submitted reports on the following divisions of the work: The Present Status and Control of Milk Pasteurization, Mr. H. A. Whittaker; The Effect of Pasteurization on the Composition of Milk, Mr. Albert F Stevenson; The Mechanical Features of Pasteurization Plants and Responsibility of Operation, Mr. S. M. Heulings; The Analytical Control of Pasteurization Plants, Dr. H. D. Pease; and The State and Municipal Supervision of Milk Pasteurization, Mr. Mayo Tolman. These reports were re-arranged and co-ordinated by the Chairman, Mr. H. A. Whittaker. Mr. R. E. Irwin was made an associate member of the Committee in October, 1919, after the present report was prepared. He has carefully reviewed the report and offered certain suggestions.

II. The Present Status of Milk Pasteurization.

Inquiries were sent to all states and territories of the United States and the provinces of the Dominion of Canada requesting information regarding the number of pasteurization plants in operation and the control exercised over the pasteurization of milk for human consumption. Sixty-three questionnaires were sent out to which 43 replies were received.

(1) Number of Plants in Operation. In regard to the number of pasteurization plants, fifteen replied that no record was available, while twenty-eight provided information which showed a total of 1,750 plants in operation. In view of the fact that the 28 replies were from various sections of the United States and Canada, and might be considered as representative of the entire territory, it may be estimated that there are roughly 4,200 pasteurization plants in operation for the treatment of milk for human consumption in these countries at the present time.

(2) State, Territorial, and Provincial Control. The information received on state, territorial, and provincial control of pasteurization showed that ten had either laws or regulations on the subject, while thirty-six had no legal authority. In reply to whether the state, territorial or provincial control was effectual, only two stated that it would be considered satisfactory in their respective jurisdictions.

(3) Municipal Control. In response to the question of what attempt was made by municipalities to control the pasteurization of market milk, twenty-five replied that certain municipalities in their state or province were exerting control, while eighteen stated that no attempt was made to maintain any supervision. Information obtained on the control of milk pasteurization directly from twenty-one of the largest cities in the United States showed that nine had milk ordinances, three were governed by state laws, and nine did not have any legal authority. The method of supervising pasteurized milk in the cities having legal authority consisted of inspection of the pasteurization plants at various intervals and the examination of samples of milk. In one instance, the city ordi-

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nance required the approval of plans on the building or that part of the building intended for pasteurizing milk or cream. The city ordinances covering pasteurization varied in practically every detail. This was especially noticeable in regard to the temperature and holding period required for pasteurization by the holding method. The temperature requirements ranged from a minimum of 140 degrees F. to a maximum of 165 degrees F., the holding period from 20 to 30 minutes, and the temperature to which the milk should be cooled after holding from 45 to 50 degrees F.

(4) Definitions of Pasteurization. A questionnaire was sent to four departments of the United States Government and to every state board or department of health in the United States asking whether they had officially defined the pasteurization of milk. Three of the Federal departments replied that their departments had officially defined pasteurization and furnished definitions covering the process. Each department defined the holding method of pasteurization. All of the definitions differed as to the wording of the temperature and time requirements which made them substantially at variance when applied to the control of the process. Thirty-seven states replied to the questionnaire, and of this number ten reported that pasteurization had been defined in their respective states. An analysis of these definitions showed that only two states had nearly uniform definitions while the others varied as to temperature and time requirements. It is apparent from the foregoing statements that there is very little uniformity in the Federal and the state definitions of pasteurization in this country.

(5) Apparent Lack of Control and Uniformity of Methods. It appears from the information obtained on the state and municipal control of pasteurization plants, that in general there is very evident lack of supervision from a health point of view. There are some instances where it would appear that active steps are taken towards control, but in many cases, through lack of legal authority, appropriations, or organization, the work is not satisfactorily carried out. In state work there are a number of instances where there is an apparent lack of co-ordination of the activities that make possible the proper supervision of pasteurization while in many others the public health aspect of the problem has not been given any consideration. The lack of uniformity in the definitions of pasteurization throughout the United States leads to needless confusion and controversy which could be obviated by establishing a definite standard.

(6) Absence of Public Understanding of Pasteurization. There is quite an evident lack of understanding on the part of the public regarding the actual meaning of pasteurization at the present time. Investigations, in one state, where an intensive survey of milk pasteurization has been undertaken during the past year, show that many health authorities are generally accepting any kind of heat treatment as a satisfactory health measure, while in many of these instances the process is being carried on to prevent the souring of the milk and for advertising purposes rather than to improve the sanitary quality of the milk. There is a very obvious need for an educational campaign to enlighten the public on the meaning of milk pasteurization.

III. The Effect of Pasteurization on the Composition of Milk.

In the early days of pasteurization, disagreement prevailed among those conversant with the subject, regarding the effect of heat on the various

substances which are present in milk, and also on the value of pasteurized milk as a food for infants. The disagreement resulted in a very extensive study of the subject by an eminent chemist who established more or less permanently certain points.

(1) Effect of Heat on Milk. The chemical changes produced in milk solids by heating at different temperatures for varying lengths of time were determined. North published in 1911 a summary of the literature on the subject, together with considerable new work done in his laboratory. This summary appeared in the form of a set of curves, which showed graphically the effect of heating milk on the chemical composition of its constituents. This diagram was accepted by the Commission on milk standards of the New York Milk Committee and was published by them in their first and second reports.* It points out that the inorganic solids in milk are very little changed by heating to 169 degrees F. for 40 minutes or less; that the albumen is much more sensitive than the salts, but will resist a temperature of 148 degrees F. for 40 minutes; that the enzymes are the most easily destroyed constituents of the milk, but are still effective after the milk has been heated to 145 degrees F. for 30 minutes The physical condition of the cream is the most sensitive indicator of over-heating, and the cream line is destroyed before any of the milk solids are chemically affected. In brief, the ordinary temperature and time of heating used in commercial pasteurization, namely, 145 degrees F for from 20 to 30 minutes, seemingly has little, if any, effect on the composition of the milk so far as we are able to determine by chemical analyses.

(2) Infant Feeding Experiments. The results of these chemical tests did not satisfy many of the medical profession, who were familiar with infant feeding. They still claimed that pasteurized milk did not produce the same effect on some infants as did clean raw milk. This criticism of pasteurized milk was, to a great extent, temporarily quieted by the experience gathered from the immense feeding experiments conducted by the New York Milk Committee in its infant feeding stations, and by similar work done at milk dispensaries in Washington. The results of these experiments seemed very conclusive. Thousands of children were fed pasteurized milk daily for three years at the New York stations, and the average daily increase in weight of the babies thus fed was equal to the average increase in weight of the babies of like age fed clean raw milk, and no cases of rickets or scurvy developed. The one objection to this evidence seems to be that these children were not always under observation of the station nurses, and other foods may have been given them, which in some way might counteract the effect of the heated milk.

Within the last few years the controversy has been again reopened and much more definite data has been presented. It has been shown that proper food substances contain exceedingly small amounts of compounds which are absolutely necessary for the maintenance of healthy life. These essential compounds have been styled vitamines. There are several classes of vitamines. One class is responsible for the growth of organisms, and when absent from the diet of the young, growth is not normal. Another class is absolutely essential for the maintenance of ordinary metabolism, and without which deficiency diseases such as scurvy and rickets develop. Any process which breaks up or destroys either of these compounds in an arti-

(1) Reports of Commission on Milk Standards appointed by the New York Milk Committee. Public Health Reports, Vol. XXVII, No. 19, and Vol. XXVIII, No. 34, August 22, 1913.

cle of food should be avoided. These vitamines have not been definitely isolated from foods, and little is known of them in a pure state. Their presence or absence can only be determined in a food substance by its effect on animals, when used exclusively as a food by them.

Experiments have been conducted by a number of scientists to determine the effect of heat on the vitamines contained in milk. It has been shown that the growth producing vitamine occurs in the butter fat.* It is very resistant to heat, and is not affected by ordinary pasteurization. The antiscorbutic substance is much less resistant to heat. For this reason its properties have been carefully studied. The best known work along this line was done by Hess in New York.** Since 1912, he has had a considerable number of cases of scurvy develop in infants being fed on pasteurized milk in an institution where the diet could be absolutely regulated. At first milk was used which was pasteurized in the hospital at a temperature of 165 degrees F. for 20 minutes. During the past year, however, pasteurized milk which has been heated to 145 degrees F. for 20 minutes at the plant of a New York dealer was used. Several children developed mild but recognizable cases of scurvy on this diet, while others in the same ward, fed on an identical diet, did not contract the disease. Raw milk was then substituted for the pasteurized, all other conditions remaining the same, and in two weeks the scorbutic symptoms wholly disappeared. Hess, therefore, concludes that pasteurized milk plays an important role in the production of the disease, but is not the sole factor. There seems to be a sufficient amount of antiscorbutic substance in cow's milk to prevent the development of infantile scurvy, but this substance is destroyed to such an extent by pasteurization that it is not safe to feed an infant, solely, a diet of pasteurized milk. This discovery need work no practical hardship and should not be used as an argument against pasteurization. An ounce or two of fresh orange juice or potato water given daily entirely eliminates all chance of contracting the disease for these substances contain the vitamine in considerable quantities. Some such substance should be given, however, to an infant fed on pasteurized milk.

Work of a similar nature* has been done by feeding small animals, such as guinea pigs, a pasteurized milk diet. It was found that such animals soon died. Control pigs fed on raw cow's milk likewise were unable to exist. This work, although much discussed and rather generally used as an argument against pasteurization, is of little value. It only substantiates the already known fact that guinea pigs cannot exist on any form of cow's milk alone. This work is cited simply to emphasize the fact that feeding experiments conducted on children constitutes the only reliable source of information regarding the suitability of any particular diet for infant feeding.

(3) Infant Feeding vs. Communicable Disease. Hess' work should not be interpreted in any way as an argument against pasteurization. Parker** summarizes the situation well when he says: "It is now recognized that in our large cities it is not feasible to bring the whole milk supply up to the standard of that required for infant feeding. The procuring of a supply

*McCollum, E. V., and Davis, M. "Observation on the Isolation of the Substance in Butter Fat which Exerts a Stimulating Influence on Growth." Journal Biol. Chemistry, Vol. 19, p. 245.

**Hess, A. V., Infantile Scurvy. The Blood, the Bloodvessels and the Diet. Am. Jour. of Diseases of Children, Vol. VIII, Dec. 1914, pp. 386-405. Hess, A. F. Subacute and Latent Infantile Scurvy. Jour. A. M. A., Vol. LXVIII, Jan. 27, 1917, pp. 235-239.

*Moore, J. J., Jackson, L., Exp. Scurvy Produced in Guinea-pigs by Milk and Milk Products, Vol. LXVII, Dec. 23, 1916, p. 1931.

**Parker, H. N., City Milk Supply, p. 282.

of babies milk is a special problem of city milk supply, and this should be remembered in relation to pasteurization. Pasteurization protects babies from diarrheal diseases, typhoid fever, diphtheria, scarlet fever, and from bovine tuberculosis and if, in the opinion of some physicians, this advantage is outweighed by other considerations, or at least in some cases makes the use of raw milk advisable, it does not follow that for these reasons older children and adults should surrender the protection from communicable diseases that the use of pasteurized milk affords."

IV. THE PROCESS OF MILK PASTEURIZATION.

The Committee questions whether this is the place to undertake the presentation of the origin and development of the heating proceses which have resulted in the adoption of the modern systems of the practical pasteurization of milk. It does not consider it necesary to refer extensively to the entire series of laboratory studies resulting in the expression of opinions on the destructive action of heating upon cultures of pathogenic bacteria or their thermal death-points.

(1) Holding System Required. The Committee feels that it has been sufficiently demonstrated that the so-called "holding" system of pasteurization of milk intended for prompt consumption in the fresh state is so much superior to any other type of method as to warrant the limiting of its activities to that general system. There are practically two stages or operations in every form of application of the "holding" system, i. e., the heating and the holding. This applies whether the milk is heated in one apparatus and held in another, or is heated and held in the same tank, coil, or bottle.

The process of pasteurizing milk consists of four essential parts: (a) the temperature to which the milk must be raised, (b) the length of the time period for which the milk must be held above the minimum temperature, (c) the time in which the milk must be cooled after the boiling period, and (d) the temperature to which the milk must be cooled.

To the temperature and time required must be added either temperature or time, or both, as a factor of safety against irregular functioning of the apparatus used to pasteurize the milk. Some types of apparatus require a far greater factor of safety than others. Regardless of the means employed to apply the pasteurizing treatment to the milk, each and every drop of milk must be raised to or above the minimum temperature. The holding time must be as long or longer than the minimum time fixed for the holding period. No averaging of temperature or of the holding time can be permitted—that is, if some of the milk is heated to a higher temperature, the remainder must not be heated to a lower temperature, or if some of the milk is held beyond the holding time the remainder must not be held for a shorter time.

(2) Pasteurization Process Defined. As the pasteurization of milk is a public health measure, that determination of time and temperature which insures the greatest protection from any possible pathogenic infection is the standard to be fixed for milk for public consumption. The Committee believes that the process of pasteurizing milk for human consumption should consist of subjecting the milk to a temperature not lower than 145 degrees F. for not less than 30 minutes. It feels that the scientific evidence already presented from studies on the process of pasteurization justifies the requirements above stated for pasteurized milk, and that by fixing only a minimum temperature and time, sufficient latitude is

allowed for any fluctuation above the minimum points that may be required in commercial practice. There are many other factors other than temperature and time requirements that enter into the problem of the efficient pasteurization of milk which are discussed under other subjects in this report.

V. THE MECHANICAL FEATURES AND THE OPERATION OF PASTEURIZING PLANTS.

The material included under this subject is limited to pasteurizing apparatus and its operation; the matter of the building, and of can fillers and cans, bottle fillers and bottles, can washers, bottle washers, sterilizers, cap sterilizers, bottle inspection, cap storage, etc., forming important parts of milk pasteurization, are not considered. Owing to the wide range of capacities and importance of local conditions, it is impossible to make any specific lay-out that would apply to even a majority of pasteurizing plants. There are, however, certain general conditions that apply to all plants and all plant operation, to all pasteurizing apparatus and the machinery co-ordinating therewith, to the sterilization of containers, to the treatment of the milk, and to the handling of the milk after treatment, in order that the output of the plant may be entitled to be sold under the marking or label of "PASTEURIZED."

(1) Methods of Pasteurization. Milk may be pastuerized in the bottle or have the treatment applied by means of apparatus, which may be of what is known as the batch or intermittent type, or as the open or closed continuous type. The apparatus generally used for applying the pasteurization treatment is either of the vat type, that is, (a) a vat in which the milk is heated, held and cooled, or (b) a combination of a heater, a vat or series of vats for holding the milk static and a cooler, or (c) a combination of a heater, a series of vats for holding the milk flowing and a cooler,

In any of the above mentioned types of apparatus the treatment may be applied, and pasteurization effected by a competent operator, providing the apparatus is free from inherently dangerous defects.

It is entirely practical to construct the intermittent or continuous type of pasteurizing apparatus so that every drop of milk and every particle of foam developed by the movement of the milk will have passed through the complete course of the apparatus, and that when milk is supplied to such an apparatus at the proper volume per time period, and the necessary temperature maintained, the milk delivered from the discharge outlet will have had pasteurizing treatment.

(2) Pasteurization in the Bottle. Milk that is pasteurized in the bottle must have the full minimum treatment applied, and care must be used in taking the milk temperature so that the instrument is not affected by the hot milk at the top of the bottle, or by the heating medium. The time of the holding period does not start until all of the milk in all the bottles is at the minimum temperature. This temperature must not be lowered until the end of the holding period, and the holding period must not be shortened. If any temperature recording instrument is used, care must be taken to see that the heat of the apparatus does not cause a higher reading than the actual temperature of the milk. If water is used in cooling, care must be taken to prevent it from being drawn into the bottle by the contraction of the milk. Bottles used for milk to be treated therein must be washed and sterilized before being filled with milk, as the pasteurizing temperature is not sufficient to sterilize the bottles that have been returned to a milk plant, often

from dumps and rubbish heaps, and frequently containing very objectionable and highly dangerous material.

(3) Pasteurization with Intermittent and Continuous Flow Apparatus. Regeneration in a pasteurizer is an exchange of temperatures between the hot and the cold milk, and is obtained by an apparatus that either brings the flow of hot and cold milk on either side of the metal surface whereby the hot milk is cooled by the cold milk and the cold milk is heated by the hot milk, or by an apparatus that circulates water first through the cooler, which becomes heated by taking up the heat of the hot milk. This water, after the addition of the steam required to bring it up to a temperature necessary to heat the milk, is circulated through the heater and raises the milk to the pasteurizing temperature. The economic advantage of regeneration is apparent, when it is considered that water at 38 degrees F. may be run into a cooler and be discharged from the cooler at 120 degrees F., all the steam then required to heat the milk is that necessary to raise this water from 120 degrees to 150 degrees, at which temperature the water will heat the milk to 145 degrees. To accomplish this it is necessary to have properly constructed and proportioned apparatus. This system is of advantage only in milk plants that have a run of several hours' duration. If milk is being held in the holders for thirty minutes for the first half hour of the run, there is no milk to cool, and on the last half of the run, there is no milk to heat. Regeneration is an economic advantage only, and of no advantage to the milk. It is a decided disadvantage, when the apparatus is so constructed, that the milk is forced through an extra device that has gasketed closures or troublesome cleaning features. Apparatus in which any possible mixing by leaks or seepage between the hot milk and the cold milk in the milk channels of the apparatus is improper in construction and may destroy the effects of pasteurization.

In a continuous flow pasteurizing apparatus, it is important to have the supply of milk flow to the heater at a uniform predetermined rate per hour, proportionate to the capacity of the apparatus, in order to maintain the holding time, the heating temperature and the cooling in co-ordination with uniform flows of the heating and cooling mediums. This uniform supply flow cannot be properly maintained by a pump, for, owing to the variations in slippage and varying quantity of air in the milk, a pump will not always discharge the same amount of milk when running at the same rate of speed.

(a) Flow Controllers. A device termed a "flow controller," consisting of a small metal tank about thirty inches in diameter by twelve inches deep, with the milk supply pipe projected through the side and having a float to maintain a constant depth of milk in the tank and a fixed outlet of proper area to discharge the amount of milk wanted per hour, will maintain a constant flow when the supply is sufficient. The fixed outlet must discharge into an open connection to prevent syphonage. In the pasteurizing installation the "flow controller" is placed above the heater to which the milk flows by gravity.

(b) Heaters. It is important to have the heater for the milk contain the necessary heating surface to raise the temperature of the milk to 145 degrees F., when running at full capacity and using water not more than five or six degrees higher as the heating medium. With such a heater, overheating cannot take place when the temperature of the water is properly controlled, even if the flow of the milk is reduced for a time, as is often the case in plant operation. A heater with a relatively

small surface in proportion to the capacity claimed for it must utilize a heating medium of much higher temperature in order to heat this quantity of milk. While such a heater will discharge milk of apparent uniform temperature, it is actually only an averaging temperature and the cream of that part of the milk that is overheated will not raise in the bottle.

When possible, the heater should be installed above the holding tanks, so that the milk may flow by gravity. Milk heaters should have, if any, as few joints or loose parts as possible, for joints and unions are likely to leak and cause loss of milk. A deposit from the milk forms on the heating surface that must be removed every day; therefore, the use of a device for heating milk that cannot be scrubbed clean should be prohibited.

The instrument for controlling the temperature of the milk discharged from the heater and the instrument for recording the same are hereinafter referred to.

(c) Holders. The reliable operation of the holding device of the pasteurizing apparatus is most important if efficient treatment is to be obtained.

In the systems using a vat or series of vats in which the milk stands during the holding period, a part of the milk may be held much longer than the holding period; while this is no disadvantage, it is a condition often overlooked. The foam on the top of the milk in the tank may lower in temperature, or the draw-off valves may leak and allow seepage of the not fully heated milk. If the heating surface of these vats is supplemented by coils, these coils are troublesome to clean, and if the coils rotate, stuffing boxes are inevitable. In the vat system it is important to have either sufficient insulation of the heat maintained by circulation of controlled temperature water, to prevent the lowering of the temperature of the milk and the foam. It is also necessary to have a safety outlet in the discharge pipe from the vats that will permit any milk that may leak through the valve before the end of the holding period to run out onto the floor.

In the system using a series of holding tanks for continuous operation, there should not be less than three tanks to the series and set vertically. They must be insulated except when provided with controlled temperature water jackets. One and one-half inches of well-packed hair felt between metal castings is sufficient insulation. These tanks must be provided with a feeder that will distribute the inflowing milk into small streams at right angles to the vertical flow of the milk in the tank and a collector for the outgoing milk that will collect the milk through several orifices. The milk from the heater is supplied to the first tank of the series, from the first tank to the second, and so on throughout the whole series.

The milk content of the series of continuous flow holders should be the same as the maximum hour capacity of the heater; this will give with 50% efficiency a net holding time of thirty minutes. A theoretical holding period of one hour, and with an efficiency of 75%, will give a net holding time of forty-five minutes, an excess holding of 50%, or fifteen minutes over the minimum holding time of thirty minutes. An excess holding time only requires an increase in the size of the tanks, which adds but little to the original cost and the additional cleaning is very small, with no extra cost for operation. This excess holding time over the thirty minutes' minimum is a factor of safety in pasteurization that may well justify its general adoption on all

The drain outlet from the bottom of the continuous flow holders must not be connected directly to the pipe lead-

ing to the cooler, but should be connected to a "flow controller" same as hereinbefore described that will not permit the milk being drawn off at a more rapid rate than the rate of filling. This "flow controller" must not have a connection with the cooler during the continuous operation, so that in case any of the valves in the discharge of the holding tanks should leak, which is likely to happen at any time, the milk that is seeping through would not pass to the cooler and infect the finished product. After the continuous operation of the milk flowing direct from the holding tanks to the cooler is finished, the "flow controller" at the discharge of the holding tanks is connected to the cooler and the milk drawn off.

The temperature recording instrument in the discharge of the continuous flow holding tanks records the temperature of the held milk, which must be at or above the minimum holding temperature, and also shows the time of drawing off the tanks by the interruption of the continuous flow.

Continuous flow holding tanks have no moving parts or moving valves, stuffing boxes or coils, or pipe fittings, or connections under pressure; they are easily cleaned and sterilized.

(d) Coolers. The cooler for cooling milk, after the holding period, forming a part of either the intermittent or continuous types of pasteurizing apparatus should have the necessary cooling surface to cool the volume of milk per hour to within two or three degrees of the temperature of the water circulated for cooling. It is preferable to use water for cooling instead of low temperature brine or direct expansion of ammonia. With the brine or direct expansion ammonia, it is almost impossible to prevent a part of the milk from being frozen. The water for the cooler may be cooled by direct expansion, or by low temperature brine.

In a continuous pasteurizing apparatus, milk heaters and coolers, when properly constructed and proportioned in relation to the quantity of milk to be heated per hour, require the volume of water circulated to exceed the volume of milk by about 5 per cent. The circulating water cooled from a normal temperature to 38 degrees F. will cool the milk at approximately 42 degrees F., and with milk from the holder at 145 degrees F., the circulating water will leave the cooler at 120 degrees to 125 degrees F., obtaining all the advantages of regeneration. This water, when passed through a water heater, using either exhaust or live steam, or both, must be raised to about 149 degrees to 150 degrees F., which will, when circulated through the heater, heat the milk to 145 degrees F. This water, when discharged from the heater, can be run to the water storage tank of the building and used for general purposes.

Sterilization of the cooler is necessary, and the cooler must be constructed to stand this process. To accomplish sterilization, steam at 10 pounds pressure should be turned into the inside of the cooler and the apparatus maintained under these conditions for the time necessary to produce sterilization as determined on the condensation water drips from the bottom of the cooler. In an open surface cooler, the steam is turned into the water thoroughfares after emptying, and maintained under these conditions for from one to two hours. The stress on a cooler caused by sterilization comes more from uneven expansion than from pressure and a steam pressure of 10 pounds is ample. It is difficult to sterilize a cooler not constructed to withstand the application of this amount of pressure, and the best way of accomplishing what can be done in

the sterilization of such a cooler is to flow boiling water down over it; even the expansion caused by this heat may buckle the surface and prevent proper sterilization. The sterility of a cooler can be determined by flowing sterile water over it after the cooler has been allowed to cool.

All open surface coolers should have removable metal covers to protect the milk, or should be installed in a properly constructed room that is not used for any other purpose. Milk should be fed to open surface coolers through a perforated pipe that does not permit foam to gather, as the collection of foam is often favorable to large bacteria growths.

From the cooler the milk flows to a cold milk tank of relative small dimensions that has an agitator to keep the cream from raising. It is not necessary to insulate this tank under usual conditions. From this tank the milk flows to the can filler and the cans, and to the bottle filler and the bottles.

(e) Pumps. Milk pumps should be eliminated from milk plants wherever possible; where their use is necessary it is preferable to use a pump large enough to handle the milk at a moderate speed. As the pump must come apart every day for cleaning, pumps with few parts that are accessible for cleaning and are put together with strong bolts, are the best type. The stuffing boxes on either the piston or rotary type pump must be opened up, cleaned and packed with clean packing every day. Off flavors in the milk are sometimes caused by the putrid milk that accumulates in the stuffing box, corrodes the metal and seeps into the milk.

Milk pumps should not be installed to pump milk after it has been heated if this can possibly be avoided, since this practice increases the liability of contamination.

(f) Stuffing Boxes. Stuffing boxes in vats, tanks or other apparatus are to be avoided wherever possible, and if used, must be cleaned and packed clean every day. If not kept in first class order, they are likely to cause corrosion and off flavors in the milk.

(g) Fittings and Valves. All machinery, apparatus, pipes and pipe fittings, and valves used for pasteurizing milk, must be so constructed as to permit every part with which the milk comes in contact of being opened up and scrubbed with a brush. There must be no cracks, crevices or inaccessible corners, nor must there be any absorbent or spongy material used in construction. Gaskets should be eliminated, but if they are necessary in some types of apparatus, heavy paper gaskets should be used and renewed daily.

(h) Metals Used in Construction of Apparatus. Apparatus constructed of copper, tinned and with tinned bronze castings, has given satisfaction in many plants, both before and after the tin has worn off. Tinned copper pipe, and red brass fittings for milk cocks, have given satisfactory service. Any metals, alloys or combination of metals that produce electric action when subjected to the condition of a milk plant should be avoided, as the decomposed metal may cause off flavors in the milk.

(i) Temperature Control Apparatus. Temperature control of heating the milk for pasteurization may be obtained by using the well known and practical temperature controllers on the market; the sensitive part of the instrument is preferable placed in the water circulation to the milk heater, when a suitable type of heater is used, or it may be placed in the hot milk discharged from the heater. In all cases small valves in the steam line should be used, as their opening and closing does not cause such fluctuation in temperature. In almost all cases steam

is required all the time so that a by-pass should be used and nearly enough steam allowed to pass through this by-pass valve to maintain temperature. The controller will then only have to act on the additional steam required, and may be comparatively small, and when closed does not cut off all steam from the heater.

All temperature controllers must have a constant steam pressure to operate properly; therefore, it is important to use at least one efficient steam pressure valve between the boiler and the controller instrument. In many cases where there is a large fluctuation in the boiler steam pressure, two reducers can be used to advantage in obtaining a final constant pressure for the controller.

(j) Temperature Recording Instruments and the Interpretation of Recording Charts. The only means that the operator of a pasteurizing plant or the health authorities have of knowing the treatment to which the milk has been subjected, excepting by constant testing with the thermometer, are the chart records made by reliable time and temperature recording instruments, that have their bulbs placed in such a manner that the bulb is not influenced by the temperature of the apparatus. Instruments sensitive to the changes of temperature and quick to record such changes should be used instead of sluggish instruments. All instruments should have pins close to the center post that holds the charts from inadvertently rotating, or from being intentionally rotated by hand to produce fake records.

Temperature recording instruments are not thermometers; they record temperatures after being adjusted to correspond with a thermometer, and require frequent testing to assure correct records.

The operator in personal charge of each pasteurizing apparatus should have a calibrated thermometer and should be held responsible for the correctness of the recording instruments and of the charts. Each chart should have printed on it a statement certifying to the correctness of the chart, to be signed by the operator, with a place for date and identification mark of the apparatus of which chart is the record. On the chart of the holding tanks there should be a statement to the effect that the pump speed (if a pump is used) has not been accelerated during the run, and that the holding time has not been shortened. The operators should be held to rigid account for the correctness of the charts on which they have signed the certifications.

All charts for recording the time and temperature of milk treatment must be set on the instrument dial at a uniform time so that the time elapsed from one treatment to another can be checked and determined. All the charts must show the time of the operation from start to finish.

The chart showing the temperature record of the milk in an apparatus where the milk is heated, held and cooled in a vat, must show the time, the temperature of the milk when it starts to raise, the time when the milk reaches its high point, the temperature maintained during the holding period, the time when the cooling temperature commences, and the temperature to which the milk is cooled. If the milk is cooled by a separate cooler, the chart on the cooler will record the cold milk temperature, and the end of the holding period which is indicated on the chart as the time of the start of the cooling operation.

The chart showing the heating temperature record of the milk in a continuous flow apparatus, must show the time and temperature of the milk when the heater starts to discharge, the temperature of the milk as it is discharged

from the heater throughout the run, and the time of the finish of the discharge of milk from the heater. The chart showing the holding time and temperature record of the milk in a continuous flow apparatus, must show the time at which the first milk is discharged from the holders, its temperature, the temperature of the milk maintained through the run, and the time of the finish of the run. The sensitive bulb of the recording instrument must be placed in the continuous flow discharge pipe of the holding tanks and not in the pipe through which the milk flows when the tanks are being emptied.

An apparatus having a series of tanks that are emptied at the end of the run, the interval between the finish of the run and the starting of emptying the first tank, must be clearly indicated on the chart recording the temperature of the cold milk; likewise, the interval between emptying the other tanks of the series will also be indicated on the cold milk chart; this is caused by interruptions to the flow of the milk in emptying the holders.

It is of the utmost importance to check the temperature of the first milk discharged from the holders, if this milk does not show 145 degrees or over when the milk supplied from the heater was 145 degrees in a properly constructed apparatus, it indicates that the holders were not properly preheated before beginning the operation. This underheated milk may defeat efficient treatment, as all of the milk discharged below 145 degrees may carry a seeding with it and infect the subsequent apparatus through which it flows to the detriment of the milk of proper temperature following it.

The charts showing the records of the temperature of the cold milk in a pasteurizing apparatus, must show the time and temperature maintained through the run, the temperature to which the milk was cooled during the run and the time of the finish of the run.

To check up the holding period of a milk run through a continuous flow pasteurizing apparatus from the chart records, the elapsed time after the time of the discharge of milk from the heater to the holders, and the time of the first discharge of milk from the holders, will be the theoretical holding time. If the flow of milk to the apparatus is uniformly maintained throughout the run, the theoretical holding time for the run will be the same; if, however, after the complete apparatus is in operation and the theoretical holding time is established on the chart record, the pump speed is accelerated or the flow of milk increased by any means, the holding time will be shortened, and effective treatment lessened. Therefore, it is of importance to install the pasteurizing apparatus in such a manner that the flow of milk per hour cannot be increased beyond the rated capacity of the apparatus.

The time of emptying the holding tanks can be checked by noting the elapsed time between the finish of the continuous flow from the holders, as shown on the holder chart, and the time of finishing the run of cooling the milk, as shown on the cooler chart. If the holding time of the milk through the run cannot readily be determined from the charts for any reason, the quantity of milk pasteurized should be taken from the plant records and the quantity of milk run through per hour determined, computing the time between the starting and finish of the holding operation, deduct from this any time lost owing to interruption to the flow (these interruptions will appear on the charts) and check the quantity of milk per hour actually run through the apparatus with its rated capacity.

Apparatus may be so constructed that it will do efficient pasteurization and the milk develop a satisfactory cream line when the quantity of milk handled does not require the raising of the temperature of the heating medium to more than a few degrees above the temperature to which the milk is to be heated. When more milk than can be properly handled is forced through such an apparatus, the heating medium must be raised in temperature to maintain the temperature of the milk, with the result of impairing, if not eliminating, the cream line.

(k) Cleaning and Sterilizing Apparatus. The whole pasteurizing apparatus must be thoroughly cleaned every day; nothing but thorough cleaning should be tolerated regardless of any reasons for careless cleaning that may be offered. Every particle of grease film must be removed from the apparatus as grease film is very favorable to bacteria growths.

Immediately after the finish of the run, the milk should be rinsed off with cold water and every part of the apparatus opened up and scrubbed with brushes and cleaning powder. Where, owing to the heat, a deposit from the milk has formed, this deposit must be cleaned off by using an abrasive cleaning powder, if necessary, and all thoroughly rinsed, first with cold water then with hot water.

Wash sinks preferably of metal, long enough to take the milk pipe and having drainage racks for rinsing, should be placed conveniently so that all milk pipe, milk fittings, cocks, etc., which must be taken apart at every point every day, can be placed in these sinks and soaked for an hour or so before scrubbing. After each and every part has been scrubbed clean and bright, it must be rinsed with clean cold and then hot water, and the apparatus assembled.

After the apparatus has been put together and is ready for operation, it must be sterilized. To sterilize, connect the steam supply at 10 pounds pressure to the various parts of the apparatus so that the steam will reach every part, using great care to drip off all water of condensation which would accumulate in any part and prevent the steam from heating it up, and also cause uneven expansion producing great strains in the apparatus. If high pressure steam is used for sterilizing, its greater velocity and higher temperature causes much more rapid heating of that part of the apparatus with which it comes in direct contact, and consequently. greater expansion of the metal at that point, setting up physical strain that will reduce the durability of the apparatus. In any part of the apparatus that will not stand pressure, lids should be closed down as tightly as safety permits, so that the steam may be confined as much as possible. Metal pipe or metal hose should be used for sterilizing steam connections to any part of the apparatus with which the milk comes in contact as rubber hose disintegrates and deposits black specks which will appear in the milk.

It usually takes from two to three hours to sterilize completely an apparatus with 10 pounds of steam. To determine whether sterilization has been effected, collect samples of the condensation water from the various parts of the apparatus and if bacteriological examination shows these to be sterile, the apparatus is in good order.

The heater and cooler should be sterilized after the washing is finished, or long enough before the milk is run to become cool before any cold water is turned into them. The holding tank may be sterilized at the same time as the heater and cooler, but must not be permitted to cool down below the pasteurizing temperature when the milk is run. If, owing to the conven-

ience of plant operation, these tanks do cool down they must be heated up to the pasteurizing temperature before the milk is allowed to flow in.

Chemicals that can be employed as a germicide in milk should not be used as a part of the cleaning of pasteurizing apparatus. The pasteurizing treatment of milk is a physical treatment of the application of heat to the milk, and in this, chemicals play no part. Chemicals used as a part of this cleaning can be removed only by thorough draining and rinsing, and a careless and inefficient rinsing would leave a residue of chemical in the apparatus that may of itself or its corrosive action on the metals of the apparatus, cause a condition in the milk flowing into it that might have a serious effect on the milk consumer. The use of such chemicals in cleaning pasteurization apparatus should be prohibited.

(4) Some Dangerous Defects in Pasteurization Apparatus. Pasteurization apparatus is inherently dangerous that shows the following defects:

(a) That has a milk pipe leading from the bottom of the heating or holding tank or tanks to the cooler, either direct or through a pump that depends on a milk cock in this pipe as a stoppage or safeguard, to prevent the raw milk or the partially heated milk, or heated but not held milk, from flowing to the cooler, mixing with and infecting the finished product. No milk cock is secure against leaks since a milk cock may be tight for months and may leak through at any time. A bristle of a brush, a thread, a bruise in cleaning, or uneven contraction after expansion, may cause a seepage leak that utterly destroys the efficiency of pasteurization which the apparatus is supposed to effect, and this may happen without the knowledge of the most careful operator.

(b) That utilizes a rotating valve having connections from the heater to the holding tanks and to the cooler. With such a valve, abrasion or cutting may occur at any time without the operator's knowledge, which will form a channel between the connections leading to the various tanks that may shorten the holding time. Should a channel in the valve be cut from the holding tank connections to the cooler connection, the heated, but not held milk, will pass directly to the cooler and mix with the finished product. Efficient pasteurizing apparatus can be constructed without using valves or mechanisms with which the milk comes in contact and that require lubrication with vaseline or other kinds of grease.

(c) That has a series of tanks for holding the milk through which the milk flows continually, that does not consist of enough tanks to prevent a current from forming and flowing through the whole system, and that does not have in each tank a device to diffuse the inflowing milk and a device to collect the outgoing milk that prevents the formation of currents in the milk. All holding systems of this type should have at least three tanks and their net efficiency should be proven by color tests.

(d) That has a by-pass pipe from the raw milk tank to the cooler, cutting out the heater and the holder, or from the heater to the cooler, cutting out the holder; such a pipe is not necessary and might be used as a time saver and is an invitation towards illegal and dangerous methods.

(e) That is connected up to use the same pump to empty the holding device at the end of the run that is used to pump the raw milk. An apparatus that requires a pump to force the raw milk through the heater, holder and cooler, when set on an approximate level, will leave the holder full of milk at the end of the run. This milk should be pumped through the cooler

by means of a separate pump. If the raw milk pump is used, it will infect the heated and held milk unless it is washed and sterilized during the operation. Pasteurizing apparatus should be so installed that all parts will drain through the whole system at the finish of the run, otherwise, the milk left in the apparatus will be neither raw nor pasteurized and must be utilized as a by-product. The public are placed in jeopardy of receiving watered milk when water is used to force the milk out of the apparatus at the finish of the run and such practice should be prohibited. A proper installation of apparatus will obviate this condition.

(f) That, in which foam developed by the milk movement, floats on the top of the milk while the milk is standing in the holding device, and owing to the lack of effective insulation of temperature maintenance, the temperature of the foam will drop below the minimum holding temperature, which is the same in result as lowering the milk temperature and defeats efficient treatment.

(g) That, on account of pipe connections or other causes, permits any lowering of the temperature of the milk in transit from the heater to the holder, or lowering of the temperature of any part of the milk while in the holder.

(h) That does not have means to prevent the emptying of continuous holders at the finish of the run at a more rapid rate of flow than the rate of filling, thereby shortening the holding time of the milk in the tanks at the finish.

(i) That does not have an absolutely safe device in the discharge outlets from the holding tanks to the cooler so that any leakage or seepage through the milk cock in the pipe emptying the holding tank, will flow out onto the floor in view of the operator, so that the leak may be remedied, instead of flowing to the cooler there mixing with and infecting the finished product.

(j) That has a steam or motor driven pump, maximum speed of which will force the milk through the holding device at a rate that reduces the holding time of the milk below the minimum, or a pump in which the rate of flow depends on the judgment of the operator to maintain the proper speed in order not to supply milk to the holding device at such a rate as will reduce the minimum holding time. A pump on account of variations in slippage and air in the milk does not produce a constant flow at a constant speed.

VI. ANALYTICAL CONTROL OF PASTEURIZATION PLANTS.

This phase of the report includes a discussion of the physical, chemical or physico-chemical and biological methods and procedure used to determine the effectiveness of the pasteurization processes.

(1) Physical Methods. The purely physical or mechanical methods of supervisory control have been considered already but it may be well to mention some of the important considerations requisite to the adequate testing of the efficiency of time and temperature recording devices.

(a) Testing of Time and Temperature Recording Devices. Thermometer tests on recording instruments to show: comparisons at lowest temperature on chart, at highest temperature on chart, at 130 degrees to 160 degrees F.; determinations to show rapidity of action to sudden changes of temperature, slight changes of temperature, changes 140 to 155 degrees F.; effects on temperature recording by changes in depth of bulb and clock located as follows: bulb low-clock high, clock low-bulb high, bulb and clock level; characteristic of instruments — printed chart lines may be too close, too heavy, in-

correct charts, revolve eccentrically, not adapted to this form of recording, recording lines may be too heavy, too light, may be blurred, poor paper, poor ink, difficulty of adjustment, easily faked by spinning of chart, by falsely adjusting pen.

(2) Chemical Methods. No purely chemical test capable of differentiating between pasteurized and unpasteurized milk have as yet been developed to a satisfactory point and certainly none have been presented which would give results which would warrant any conclusion that an appropriate heating as to time and degree had or had not been applied.

(3) Physico - Chemical Methods. While Frost and others have presented methods for determining by microstaining reactions whether a given sample of milk has been subjected to a heating process, such procedures have been far from giving results which could be utilized in even estimating the degree of temperature attained or the duration or the thoroughness of its application and yet upon these factors rests the efficiency of all pasteurization processes.

(4) Biological Methods. In any system designed to measure accurately the effectiveness of the pasteurization process, the main and special object of the elimination of disease-producing parasites as distinguished from the reduction in numbers of bacteria must always be kept to the front. Failures to give proper relative values to these two results occur commonly by reason of the past general utilization of the count of the total number of bacteria in a cubic centimeter of a raw milk as an adequate measure of the sanitary quality of that product. However, the almost insuperable difficulties surrounding any attempt at testing a practical system by the use of milk actually innoculated with pathogenic bacteria, has left the investigators in a degree of uncertainty with no choice but to interpret the results of appropriate tests on the forms of bacteria commonly found in milk in the light of the probable effects on those having the power of producing disease in man.

The usual biological determinations aim, therefore, at the demonstration of the direct and indirect effects on the degree, duration, and thoroughness, of the heating and cooling operations upon the bacterial flora of the raw product.

In view of the relatively large amounts of milk handled even in the very shortest operative runs of even the smallest forms of practical pasteurization systems, it is obvious that substantially all control observations must be made while the system is in practical use—that is, the demonstrations of efficiency involving tests of the product, must be made upon the milk as it comes to the plant in the regular course of business, although this tends to introduce a considerable number of variable factors by reason of the multiplicity of experiences and vicissitudes through which the raw milk may have passed which may have affected its bacterial flora.

(a) Biological Demonstrations of Efficiency, Procedure A.

A. Comparison made between the counts of viable bacteria in the raw and the pasteurized and cooled milks, supplemented by similar bacterial counts at various important intermediary stages.

These comparisons are frequently expressed in terms of percentage reductions in numbers of living, i. e., viable bacteria on the usual culture media.

Ayers and others have called attention to some of the fallacies involved in the use of this system of percentage reduction in numbers of living bacteria, as a means of judging the effi-

ciencies of the design and operation of heating or pasteurizing processes.

Bacterial Flora in Raw Milk. The floras of raw milk are obviously subject to extensive variations as to origin, character and condition of the bacteria and of the effects of the growth or other activities of the same on each other and on the product itself.

The bacterial flora of a milk about to be pasteurized is derived from many sources, i. e., from the cow, i. e., milk ducts and reservoirs, udder, hair, skin, feces, saliva, etc., and from the subsequent growth of the same if any in the milk; from the human beings handling and milking the cows, i. e., skin, urine, feces, saliva, etc., and from the subsequent growth if any of the same in the milk; from the surroundings of the cow and human attendants, i. e., hay, grains and other feeds, soil, water, air, etc., and from the subsequent growth if any of the same in the milk; from flies, insects and other animal contaminations and from the subsequent growth of them in the product; from the farm utensils with which the milk on previous occasions has come in contact, the flora on the surface of which may be the result of failures in proper cleaning and are, therefore, of origins already mentioned, with or without subsequent growth due to bacterial incubation on moist apparatus surfaces or in cracks and crevices; or they may be due to the direct contamination of the milk handling apparatus from any or all of these sources of milk pollution just mentioned and from the incubation of these added micro-organisms on the moist and greasy surfaces of the utensils, cans, etc.

Factors Causing Changes in Bacterial Flora. The combined floras from these various sources may have been greatly modified by the time the product reaches the pasteurization system by action of bacterial antagonisms or associated growths, the character of which may have been subject to change by the likely alterations in temperatures, by agitations and other physical states to which milk in transit is continually exposed. These may likewise be influenced by the character of the metallic surfaces with which the milk has been in contact, such as brass, copper, and inappropriate metallic combinations.

Chemical Treatment of Milk. There occur frequently outbreaks of effort to keep down the bacterial counts in raw milk by various chemical treatment of milk and of milk handling apparatus. Those in which the germicide or preservatives have been added directly to the product itself have been but short lived. They are not often indulged in at present. Those in which the germicides are utilized in the sterilization or prevention of bacterial incubation in certain forms of milk handling apparatus, such as rubber milking machine parts, are not objectionable when the product used is harmless and special care is taken to remove the germ killing or restraining solutions by thorough rinsing. Where this rinsing is not done the solution may gain direct access to the milk subsequently handled in the apparatus. As the use of such solutions is extended to the washing or sterilization of pails, cans, etc., fairly substantial amounts of even more than one solution each having its own separate chemical characteristics, may finally find their way into the raw product. Here we must consider the probable selective germicidal effect upon different types of bacteria and other micro-organisms which, if some subsequent incubation occurs, is very likely to upset that important and more or less normal "balance" between the numbers of acid producing, inert, and alkali developing groups of bacteria. Any procedures liable to suppress or eleminate the natural preponderance of the non-spore forming acid types

without seriously affecting those alkali producing species which develop spores, will be liable to cause serious conditions in the raw milks presented for pasteurization.

Action of Heat on Different Bacteria. It is well known that exceedingly wide variations in susceptibility to the destructive action of heat exists among the different species, groups and strains of bacteria and between individual numbers of the same. It is also well known that different products resulting from the growth of these variable biological agents have more or less selective destructive action upon each other, especially when temperatures approximating their thermal death points are applied. Under such conditions the utilization for estimations of pasteurization efficiency of any numerical system, such as a percentage of the bacteria in the raw milk killed by the process, gives results in which the implied accuracy of the means of expression of the results is apt to be entirely fictitious. The use of percentage reductions in numbers of living bacteria as a measure of efficiency of pasteurization is not to be recommended.

Interpretation of Results. It is evident that the results of the operations of all of those very variable factors in the production, handling and transportation of milk, up to the time of its introduction into the pasteurization system are impossible of adequate detection by any simple laboratory or other technical procedure and that they are not easy of estimation even if one has a reasonably correct and detailed history of the product.

To those very familiar with the results of bacterial investigations of pasteurization apparatus and who also are experienced in biological problems in milk production and handling, in pasteurization processes, the results of bacterial counts of the raw and pasteurized products and of sample collected at important intermediary stages in the process, are of considerable value, although they are of more substantial utility when they can be correlated with the more or less detailed history of the product including the conditions to which it has been subjected prior to pasteurization. Therefore, in every investigation, having as its object the determination of the efficiency of a pasteurization system, one of the most essential features consists in the collection of a well-planned series of samples for the determinations of total bacterial counts, by use of the methods recommended by the Committee on Standard Methods of the Laboratory Section of this Association for the bacteriological examination of milk for legal purposes, i. e., those of the greatest degree of accuracy. However, in the interpretation of the results of such studies, those of adequate training and experience in this and allied fields will always give due consideration to the information available regarding the history of the product subjected to the pasteurization process.

(b) Biological Demonstrations of Efficiency, Procedure B.

B. Another procedure considers the final total numbers of living bacteria at some stage in the history of the milk subsequent to its pasteurization, as a measure of efficiency of the pasteurization system.

This plan is obviously better adapted to the estimation of the general bacterial condition or quality of the product itself than it is useful in the determination of the pasteurization efficiency. It consists essentially in accepting the results of one, i. e., the final step in the performance of a rather elaborate study, as adequately representing the results of the entire investigation. As generally used it complicates the study of the vital question of

the real effect of the heating process by introducing the results of the action of the various other factors which have altered the character of the product itself. It is true that the character of the raw product influences greatly the results of the study of the heating process, but the final results alone give at best only clues as to causes producing them. For example, a very low count milk could be passed through a bacterially clean, but otherwise very poorly designed or operated pasteurization system and yet yield a total count, which if taken alone might be acclaimed as indicative of high pasteurization efficiency. On the other hand, a high count milk might have been passed through a modern, well designed and efficiently operated pasteurizing plant and have given the same results. Still further those who make use of the final total counts as indicative of pasteurization efficiency or the lack of it, fail to consider the fact that some of the most common sources of high bacterial counts in pasteurized milk have no relation whatever to the efficiency of the heating processes applied or the thoroughness of their application, but are essentially matters of the adequacy of the previous cleaning of the cooling and bottling systems through which the product has passed after heating.

Interpretations of Results. As a means of estimating the general bacterial conditions and quality of pasteurized milks, the bacterial counts as standards are, as has been indicated, very useful, but reliance should not be placed upon them as accurate indices of the efficiency of pasteurization operations. Here again, they would be of more value if there were available for correlation with them, the fully detailed information covered by a history of the milk up to the time of heating and of testing.

(c) Biological Demonstrations of Efficiency, Procedure C.

C. Another procedure employed in the estimation of the efficiency of pasteurization operations, calls for the determination in the pasteurized product of the presence or absence and the numbers if present of bacteria of the B. Coli group. Rarely also are tests made for the presence or absence of streptococci. Still more rarely are they made for species or groups of pathogenic bacteria or other micro-organisms and then only if cultures of them have been added to the raw milk intentionally under experimental conditions, or if they have been found to be present in the raw milk.

It is obvious that in all of these cases too, it is quite essential to have available for comparison the results of quantitive determinations of the presence of the same types in the raw milk, for otherwise any negative results in the tests of the pasteurized product have but little significance.

These quantitive determinations in the raw and the pasteurized products yield far more valuable results for purposes of judgment of the effectiveness of the heating process than the comparative total bacterial counts on the same products, for the reason that members of these groups are more uniform in their respective susceptibilities to heat, and in fact to all alterations of environment, than are the various types of bacteria of all sorts. Moreover, these groups and species number among their members, races and strains which are themselves possessed, at times at least, with the power of producing human disease. They furnish therefore the means for a closer judgment of the probable effects of the processes studied upon the wider range of pathogenic bacteria of the non-spore forming types if the latter had happened to be present in the product under the conditions of the

heating. Not until recently, however, has science been in the position to appreciate the value of the results of such tests when made on a routine basis. The question of the relative susceptibility to heat of the various members of the B. Coli group and also the relative frequency of them in milk have been answered in part. This is also true of the members of the groups of streptococci. Extensive laboratory and some practical studies had been made by numerous workers on the thermal death points, in the presence of milk, of the various pathogenic types of bacteria but the opportunities for making tests with truly pathogenic bacteria in the raw product and the numbers there present known have been extremely few.

B. Coli as an Index of Efficiency. In one series of tests made under the direction of two members of the Committee, Dr. H. D. Pease and Mr. S. M. Heulings, strictly pathogenic types, B. typhosus (Hopkins); B. diphtherae; B. tuberculosis (both bovine and human varieties), were added in large numbers to volumes of milk possible of practical treatment in an apparatus which had been for several years in active commercial use in a fair-sized plant. Tests were applied for their presence in samples collected at all important stages in the process. Upon all such samples, quantitative fermentation tube tests were also made for the presence or absence of those forms of B. Coli which are commonly present in milk. It was possible, therefore, in this series of tests to make rather direct comparisons between the susceptibilities of each of the pathogenic types named and of the milk B. Coli forms, under the same conditions of temperature and time period of exposure. The results of these investigations are of special value as they offer strong support of the use of quantitative tests for the presence or absence of B. Coli in the heated product, as a means of indicating pasteurization efficiency. It can be stated that in no case were any pathogenic bacteria found in samples of the heated and held milks when the latter did not contain also the presence of substantial numbers of living B. Coli and in many of the samples in which B. Coli were found present in small numbers no pathogenic bacteria were detected by the most careful searching. Some of the pathogenic types and some of the B. Coli were found living after heating from 140 to 141 degrees F. and holding for 15 minutes, but none of the former were alive after 30 minutes, while B. Coli were still present in 1 c. c. tests, but were not found after 45 or 60 minutes holding at these temperatures nor with 30 minutes holdings at higher temperatures. In short, the greater tenacity of life of the B. Coli in the medium time periods of exposure to the medium temperatures was clearly indicated.

The conclusion is certainly warranted that the B. Coli forms commonly found in raw milk are at least not more susceptible to the usual temperature and time exposure conditions of pasteurization than are B. typhosus and B. diphtheriæ. In the case of B. tuberculosis, the comparison must be made between the effect of the heating process upon its power of infection of guinea pigs as compared with the growth power of the B. Coli in lactose bile tubes.

Since the time of these investigations (1911-1912) much work has been done by Rogers and by Ayers and their respective associates, on the means of separation of the B. Coli group into the two types of true B. coli and B. aerogenes and of the relative frequency of them in cow manure and cattle fodder as well as in milk.

Ayers and his associates have shown the relative occurrence of these two

forms in milk produced and handled under several of the most common commercial conditions. Neither they nor have others shown the relative susceptibilities to pasteurization temperatures of the two sub-groups. However, this information when available may be of greater scientific than practical value, for if those of one subgroup are shown to be more susceptible to the heat than the other, the practical fact would not be altered that in an efficiently performed pasteurization operation none of either of them should be found alive after the minimum standard temperatures and time periods of holding when the standard method for determination of them in 1 c. c. were used.

It is to be strongly recommended that similar quantitative tests be made upon the raw milk just before pasteurization and upon samples of the product upon the completion of each of the several important steps in the heating and cooling processes, such as samples from the first and last milks out of the heater, and one or two intermediary ones; from the first and last milk from the outlet of a continuous flow tank or pipe holding system and several intermediary ones; from the milk at the beginning and at the end of each holding period from a batch pasteurizer; from the first and last milks over the cooler and at several intervals in between.

Of special importance in this connection have been the demonstrations that substantially all market milks produced under commercial conditions and even the majority of certified milks will show positive results in the application of the standard tests for the presence of members of the B. coli group in 1. c. c. innoculations. Ayers and Clemmer have recently published more elaborate studies on the occurrence and significance of the colon count in raw milk and one of their conclusions is that "fresh milk produced under the best conditions always contains some organisms of the colon aerogenes group." While, therefore, it is recommended that in all tests to determine the efficiencies of pasteurization process by the use of quantitative determinations of the B. coli group, samples of the raw as well as those of the fully and partially pasteurized product should be collected and tested, it is evident that the results of such testing of samples of the raw and of the intermediary product are not as necessary for final judgment as are the results of the bacterial counts of the same or similar samples when only total counts are available. When a fairly detailed history of the product is available one familiar with the subject can obtain much valuable information and may even be able to express a tentative conclusion of the first, last and intermediary samples of the final heated and held product even if no such tests have been made of the raw milk.

Streptococci in Relation to Pasteurization. With respect to the use of tests for the presence of streptococci in pasteurized milk, the Committee knows of no published reports of studies conducted under truly commercial conditions. However, there have been a number of intensive investigations conducted under laboratory and semi-laboratory conditions.

Davis* made extensive studies of the streptococci found in various types of milks, i. e., certified and pasteurized, by both the flash and the holding system. His work deals chiefly with the haemolytic forms. His conclusion that of the strains of the Streptococcus lacticus some are haemolytic and some not, although neither are virulent for animals, is of importance. He found the various haemolytic forms more

*Davis, Journal of Infectious Diseases, 1916, 19, p. 236, and 1918, 23, p. 559.

often in certified milk than in the pasteurized. Some of them were of the human variety as indicated by various tests applied.

Avery and Cullen** have divided the haemolytic streptococci into two groups by means of their final hydrogen ion concentrations. Their haemolytic group includes both those of human and of bovine origins. They showed that substantially all of those of human origin reached final concentrations of pH. of from 4.8 to 5.3, whereas those of bovine types with but few exceptions and those of more or less doubtful bovine origin reached PH. of from 4.3 to 4.5. They propose a method of rapid differentiation of these two types.

The most applicable researches to the problem before us are those of Ayers, Johnson and Davis.* They investigated a wide range of organisms collected from many sources. By various tests both by cultural and animal inoculation measures they divide them into two groups. Those of Group A were all haemolytic many of them pathogenic and all had thermal death points of 140 degrees F., (60 degrees C.), or lower—chiefly lower. The other Group B., contained a very few concerning the pathogenicity of which there was some question, but they were chiefly non-pathogenic and were generally non-haemolytic, and the majority had high thermal death points.

They likewise correlated the two groups thus separated with a division through the application of the results of hydrogen ion determinations. Thus the A. series gave PH. figures of from 5.4-6, while the B. series showed from 4.5-4.7. They believe that pathogenic streptococci (Group A. type) are destroyed by the proper pasteurization of milk at 140 degrees F. (60 degrees C.) for thirty (30) minutes. They feel it desirable to have their work confirmed before coming to any definite conclusions.

The Committee feels that while enough has been done to indicate clearly that a proper application of heat to a temperature of 140 degrees F. for a minimum period of thirty minutes will destroy substantially all the pathogenic streptococci in milk still they believe as already expressed that a margin of safety for biological reasons calls for the use of higher temperatures of not lower than 145 degrees F. The Committee does not feel that there has as yet been suggested any easy and reliable bacteriological method for the determination of pasteurization efficiencies by tests for streptococci in the final product or by a comparison between the findings as to these forms in the raw and pasteurized samples.

As to studies upon other groups of bacteria having pathogenic powers, there is but little requiring comment. In so far as bacteria of known pathogenic properties is concerned, the opportunities for their use in determinations of the efficiencies of commercially operating pasteurization processes will be very infrequent. Of necessity these bacterial forms must have gained access to the milk accidentally and have been found present in the raw product and their approximate numbers there determined, before any failure to find them in the pasteurized samples would have significance. The epidemiological evidence as to the relative infectivities of the same milk, raw and pasteurized, would of course, be far more valuable.

The only occasions in which it would be warrantable for known pathogenic types to be used would be in instances where the apparatus was experimental in character and the milks to be treated in them were never to be offered for human consumption.

**Avery and Cullen, Journal of Experimental Medicine, Vol. XXIX, No. 2, February 1, 1919, p. 215.

*Ayers, Johnson and Davis, Journal of Infectious Diseases, 1918, 23, p. 290.

(d) Application of Tests to Demonstrate Efficiency. Up to this point, consideration of the methods and significance of the results of biological tests has been given on a more or less theoretical basis. It would be well to point out some of the most disturbing factors in the application of the tests discussed.

Tests for Sterility of Apparatus. From what has already been stated the conclusion must be self evident that the results of any study for the determination of the real pasteurization efficiency will be of little or no value if corroborated evidence is not available demonstrating the substantial sterility of the entire apparatus of the pasteurization system including the cooler, before the run starts. In fact, the first and most important element in such a study consists in the determination of the existence of such a sterile condition. When this is not possible, and it is true that many forms of commercial apparatus are substantially impossible to sterilize, it becomes necessary to collect a series of samples of waters of condensation or of previously sterilized salt solutions used as wash waters and to determine through the testing of them, the real bacterial condition of each and every important section of the apparatus before the treatment of the milk begins. This involves a considerable amount of labor and on many occasions even with such results available for consideration along with those of the tests on the samples of the raw and fully or partially treated milks, it is not possible to come to any definite conclusions as to the real efficiency of the heating processes studied.

One of the compensations arising out of this substantial difficulty comes from the fact that every study of the heating efficiency of a system must of necessity, determine the efficiency of the cleaning operations applied to each section of the apparatus at the close of the day's run.

While uncleanliness of apparatus is largely a matter of old milk, either dried on or moist and in a variable condition of bacterial incubation and while these conditions may not lead to any very direct health menace to the adult consumer of the product even when these conditions exist in the apparatus handling the already heated and held milk, still they are most objectionable from other standpoints. They tend greatly to reduce the general quality of the milk in that they promote rapid souring or other spoilage, and they tend to the development of objectionable flavors and tastes in the product. In the determination of the condition of sterility or cleanliness of pasteurization apparatus or of any milk handling utensils, the following procedures are offered as having given excellent results.

Samples of any water of condensation resulting from the steaming of the apparatus should be collected and tested both for total bacterial count and for the quantative presence of B. coli using inoculations of 1 c.c. and of smaller amounts when the washing and steaming processes have been obviously deficient. Such waters of condensation are obviously to be looked for at the lower or dependent portions of the apparatus. The points of collection should be selected also with the idea of obtaining information as to the condition of each important unit of apparatus in the system, i. e., weigh and receiving tanks, heaters, holders, cooler, fillers, etc. To collect the same it is often only necessary to unloosen the couplings in the sanitary piping at the lowest points. But in many cases the inspector must use considerable ingenuity in order to obtain sufficient amounts of such waters without contaminating them. Where there is no water of condensation the use of 200 c. c. of sterilized 85

per cent solution of sodium chloride may be advantageously employed as a wash water, with the collection of as much of it as is possible after it has flowed or been washed over the milk contact surfaces. Sterile spoons of the tea or dessert spoon size are very useful in the collection of samples of such waters when the latter are small in amount and are in otherwise inaccessible portions of the apparatus. They make it possible to remove by physical force with the wash or condensation water perceptible portions of the greasy films on the metal surfaces which almost invariably harbor excessive numbers of bacteria.

The tests should be performed by the Standard Methods of Bacteriological Water Analyses of the Laboratory Section of the A. P. H. A.

Tests for Defects in Design or Operation of Apparatus. In addition to the collection of the samples for the determinations of the sterility of the apparatus, samples of milk, foams, drips, etc., should be collected from each unit of apparatus to check up the possibilities of inefficient pasteurization treatments due to defective mechanical design or of improper operation of the unit. Attention has already been called to the more important of these possibilities in another section of the report. These samples are all in addition to those collected for the direct determination of pasteurization efficiencies. The latter should include the following and as many more as may be necessary to give a thorough picture of the effectiveness of each step and stage in the operation: Samples of the raw milk at the very start of the run and at the very close and one or more in between depending upon the history of the milk under treatment; samples of the first, last and several intermediary in the run of the milk after heating and before holding; the same after holding and before cooling and if there are several steps in this portion of the operation then samples from each should be taken; the same after cooling and before passing into the can or bottle fillers, also after leaving the fillers and before entering the cans or bottles and finally from the cans and bottles. The samples of the product in these later steps in the process are to be collected and tested with the object of determining the possibilities of reinfection of the already heated and held milk.

All such samples should be subjected to the standard procedures of the Laboratory Section of the A. P. H. A. for the bacteriological examination of milk for total counts and for the quantitative determinations of B. coli by the standard methods of bacteriological examination of water of the same Section.

Interpretation of Results on Tests of Efficiency. When the results on the cleanliness samples do not show the absence of B. coli in one c. c. tests of all drips and condensations and wash waters, and very small numbers of bacteria or none at all, the cleaning and sterilizing processes should be considered as deficient. When the results of the tests of the samples of the milk from the holding system indicate the presence of B. coli in 1 c. c. inoculations, further or careful and critical study of operations of each step and stage in the pasteurization process are called for. When the results of the tests of the samples taken at any stage after the holding show the presence of substantially larger total counts of the presence of larger numbers of B. coli than were found in comparable samples of the milk from the holding system, reinfection of the milk is indicated and the cause or causes of the same should be ascertained by further study.

(5) Conclusions on the Analytical Control of Pasteurization Plants. It is evident that the analytical control of pasteurization plants calls for the

active co-operation of engineers and laboratory investigators and that any complete or satisfactory study of even one plant calls for considerable effort. The great importance of efficiency in the ever increasing application of pasteurization processes to market milk and cream and other dairy products warrants a concentration of effort by both official and industrial agencies upon this phase of milk and dairy product supervision, even, if necessary, at the sacrifice of other inspectional or analytical investigations, the results of which do not have such a direct bearing upon the actual prevention of the transmission of disease.

VII. State and Municipal Supervision of the Pasteurization of Milk.

The Committee has carefully reviewed those parts of the milk ordinances, governing pasteurization, of practically every city of over 25,000 inhabitants in the United States, and does not feel that any one of them affords the full protection against ineffective pasteurizers and faulty operation that should be assured for so important a process. The legal authority controlling pasteurization grades all the way from ordinances that merely state that milk from tubercular cows shall be pasteurized and then do not define pasteurization, to the more complete and carefully thought out laws governing pasteurization plants for New York, San Francisco and Chicago. In the opinion of the Committee, none of them are adequate in light of careful studies of the various types of pasteurizing apparatus and the careless handling that frequently is met.

In reviewing many of the ordinances, the Committee has been impressed with the feeling that the framers of the regulations look upon pasteurization as merely a means for putting dirty milk on the market, without too greatly jeopardizing the health of the consumer. This is a great mistake, and no ordinance should be so worded as to permit a feeling to arise in the minds of the public that pasteurized milk is necessarily inferior to raw milk; in fact, if any impression is to be given by such regulations, it should be that pasteurized milk is preferable to the raw product. In the opinion of the Committee, any ordinance to be complete and effective must contain the following points: A definition of pasteurization; a statement of the temperature and time that shall be used; temperature to which milk shall be cooled, etc; the objects to be obtained, namely ultimate bacterial count, etc; regulations governing construction of the apparatus and equipment; control, namely, the organization to have supervision of the process; and finally, penalties to be imposed for failure to comply with the provision of the ordinance.

(1) Time and Temperature Requirements. The Committee feels that no milk should be considered pasteurized that has not been heated to a temperature not lower than 145 degrees F. for not less than 30 minutes. Technical investigations have demonstrated that the germicidal action of increasing degrees of heat for longer and longer time periods is progressive in character; that is to say, that temperatures substantially below 145 degrees F. have a more and more injurious action upon some of the disease producing bacteria which may be in the milk under treatment, both as the temperature is increased and as the time of its application is prolonged. While the action of these amounts of heat for these periods is progressive in character, it is the opinion of the Committee that this factor must not be considered as justifying any failure in the operation of any form of apparatus or system to apply the appropriate degree of heat for the full time period to every particle of the product. In other

words, this factor does not justify the averaging of the temperatures of different portions of the product under treatment at any particular time period. It is the opinion of the Committee that a temperature of 145 degrees F. applied for 30 minutes to every particle of the milk under treatment consitutes a process which can be applied under appropriate practical conditions without endangering what has been popularly known as the "cream line" of the product. It should be specified that pasteurized milk shall be promply cooled to and maintained at 50 degrees F. or below, a principle which should be applied to the handling of all milk.

(2) Department to Control Pasteurization. The Committee feels that the control of pasteurization plants in the various states is properly a function of the state health departments, except in those cases where the municipal health department has a division for milk work exclusively, organized on an efficient basis that will give the municipality competent supervision and control of not only all the milk within the municipality but also its production, preliminary handling and transportation. This division having a superintendent and assistants with an organized staff of inspectors for the inspection of the sources of the supply and all creameries or shipping stations through which the milk passes; also bacteriological and chemical laboratories in charge of technical operators and assistants; an engineering staff in charge of a chief engineer, competent to pass on the construction of dairy buildings and apparatus and direct the operation of the apparatus and also a system of rules and regulations, that when enforced will insure safety to the public in the matter of the milk supply. It is probable that a majority of the existing pasteurizing plants will fall under municipal control, but gradually more and more plants are being constructed in outlying districts to which municipal authority cannot well extend, and these should be covered by the inspection service of the state health departments, and should be under their authority. At present in several states the supervision of pasteurizing machinery is a function of the department of agriculture. It is the opinion of the Committee that the control of a process so fundamentally allied with health as is the pasteurization of milk, lies properly with the health authorities, rather than with the department of agriculture.

The state health department should promulgate minimum standards for the control of milk pasteurization, which may be supplemented by the municipalities to fit their peculiar needs, but in no case shall the minimum standards of the municipality be less than those for the state at large. Again the health departments of the various states are equipped to undertake the medical, engineering and laboratory work that is absolutely essential, if proper supervision and control of the design, construction, and operation of milk pasteurization plants is to be maintained. The prosecution of failures to pasteurize milk properly is naturally a function of the health department.

(3) Requirements Regarding Apparatus. A special permit should be required by the state department of health or by the municipality having an adequate health department for the erection or alteration of a pasteurizing plant, and the issuance of this permit should be based upon plans, specifications and detailed drawings, submitted to that office for their permanent files. This recommendation is in line with the procedure already adopted for water supply and sewerage systems. These drawings should be carefully studied in the light of the information relating to the defects and their dangers commonly found in pasteurizing

plants as brought forth in the preceding pages of the report of this Committee, and the permits withheld if there is any possibility that defects in the design may jeopardize operation. Not only must the design of the proposed pasteurization plant be free from defects, but provision must also be made for accurate control of the process, and for the securing of complete records of the operation of the plant, as shown by recording temperature and time devices on the heater holders, and cooling apparatus.

(4) Qualifications of Plant Operators. The pasteurization of milk requires not only a proper building and efficient apparatus for treatment of the milk and sterilization of the containers, but, also, competent, efficient and reliable operation of the apparatus. This requires that the prospective operators should have special training at a dairy school or under an experienced and competent operator of a pasteurizing plant, before being allowed to assume the charge of a plant. To bring these operators closely under legal control, no person should be permitted to have supervision of the pasteurization of milk or to operate milk pasteurizing apparatus without having passed an examination conducted by the health authorities. This examination should show that the person is not only competent to operate the apparatus, but is also familiar with the laws relating to this work and the responsibility of the supervisor or operator to the public.

A license at nominal cost, renewable annually, should be issued by the health department to person desiring to engage in this work and who show they are qualified to perform it. The suspension or revocation of such a license for careless or improper work on the part of the licensee should render it impossible for such person to be employed in any dairy work within the jurisdiction of the health department issuing the license until reinstatement.

(5) Bacterial Results on Pasteurization. There is probably no one phase of the pasteurization problem that is occasioning more thought and controversy than that of the bacterial results to be demanded. The Committee feels that this is a problem to be studied in each community, and the limit set as a result of such investigation. Climatic conditions, length of haul, type of conveyance as by auto truck, refrigerator car, a common baggage car, and many other factors, each affect the bacterial content of the raw milk, and with it the content of the final product, so that the Committee does not believe it possible to specify a minimum standard that can be used nationwide. Any such standard would permit of careless operation of pasteurizers in some cities, and with it the placing of inferior milk on the markets; while in other cities it might become either a question of violating the ordinance or going without this article of food.

The ordinances of several cities specify a 99 per cent removal. Nothing could be more ridiculous, for in cases of raw milk with a low bacterial content it is impossible to secure a 99 per cent removal; while with a very high content a removal of a greater percentage is readily affected, and 99 per cent represents an inferior product, and yet the percentage required can be met even with faulty operation.

(6) Penalties for Violations. To insure compliance with an ordinance it is unfortunately necessary to provide penalties for failure to do so. The severity of penalties varies more or less throughout the country, and it is best that this feature of any milk ordinance be prepared to conform with practice in that district. The Committee feels that, in order to insure convictions for violations of those fea-

tures of the ordinance governing the character of the milk and its bacterial content, the regulations should be specific on how samples shall be collected and handled. For example, in some regions the agent of the health department takes forth a few cubic centimeters out of the bottle with a sterile pipette, and takes this sample to the laboratory in a sterile container. The dealer thus always has the opportunity to claim that the pipette or the container were contaminated, or that the wind was blowing street dust over everything, while the sample was being collected. The Committee is inclined to favor the purchase of an entire bottle of milk direct from the delivery wagon, and sending this in an iced container to the laboratory for analysis, for then the dealer cannot complain that contamination was introduced in sampling, but on the other hand it does not allow the dealer an opportunity to get a check on the same sample.

Milk that is sold as raw or without any marking or labeling whatsoever, is notice to the public that the milk should be cooked before use in order to protect themselves against any possible infection that may be carried by the milk. Milk that is sold as "pasteurized" conveys to the public the impression that no cooking or further safeguard is required. Any use of the word "pasteurized" or of any designation or marking that may create in the mind of the public the impression that the milk has been pasteurized when it has not had efficient pasteurizing treatment, or has been exposed to possible infection by having had the package tampered with, or bears an incorrect date or any other incorrect data, should be made by law a criminal use of the word "pasteurized." This should subject the person or persons guilty of such crime, or the person or persons by whose direction, or connivance such crime was consumated, to prosecution and such heavy legal penalties, as will insure full protection to the public health in the matter of pasteurized milk.

The word "pasteurized" when applied to milk, should be limited by law to designate raw milk that has been produced and handled under sanitary conditions, that is of good bacteriological, chemical and physical condition, before pasteurization, that has had efficient pasteurizing treatment under competent and responsible operators and supervision, that immediately after pasteurization and in the container when delivered to the consumer, does not have an excessive bacterial content, and that has not been exposed to unnecessary risk of infection after pasteurizing treatment. These requirements should be fulfilled in order that the public will not be deceived when they see the word "pasteurized" on a package of milk.

The opening of a package containing pasteurized milk in any place not licensed by the health department as a milk plant or store, or by any person not authorized by the health department to handle milk, previous to its delivery to the consumer, is an unnecessary risk of infection and should be prohibited.

The sole object to be attained by pasteurizing milk is the elimination of any pathogenic organisms that the milk may contain. Therefore, the treatment must be obsolutely efficient, and unnecessary risk of infection after treatment must not be permitted. Pasteurization is under no circumstances to be considered as a renovating process; unhealthy operators, insanitary surroundings, and careless or dirty methods of handling the milk, must not be tolerated.

Summary.

Information collected throughout the United States and the Dominion of Canada shows that there are approximately 4,200 pasteurization plants in operation in these countries at the present time and that only a very limited number of these installations are controlled from a public health point of view. There is little uniformity in the definitions of milk pasteurization used by Federal, state and municipal branches of government which leads to much confusion as to the proper meaning of "pasteurized milk". There is also a very apparent lack of understanding on the part of the public regarding the actual meaning of pasteurization and the reason for its general application.

The results of scientific workers on the effect of pasteurization on the composition of milk indicate that there is little, if any, change in the chemical composition so far as can be determined by chemical analysis. A large amount of experimental work has been conducted on the undesirable effects caused by pasteurization on milk that is to be used for infant feeding. Some conflicting opinions have resulted from this work but it is now generally recognized that any ill effects from the use of such milk for infant feeding can be easily remedied by the addition of certain common substances such as orange juice and potato water. The protection that pasteurization affords older children and adults from communicable diseases far overshadow any of the easily remedied ill effects associated with infant feeding.

The evidence presented on the various methods used for the pasteurization of milk indicate that the "holding" system is so much superior to any other as to justify its universal application for the pasteurization of milk to be used for human consumption. The process of pasteurization of milk should consist in subjecting the milk to a temperature not lower than 145 degrees F. for not less than 30 minutes.

The mechanical features of pasteurization plants has not been given sufficient attention by many of the departments supervising the pasteurization of milk, and defects in pasteurizing apparatus are found in many plants now in operation. Defects may be found associated with nearly every part of pasteurization apparatus which can be eliminated by proper design, construction and operation of the plant. It is possible to construct a commercial pasteurization plant on a practical basis without inherently dangerous defects. Such a plant when properly operated should produce a pasteurized milk which is safe for human consumption.

The analytical control of pasteurization plants is a subject that has been given considerable attention by health authorities and many methods have been studied for determining the efficiency of pasteurization plants and the various apparatus associated with the pasteurization of milk. The methods discussed in this report include the physical, chemical, physico-chemical and biological. The physical methods involving the testing of sensitive instruments used to control the process, and biological tests to study the efficiency of plants and the quality of their effluents appear to be the best of those already devised and applied on a practical basis. It is clearly evident that the analytical control of pasteurization plants calls for the active co-operation of engineers and laboratory investigators before satisfactory interpretations can be made and efficient results accomplished.

State and municipal supervision of pasteurization should involve certain fundamentals for the protection of the public health. It appears to be gener-

ally recognized at the present time that the branch of government to which this supervision work should be assigned is the health department. This makes possible the correlation of this work with other health activities that are primarily directed towards the suppression of disease. The state should at least set minimum standards for the control of milk pasteurization which may be supplanted by the municipalities to fit their particular needs. The supervising health department, organized for the control of milk pasteurization, should have available engineering, laboratory, and medical service. The approval of the health department should be required on the system and equipment of each plant producing pasteurized milk. A large part of the supervision should be concentrated on the construction, operation and management of the plant and its equipment. The operators of such plants should have had proper training and be licensed by the health department. Milk should not be sold as "pasteurized" unless its production and sale is properly legalized and supervised by competent health authorities. Laws and ordinances governing pasteurized milk should be specific in every detail and should provide adequate penalties for offenders.

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STANDARD METHODS

FOR THE

BACTERIOLOGICAL EXAMINATION OF MILK

THIRD EDITION

AMERICAN PUBLIC HEALTH ASSOCIATION
169 Massachusetts Avenue
BOSTON, MASSACHUSETTS
1921

STANDARD METHODS

FOR THE

BACTERIOLOGICAL EXAMINATION OF MILK

THIRD EDITION

REVISED BY COMMITTEES OF THE LABORATORY SECTION OF THE AMERICAN PUBLIC HEALTH ASSOCIATION, AMERICAN DAIRY SCIENCE ASSOCIATION, INTERNATIONAL ASSOCIATION OF DAIRY AND MILK INSPECTORS, AND MEMBERS OF COMMITTEES FROM THE SOCIETY OF AMERICAN BACTERIOLOGISTS AND AMERICAN ASSOCIATION OF MEDICAL MILK COMMISSIONS.

ADOPTED BY THE LABORATORY SECTION OF THE AMERICAN PUBLIC HEALTH ASSOCIATION IN SEPTEMBER, 1920, AT SAN FRANCISCO, CAL.

AMERICAN PUBLIC HEALTH ASSOCIATION
169 Massachusetts Avenue
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1921

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PREFACE TO THIRD EDITION

The first edition of the Standard Methods for Milk Examination was prepared by a committee consisting of F. H. Slack, Chairman, W. H. Park, B. H. Stone, H. L. Russell, C. E. Marshall, and F. C. Harrison, and was issued by the American Public Health Association in 1910. A second edition of this report was prepared in 1916 by a committee consisting of M. P. Ravenel, Chairman, J. A. Anderson, H. W. Conn, W. H. Park and R. S. Breed with B. H. Stone and W. R. Stokes as adjunct members. This committee worked in coöperation with committees from the Society of American Bacteriologists and of the American Dairy Science Association.

The present third edition report has been prepared by a committee consisting of W. H. Park, Chairman, M. P. Ravenel, R. S. Breed, J. A. Anderson and H. A. Harding with B. H. Stone and W. R. Stokes as adjunct members. This committee has sought and obtained the coöperation of still other associations interested in the sanitary control of milk in an effort to make the report truly representative of American laboratory workers.

A summary of the more important changes from the second edition follows:

1. The scope of the report has been broadened to cover the sediment test and the examination of milk for the presence of long chain streptococci.
2. A summary of required procedures is given at the end of the report.
3. Encouragement is given for further investigation of promising new laboratory methods.
4. Official recognition is given to microscopic counts made directly from unpasteurized milk.
5. Methods for determining the H-ion concentration of agar media are given.
6. It is recommended that the practice of speaking of agar plate counts as showing the number of "bacteria" per cc. be discontinued and that a more accurate form be used.
7. It is insisted that punitive actions should be based upon the average results from a series of samples, and that the routine counts should be verified by suitable procedures when actions based on their use are likely to be questioned.

STANDARD METHODS FOR THE SANITARY ANALYSIS OF MILK

AMERICAN PUBLIC HEALTH ASSOCIATION

LABORATORY SECTION

The rapid development in the use of laboratory methods for controlling the quality of milk, makes a third revision of the Standard Methods necessary In the second report[1] of this committee, it was pointed out that two types of bacteriological analysis of milk were required. These were distinguished as routine and research methods. In the years that have passed there has been an increasing appreciation of the need for such a distinction, and less tendency on the part of research workers to use the routine type of analysis for investigations in which the greatest possible accuracy of counts is more important than moderate accuracy combined with a rapid and relatively inexpensive technique.

1. *Routine Milk Analyses.* This type of analysis is designed for general control of the public milk supply, and for the purpose of grading milk. For routine analysis the work must be capable of being done quickly and cheaply with fair uniformity of results. Some opposition to the methods outlined in the previous report has existed because of the willingness of the committee to meet this need. This objection has been considered but it is still felt that the necessity for such quick and inexpensive methods must be recognized. No such rapid progress in milk control work could have been made as has been made since 1916, if a more time consuming procedure had been outlined as the standard procedure.

Numerous investigations[2] to be sure have shown that higher counts may be obtained from agar counts where lactose, or meat infusion, or both are added, or the count may be increased by prolonged incubation. The expense of such procedures in time or in materials would greatly reduce the number of analyses that a routine laboratory could make, and the quickness with which results could be utilized. Hence they limit its usefulness. Long experience in some of our most important control laboratories shows that the results secured by the agar plate technique described in this report give a sufficiently accurate proportional fraction of the true count to be of value in milk control. Therefore it seems unnecessary to require the more refined technique for routine work; but the methods used should be uniform in different laboratories.

It is not necessary in public health work that the counts used should represent the actual number of bacteria present or even that they should represent the greatest number of colonies that could be developed on agar media, if there is a fairly accurate knowledge of the percentage developing. The standards set for various grades of milk in the usual ordinances recognize the fact that the counts obtained by the standard procedure

do not represent the highest count that can be obtained.*

It has been abundantly demonstrated that no plate count, with any technique yet devised, gives the total number of bacteria in milk. Hence, the standard plate method should not be expected to show all the bacteria possible, but rather to furnish an artificial means by which different laboratories can get reasonably accurate comparative results. Because some method gives a higher count than the standard technique does not prove that it is better for control work. The best count for this purpose would be the one giving the most uniform percentage of the actual number of bacteria. Until the evidence is forthcoming to show which method is best from this standpoint, there is ample justification for recommending media that can be prepared as easily and inexpensively as possible, although other media may give higher counts.

2. *Verification and Research Methods.* For certain specific purposes it is recognized that the routine methods here described do not give the full information or the grade of accuracy required. For investigational purposes or wherever it may become necessary in a routine laboratory to verify routine analyses for legal purposes or for other reasons, more accurate methods should be used. These uses are likely to become even more distinct than they are at present as the grading of milk comes to be more generally observed in commercial practice. In the first report issued[4] these different uses for bacterial counts from milk samples were not recognized because the bacteriological control of the milk supply had scarcely started and no one could anticipate what it might demand.

*The most notable exceptions to this statement are the ordinances for Boston and several nearby cities.

METHODS DESCRIBED

In spite of numerous attempts to find satisfactory and simple biochemical tests or tests for specific bacteria that will give results as valuable as those secured from bacterial counts none have been found of such value that they have come into general use. For this reason they are not included in the present report; but this conservatism should not be used to discourage investigation along these lines.

The sediment test has been included because investigations have made it clear that the relation between milk containing visible dirt and milk containing large numbers of bacteria is not necessarily a close one. If sole reliance is placed on bacterial counts in the grading of milk, it occasionally happens that dirty milk, or milk that is undesirable for other reasons, is given a stamp of approval. Even the use of the sediment test in addition to the bacterial analysis does not certainly prevent this, as dirt is easily removed from milk by straining or clarification. Moreover, neither the ordinary bacterial analysis nor the sediment test reveal the presence of pathogenic bacteria, or undesirable oders or flavors.

The most important new developments in the five years since the last report was issued, have been the increasing use of modifications of the agar plate technique intended still further to simplify and shorten it, in improvements in adjusting the reaction of agar media, and in using microscopic examinations of fresh milk as a means of controlling its quality. The newer developments in the technique of counting bacteria have resulted in making it possible to

get a more correct conception of the size and the causes of the errors in counts as made with the ordinary technique. As these investigations have produced data showing that agar plate counts are sufficiently accurate amply to justify their use in grading milk, much uncertainty regarding the justification for their use has disappeared.

The development of methods for counting bacteria in milk by direct observation under the microscope has also served to differentiate more sharply the various purposes for which bacteria counts are useful. Thus the microscopic method has found a large field of usefulness as a method of judging the quality of fresh milk as delivered at milk receiving or pasteurizing stations.

It serves this purpose so satisfactorily that this method is recognized as an official procedure in this report. On the other hand there is no method for judging the efficiency of pasteurization by examination of a series of process samples that can replace the generally used agar plate technique. Likewise because of the extensive use of pasteurization, the agar plate count remains the standard method for judging the number of living organisms present in milk as delivered to the consumer. In small places where pasteurization has not been introduced, the microscopic examination may be used, if preferred, as a means of judging the quality of milk as delivered to the consumer.

Collection of Samples for Bacteriological Counts

Although the technique of the plating method is fundamentally different from that involved in microscopic counting, microscopic counts are readily made from the same samples as those used in making agar plates. As the precautions necessary for securing a fair sample are identical, the method of collecting samples for both methods are described under a single heading.

All collecting apparatus, glassware, pipettes, collecting tubes, bottles, etc., shall be sterilized at a temperature of at least 175° C. for one hour.

Each sample shall consist of at least 10 cc. of milk. Before taking the sample the milk shall be mixed as thoroughly as possible. If the original container can be inverted the mixing of the milk should be done by inverting it several times. If this is impossible, the milk should be stirred with some sterile stirrer. Any stirrer already in the container may be used. If there is none in the container, the sampling pipette (or any other sterile article) may be used; but it shall be used for one container only until it is again sterilized.

A sample merely poured from a large can is not a fair sample unless the milk in the can is thoroughly stirred. Neither is a sample of mixed milk, taken after it is poured into an unsterilized weighing vat, a fair sample from which to judge the quality of the milk before it was poured into the vat. The sample shall be taken from cans by means of a glass or aluminum tube with straight sides, long enough to reach the bottom of the original container and inserted, not too rapidly, with the top of the tube left open. This will result in the tubes containing a cylindrical section of the milk from top to bottom of the can. The finger then placed on the top of the tube will make it possible to withdraw the tube full of milk and transfer it to the sampling bottle. The sampling bottle should be large enough to hold the entire contents of the tube, all of which must be reserved as the sample. Each tube shall be used for collecting a single sample only, and must be washed

and sterilized before it is used again. If the sample is taken from a bottle, the bottle should be first shaken to ensure thorough mixing and the milk may be poured into the sample bottle, although it is better here also to use a sampling tube.

If the temperature of the milk is desired, it should be taken from a different container from that used for the bacteriological sample, or after the bacteriological sample has been withdrawn. All records shall be made immediately after taking the sample. The milk sample shall be placed in a properly labeled bottle. The most convenient kinds of sample bottles are glass stoppered, or those closed with a cork lined screw cap. Cotton plugs are not a satisfactory method of closure. The sample bottles shall be placed at once in a carrying case containing cracked ice, so that the milk is promptly cooled to near the freezing point.

The samples shall be transferred to the laboratory as quickly as possible and shall be plated with as little delay as possible. The samples placed in cracked ice and water may be kept for several hours (12) without an appreciable increase in bacteria. If the plates are not made within four hours from the time of collection, the number of hours that did elapse should be stated in the report. If the milk is kept at 40° C. a slight and somewhat variable increase may be found in twelve to twenty hours. Up to twenty hours this will not be more than 20 per cent in normal cases. The larger increases may be expected in milk which has been stored at low temperatures for some time previous to sampling.[6] Continued shaking of the milk during its transit to the laboratory tends to break up the clumps into smaller masses and so increases slightly the number of colonies.

In the case of samples to be used for direct microscopic examination, icing of the samples may be dispensed with under some conditions where it is possible to add preservatives (formaline 2 to 3 drops of a 40 per cent solution of formaldehyde for each 10 cc. of milk)[7] to the samples as taken. Samples containing preservatives that have been allowed to stand until the cream is compact are not satisfactory, and are likely to give a lower count than fresh samples.

(A) MACROSCOPIC COLONY COUNT (PETRI PLATE METHOD)

Composition of medium.

Standard beef extract agar* shall be used for all routine work and shall contain the following ingredients:**

Agar (oven dried)	1.2%
or	
Agar (market)	1.5%
Beef extract	0.3%
Peptone	0.5%
Distilled water	

The beef extract shall be Liebig's where this is obtainable, or some other brand giving comparable results.

Witte peptone, if available, can be used with assurance that the reaction of the medium will be neutral (pH = 7.0); other brands — such as Armour's, Digestive Ferments Co.'s, Park, Davis Co.'s, — although more acid can often be used for milk analysis without necessitating change of reaction; and nearly any good commercial peptone may be used with comparable results

*Beef infusion may be substituted for beef extract in those laboratories where past records are based on the use of beef infusion agar; but in the interest of uniformity, it is urged that beef extract be used.

**This medium is essentially the same as that recommended in the last edition of the Standard Methods of Water Analysis except for the reaction preferred.

provided special attention be given to the H-ion concentration of the medium.

The agar must be of the best quality. If oven-dried at 105° C. just before using, take 1.2%; if used just as obtained in the market without oven-drying, use 1.5%. Remove salts and any dirt present by soaking, washing and draining. Distilled water is to be used for dissolving the ingredients.

Reaction.

A medium consisting of the above ingredients, including a suitable peptone, ordinarily has a reaction between pH = 6.2 and 7.0. If within these limits, the reaction requires no adjustment for milk analysis. The most desirable reaction is about pH = 6.5 to 6.6; but any reaction between pH = 6.2 and 7.0 is allowable. No change in reaction should be made without carefully determining the H-ion concentration of the finished medium by the method described below.

Inasmuch as the range of H-ion concentration recommended for water analysis[8] is pH = 6.8 to 8.4, it is permissable, if desired, to use a single agar for both purposes with a reaction of pH = 6.8 to 7.0. If Witte's peptone is used in the above formula, this will ordinarily be the reaction without adjustment.

Each batch of finished medium should be tested before use as to its final reaction after sterilization. This test is to be made as follows:

Put 4 cc. of distilled water at 30 to 40° C. (*not warmer*) in a test tube. Add 1 cc. of the agar to be tested and then 10 drops of the indicator, brom thymol blue* (0.04 per cent solution in 95 per cent alcohol). The resulting color should be either a yellowish green or vary to a deeper shade of grass green. To one whose eye is trained this shade of color is sufficient.

These shades may be accurately determined by means of the buffered solutions[9] of Sörensen or of Clark and Lubs.

However, they may be approximately determined by comparing the tube of agar containing the indicator with a set of color tubes after the method of Barnett and Chapman.[10]

Select 12 test tubes of even caliber and place in two rows of 6 each. In each tube of one row put 5 cc. of a dilute alkali (as, for example, twentieth normal sodium hydroxid). In each tube of the other row put 5 cc. of very dilute acid (one drop of concentrated sulphuric or of hydrochloric to 100 cc. of distilled water is sufficient). Avoid stronger acid.

Add indicator to the tubes as follows:

Acid tubes	Alkali tubes	H-ion concentration
9 drops	1 drop	pH 6.2
8 drops	2 drops	pH 6.4
7 drops	3 drops	pH 6.7
6 drops	4 drops	pH 6.9
5 drops	5 drops	pH 7.1
4 drops	6 drops	pH 7.3

The tubes are to be viewed in pairs of acid and alkali, each pair containing the sum of ten drops of indicator.

If preferred, double these quantities may be used throughout and the indicator measured in fractions of a cubic centimeter instead of drops. That is, two cc. of agar should be taken for testing. This should be added to 8 cc. of distilled water. One cc. of indicator should be used. In comparing with the Barnett and Chapman tubes, use 10 cc. of dilute acid or alkali in each tube, and add the indicator in tenths of a cubic centimeter instead of in drops.

All of the test tubes used in this

*Prepared by Hynson, Wescott & Dunning, Baltimore, Md.

determination must be of the *same diameter* and of clear glass.

Another indicator, brom cresol purple,* (0.04 per cent solution in 95 per cent alcohol) may be used as an alternative for brom thymol blue. Its use is especially desirable if the reaction of the agar is more acid than pH = 6.4, because brom thymol blue is not very sensitive at this point. Brom cresol purple, on the other hand, is not sensitive at pH = 7.0 and therefore cannot be used if the medium is of neutral reaction.

The pH values corresponding to the color pairs (acid and alkali) prepared by the method of Barnett and Chapman have been worked out by Medalia.[11] The color of brom cresol purple is a deep shade of purple at pH = 6.8 with increasingly lighter shades to pH = 6.2. At pH = 6.0 the color is of a grayish hue not easily confused with that of pH = 6.2.

Adjustment of reaction.

If the correct color of the indicator does not appear in the agar as tested, add dilute NaOH (e. g. N/20) from a burette until the shade is obtained which represents the desired H-ion concentration, that is between pH = 6.8 and 7.0. Fifty times the amount of N/20 NaOH added from the burette equals the amount of normal NaOH to be added to one liter of the medium if 1 cc. of the agar is being tested. When testing 2 cc. of agar, multiply by 25 instead of 50.

In this adjustment, it is permissable to use any strength NaOH, but the strength of that added to the medium must be an exact multiple of the strength of NaOH used in titration; if the ratio is not 1:20 proper allowances must be made.

*Prepared by Hynson, Wescott & Dunning, Baltimore, Md.

Method of preparing agar.

The important point is to secure an agar of the correct reaction and composition which contains no troublesome precipitates. Methods of cooking and filtering to accomplish this vary with the ingredients used. Those suggested below have been found satisfactory in practical use; but other methods securing the same results are allowed. White of egg, however, must not be used for clarification.

The finished medium may be tubed or bottled, placing 10 cc. in each tube or 55 cc. (enough for five plates) in each bottle.

Sterilization shall be accomplished by heating in the autoclave for 20 minutes after the pressure reaches 15 lbs.; or after the agar is completely melted, heat in flowing steam on three successive days for 20 minutes each day.

All glassware and all apparatus such as kettles, funnels, and filtration flasks, must be kept scrupulously clean by running hot water over or through them after use before the agar has had time to harden. There is danger otherwise of dried particles of agar chipping off and giving rise to sediment in future batches of agar which in the poured plates may be mistaken for colonies.

Procedure No. 1. Mix all of the ingredients together cold. Heat in an autoclave at 15 lbs. pressure for 40 to 90 minutes according to the quantity of medium being made in each batch. Allow the autoclave to cool very slowly so as not to disturb the sediment. Decant through a cotton filter taking care not to pour the sediment on the cotton until the bulk of the liquid has passed through.

This simple procedure with certain brands of peptone and grades of agar gives excellent results.

Procedure No. 2. Where large quantities of agar are to be prepared the following procedure has been found useful. Prepare two separate solutions:

MIXTURE A

Beef extract 0.3 per cent of total quantity of medium to be made.

Peptone 0.5 per cent of total quantity of medium to be made.

Distilled water 40 per cent of total quantity of medium to be made.

Place in a kettle. Weigh kettle with contents. Heat on stove to boiling, and boil five minutes. If absolutely necessary to adjust reaction (see Reaction) do so at this point and boil again. Make up with hot distilled water that lost by evaporation. Do this by weight. Filter through paper or paper pulp in a Buchner funnel (see below).

MIXTURE B

Agar oven dried 1.2 per cent (market 1.5 per cent) of total quantity of medium to be made. Soak and wash under tap in sieve. Weigh before and after soaking to determine quantity of water absorbed. Distilled water 60 per cent of total quantity of medium to be made, minus that absorbed by the agar during the washing.

Mix A and B (agar not yet melted). Heat mixture over stove, stirring at frequent intervals until agar is entirely melted. Then boil and stir constantly for 20 minutes. Make up by weight water lost by evaporation by adding hot distilled water. Keep kettle of agar in chamber of flowing steam while preparing funnel for filtering.

Filter through cotton until clear. For 10 liter amounts it is suggested that either a Sharples centrifuge or a nine inch Buchner funnel with a suction pump be used. The ordinary filtration pump attached to a water faucet producing about 11 inches of vacuum gives good results.

Prepare paper pulp by soaking scraps of ordinary filter paper for 36 to 48 hours in a large wide-mouthed bottle. The paper and water should be in the ratio of six sheets of soft absorbent filter paper (20 by 20 inches) to 2½ liters of hot water. Moisten the paper and tear it into fragments about ¼ to ½ inches square. Shake vigorously at intervals to make the suspension fine and uniform. When ready to prepare the nine inch funnel, take 400 to 500 cc. of the paper pulp and dilute it with about three liters of very hot water. Cut a piece of surgeon's lint (or cotton flannel) to fit the bottom of the funnel exactly. Rinse the funnel with hot water. Place in it the lint with the fleecy side uppermost. Pour in the hot paper pulp suspension carefully so as to cover the lint with an even layer about ⅛ to ¼ inch thick. Over this lay a disk of filter paper. Place a four liter suction flask under the funnel and apply the suction to draw the water into the filtration flask until the pulp is firm, yet somewhat moist. The agar will not go through too dry a filter.

The funnel and the paper pulp must be hot when the agar is poured in carefully and slowly, striking the disk of filter paper which prevents the breaking of the surface of the paper pulp. Discard the first 100 cc. of agar which come through as they contain some of the water from the pulp.

Even in the first filtration the agar should come through very clear. Keep the remainder of the unfiltered agar hot in flowing steam while the first part is running through the filter.

Ordinarily the temperature of the agar in the funnel is 80° to 85° C. but

the last portions will come through well as low as 50° to 55° C.

Keep the filtered agar hot in flowing steam while preparing a second funnel in the same way as the first. Then filter as before.

Plating.

For miscellaneous milk samples, the character of which is not known, three dilutions shall be made; 1 : 100, 1 : 1,000 and 1 : 10,000. Where the character of the milk is known, the number of dilutions may be reduced. If the milk is pasteurized, certified or known to be fresh, and of high grade, the 1,000 and 10,000 dilutions may be omitted. If the milk is known to be old and of high bacterial count, the 100 and 1,000 dilutions may be omitted, and dilutions in excess of 10,000 prepared. In no case shall less than two plates be made from each sample. Where two satisfactory plates are obtained it is advisable to count both of them.

The water used for dilutions may be placed in dilution bottles (99 cc., 49.5 and 9 cc. are convenient sizes) and sterilized for one hour in an autoclave at 15 lbs. pressure. The bottles should be marked so that it can be determined that they have neither gained nor lost water during or subsequent to sterilization. Or, the water may be sterilized in bulk, if kept in a properly guarded container, and subsequently measured directly into the dilution bottles with sterilized pipettes.

The dilution bottles should have glass or cork stoppers, or some other type of closing that makes shaking possible. Cotton plugs are a less satisfactory method of closure because a small portion of the dilution water will soak into the cotton[12].

Straight sided pipettes graduated to deliver 1 cc. are best. They may be either the two mark or the one mark style. In either case, the errors of measurement are caused more by faulty calibration or by faulty manipulation of the pipettes than by the particular form of pipette used. In using two mark pipettes, great care must be taken to see that the quantities used are exactly 1 cc., while many one mark pipettes in use are calibrated to contain 1 cc. rather than to deliver 1 cc. Breakage of tips of the latter type of pipette also causes errors[13].

In making dilutions the original sample and each dilution bottle shall be rapidly shaken 25 times, each shake being an up and down excursion of about one foot[14] (entire shaking not to take longer than about seven seconds). After the final dilution fill a pipette to the mark and allow contents to run into an empty petri dish, the end of the pipette touching the dish as the liquid runs out. If the pipettes are of the one mark style be sure that they are so manipulated as to deliver a full cubic centimeter. Use care to raise the cover only as far as necessary to insert the end of the pipette.

Pipettes should be placed immediately in water after using to make subsequent cleaning easier.

The flasks (or test tubes) of agar shall be melted in boiling water or steam and after melting shall be cooled to a temperature of between 40 and 45° C. before using.

Pour about 10 cc. of the melted agar in each inoculated petri dish, and by a gentle rotary motion thoroughly mix the agar and the diluted milk. As nearly as possible the same amount of agar should be poured into each petri dish so that the depth of agar will be uniform in all. If desired 10 cc. may be measured out from the flask with a sterile pipette.

It is important that the plating shall be completed as rapidly as possible. The work should be so planned that no more than 15 minutes shall elapse after the dilution of the milk and before the agar is poured into the petri dishes; and in no case shall the interval be allowed to exceed 20 minutes.

After the agar has been thoroughly hardened, place the petri dishes in an incubator. The danger of spreaders may be reduced either by the use of clay tops or by inverting the plates as preferred.

Incubation.

Only one period of incubation, and one temperature is regarded as standard, 48 hours at 37.5° C. Piles of plates should not be packed too closely together and in crowded incubators ventilation should be provided.

Counting plates.

If among the different dilutions there are plates containing from 30 to 300 colonies these should be counted[15], and the number, multiplied by the dilution, be reported as the final count. All colonies on such plates should be counted, and the numbers from the different plates averaged. If there are no plates within these limits, the one that comes the nearest to 300 is to be counted. No plate that contains less than 20 colonies shall be counted, unless it happens that there are no other plates. If the number of colonies on the plates to be counted are in excess of 300 per plate, a part of the plate may be counted and the total number estimated; but such plates are admittedly overcrowded and the counts are less than they should be.

Counting shall be done with a lens, and all recognizable colonies included. A lens magnifying 2½ diameters (or what the opticians call a 3½ x lens) is recommended for general use. In case any particles visible by this method are of doubtful nature they should be examined with a compound microscope to determine whether they are colonies or dirt specks.

Common sources of error in counts.

Agar plate "counts" per cc. are to be regarded as estimates of numbers rather than as exact counts, since only a portion of a cubic centimeter is used in preparing the plates. As such they are (like all estimates) subject to certain well known and recognized errors whose size can be largely controlled by the care taken in the analysis. Among these errors are: (a) Failure of some of the bacteria to grow because the incubation temperature, or the composition or reaction of the medium, is not suitable. (b) Inaccuracies in measurement of the quantities used. (c) Mistakes in counting, recording data, computing results and the like. (d) Incomplete sterilization or contamination of the plates, dilution waters, etc. The possible errors caused by these things makes it highly important for all routine laboratories to follow carefully a standard procedure.

Recent investigations[16] make it clear that these largely controllable errors, are not so likely to cause serious misconceptions of the accuracy of results as are the errors due to the fact that bacteria in milk usually cling together in groups of from two to many hundreds of individuals. These groups are only partially broken apart by the shaking given in preparing the dilutions so that at best the counts from the agar plates represent the number of isolated individuals and groups of two or more bacteria that exist in the final dilution water. Thus the colony counts from the plates are always much smaller than the total number of bacteria

present. This error would not be troublesome if the groups were of constant average size; but the best information available shows that the groups in ordinary market milk commonly vary in size so that they contain an average of from 2 to 6 individual bacteria. Some samples contain groups of even smaller size than this, while others, such as those bearing long chain streptococci, may show groups containing an average of 25 or even more individual bacteria. The irregularity of this error (whose size is not indicated in any way by the appearance of the plates) should be kept in mind in interpreting the results obtained.

Reports.

Because of the fact that agar plate counts only represent a fraction of the total number of bacteria present, they should not be reported as showing the "number of bacteria per cc." Accurately speaking the counts from agar plates give the estimated number of colonies that would have developed on standard agar per cc. of milk if an entire cubic centimeter of milk had been used for inoculation. Because this statement of fact is cumbersome, and also because a certain ratio exists in each case between the colony count and the total number of bacteria, it has become a common practice to speak of the plate counts as showing the number of bacteria per cc. This is very confusing now that microscopic methods of counting have been developed which permits counts of the actual bacteria to be made. These counts average approximately five times the size of the counts as made by the standard agar plate technique.

It is therefore recommended that all agar plate counts obtained by the standard technique shall not be stated in the form "2,000,000 bacteria per cc." but rather as follows: "official plate count, 2,000,000." This latter form of expression shall be considered an abbreviated method of saying: "a count of 2,000,000 colonies per cc. as obtained by standard methods." Moreover analysts shall be careful to avoid giving a ficticious idea of the accuracy of the official plate count. There is ample justification for thinking them sufficiently accurate to justify drawing conclusions as to the general quality of a given sample of milk, and when a series of samples from the same source are examined the average result may permit much more specific conclusions to be drawn with confidence.

Specific data showing the actual percentage error in these counts has been difficult to obtain, and has only recently been obtained by means of comparisons made between microscopic and agar plate counts. The conclusions reached by Breed and Stocking,[17] are that the margin between two plate counts made from similar samples of market milk must be as great as one to five before it becomes a practical certainty that the larger count actually represents the larger number of bacteria.

It is, however, self evident that between any two samples the one having the higher count probably contains the greater number of bacteria, and this probability can be made a practical certainty by the examination of a series of samples. It is therefore required that a series of samples, preferably four or more, be examined before judgment shall be rendered as to the general quality of a given milk supply. Under no conditions is the practice sanctioned of publishing exact counts from indi-

vidual samples as showing the quality of a given milk supply.

All laboratories conforming to standard procedure will keep a record of the exact number of colonies developed on the plates that are counted; but will render their reports in round numbers only. Never use more than two significant left hand digits in any report, raising the number to the next highest round number in any case; but never lowering it. Those wishing to be still more conservative may use a form of report such as "official plate count less than 10,000," "official plate count between 10,000 and 30,000," and the like.

(B) MICROSCOPIC COUNT OF BACTERIA (BREED METHOD)

Various methods for counting bacteria in milk by microscopic examination have been described, but the method that is commonly described as a **direct microscopic examination**[18] of a dried film of milk has been found to be the simplest and most reliable method of counting the bacteria as they exist in the milk itself. It is recognized in this report as a standard or official technique of equal standing with the colony count from agar plates where used for judging the quality of unpasteurized milk.

Apparatus required.

In addition to a microscope, microscopic slides, stains, etc., the only special apparatus required is a capillary pipette which discharges 1/100 cc. of milk. The most satisfactory form of pipette is made from a straight piece of thick walled capillary tubing with a bore of such a size that the single graduation mark is from 1½ to 2½ inches from the tip. The tip shall be blunt and of such a form that it will discharge the milk cleanly without running back on the side of the tip. Pipettes of this type are now listed by all of the usual supply houses. The pipettes shall be calibrated so as to **deliver** 1/100 cc., not to **contain** 1/100 cc. Because there are many inaccurately calibrated pipettes on the market, the calibration of all pipettes shall be tested by weighing the amount of milk discharged on chemical balances. The weight for milk should be .0103 grams.

Only a single pipette is needed in making a series of tests, provided this is kept clean while in use. In this kind of work cleanliness of glassware is more important than sterilization. Clean towels may be used for wiping the exterior of the pipettes while making the microscopic preparations, and their bores may be kept clean by rinsing them in clean water between each sample. The small amount of water left in the bore may be rinsed out in the milk sample under examination. This method of procedure, while adding a small number of bacteria to each sample, introduces only a theoretical error, tests showing that such bacteria cannot subsequently be detected, and make no difference in the final result. After use, the pipettes should be kept in a glass-cleaning solution, such as the commonly used mixture of sulphuric acid and potassium bichromate.

Routine laboratories will find it convenient to use larger microscopic slides than the ordinary 1 by 3 inch slide. The largest slides that have been found to be conveniently examined with the use of a mechanical stage are cut 2 by 4½ inches. Such slides may be stored in ordinary card catalogue cases and may be very cheaply prepared from thin window glass or old photographic negatives. A margin

of ground or etched glass on the longer edges of the slide about ¼ inch in width allows lead pencil labeling. The margins may be ground with an emery wheel, or they may be etched with hydroflouric acid. The cost of these home made slides ought to not to exceed 2 to 3 cents each, whereas the similar slides listed by supply houses cost much more than this. A special guide plate (size 2 by 4½ inches) marked off with 16 square centimeter areas is also needed. This can be obtained from regular supply houses.* Only one of these is needed as it used is as a guide plate underneath the slides on which the milk preparations are made.

Preparation of films of dried milk.

After a thorough shaking of the sample, 0.01 cc. of milk or cream shall be deposited upon a clean glass slide by means of the pipette above described. Spread the drop of milk uniformly over an area of one square centimeter by means of a clean, stiff needle. This may be most conveniently done by placing the slide upon the guide plate just described, or upon any other form of guide plate of glass or paper which is ruled in square centimeter areas. The marks showing through the glass serve as guides. After spreading, the preparation shall be dried in a warm place upon a level surface protected from dust. In order to prevent noticeable growth, this drying must be accomplished within five to ten minutes; but excessive heat must be avoided or the dry films may crack and peel from the slide in later handling.

After drying, the slides are to be dipped in xylol, or any other suitable fat solvent, for a sufficient time to remove the fat (at least one minute), then drained and again dried. After this, the slides are to be immersed in 90 per cent grain or denatured alcohol for one or more minutes, and then transferred to a fresh aqueous or carbolic acid solution of methylene blue (about 1 per cent, exact strength unimportant) that has previously been tested and found to stain the bacteria satisfactorily in milk preparations. Some methylene blue now on the market in powder form is very unsatisfactory in that solutions will dissolve the milk films, or will wash them with an even blue color in which the bacteria fail to show distinctly. Old or unfiltered stains are to be avoided as they may contain troublesome precipitates.

The slides are to be left in the stain until overstained. They are then to be rinsed in water and decolorized in alcohol. The decolorization takes from several seconds to a minute or more, during which time the slide should be under observation, in order that the decolorization may not proceed too far. When properly decolorized the background of the film should show a faint blue tint. Poorly stained slides may be decolorized and restained without apparent injury. After drying, the slides may be examined at once, or they may be preserved indefinitely.

Standardization of the microscope.

The microscope used must be so adjusted that each field covers a certain known fraction of the area of a square centimeter. This adjustment is simple if a micrometer slide, ruled in hundredths of a millimeter, is at hand (sometimes called a stage micrometer as it is used under the objective on the stage of the microscope). The microscope should have a 1.9 mm. (1/12 inch) oil immersion lens, and an ocular

*Listed by the Will Corporation, Rochester, N. Y.

giving approximately the field desired (for example a 6.4 x ocular). It should also be fitted with a mechanical stage. If the large slides described above are used, this must be a special stage allowing a larger area of the slide to be examined than can be examined with the usual mechanical stage.*

To standardize the microscope, place the stage micrometer on the stage of the microscope, and by selection of oculars or by adjustment of the draw tube, or both, bring the diameter of the whole microscopic field to .205 mm. When so adjusted, each field of the microscope covers an area of approximately 1/3000 cm^2. (actually 1/3028 cm^2). This means that the dried milk solids from 1/300,000 part of a cc. of milk are visible in each field of the microscope. Therefore if the bacteria in one field only are counted, the number found should be multiplied by 300,000 to give the estimated number of bacteria per cc. In practice, however, more than a single field is examined so that the number used for multiplication is smaller than this.

As the microscopic examinations must be made with greater care where the bacteria are relatively few in number, it is required that, in grading low count milk, a special ocular micrometer* with a circular ruling divided into quadrants shall be used. In using this micrometer, the microscope shall be so adjusted that the diameter of the circle on the eye piece micrometer shall be .146 mm. In this case the amount of dried milk solids examined in each field of the microscope is 1/600,000 part of a cc. of milk. The limitation of the examination of the slide to the central portion of each field, avoids using the margins of the field where definition is hazy, and lessens the danger of overlooking bacteria. Likewise the magnification used is greater than that used where the whole field is examined.

* May be secured from the Bausch and Lomb Optical Co., Rochester, N. Y.

Counting and Grading Milk.

The number of fields of the microscope to be examined varies with the character of the milk, and with the character of the data desired. Experience has shown that where the purpose is primarily to detect and eliminate the worst milk from ordinary market milk supplies, it is entirely permissible to use the entire field of the microscope for examination. At least thirty representative fields of the microscope should be examined for each sample of milk. Where the average number of individual bacteria (not groups of bacteria) is less than one per field, it may be assumed that the milk will ordinarily give an official plate count of less than 60,000 per cc. Where the number is less than 100 in 30 fields (average of less than 3 1/3 bacteria per field) it may be assumed that the official plate count will be less than 200,000 per cc. Where less than 1000 per 30 fields (average of less than 33 1/3 per field) is may be assumed that the official plate count will not exceed one to two million per cc.

Where counts are made in order to enforce more stringent standards, as at Grade A plants[19] or as a basis for premiums on milk giving an official plate count of less than 10,000 per cc., the special eyepiece micrometer described above shall be used and the microscope so adjusted that only the central portion of each field is examined for counting. Where less than 5 bacteria are found in 60 fields (average of less than 1/12 of a bacterium per field) it may be assumed that the milk

* Listed by the Bausch and Lomb Optical Co.

would ordinarily give an official plate count of less than 10,000 per cc. The grading of milk of this type must be done with especial care as persons inexperienced with microscopic work have been found readily to confuse extraneous objects with bacteria, in milk containing very few organisms. Where the number is less than 30 per 60 fields (average of less than ½ a bacterium per field), it may be assumed that the official plate count will be less than 60,000 per cc. Where the number is less than 100 per 60 fields (average of less than 1 ⅔ bacteria per field), it may be assumed that the official plate count will be less than 200,000 per cc. Where the number is less than 1,000 per 60 fields (average of less than 16 ⅔ bacteria per field), it may be assumed that the official plate count will be less than one to two million.

The standards given are computed (with the exception of the poorest grades) on the assumption that the official plate count will normally **average** 1/5 of the total number of individual bacteria present. As many cases will be found which diverge markedly from the **average,** it is self evident that this average represents only an approximation to the real conditions in any specific case, so that in some cases the microscopic grading will be more severe than that based on the plate counts, and vice versa. There is still a lack of sufficient data from which to judge fairly which system of grading is the more accurate. The indications are, however, that where the work is done with equal skill and care, and the allowances indicated are made, a reasonably close agreement in grade will be secured[20]. This fact is highly reassuring as to the general accuracy of both systems of grading.

In the routine grading of milk by the microscopic method it is not expected that exact counts will be made. A high grade milk will show field after field of the microscope in which no bacteria are seen, while a poor grade of milk will show numerous bacteria in every field examined. It is only where the number of bacteria present is close to the border line between grades that counts need to be made. The examination, however, must be sufficiently thorough to make sure of the grade as specified above.

In order to ensure careful work in grading, it is required that laboratories conforming to standard procedure shall preserve microscopic preparations until a reasonable period has elapsed after the reports are rendered to the person or persons whose milk has been examined. It is an excellent custom occasionally to have the grading done by one analyst repeated by a second analyst, particularly in those cases where punitive actions are to be based on the reports made.

Common Sources of Error in Count.

Routine microscopic counts, like all bacterial counts, are to be regarded as estimates of numbers only. They cannot be made with absolute accuracy even with the most careful technique. Errors will arise from inaccuracies in measurement of the minute quantities of milk examined at any one time, from faulty staining or preparation of slides, from mistakes in observation and the like. These limitations, while important, are not difficult to overcome in sufficient measure to make microscopic grading a satisfactory method of controlling the quality of unpasteurized milk. As it is only in this way that counts of the bacteria themselves can be

made, it must be recognized that accurately carried out microscopic counts of individual bacteria give the truest picture of the actual conditions of raw milk that can be obtained with any technique.

Where there is reason to fear the presence of large numbers of dead organisms, as for example in pasteurized milk, it is improper to place reliance upon microscopic counts. Valuable information may, however, sometimes be obtained by making both plate and microscopic counts from samples of pasteurized milk.

Reports.

As only a few ordinances[21] have yet been adopted in which both official and microscopic count standards have been given, the form of report used will need to be adapted to the circumstances under which each laboratory is working. Specific counts should not be given under normal circumstances, and care should be taken to avoid making finer distinctions in grade than are justified by the accuracy of the grading. A series of samples should be examined in all cases before rendering judgment as to the quality of any milk supply.

(c) MICROSCOPIC COLONY COUNT (FROST METHOD)

Although this technique is not recommended at this time as a standard of official technique, it is described in this report because of the need for more extensive comparative investigations upon which to base judgment as to its real merits. The technique in question has been described by its author as follows:

"An area of four square centimeters is marked off on an ordinary microscopic slide with a wax pencil. The slide is sterilized in a flame, and then 0.05 (1/20) cc. of the milk to be examined is placed on it with an accurately calibrated pipette. An equal amount of sterile nutrient liquefied agar, at 42-45° C., is added and the two drops thoroughly mixed with a sterile loop and carefully spread over the area marked off. The mixture is allowed to harden and then a 'little plate' culture is formed.

"The bacteria in the milk are allowed to grow into colonies by keeping the preparation in a moist sterile chamber for a few hours. The period of incubation should be long enough to allow the bacteria to grow into distinct colonies although they may not be visible to the naked eye. In practice it seems best to allow eight hours at 37.5° C. although good sized colonies are frequently formed in four hours. On the other hand, if more convenient, they may be allowed to grow 16 or more hours before they are counted. In order to count the colonies most readily the plates are thoroughly dried at a little less than 100° C., treated with a 10% solution of glacial acetic acid in 95 per cent alcohol, and stained with a methylene blue or carbolthionine solution about ¼ its usual strength. In this way the colonies are deeply stained while the background is colorless.

"The colonies are counted under the microscope. Usually this can be done with the 16 mm. (2/3 inch) objective, although where the colonies are small or very numerous the higher powers may be used, e. g. the 4 mm. (1/6 inch) or the 1.9 mm. (1/12 inch) oil immersion lens. The factor needed to convert colonies per microscopic field into the number of colonies that would develop per cc. of milk must be determined for each microscope and each combination of lenses of the

same; but roughly, when a 10 x eyepiece is used, one colony under a 16 mm. lens means 4,000 colonies per cc. of milk, and the colonies in an average field of a 4 mm. lens should be multiplied by 100,000, and under a 1.9 mm. or oil immersion lens by 400,000. At least five representative fields should be counted and averaged.

"Dilutions may be avoided with milks expected to contain several million bacteria per cc. by using 0.01 cc. A drop of sterile milk should be added before mixing with the agar in order that the composition and consistency of the medium may not vary greatly from that used with the ordinary dilution. Some special apparatus has been found to be desirable."*

This is essentially an agar plate technique in which the count of colonies can be secured within a shorter period than that used in the standard plating technique. This possibility makes it highly desirable that further studies be made with it. Some comparative counts have already been made by the author[22] and others who have used the method report to the Committee that they find counts similar to those obtained by the author. The fact that a microscope is used in making the counts has caused some to confuse this technique with that just described as the Breed method. The two are essentially different in that in the one case, the actual bacteria are counted as they exist in the milk, while in the other case the count is a count of colonies of bacteria which have grown either from isolated bacteria or from clumps of bacteria. The counts obtained by the Frost technique, like those from the standard technique ought not to be described as showing the number of individual bacteria originally present in the milk.

Because the counts obtained with the Frost technique do not agree exactly with standard counts, this technique cannot be recommended as a standard technique at present. This fact ought not, however, to be interpreted as expressing a view on the part of the Committee that the counts obtained by experienced workers are any less accurate than those obtained by the standard technique. Very little data has thus far been gathered even in comparative studies, and none is of sufficient extent or accuracy to warrant making a mathematical analysis of it in order to establish the true accuracy of the counts.

(D) VERIFICATION AND RESEARCH METHODS

Because of the fact that the Committee on Technique of the Society of American Bacteriologists has undertaken the study of methods of making bacterial counts for research purposes, it is not necessary to discuss further the use of standard methods as research methods. The standard methods are designed for use in routine analytical work and should also be used in those cases where investigations involving routine milk control are under consideration. They may also be suitable for use in other cases, but ordinarily will not be found to give the grade of accuracy expected in research work.

There is in all routine laboratories a very important use for methods giving more accurate data than can be obtained from the use of the routine count. These may be termed verification methods; and they should be used in all cases where administrative actions are taken which depend upon the analytical results[23].

*Listed by the Central Scientific Co., Chicago.

The simplest form of verification for official plate count in the case of raw milk is to make a count from the same sample of milk by direct microscopic examination, and vice versa. If the counts found from the second examination are such that they are readily understandable under the known conditions, a very large part of the uncertainty existing in regard to the first count is eliminated at once. Under other conditions it may be found advantageous to verify the routine plate counts by making plate counts in which additional dilutions, or plates are used. Likewise more careful microscopic counts may be obtained either by examining duplicate preparations from the same sample of milk, or by making a more careful examination of the original preparation than that made for routine purposes.

If procedures of this sort were more common in bacteriological laboratories, control officials would have much firmer ground upon which to defend their actions in court if necessary.

(E) SEDIMENT TEST

Because much of the dirt that appears as a visible and insoluble sediment in milk is accompanied by relatively few bacteria in proportion to the number derived from other sources, it frequently becomes desirable to use a test that will reveal the amount of visible sediment in the milk. For this reason the committee has included a description of the commonly used sediment test[24]. In this way, valuable information may be obtained and the occasional approval of a dirty milk prevented. As the sediment test becomes valueless if the milk has been subjected to thorough straining or clarification, or both, this test finds its greatest use at milk receiving and pasteurizing plants.

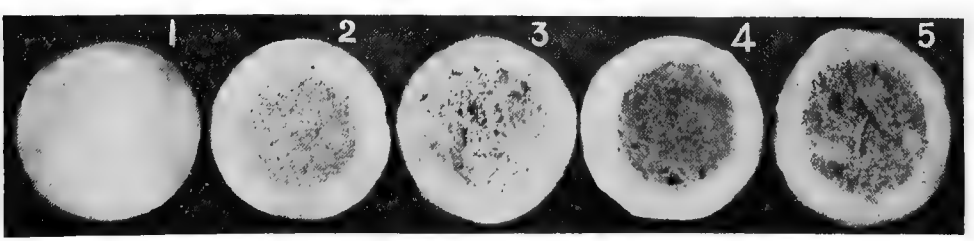
Technique.

After thorough stirring of the milk in the can, or by inverting and shaking a bottle, quart (or pint) samples of milk shall be strained through cotton disks placed over openings 1 inch in diameter. This may be done satisfactorily by means of any one of several types of apparatus such as the Schroeder, Lorenz Model,* and Dairymens Mfg. Co. It will hasten the process of filtering in many cases, if provision is made for warming the milk.

Method of recording results.

In order to make comparisons between the work of different analysts, standard gauges are given. A set of these are photographed and given in Figure 1. These gauges represent the amount of dirt obtained by filtering quart samples of milk to which have been added 2½, 5, 7 and 10 milligrams of the material which usually finds its way into milk. The standards given are intended for use where quart

*Creamery Package Mfg. Co.



samples are used. Where pint samples are used, standards corresponding to the smaller quantity of milk should be used.

Preparation of disks for filing.

After the cotton disks are removed from the filter they are placed on white blotting paper and sprayed with a strong disinfectant such as corrosive sublimate. Good results are secured by using an ordinary throat atomizer provided the caution is observed of not using corrosive sublimate in contact with metal. The disks are then allowed to dry. The drying process may be hastened by placing them in a hot air oven at a drying temperature carefully protected from dust and flies. When dry they should be compared with the gauge and the results recorded. They may be filed by placing in paraffin envelopes, or by attaching the disks to heavy paper or cardboard which are then placed in envelopes of suitable size. If the transfer is made to the permanent record sheet before the disks are entirely dry, the dried milk will serve as a very satisfactory glue for attachment.

Reports.

Experience has shown that one of the most effective ways of reporting results to dairymen or dealers is to return a half disk or duplicate disk to them with a statement as to the way in which it was obtained. The commonest criticism that can be applied to the comments sent with such reports is that they frequently make claims regarding the influence of dirt upon the bacterial count which are not substantiated by investigation[25]. Dirt of any sort is sufficiently objectionable in milk or any other food to be condemned solely on the ground that it is dirt; and it only weakens the objection to attempt to bolster it by doubtful claims as to the exact correlation between bacteria and dirt.

The general effect of the use of the sediment test has usually been to cause quickly a noticeable reduction in the amount of visible sediment. However this is accomplished in too many cases by greater care in straining, clarification and the like, rather than by greater cleanliness in the handling of the milk. As "clean" milk is greatly to be preferred to "cleaned" milk some have objected to the use of the sediment test. However, a proper combination of dairy inspection with the sediment test, may be made a very valuable means of securing clean milk.

(F) DETECTION OF SPECIFIC PATHOGENS IN MILK

There is no part of the field of sanitary analysis of milk where routine laboratory methods have so failed to meet the need of the control official as at this point. Some notable attempts have been made to secure the elimination of the bacillus of bovine tuberculosis from market milk supplies through routine laboratory examinations of milk samples; but none have been found to be sufficiently practical to have been widely followed[26]. Other pathogenic organisms, such as those of typhoid fever, are rarely sought for in milk, though methods for detecting this organism have been suggested[27]. In all of these cases, it has become necessary to rely on elimination of the pathogens in market milk supplies through pasteurization, or by veterinary inspection of the herds, and medical supervision of dairy employees.

Several of our important control

laboratories are, however, using a laboratory method for the elimination of long chain streptococci derived from inflamed udders. Certain precautions must, however, be used in this case as false interpretations of findings are easily possible. The long chain streptococci are readily found by microscopic examination of dried films of milk or of sediments from centrifuged samples of milk. Perhaps the two most frequently used routine methods are the Breed method already described, and the Stewart-Slack method described in detail in the first edition issued by the American Public Health Association[28].

The use of these methods for this purpose has shown that even the presence of large numbers of long chain streptococci may be of little significance where there has been opportunity for their growth after the milk has been drawn. Streptococci of the long chain type occur frequently in apparently normal udders, and may even occur in very large numbers where threre is no clinical evidence of inflammation[29]. Nevertheless, where samples of milk can be taken from individual cans as delivered within 6 hours after milking, it has been found that it is almost invariably possible to find a cow suffering from an inflamed udder if the count of individual cocci in long chains is in excess of 1,000,000 per cc. Such milk usually contains leucocytes in excess of 1,000,000 per cc.; but this relationship is not an invariable one. Because of the presence of alkaline substances from blood serum, milk from cows with inflamed udders usually has a pH value greater than 6.8 and may also contain detectable mucin fibers[30].

Where milk is centrifuged and the sediment examined, even greater caution should be used in drawing conclusions, as the concentration of material may cause insignificant numbers of these organisms to be regarded as significant. In this connection it should be remembered that many entirely satisfactory butter starters are composed of streptococci which occur in fairly long chains. These supposedly saphrophytic streptococci cannot be distinguished from the udder streptococci through microscopic examination alone.

Under these conditions, the laboratory findings should in every case be confirmed by clinical examination of suspected herds before action is taken.

SUMMARY

In order to separate required procedures from the necessary discussion, a short summary of the former has been prepared as follows:

Collection of Samples. Milk to be thoroughly mixed, and sampled with a sterile, straight sided tube (thief) long enough to reach to the bottom of the can or bottle. Each sample is to be at least 10 cc. and is to be kept in a tightly stoppered bottle (closure with cotton plugs is not permitted). Samples are to be iced and if plates are not made within four hours of the time when the samples were taken, the time elapsing is to be reported.

macroscopic colony count (petri plate method).

Composition of Medium

Agar	1.2% if oven dried, or 1.5% if undried.
Beef extract	0.3%
Peptone	0.5%
Distilled water.	

The reaction of the medium is to be between pH = 6.2 and pH = 7.0. If necessary to adjust the reaction, special attention is to be given to the H-ion concentration, making use of one of the indicators, brom thymol blue or brom cresol purple.

Three dilutions are to be made in plating: 1 to 100, 1 to 1,000 and 1 to 10,000 — unless the quality of the milk is such that the highest or lowest of these is known to be superfluous. In no case are less than two plates to be made from each sample. Each sample bottle and dilution bottle is to be shaken 25 times with an up and down motion of about one foot, in not more than seven seconds. After dilution of the milk, the agar is to be poured into the plates within 15 minutes.

Incubation shall be at 37.5° C. for 48 hours.

The plates used for counting are to have, if possible, between 30 and 300 colonies each. If there are no plates within these limits, the one having nearest to 300 is to be counted. Counting to is be done with a lens magnifying 2½ diameters. The exact counts from each plate are to be recorded, but not more than two significant left hand digits are to be used in making the final report.

Results are to be expressed, not as so many "bacteria per cc." of milk; but as "colonies per cc." or better as "official plate count" so much per cc. The practice of publishing counts from individual samples of milk as showing the quality of a given milk supply is not sanctioned, and it is required that a series of samples be examined before rendering judgment in regard to any milk supply.

Microscopic Count of Bacteria. Milk to be taken in a capillary pipette discharging 0.01 cc. and dried over an area of 1 sq. cm. on a microscopic slide. It shall be stained in methylene blue, after washing out the fat in xylol and fixing in alcohol. The number of bacteria per cc. is to be estimated by counting those within a given area in a microscopic field, this area having been carefully measured and its ratio to a square centimeter determined. at least 1/10,000 part of a cc. of milk is to be examined, and if the milk is of high grade this must be done under favorable conditions for accurate counting. The ratio to be used in comparing the "official plate counts" with the counts of bacteria per cc. as shown under the microscope, is provisionally placed at 1 to 5. Preparations are to be preserved a reasonable time after the reports have been sent to the person or persons interested.

Sediment Test. Quart (or pint) samples are to be filtered through cotton disks, one inch in diameter. Five degrees of dirtiness are to be recognized, correspondingly respectively to milk containing 0, 2.5, 5, 7, and 10 milligrams of dirt per quart (corresponding allowances to be made if pint samples are used).

Detection of Specific Pathogens in Milk. The only pathogenic organisms ordinarily sought in routine analytical work are the streptococci associated with udder inflammations. They may be detected either on the same slides as made for the microscopic count of bacteria, or on slides smeared with the sediment obtained by centrifuging the milk. Proper precautions are to be observed in distinguishing between merely parasitic or saphrophytic streptococci and those causing truly pathological conditions. Laboratory findings are to be confirmed by clinical examinations.

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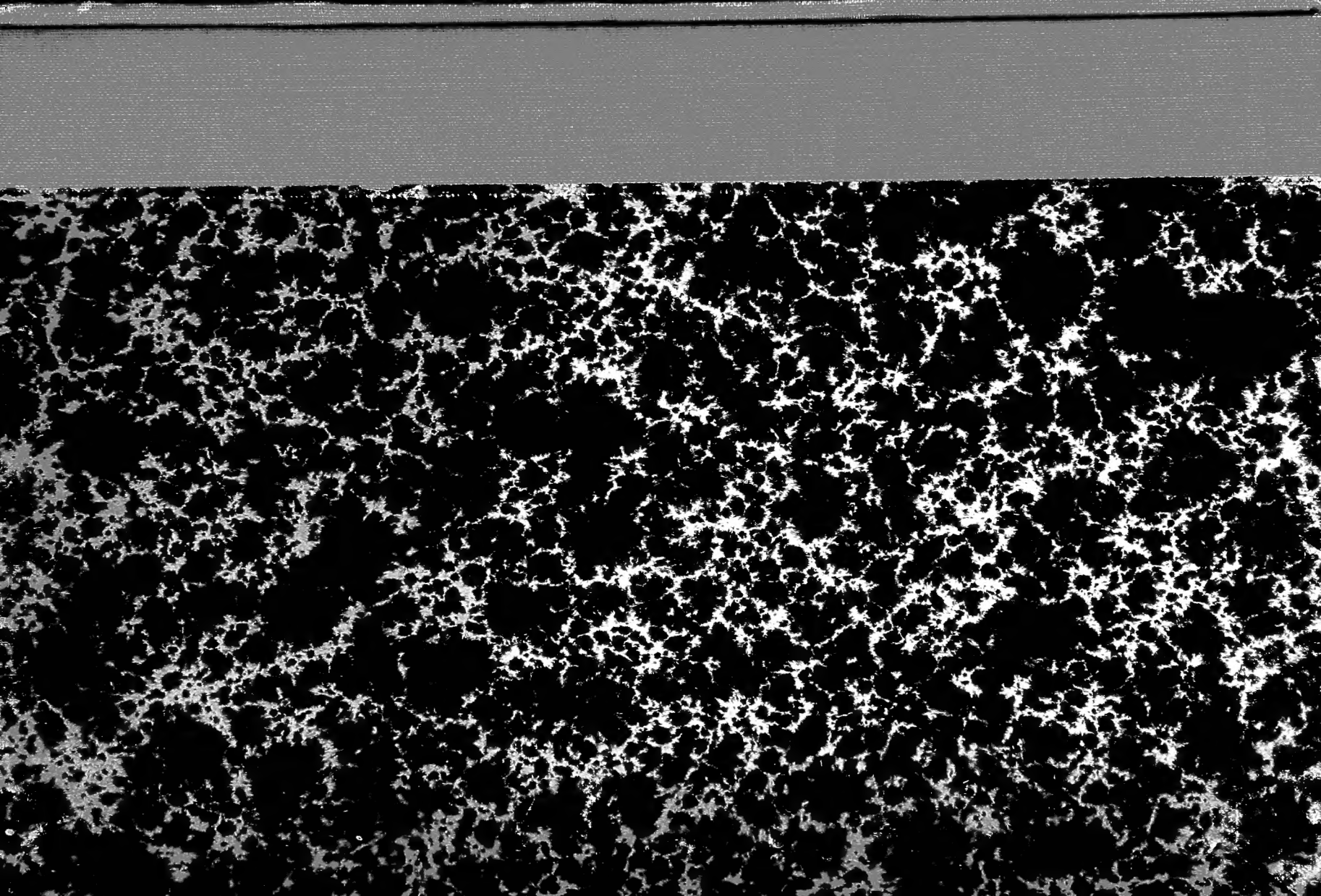
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