Oklahoma Agricultural Experiment Station,

BULLETIN NO. 40.

MAY, 1899

Bacteriology of Milk.

L. L. LEWIS.

BULLETINS OF THIS STATION ARE SENT FREE TO RESIDENTS OF THE TERRITORY ON REQUEST.

STILLWATER, OKLAHOMA.

Oklahoma Agricultural and Mechanical College.

Agricultural Experiment Station.

BOARD OF REGENTS.

HON. C. M. BARNES, GONERNOR, ex-officio,	GUTHRIE.
HON. F. J. WIKOFF, PRESIDENT,	STILLWATER.
HON. C. J. BENSON, TREASURER,	SHAWNEE.
HON. J. C. TOUSLEY,	WEATHERFORD.
HON. J. D. BALLARD,	WEATHERFORD
HON. J. P. GANDY,	ALVA.

STATION STAFF.

GEORGE E. MORROW, M. A.,	DIRECTOR AND AGRICULTURIST.
HENRY E. GLAZIER,	VICE-DIRECTOR AND HORTICULTURIST.
GEO. L. HOLTER, B. S;	CHEMIST.
E. E. BOGUE, M. S,,	BOTANIST AND ENTOTMOLOGIST.
L. L. LEWIS, M. S., D. V. M.,	VETERINARIAN.
JOHN FIELDS, B. S.,	ASSOCIATE CHEMIST.
J. H. BONE,	ASSOCIATE AGRICUTTURIST.
OSCAR M. MORRIS, B. S.,	ASSISTANT IN HORTICULTURE.
A. G. FORD, B. S.,	ASSISTANT IN CHEMISTRY.
H. M. HAND,	CLERK.
MISS G. M. HOLT,	STENOGRAPHER.

BACTERIOLOGY OF MILK.

SUMMARY.

The number of bacteria in milk will vary greatly when every precaution is taken to exclude them; some samples showing two or three times the number of others when apparently the milking was done under the same conditions.

The largest number of bacteria is found in the first milk and this should be kept separate from the remainder of the milk.

Pasteurizing should be practiced in all cases when milk is to be kept a few hours before using. A temperature of 70° C. for fifteen minutes will give good results without affecting the taste or physical character of the milk.

Milk not pasteurized required twenty-five hours to develop sufficient lactic acid to neutralize 1.5 c.c, of sodium hydroxide. Milk pasteurized fifteen minutes at 60° C. required thirty hours and milk pasteurized fifteen minutes at 70° C. required forty-six hours to develop sufficient acid to neutralize 1.5 c.c. of the soda solution.

BACTERIOLOGY OF MILK.

Milk like other albuminous foods undergoes decomposition readily because it is a favorable medium for the development of microscopical organisms or germs, and any means preventing the entrance of these into or destroying them in the milk will add to its keeping qualities. Bacteria are found in the air, water, and in fact almost everywhere, and in practice it is impossible to prevent them getting into milk or other foods where conditions are so favorable for growth.

The relation of bacteria to milk is of more practical importance than with any other food product, because of the extensive use of milk as a food by adults and more especially by children, and because it is a good medium for the growth of more species of bacteria than any other food. Souring or fermentation of milk is due to the presence of bacteria. The lactic acid fermentation is the most common change occurring, and is so constant that it is sometimes spoken of as normal, not in the sense that milk will itself undergo this change but because the bacteria causing it are so uniformly present. When all bacterial life is destroyed by heating or preservatives, milk will remain sweet for an indefinite length of time and its keeping qualities are increased in proportion to the fewness of bacteria present except in some cases where a single species may withstand a high degree of heat and still cause coagulation of the casein almost as rapidly as the germs which cause souring in milk not pasteurized.

BACTERIA.

Bacteria are the lowest forms of vegetable life. They are one-celled plants and are to be found everywhere where conditions are favorable to their growth, and many will live but not multiply under very unfavorable conditions. Warmth and moisture with some organic matter for food are the conditions ecessary for growth, and these conditions are very favorable in milk. Souring of milk is due to the development of bacteria and when their development is interfered with by heat or cold, the milk remains sweet for a greater length of time. Ordinarily the handling of milk in unclean vessels injures the keeping qualities more than the bacteria found in milk at the time of milking. Particles of sour milk left in the milk cans soon contaminate the entire can of milk.

The following table from Freudenreich's Dairy Bacteriology shows the effect of clean and unclean vessels:

When kept at 68° to 75° F. the milk turned—

	IN STERILIZED VESSELS.	IN NON-STERILIZED VESSELS.
Experiment 1	after 46 hours	after 24 hours.
Experiment 2	after 96 hours	after 48 hours.
Experiment 3	after 72 hours	after 24 hours.
Experiment 4	after 130 hours	after 65 hours.
Experiment 5	after 86 hours	after 48 hours.
Experiment 6	after 104 hours	after 66 hours.
Experiment 7	after 46 hours	after 18 hours.
Experiment 8	after 80 hours	after 48 hours.

Many of the bacteria which enter the milk from the hands of the milker, from the udder of the cow and from dirty strainers can be kept out by following a few simple rules of cleanliness.

Ordinarily the bacteria found in milk are not harmful but are those which produce lactic acid fermentation, as these bacteria find the most favorable conditions of growth here. There are not very many species of bacteria noticed in making cultures from milk unless there is contamination from other sources. But it is not impossible for injurious bacteria to find their way into milk, producing disease in the consumer or changes in the color, taste, or consistency of the milk. Disease producing bacteria may frequently find their way into milk. The most important of these is the bacillus of tuberculosis which may find its way into milk from the udder, as tuberculosis is a very common disease among cattle. The germs of diphtheria and typhoid fever may accidentally contaminate milk and the disease spread in this way. For this reason every care should be taken to avoid the possibility of contaminating milk with such bacteria, and where there is a possibility of such contamination the milk should be heated to a temperature sufficient to destroy the germ.

Thermal death point of disease producing bacteria. (Sternberg).

Bacillus of tuberculosis	65 C. 30 min.
Bacillus of tuberculosis	68 C. 15 min.
Bacillus of tuberculosis	75 C. 10 min.
Bacillus of typhoid fever	65 C. 10 min.
Bacillus of diphtheria	58 C. 10 min.

The chief sources of bacteria found in milk besides those found in the milk duct and udder are the dust from the coat of the animal, dust of the stable, hands and clothing of the milker, and from unclean pails. Contamination from these sources may be lessened very greatly by ordinary care. The coat of the cow affords a good place for the collection of dirt which becomes attached to the animal while lying down and contains large numbers of bacteria, and this dirt is constantly falling into the pail unless the parts are brushed before milking. The hands and clothing of the milker are the source of many bacteria. The air of the barn is filled with them and dust created by feeding or by currents of wind increase the number. A very important source of bacteria which might be overlooked is in the fore milk. There remains after milking some milk adhering to the end of the teat and in the milk duct. This affords a medium of growth for bacteria and they soon pass from the end of the teat through the milk duct into the udder. By milking the first milk on the ground the number of bacteria in the milk may be greatly reduced without the loss of much milk.

There are not very many species of bacteria found in milk under ordinary conditions and of those that are found the species which produce lactic acid fermentation or souring is the most common, and with the exception of disease producing germs are the most important. It was at one time believed that there was only one species of bacteria which produced lactic acid fermentation and this was called bacterium lactis by Lister. Bacteriologists have not always been able to identify this species as the only cause of souring of milk. This has led to the description of a number of species which different investigators have found would produce lactic acid fermentation. When there are no bacteria present in sufficient numbers to interfere with the action of the true fermentive or souring bacteria, the milk passes through a certain series of changes Soon after it has begun to "turn" it forms a soft, coagulated mass with very little whey and no unpleasant odor, and does not develop any unpleasant odor unless allowed to stand for some time after souring.

There are undesirable species of bacteria found in nearly every sample of milk but generally not in sufficient numbers to be of any consequence, but if in sufficient numbers undesirable changes may result.

Milk often acquires a bitter taste that renders it unfit for food. This may in some cases be due to feed but such results may come from the growth of bacteria; changes in color and unpleasant odors are most frequently of bacterial origin. As most of these undesirable changes come from bacterial infection it is possible to exclude them by exercising care. These bacteria may occur in the hay or bedding, may contaminate the milk through dust particles, or they may be in particles of dirt attached to the udder; but whatever their source they may be excluded to such an extent as to not produce noticable changes in the milk.

COMPOSITION OF MILK.

Milk is a perfect emulsion when first drawn, the particles of fat being evenly distributed in a serum containing sugar, casein and ash ingredients. The chief muscle forming element is the casein which is the body of cheese and is the representative of proteids in other foods. Fat and milk sugar are the next in abundance, except water which forms the greater part. Fat is the most important part commercially as it is the source of butter and is a valuable part of cheese. The fat varies considerably in different samples of milk, but milk from any herd of cows should have not less than 3.5 per cent. to 4 per cent. of fat. The amount in any case should not fall below 3 per cent. of fat. It is a very rich sample of milk that will test more than 6 per cent. of fat.

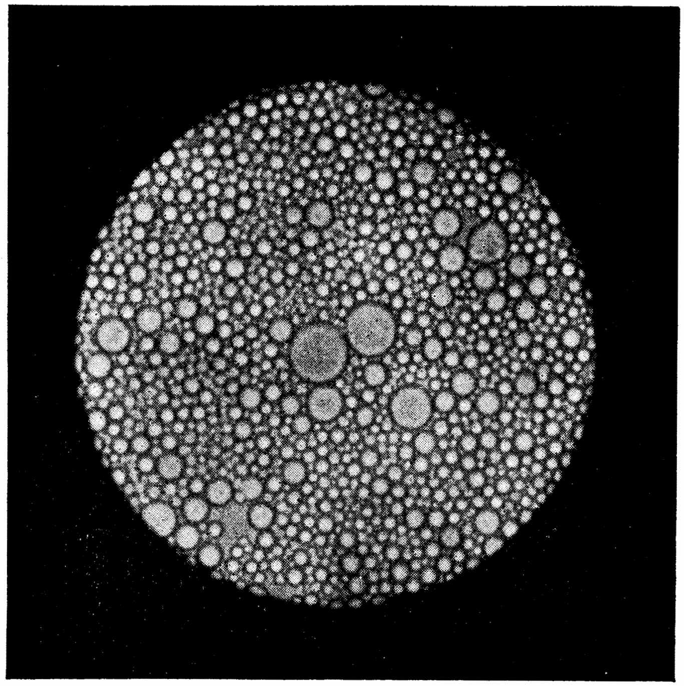

Fig. I. Photo-micrograph of Milk Showing Oil Globules.

Table showing the composition of milk. (Konig).

Water	87.2
Solids	12.8
Casein	3.0
Albumin	.5
Fat	3.7
Lactose	4.9
Salts	.7

PASTEURIZING MILK.

Pasteurizing milk consists in heating it to a temperature considerable below the boiling point, but not high enough to affect the proteids. If milk to be pasteurized is heated more than once it is called the intermittent method of pasteurizing. All forms of bacteria are not killed by pasteurizing as some in the spore condition will resist the temperature of boiling water. The principal objection to the intermittent method is the time necessary for its completion, as not less than six to eight hours should separate the times of heating. The heating of milk to a sufficient temperature to destroy all bacteria gives a cooked taste which is disagreeable to many. Heating the milk to 70 degrees C. for fifteen minutes is sufficient to destroy most of the bacteria, and if cooled immediately after, the cooked taste is not noticeable.

For commercial purposes the intermittent method is not practicable; the highest degree of heat should be used for as great a length of time as possible without altering the physical character of the milk. A temperature of 75 degrees C. for ten or fifteen minutes gives a cooked taste and odor to the milk, but a temperature of 70 degrees C. can be used if the milk is cooled afterwards.

The method used in these experiments was to first heat the water several degrees above the desired temperature, then place the bottles of milk in the water, having the surface of the water on a level with that of the milk. By this means the milk soon reached the desired temperature and can be kept at an even temperature for any desired length of time.

The Arnold steam sterilizer is the form of apparatus in general use for pasteurizing and sterilizing and is very convenient for this purpose. The milk may be steamed in closed bottles for about twenty minutes for quart sizes, cooled slowly until they can be placed in cold water, then kept cool until used. Cold is perhaps the most common agent in use in keeping milk sweet. Cold does not destroy any of the bacteria present, but prevents or retards their development, depending on the temperature used.

Pasteurizing at 60 degrees C. ten minutes will keep milk sweet for six to twelve hours longer than milk not pasteurized if it be kept cool after heating, but if allowed in a temperature above 65 degrees C. it is of no advantage. Cultures from milk heated to 60 degrees C. for ten minutes showed a very small difference in the number of germs, while tests of milk pasteurized for fifteen minutes at 60 degrees C. showed a decrease in the number of one-third to one-half, before and after heating, but the bacteria are very slow in developing from the pasteurized milk; sixty to eighty hours were necessary for their development while in the cultures from milk not pasteurized forty to sixty hours were necessary.

Chemical agents have been used extensively for preserving milk but are in general to be avoided. First because the minimum amount required to keep milk sweet a few hours longer than it would normally remain so is not used; and, second the use of milk containing the amount generally used is thought to be injurious. Formol was used in part of these experiments, both with and without pasteurizing and it was found to add so materially to the keeping qualities of the milk when used in very small amounts that the results are given along with this work The action of formol on tissue is to harden it but whether, when used in the proportion of one part formol to 30,000 of milk or one to 15,000, it is of sufficient strength to interfere with the normal action of the mucous membrane of the stomach and in testines is not known and could only be determined by a series of experiments.

In using formol as a preservative the milk was heated only once, formol added and the milk kept in closed bottles. The high temperature of the milk at the time the formol was added aided the action of the preservative.

SAMPLING THE MILK.

When tests were made to determine the number of bacteria in the first, middle and last milk, the sample was milked directly into a sterile tube which was closed immediately and tests made in ten to fifteen minutes. When samples of the entire milk were used they were taken from the pail, placed in sterile tubes and used in ten to fifteen minutes. In determining the number of bacteria the milk was diluted with sterilized water and one or two drops of the dilution placed in agar tubes and petri dishes poured. In dropping the milk into the agar, pipettes were used which dropped 30 drops per cubic centimeter. The pipettes used in dropping the solution of formol also dropped 30 drops per cubic centimeter, this giving a basis for calculating the number of bacteria per cubic centimeter in milk and the proportion of formol to milk.

In determining the time that formol or pasteurizing would keep milk sweet, the samples of milk were tested at short intervals by using a tenth normal solution of sodium hydroxide and an alcoholic solution of phenothalein. Five cubic centimeters of milk were used at each test and when sufficient acid was developed in the five cubic centimeters to neutralize 1.5 cubic centimeters of the soda solution the sample was thrown out. This degree of acidity is scarcely perceptible to the taste and a number of tests showed that five cubic centimeters of fresh milk would contain sufficient acid to neutralize .8 cubic centimeters of the sodium hydroxide solution. From this it will be seen that the condition of the milk at the end of the time given in the tables is not that of sour milk or milk that is "turned."

Only the series carried through at the same time are comparable on account of the variation of the temperature in the laboratory, and only samples carried through at the same time are given in the same tables. In all cases where milk was pasteurized or had formol added the samples contained 100 cubic centimeters each. They were taken from milk after it was brought to the dairy room and placed in sterilized bottles.

NUMBER OF BACTERIA IN MILK.

The first milk from the udder contains more bacteria than any other portion of the milk, as the bacteria multiply rapidly in the milk duct and are more abundant in the milk near the opening from the teat than any other portion of the udder. Many tests from the first milk gave no results as the plates would be overgrown by various forms of moulds which would cover the dish before the colonies of bacteria would have time to develop. Where the colonies could be counted the number of bacteria in the first milk varied from 90,000 per cubic centimeter to 803,520 per cubic centimeter. The germs rapidly decrease in number until in the middle milk the average of a number of tests showed 4,640 bacteria per cubic centimeter. The last milk may be obtained free from germs and a number of tests showed the number of bacteria to vary from 720 to 1,680 per cubic centimeter and 30 per cent of the dishes poured remained sterile.

The table shows the rate of increase of bacteria in milk kept at a temperature of 33 degrees C. The numbers given are the averages of several tests:

	BACTERIA PER CUBIC CENTIMETER.
At time of milking	48,200
One hour after milking	110,000
Two hours after milking	125,200
Three hours after milking	361,800
Four hours after milking	526,500

When the milking was done in the open air the number of bacteria found per cubic centimeter was very much less than when the work was done in the barn. The average of four tests in the open air was 21,600 bacteria per cubic centimeter as compared with 56,500 as average of four tests in closed barn; sample taken from ten to fifteen minutes after milking. No precautions were taken to lessen the number of bacteria, but the work was done as the average dairy man would do the work. When care was taken to have everything as clean as possible and the first milk thrown away the number of bacteria was 2,325 bacteria per cubic centimeter.

The table shows results of adding formol to milk not pasteurized, pasteurized fifteen minutes at 60 degrees C. and for fifteen minutes at 70 degrees C. The hours given is the time required for sufficient lactic acid to develop to neutralize 1.5 cubic centimeters of tenth normal solution of sodium hydrioxide:

Milk not pasteurized.—

SAMPLE NO.	AMT. OF MILK.	PROPORTION OF FORMOL TO MILK.	HOURS.
1	100 cubic centi	none	31
2	100 cubic centi	1 to 30,000	74
3	100 cubic centi	1 to 15,000	97
4	100 cubic centi	1 to 10,000	142

Milk pasteurized fifteen minutes at 60 degrees C.—

SAMPLE NO.	AMT. OF MILK.	PROPORTION OF FORMOL TO MILK.	HOURS.
1	100 cubic centi	none	36
2	100 cubic centi	none	44
3	100 cubic centi	1 to 30,000	76
4	100 cubic centi	1 to 30,000	72
5	100 cubic centi	1 to 15,000	120
6	100 cubic centi	1 to 15,000	96

Milk pasteurized fifteen minutes at 70 degrees C.—

SAMPLE NO.	AMT. OF MILK.	PROPORTION OF FORMOL TO MILK.	HOURS.
1	100 cubic centi	none	47
2	100 cubic centi	none	45
3	100 cubic centi	1 to 30,000	78
4	100 cubic centi	1 to 300 000	89
5	100 cubic centi	1 to 15,000	120
6	100 cubic centi	1 to 15,000	133

In the pasteurized samples numbers one, three and five were immediately cooled to 18 degrees C. then kept at room temperature with numbers two, four and six. It appears that very little advantage is gained by cooling pasteurized milk unless the temperature is kept low.

The following will show the decrease in the number of bacteria after heating:

Milk not pasteurized.—

SAMPLE NO,	BACTERIA PER CUBIC CENTIMETER.
1	54,000
2	55,080
3	51,300

Milk pasteurized fifteen minutes at 60 degrees C.—

SAMPLE NO.	BACTERIA PER CUBIC CENTIMETER.
1	35,100
2	39,460
3	28,080

Milk pasteurized fifteen minutes at 70 degrees C.—

SAMPLE NO.	BACTERIA PER CUBIC CENTIMETER.
1	2,860
2	720
3	1,400

When formol is added in the proportion of one part to 10,000 of milk, pasteurizing does not increase to any extent the keeping qualities of milk but when added in the proportion of one part formol to 30,000 parts of milk pasteurizing 15 minutes at 70 degrees C., it remains sweet from 45 to 50 hours longer than samples of the same milk receiving no formol and from 55 to 65 longer than milk not pasteurized.

This table shows time necessary for casein to coagulate at a temperature of 33 degrees C.:

SAMPLE NO.	AMT. OF MILK.	PROPORTION OF FORMOL TO MILK.	HOURS.
1	100 cubic cent	none	20-30
2	100 cubic cent	1 to 30,000	30-60
3	100 cubic cent	1 to 15,000	55-70
4	100 cubic cent	1 to 10,000	75-85

The results given in all these tables are averages of a number of tests.

A microscopical examination of the colonies developing from milk pasteurized for fifteen minutes showed that only a few species of bacteria withstood this temperature and a part of these when placed in sterilized milk had no apparent effect. When the milk was pasteurized the second time at 70 degrees C. for fifteen minutes, from two to five different bacteria were found but only two had any effect on the milk.

No study was made of the bacteria in non-pasteurized milk. When plates were poured immediately after pasteurizing the second time they frequently remained sterile, showing that in some samples the number of bacteria per cubic centimeter was very much less than is shown in the table for that particular sample.

BACILLUS NO. I. FIG. I.

Habitat.—Found in milk after second pasteurizing at 70 degrees C. for fifteen minutes.

Morphology.—Non-motile. An elongated bacillus with rounded ends, 2*u* to 4*u* in length. 1*u* to 5*u* in thickness. Tendency to thread formation in fresh cultures, especially in agar. Forms spores in cultures six to eight hours old. Preparations from old cultures show very few bacilli but an abundance of spores. Stains readily with all aniline stains, gentian violet giving best results. Grows rapidly at temperatures from 25 to 38 degrees C. Thermal death point not determined.

Agar Agar.—Grows rapid, colonies white, surface elevated, most and glistening.

Potato.—Growth rapid and spreading, surface elevated, white and dry.

Bouillon.—Fluid becomes turbid after twenty-four hours. A stringy sediment fall to the bottom of the tube and, if left undisturbed, a white surface forms which sinks to the bottom in flakes when broken up. After standing for a week the bouillon becomes clear and sediment ropy.

Fermentation tubes.—Lactose bouillon. No gas formed. Germ ærobic, growth only in open arm and similar to bouillon.

Gelatin.—Growth rapid, liquifies gelatin. Funnel fusiform, sediment stringy, surface membrane white.

Milk.—Coagulates milk very slowly unless the temperature is above 35 degrees C. It will coagulate in 75 to 90 hours. Curd soft and does not become changed to whey.

BACILLUS NO. II. FIG. II.

Habitat.—Found in milk after second pasteurizing at 70 degrees C. for fifteen minutes.

Morphology.—Short thick bacillus occurring in pairs. Length varies from 0.5*u* to 1.5*u*, stains irregularly and is very difficult to stain. Best results from using Loffler's alkaline methylen blue. Bacillus seen in groups in preparations from agar as if bound together by gelatinous material. Forms spores.

Agar Agar.—Colonies on agar (Fig III.) plates show a number of concentric circles. Appearance varies slightly after a series of cultures have been made. In old cultures the inner rings disappear and surface shows radiating fibrillæ extending from central elevation to outer half of colony. Beaded growth along line of puncture.

Bouillon.—Cloudy after 24 hours, no surface growth. In old cultures the medium is clear with a very stringy sediment on bottom. Growth in fermentation tubes same as in bouillon. No gas.

Milk.—Coagulates milk in 60 to 75 hours, curd soft, becoming converted into whey.

Potato.—In twelve hours after inoculation growth is well marked in form of glistening, white elevation; this soon spreads to the entire surface of the potato, becomes slightly yellowish in color; surface remaining moist and glistening.

Cochineal Agar.—Old cultures become purple.

Gelatin.—Liquifies, gradually extending down the tube from the surface. Fluid cloudy, a thin, white growth forming on the surface.

BACILLUS NO. III. FIG. V.

Habitat.—Found in milk after first pasteurizing at 70 degrees C. for fifteen minutes.

Morphology.—Bacillus 1*u* to 2*u* in length, rounded ends. Motile, and forms spores in cultures 24 hours old. Chain formation marked in fresh cultures. Stains with aniline dyes.

Agar Agar.—Growth white at first but becomes a dirty, brownish color after six or seven days. Colonies on plates 24 hours old are granular, circular (Fig. IV.) and edge of colony in tire. In old colonies outer half radiate. In stab cultures growth confined to line of puncture.

Milk.—Coagulates milk after 36 to 48 hours when large numbers are introduced, at temperature of 32 degrees C. Soft curd, whey increases until there is only a small amount of curd in bottom of tube.

Potato.—Growth rapid and spreading, surface elevated, smooth and moist but not glistening. Color yellowish or cream color.

Cochineal Agar.—Growth extends over surface of tube showing color of agar below. In old cultures the center becomes deep purple. which gradually extends to edge of tube.

Lactose Bouillon.—After two days fluid in closed end of tube cloudy. Similar in other respects to growth in bouillon. No gas. After six days all cloudiness disappears, sediment flocuent. No marked surface growth.

Gelatin.—Liquifies gelatin rapidly, funnel saccate. fluid turbid, sediment stringy; no surface membrane.

BACILLUS NO. IV. FIG VI.

Habitat.—Found in milk after first pasteurizing at 70 degrees for fifteen minutes.

Morphology.—Actively motile, short thick bacillus with rounded ends, dimensions .5*u* to 1*u* in length. Does not form chains or clusters. Stains with ordinary aniline dyes and with carbol fuchsin.

Agar Agar.—In plate culture colonies from this bacteria are small and round, have a smooth surface (Fig. VII.) and a very characteristic pearly luster. In pasteurized milk allowed to stand several days after souring this would frequently be the only specie developed from the cultures. Old colonies slightly elevated at center, outline irregular with the luster not so noticeable. In stab cultures an abundant growth along the line of puncture.

Fermentation tubes.—Lactose bouillon, entire media cloudy after twelve hours, produces gas. No surface pellicle forms.

Potato.—No growth noticeable after twelve hours. After three days a thin white growth appears, surface smooth and moist, growth not spreading.

Milk.—Coagulates milk in 48 to 60 hours at temperature of 32 degree C. Curd soft and not converted into whey, Odor similar to that of ripe cheese.

Gelatin.—Liquifies gelatin, first along the line of puncture gradually extending to edge of tube. Forms a white surface growth. A thick white sediment settles on the bottom of the tube.

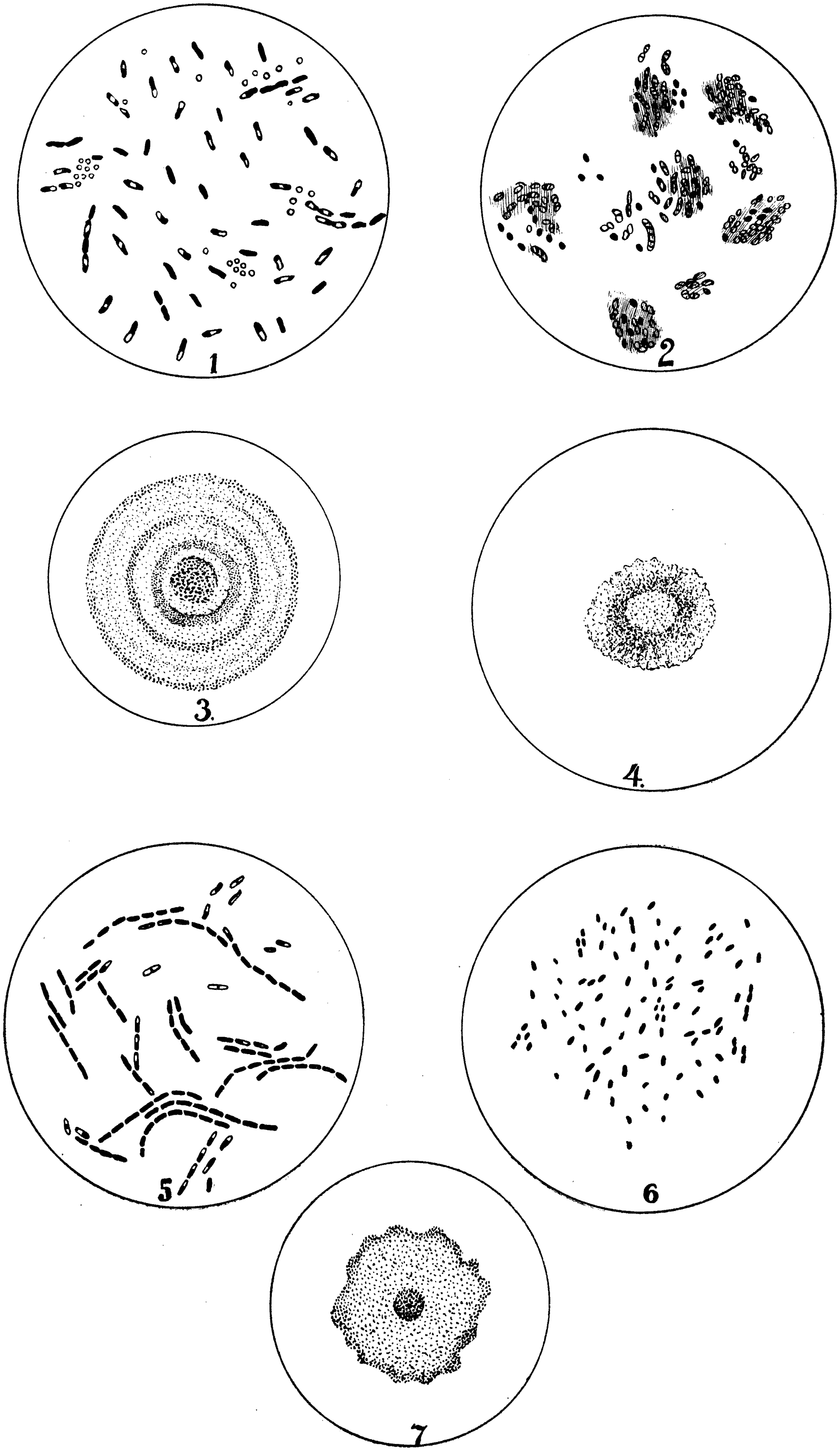
1
2
3.
4.
5
6
7

PLATE I.

FIGURE 1.—Bacillus No. 1.
" 2.—Bacillus No. 2.
" 3.—Colony of bacillus No. 2 on old agar agar plate.
" 4.—Colony of bacillus on agar agar eight days old.
" 5.—Bacillus No. 3 from agar agar.
" 6.—Bacillus No. 4.
" 7.—Colony of bacillus No. 2 on agar agar four days old.