BACTERIOLOGICAL STUDIES ON THE COAGULATION OF EVAPORATED MILK

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DAIRY SECTION

AMES, IOWA
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INTRODUCTION

The value of milk as a food has led to many attempts at its preservation until at the present time various kinds of dried and canned milks are manufactured. The canned milks, because of the water contained in them, are open to bacteriological changes unless the action of microorganisms is overcome by either killing those present or by making the conditions unsuited to their growth.

The sterilization of canned milk, although usually not difficult, is somewhat complicated by the extreme variations in the heat resistance of organisms; exposures which are entirely satisfactory at one period may be inadequate at another because a different bacterial flora, including types extremely resistant to heat, is being dealt with. Similar variations in the resistance of organisms to preservative agents like sugar undoubtedly occur. Because of these variations in the resistance of organisms, canned milks, supposed to be satisfactorily preserved, occasionally undergo undesirable bacteriological changes. When such changes do occur it is evident that the causative organisms are somewhat unusual.

This bulletin deals with an outbreak of curdled milk that was reported by one of the Iowa condenseries putting out evaporated milk, which is supposed to be preserved by the heat to which the filled cans are subjected.

HISTORICAL

Canned milks have been studied bacteriologically, from both the standpoints of the numbers of microorganisms present and of the types of organisms causing certain changes. A review of the literature dealing with bacteria in canned milks is made somewhat difficult by the lack of care in stating the type of milk studied.

BACTERIOLOGY OF NORMAL CANNED MILK

Coutts in a recent (1911) review of the literature on con-
densed milk has dealt with the numbers of bacteria present and Andrewes\(^2\) still more recently (1913) has considered the same subject. From these reviews it is evident that sweetened condensed milk, whether made from whole milk or skim milk, apparently always contains living microorganisms. A number of investigators have reported this type of milk to lack sterility and a few have reported the numbers present, which vary from something under 100 to over 100,000 per c.e., depending to a certain extent on the conditions of plating and incubation. Cocci of various types have been perhaps the most common organisms reported but a number of other types have also been isolated. While the process of condensation markedly reduces the number of bacteria, the organisms of condensed milk, in certain cases at least, come in part from the original milk.

Evaporated milk, altho supposed to be sterilized in the cans, has been reported to contain microorganisms in some cases. Heggs\(^3\) in one sample found 5450 per c. c. and Dold and Garratt\(^4\) in another found 40 per c. c. Gordon and Elmslie,\(^5\) who examined four cans of milk, found none of them sterile, altho "no organisms were demonstrated that were likely to have existed in the original milk before condensation." They considered that the organisms found were introduced from the air after condensation. Andrewes, however, invariably found unsweetened canned milks sterile on culture.

Coutts has considered the growth of bacteria in sweetened condensed milk and referred to the work of Sandilands who got only slight increases in this material and to the work of Heggs who got only slight increases with most brands but found with one brand an increase from 21,800 to 114,700 per c.c. in three days at 22° C. Andrewes has more recently found that in sweetened condensed milk \textit{Staphylococcus aureus} increased greatly, and his experiments "suggest that condensed milk is almost a differential medium for the growth of \textit{Staphylococci}.''

**BACTERIOLOGY OF ABNORMAL CANNED MILK**

Undoubtedly the most common change in cans of milk is a swelling of the package. Cassedebat\(^6\) in his studies on blown tins of milk failed to find either aerobic or anaerobic bacteria and concluded that deteriorations in condensed milk are of a chemical or physical character and not of bacterial origin. Pethybridge\(^7\) found yeasts to be the cause of blowing in cans of sweetened condensed milk. Dodge\(^8\) was unable to isolate from blown cans of

\(^2\) Andrewes; The Jour. of Path. & Bact., 18: 169. 1913.
\(^3\) Heggs. See Coutts, footnote 1.
\(^4\) Dold & Garratt. See Coutts, footnote 1.
\(^6\) Cassedebat. See Coutts, footnote 1.
\(^7\) Pethybridge; The Econ. Proc. Royal Dublin Soc. 1: 306.
condensed milk an organism which he believed was responsible for the trouble. From experiments with butyric and lactic acids he concluded that it was probable that in the cans of spoiled milk the gas was not formed by the bacteria directly but was formed by electrolytic action between the metals of which the cans were composed and the acid generated by the growth of the bacteria in the milk before the later was condensed.

Grieg-Smith studied the cause of the coagulation of condensed milk and found it to be a coccus; he also found that the presence of lime salts accelerated the thickening.

The Vermont Agricultural Experiment station has recently published a short paper on the coagulation of supposedly sterilized milk which is an abstract of a thesis written at the University of Vermont. The original paper shows that the trouble never was observed until after the milk had been sterilized and that, when present, it was first noted in from 12 to 24 hours after heating. The milk curdled without souring, after which the curd was apparently digested leaving a whey-like liquid above, with thick masses of slimy curd and precipitate in the bottom. B. subtilis was the organism most actively figuring in the change.

**HISTORY OF THE TROUBLE**

An outbreak of condensery trouble was recently brought to the attention of the dairy section of the Iowa Agricultural Experiment station. The monetary loss was considerable and, altho the trouble was partly overcome, it seemed advisable to study the organism or organisms responsible, because of the extent of the trouble and also because the manager of the plant characterized the trouble as out of the ordinary.

While a very small percentage of the spoiled cans showed a bulging due to the formation of gas, the typical change did not involve any such condition, but was merely a coagulation. The cans that showed a bulging were not considered in the investigation made. At the time the trouble was first encountered the exposures used varied with the milk, but were around 230° to 233° F. for from 30 to 36 minutes. The exposures were increased (234° to 236° F. for from 30 to 36 minutes) and, altho the trouble decreased, the loss was still enough to be of considerable consequence. It is reasonably certain that some of the cans that spoiled received an exposure of 236° F. for 36 minutes. The May and June milk gave the greatest percentage of spoiled cans altho there was also considerable loss later in the summer. The heated cans were sometimes kept in large piles near the sterilizer and during part of the period when the trouble was encountered the temperature here was comparatively high.


On arrival at the condensery, the milk was put into a vat and ice for cooling added direct. Some of the milk was of a poor quality altho it was delivered daily; a certain amount was refused at the plant but the manager found it impossible to be strict in his demand for an improvement in quality because, on account of the very great demand for milk in the neighborhood, it would have resulted in the loss of a considerable portion of the supply.

Considerable care was exercised in the cleaning of the pipes and vats at the plant and a great deal of time was spent daily on the filling machine so that it does not seem probable that the source of contamination could have been at the plant. The poor condition of the milk, however, was a noticeable feature of the condensery having the trouble.

The canned milk was held at the condensery for at least 10 days before it was sent out and of course had not coagulated after the period of holding. It is impossible to state the time elapsing before the milk coagulated under practical conditions, because of the lack of examinations of the packed material. Milk commonly, however, was not returned until some little time after it had been sent out.

More trouble was experienced with the 6 oz. cans than with the 16 oz. cans. It is, however, impossible to make any definite statements regarding the resistance of the organisms in the different sized cans because only one size was packed on any one day, and accordingly milk from the same batch was never heated in the different sized cans. The old batches of canned milk in which there were some spoiled tins were opened and the normal milk returned to the vacuum pan. Only one opener was used and there was a good opportunity for the contamination of the milk of the normal cans after it had been used on a spoiled can. When this was pointed out to the people in charge of the plant as a possible source of the contamination, they stated that all of the old canned milk was put into batches going to fill 16 oz. cans and that comparatively little trouble was experienced with that size, so they felt that the procedure described was of but little significance as a cause of their trouble.

RESULTS OF THE LABORATORY STUDY

A considerable number of cans of the spoiled evaporated milk were examined in the laboratory. On shaking most of these, it was evident that the contained milk had coagulated as the contents moved in a mass from one end of the can to the other. On opening such cans, a small amount of expressed whey was commonly present and the coagulum was found to be very firm, altho not firm enough to retain its shape when the end was cut from the
can and the contents slipped out. With such treatment an irregular heap of the material, much higher at the middle than at the edge, was obtained. A few of the cans had much the sound of a normal can when shaken and, on opening, the coagulated material was quite flaky and a considerable amount of whey had been liberated. In all of the spoiled cans observed, the tin next to the milk had the same appearance as in sound cans.

The spoiled condensed milk had a sweetish, cheesy odor not at all disagreeable and resembling to a certain extent the odor of Swiss cheese; it was not in the least suggestive of putrefaction. The odor of heated milk was, of course, very evident.

The flavor of the milk was faintly sour and slightly cheesy, but not at all disagreeable. The acid flavor did not resemble the acid flavor of cultures of *Bact. lactis aoidi* altho the pronounced heated flavor present undoubtedly masked to a considerable extent the other flavors.

The acidities encountered in the cans of coagulated material were quite uniform. Table I presents the acidities, calculated as lactic acid, found in a number of cans of the spoiled milk at the time of opening.

**TABLE I. ACIDITIES (CALCULATED AS LACTIC ACID) IN THE CANS OF SPOILED EVAPORATED MILK**

<table>
<thead>
<tr>
<th>Date of Opening</th>
<th>% Acidity</th>
</tr>
</thead>
<tbody>
<tr>
<td>August 20, 1914</td>
<td>.97</td>
</tr>
<tr>
<td>August 21, 1914</td>
<td>1.05</td>
</tr>
<tr>
<td>August 26, 1914</td>
<td>1.05</td>
</tr>
<tr>
<td>September 3, 1914</td>
<td>.95</td>
</tr>
<tr>
<td>September 9, 1914</td>
<td>1.00</td>
</tr>
<tr>
<td>Average</td>
<td>1.027</td>
</tr>
</tbody>
</table>

Table II gives the acidities (calculated as lactic acid) of three cans of the spoiled milk from different lots and for comparison the acidities of sound cans from the same lots.

**TABLE II. COMPARISON OF ACIDITIES (CALCULATED AS LACTIC ACID) IN CANS OF SPOILED AND NORMAL EVAPORATED MILK**

<table>
<thead>
<tr>
<th>Number</th>
<th>% Acidity in Spoiled Milk</th>
<th>% Acidity in Sound Milk</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>.98</td>
<td>.41</td>
</tr>
<tr>
<td>2</td>
<td>.95</td>
<td>.44</td>
</tr>
<tr>
<td>3</td>
<td>.94</td>
<td>.39</td>
</tr>
</tbody>
</table>

From tables I and II it is evident that the curdling of the milk is either accompanied or followed by a considerable increase in the acidity of the milk. The cause of the curdling (whether due to acid or to a rennet-like body) will be discussed later.

Stained smears made from the coagulated material generally showed a rod-shaped organism in rather small numbers, but in two instances no organisms were observed during a search of
about 10 minutes. The use of material from one of these cans in inoculation experiments is discussed later. The organisms were commonly single, altho small chain groupings were occasionally encountered, and they had an appearance suggestive of spores. The spores were by no means distinct, however, and without a heat test their presence could not be definitely determined.

The sound canned milk, when examined microscopically after staining with Gram stain, using carbol-fuchsin as a counter stain, failed to show any organisms during a search of from 10 to 15 minutes. In two instances a can of sound milk and a can of spoiled milk from the same lot were examined microscopically, using Gram stain with a counter stain. In the first instance Gram + organisms were found in the spoiled milk in 15 seconds while a 10 minute search failed to reveal any in the sound material; in the second instance Gram + organisms were observed in the spoiled milk in 10 seconds while here again a 10 minute search showed none in the sound milk.

The inoculation of tubes of either litmus or plain milk with the coagulated milk resulted in their coagulation, while inoculations from cans of sound milk failed to produce any change. The microbial nature of the trouble was quite clearly shown by taking a can of normal milk and a can of spoiled milk from the same batch and making inoculations from each into tubes of litmus milk; in all cases the tubes inoculated with the spoiled milk coagulated on incubating while the tubes inoculated with the sound milk underwent no change. Material from a can of the curdled milk in which no organisms could be found microscopically during a short search gave a growth when inoculated into tubes of litmus milk; this simply shows the greater delicacy of inoculation experiments as compared with microscopical examination for the detection of organisms. Inoculated tubes were held under various conditions; some were corked, others had only cotton stoppers, while others were flooded with sterilized oil, but no appreciable difference in the time required for coagulation was observed.

Plates poured from the original material, using the ordinary amounts for such isolation work, gave colonies only in such small numbers that it was felt they might be contaminating organisms. This point will be taken up later.

The milk that coagulated after inoculation showed rod-shaped organisms that had somewhat the same general appearance as the organisms in the original cans, altho they were much more abundant than in an old coagulum. The striking thing regarding the coagulation of the inoculated tubes was the extreme slowness with which it occurred, altho at 37° C. the change was more rapid than at room temperature. The slow growth even at 37° C. led
to the incubation of freshly inoculated tubes at 55° C. At this temperature the inoculated milk curdled much more rapidly than at room temperature or 37° C. and the usual firm coagulum was also formed. Table III gives the time of coagulation of tubes of milk at the various temperatures; the comparative inoculations were made with a standard loop or wire and while not exactly the same, they were very nearly so. In trials 1, 2, and 3, young milk cultures of the organism served as a source of the inoculation material, in trials 4 and 5, the inoculation material came from the original cans of spoiled milk, while in trial 6 the inoculation material came from an agar colony. Experiments other than those reported gave essentially the same results.

### Table III. Comparison of the Time of Coagulation of Inoculated Milk Held at Different Temperatures

| Temp. of Holding | Time of Coagulation
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Trial 1</td>
</tr>
<tr>
<td>Room Temp.</td>
<td></td>
</tr>
<tr>
<td>37° C.</td>
<td>22 days</td>
</tr>
<tr>
<td>55° C.</td>
<td>3 days</td>
</tr>
</tbody>
</table>

At room temperature wide variations in the time required for the curdling of inoculated tubes were encountered, depending presumably on the prevailing temperature. During the warm summer weather, growth in litmus milk was commonly evident (by the reduction of the litmus) in from 10 to 20 days and coagulation followed soon after. Later in the year, however, growth was evident in from 20 to 30 days and coagulation after it had begun in the bottom of a tube progressed very slowly.

The inoculation of normal cans of condensed milk was also carried out. Small holes were punched in the cans with a sterilized piece of metal, at a point where the tin had been thoroughly flamed. After the inoculation of the material, the opening was sealed with solder or with sealing wax. The sealing wax was quite satisfactory at room temperature but at higher temperatures there was a tendency for it to soften.

The change produced in these cans of milk, whether the inoculation material was from a can of spoiled milk or from a culture, was uniformly a coagulation, without any evidence of gas formation, that eventually resulted in a compact firm curd fully as firm as the original curds. The curds in these cans would retain their general shape when slipped from tins that had had an end removed, undoubtedly because they had not been subjected to the agitation incident to shipping as the original cans had, and they could also be split open and each portion retain its shape. (See figs. 1 and 2.) In these artificially infected cans the tin was bright and the odor and taste were much the same as in the original cans. A small amount of whey was commonly found on top of the coagulum. The acidities of coagulated
milk from such cans agreed very well with the acidities found in the original cans altho with the shorter incubations the acidities were lower as would be expected. Table IV presents some of the acidities (calculated as lactic acid) together with the temperature of holding and the time elapsing between the inoculation and the titration of the milk.
**TABLE IV. ACIDITIES (CALCULATED AS LACTIC ACID) DEVELOPED IN INOCULATED CANS OF EVAPORATED MILK**

<table>
<thead>
<tr>
<th>Temperature of Holding</th>
<th>Time of Holding</th>
<th>% Acidity</th>
</tr>
</thead>
<tbody>
<tr>
<td>55° C</td>
<td>5 days</td>
<td>.50</td>
</tr>
<tr>
<td>55° C</td>
<td>15 days</td>
<td>.55</td>
</tr>
<tr>
<td>55° C</td>
<td>9 days</td>
<td>.76</td>
</tr>
<tr>
<td>55° C</td>
<td>15 days</td>
<td>.77</td>
</tr>
<tr>
<td>37° C</td>
<td>6 days</td>
<td>.75</td>
</tr>
<tr>
<td>37° C</td>
<td>8 days</td>
<td>.94</td>
</tr>
<tr>
<td>37° C</td>
<td>9 days</td>
<td>.94</td>
</tr>
<tr>
<td>37° C</td>
<td>12 days</td>
<td>.89</td>
</tr>
<tr>
<td>Room Temp.</td>
<td>24 days</td>
<td>.59</td>
</tr>
<tr>
<td>Room Temp.</td>
<td>48 days</td>
<td>.92</td>
</tr>
<tr>
<td>Room Temp.</td>
<td>29 days</td>
<td>1.03</td>
</tr>
<tr>
<td>Room Temp.</td>
<td>51 days</td>
<td>.70</td>
</tr>
</tbody>
</table>

The time of coagulation of the milk in cans is somewhat difficult of determination because the condition of the milk must be judged simply by shaking. What were apparently wide variations were encountered in duplicate inoculations. At 55° C. cans inoculated with the original material were usually curdled in 4 or 5 days while cans inoculated with pure cultures curdled in 5 or 6 days. It is probable that variations in the amount of inoculating material were responsible for the slight differences. At 37° C. cans inoculated from the original material required from 9 to 14 days before coagulation was evident while at room temperature from 19 days on were required depending largely on the temperature.

The increased time required for coagulation of the inoculated canned milk as compared with inoculated tubes suggested that possibly a free access of air was an advantage to the organism. Accordingly, tubes of litmus milk were inoculated and some of them sealed as near the milk as possible. The sealed tubes coagulated in approximately the same time as the cotton stoppered tubes in a number of different experiments.

The inoculation of tubes of milk with material taken from sound cans has already been mentioned. Cans of milk were also inoculated with such material but in no case was coagulation secured even after holding for long periods of time at a favorable temperature.

Altho the old coagulated milk constantly showed an acidity of approximately 1% some of the inoculated cans were found to be curdled without having any very great amount of acid present. The inoculation of a number of tubes of plain milk and their titration after varying periods of incubation was then carried out. Table V gives 2 series of titrations, one on tubes held at 37° C. and the other on tubes held at 55° C. The time of coagulation was observed with each series.

Table V shows that at 37° C. coagulation had taken place in the bottom of the tube when the acidity was approximately .38% and that at 55° C. coagulation had taken place when the acidity
TABLE V. ACIDITY CHANGE IN MILK (ACIDITY CALCULATED AS LACTIC ACID)

<table>
<thead>
<tr>
<th>Time of Incubation in Days</th>
<th>Acidity at 37° C.</th>
<th>Acidity at 55° C.</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>.171</td>
<td>.171</td>
</tr>
<tr>
<td>1</td>
<td>.189</td>
<td>.270</td>
</tr>
<tr>
<td>2</td>
<td>.288</td>
<td>.360 coag. complete</td>
</tr>
<tr>
<td>3</td>
<td>.378 coag. at bottom</td>
<td>.531</td>
</tr>
<tr>
<td>4</td>
<td>.489</td>
<td>.687</td>
</tr>
<tr>
<td>5</td>
<td>.639 coag. complete</td>
<td>.657</td>
</tr>
<tr>
<td>7</td>
<td>.918</td>
<td>.657</td>
</tr>
<tr>
<td>9</td>
<td>.927</td>
<td>.648</td>
</tr>
<tr>
<td>11</td>
<td>.954</td>
<td>.747</td>
</tr>
<tr>
<td>13</td>
<td>.963</td>
<td>.738</td>
</tr>
<tr>
<td>16</td>
<td>1.071</td>
<td>.738</td>
</tr>
</tbody>
</table>

was .36%. From these results it is evident that the coagulation in ordinary milk is not due to the acidity primarily, but occurs when the acidity is too low to account for such a change.

Altho the acid development starts off somewhat more rapidly at 55° C. than at 37° C., the acidity developed at 37° C. is soon greater than that developed at 55° C. and the final acidity is likewise higher at 37° C. The results presented in table IV also show higher acidities at 37° C. than at 55° C. This condition introduces the question of the influence of temperature on the enzymes produced by an organism, as well as the question of the influence of temperature on the enzymes that are elaborated. The fact that growth on agar is more rapid at 55° C. than at 37° C. is evidence that there is actually a more rapid multiplication of the organisms at 55° C. than at 37° C. instead of simply a greater activity of the enzymes elaborated.

The lack of organisms in the old coagulated milk is easily explained if the number of organisms in a culture is followed thru a period of some little time. The number of Gram + organisms in a culture increases for some little time as would be expected, then for a period there seems to be no change in the number, and eventually there is a decrease in the number. When the number of Gram + organisms begins to decrease, Gram — organisms become evident. The Gram — organisms stain lightly with the counter stain and eventually begin to disintegrate. As has already been mentioned, even in the old coagulated material some few Gram + organisms are present, and it is probable that these persist for a very long time. It would be of considerable interest to know just what determines whether an organism will lose its ability to stain with Gram or whether it will persist for considerable periods of time in the coagulated milk in which it has grown. The small number of Gram + organisms, which are presumed to be the viable ones since the Gram — forms evidently disintegrate, explains the unsatisfactory results obtained when the original material was used for the isolation of the organism by the plate method.
Fig. 3. *B. coagulans* from 24 hr. agar slope culture (55° C.) stained with gentian violet.

There were a number of difficulties, such as the distance of the condensery from Ames, and the time required before the canned milk spoiled, which made an attempt to find the exact source of infection rather out of the question. It was evident, however, that one of the factors responsible for the trouble was the poor quality of the milk and a demand for an improvement in the supply was advised. An increase in the exposure to heat naturally suggested itself as a means of overcoming the trouble, but the quality of the milk was such that this was not easily carried out because of the tendency to firm coagulation in the presence of any slight increase in acid. The increase in the heated flavor with an increased exposure is also undesirable.

Experiments on an increased exposure were carried out by the manager of the plant. The milk heated in the ordinary way (the control) failed to coagulate, however, even when held (in the incubators in the laboratory) at favorable temperatures for the growth of the organism responsible for the trouble, so the results do not admit of any conclusions.

**DESCRIPTION OF THE ORGANISM**

The organism isolated has been studied morphologically, culturally, and bio-chemically. It is believed to be an undescribed species, and to present peculiarities enough to make it of some little interest. The name proposed for this organism is *B. coagulans* and its description is as follows:
MORPHOLOGY.

**Form** — The organism is rod-shaped.

**Size** — The organisms in the water of condensation of a 24 hr. agar culture (37° C.) when stained with Gentian Violet varied from 1.6 to 7.1 micron in length and from .5 to .7 micron in width. The organisms in milk are smaller and the organisms in old milk cultures smaller still.

**Arrangement** — In cultures of various kinds and ages single organisms are usually found altho a few short chains are not infrequent.

**Motility** — Bouillon cultures in which there is a growth show motile organisms as does also the water of condensation in agar slope cultures.

**Staining Reaction** — The organism stains readily. It is Gram + in young cultures but commonly there is great irregularity in the staining of each organism, the Gentian Violet sometimes being retained only by many small round areas in each cell. In old milk cultures the organism is commonly Gram — altho a few Gram + organisms persist.

**Spore Formation** — Old cultures as well as the material from the original cans, when examined microscopically show organisms that have the appearance of containing spores altho commonly the spores are not real distinct. They do not seem to be more than one-third the width of the organism and apparently are irregularly located. Milk cultures grown 9 days at 37° C. resisted 80° C. for ten minutes.

CULTURAL CHARACTERISTICS.

**Agar Slope** — Agar slope cultures show an echinulate, white, non-viscid, shiny growth with a heavy turbidity and sediment in the water of condensation. At 55° C. growth is quite heavy after 24 hours while at 37° C. growth is not so rapid. At room temperature it is extremely slow. At 55° C. the old cultures are a yellowish gray, and at 37° C. a dirty white.

**Agar Stab** — Agar stab cultures show a heavy, white, non-viscid, surface growth together with some growth along the line of inoculation. The growth in agar stab cultures is also heavy after 24 hrs. at 55° C., is less rapid at 37° C., and extremely slow at room temperature. The old growths are darker than the young growths and are also commonly thicker at the center.

**Agar Plate Colony** — Surface colonies are shiny, white, non-viscid and round and show an entire edge. Growth is evident at 37° C. after from 2 to 3 days and the mature colonies are 1 to 2 mm. in diameter. The subsurface colonies are round to oval, white, non-viscid and smaller than the surface colonies.

**Gelatin Stab** — Growth in gelatine at 55° C. is evident in 24 hrs. After several days there is a sediment and a thin pellicle which is not attached to the glass. At 37° C. essentially the same change occurs altho more time is required. The tubes from either temperature when put into ice water solidify rapidly, showing the absence of an enzyme capable of liquiflying gelatin.

**Bouillons** — In those bouillons in which growth occurs, it is evident as a turbidity and a sediment. In bouillon made with beef extract growth occurs in the presence of dextrose, galactose, levulose, lactose, maltose or raffinose but not in plain bouillon or bouillon containing glycerine, sucrose, mannit, salicin, or inulin. Growth, which is evident at 55° C. in 24 hrs. or less and at 37° C. in approximately 48 hrs., appears first in the monoses and is increasingly less rapid with an increase in the complexity of the material added to the bouillon. In bouillon made with beef infusion growth occurs in the presence of any of the materials mentioned above or in plain bouillon but is much more abundant
in the presence of dextrose, galactose, levulose, lactose, maltose or raffinose.

Potato — At 55° C. potato cultures show a growth in less than 24 hrs. while at 37° C. from two to three days are required. The growth in both instances is dirty white in color, shiny, non-viscid, and gradually spreads over a considerable portion of the surface of the potato.

Dunham’s Solution — No growth is evident at either 37° C. or 55° C.

Uschinsky’s Solution — There is no growth evident at either 37° C. or 55° C.

Litmus Milk — The first change in litmus milk is a reduction which begins at the bottom of the tube. After complete reduction, coagulation begins, also at the bottom. A red band commonly appears at the top of the milk and gradually increases in width. There is usually a slight contraction of the curd, with an expression of a small amount of whey, but apparently no digestion. The time of coagulation at various temperatures has already been mentioned.

Plain Milk — Aside from the color reactions essentially the same changes occur as take place in litmus milk.

BIO-CHEMICAL FEATURES.

Gas Formation — No gas is produced in the various bouillons or in milk. The change produced by the organism in canned evaporated milk is, as has already been mentioned, unaccompanied by gas formation.

Oxygen Relation — Growth occurs in both arms of fermentation tubes containing sugar bouillons that favor growth.

Reaction Change — Acid is produced in milk as has already been mentioned. In those bouillons in which there is a growth there is constantly a production of acid. Table VI gives some of the results obtained with bouillons held under different conditions, the bouillons having been made with beef extract and practically neutral before inoculation.

TABLE VI. ACID PRODUCTION IN DIFFERENT BOUILLONS
Results expressed as c. c. of N/20 NaOH required for 5 c. c. of bouillon

<table>
<thead>
<tr>
<th>Sugar</th>
<th>37° C. 7 Days</th>
<th>37° C. 14 Days</th>
<th>37° C. 16 Days</th>
<th>55° C. 7 Days</th>
</tr>
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