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Bacteriology of Butter

VIII. Relationship of *Achromobacter putrefaciens* to the Putrid Defect of Butter

By T. J. Claydon and B. W. Hammer

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AND MECHANIC ARTS

DAIRY INDUSTRY SECTION

AMES, IOWA
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SUMMARY AND CONCLUSIONS

1. By means of certain enrichment procedures and a modification of the Burri smear culture technique, *Ach. putrefaciens* was isolated from various samples of commercial putrid butter. With few exceptions it was the only organism obtained which was capable of reproducing the typical defect when it was inoculated into cream and the cream churned. The organism was not isolated by plating the samples and the predominating species on plates poured with commercial or experimental putrid butter did not reproduce the defect.

2. *Ach. putrefaciens* was most easily isolated from commercial putrid butter by inoculating the butter into thoroughly pasteurized cream, churning the cream and smearing portions of the resulting butter on agar after the defect had developed. Inoculation of the defective experimental butter into cream for production of second or third generation samples occasionally resulted in isolation of the organism when it had not been obtained previously. Incubation of the inoculated cream overnight at 10°C. facilitated isolation as also did incubation of the experimental butter and the smeared plates at 5°C to 10°C., rather than at 21°C., and certain other procedures. The age of the butter seemed to be related to the ease with which *Ach. putrefaciens* was isolated, since fresh samples yielded the organism more readily than older samples.

3. Of 58 commercial samples having the typical putrid defect, 41 (70.7 percent) yielded *Ach. putrefaciens*, and six (10.3 percent) yielded an apparent variant form. Three (5.2 percent) yielded other types of organisms capable of producing objectionable odors which were not typical of the putrid defect. From eight (13.8 percent), the causative organism was not isolated.

4. *Ach. putrefaciens* did not readily initiate growth on media, which probably accounts, in part, for the difficulty in isolating it from putrid butter; for the same reason the numbers of the organism in butter and other materials could not be counted satisfactorily.

5. *Ach. putrefaciens* was isolated from the water supply of a plant having difficulty with the putrid defect.

6. When inoculated into the cream used for churning, *Ach. putrefaciens* usually produced the typical defect in the unsalted butter in 1 day at 21°C. and in 7 days at 5°C. The defect passed through the same stages as those occurring in commercial butter showing the typical defect.

7. *Ach. putrefaciens* added to the cream or wash water in such small numbers that reisolation was difficult (presumably because...
the organism does not readily initiate growth) caused the putrid defect in the unsalted butter.

8. The pH values of a number of samples of commercial putrid butter varied from 5.8 to 6.8. When *Ach. putrefaciens* was added to lots of cream adjusted to pH values from 5.2 to 7.8, the putrid defect developed in the unsalted butter, but when the pH of the cream was 4.5 it did not.

9. The salt content of a number of samples of commercial putrid butter ranged from 1.08 to 2.41 percent. Although in experimental butter salt tended to prevent development of the putrid defect by *Ach. putrefaciens*, it was not entirely effective unless the butter was thoroughly worked.

10. Five percent or more butter culture added to the cream prevented production of the putrid defect by *Ach. putrefaciens* in experimental unsalted butter. However, some of the commercial putrid butter had been made with butter culture so that other factors may influence the relationship.

11. In experimental butter *Ach. putrefaciens* decreased in numbers as the butter aged and became increasingly difficult to regain.

12. Because of its characteristics, action in experimental butter and presence in much commercial putrid butter, *Ach. putrefaciens* was considered an important cause of the putrid defect in butter.
Bacteriology of Butter

VIII. Relationship of Achromobacter putrefaciens to the Putrid Defect of Butter*

By T. J. Claydon and B. W. Hammer

The action of bacteria is an important factor in the deterioration of butter. Organisms of various types are able to grow and produce conspicuous defects in both the unsalted and salted product. The growth of bacteria and the accompanying development of defects are greatly influenced by the temperature at which butter is held. At storage temperatures, such as -23.3°C, no growth occurs, but at temperatures as low as 0°C certain organisms are able to multiply and bring about various types of deterioration. At still higher temperatures additional organisms develop and cause a greater number of changes.

Defects suggesting extensive protein decomposition in butter have been encountered in various butter-producing countries. These include surface taint in Canada, rabbito or a disagreeable aroma in Australia, a fetid odor in New Zealand, a putrid odor in Denmark and cheesiness and putrid (or limburger) odor in the United States.

The term cheesiness as applied to butter defects includes a wide range of off flavors and odors which vary from those suggestive of roquefort cheese and of a putrid condition to those suggestive of swiss cheese and of cheddar cheese. The flavor suggesting roquefort cheese is probably due to formation of methyl ketones, such as methyl-n-amyl ketone, from acids liberated from the butterfat, but the exact compounds formed with the other types of cheesiness are not so evident, although they may be related to fat hydrolysis as well as protein decomposition.

There is no sharp dividing line between the different types of cheesiness since, like other bacterial defects, each undergoes a sequence of changes according to the growth of the causative organism. At some stage of development one type of defect may resemble another. Furthermore, since organisms capable of causing different types of cheesiness may be present at the same time, various combinations of the putrid, swiss and cheddar defects may occur in butter. In these combinations one type of defect may predominate and then be replaced by another, or a blending may result in a defect suggestive of more than one type of cheesiness. Under such conditions a definite description of a defect is difficult.

The putrid defect is the most important because of its prevalence, its objectionable characteristics and certain peculiarities in its development. Outbreaks are not confined to plants obviously having unsatisfactory sanitary conditions but often occur spasmodi-
cally where apparently adequate precautions are being taken. The defect develops in butter from cream of high quality as well as in butter from the lower grades.

Because of the serious losses the putrid defect has caused the butter industry of the United States and the necessity for further information with reference to it, the work herein reported was undertaken. The investigation involved principally bacteriological examinations of commercial putrid butter, isolations of the causative organisms, action of Achromobacter putrefaciens in experimental butter and effects of various factors on development of the putrid defect by this species.

GENERAL CONSIDERATIONS IN REPRODUCING BACTERIAL DEFECTS OF BUTTER

Commonly, defects due to growth of bacteria in butter can be reproduced by inoculating the defective butter into pasteurized cream, then churning and holding the butter, while defects due to chemical action cannot. The bacterial defects often can be carried through successive generations by churning cream inoculated with the butter just previously made. Such reproduction is evidence that the defect is caused by organisms.

In studying a butter defect with the object of proving its biological nature or of isolating the causative organism, the age of the sample is important, because in old butter many of the organisms, including those responsible for the defect, may be dead. The stage of the defect should also be considered in attempting to duplicate it since, unless the original defect is rather characteristic, it may be difficult to recognize in experimental butter.

After isolation of an organism that appears to be important, certain points should be recognized in studying its effect on butter. Such factors as temperature and time of holding, salting and working influence the development of defects by bacteria and must be considered in experimental procedures and in interpretation of results.

Most bacteria causing defects in commercial butter grow at comparatively low temperatures, 5° to 10°C. or lower. However, at such temperatures defects are produced relatively slowly and may be somewhat different than those produced by the organisms at 21°C. The time of holding butter influences the degree and sometimes the type of defect. The progressive changes that occur due to growth of the organisms may result in defects that differ somewhat from one examination to the next.

Salt inhibits growth of many bacteria, the effect varying with the concentration. In a lot of butter the salt concentration is not uniform throughout, and organisms may grow at some points and cause a defect, even though the general salt content, as indicated by the usual methods of analysis, should prevent development. Dis-
tribution of salt is closely correlated with size and distribution of the moisture droplets, which in turn are controlled largely by the amount of working the butter receives.

In unsalted butter also, working is a factor in controlling bacterial defects. With increased working the moisture droplets are decreased in size, and since the bacteria are largely in the moisture droplets, with many more droplets than there are bacteria, this results in reduction in the food available for growth of the organisms.

Some of the above conditions may not be the same with butter made in small experimental churnings as in commercial butter, and results obtained experimentally should be interpreted on this basis.

HISTORICAL

In 1931, Derby and Hammer (4) reported the isolation of an organism which they named *Achromobacter putrefaciens* from a number of samples of surface taint butter. By plating on beef infusion agar, they were unable to obtain this organism consistently from defective butter, and they attempted to develop suitable enrichment methods. Since the organism grew well at low temperatures, they inoculated the butter into litmus milk, incubated at 5°C. until reduction occurred and then plated on beef infusion agar. This procedure resulted in additional isolations of *Achromobacter putrefaciens*, although it was obtained only in small numbers. The organism was capable of producing a pronounced defect in butter at both 21°C and 5°C. Several other types of bacteria capable of producing surface taint were isolated. Although various enrichment methods were used, a number of samples of surface taint butter failed to yield an organism capable of producing the defect.

Derby and Hammer (4) considered that there was little correlation between the general types of bacteria in butter, as shown by beef infusion agar plates, and the occurrence of surface taint. While the defective butter frequently showed large numbers of microorganisms, particularly at the surface, some samples had rather low counts. The flora obtained by plating surface taint butter differed little from that of normal butter, and the predominant organisms on plates poured with the abnormal butter failed to give typical surface taint when inoculated into cream and the cream churned.

The causes of cheesy flavors of the cheddar type in unsalted butter were investigated by Herreid, Macy and Combs (5). Mixed cultures from defective butter reproduced the cheesy defects when added to the cream used for churning. The cultures also produced a defect in butter when inoculated into the water used for washing. Certain of the mixed cultures produced a putrid rather than cheesy odor in butter at some stage of the holding.

Cullity and Griffin (3) cited the work of Loftus-Hills, Scharp
and Searle in which organisms capable of producing rabbito were isolated from factory water supplies, churns and raw and pasteurized cream. Water supplies were considered to be the natural habitat. The organisms were similar to that isolated by Derby and Hammer (4) from surface taint butter. Loftus-Hills, Scharp and Searle considered that poor texture in butter favored production of the putrid condition.

Rabbito in Australian butter was discussed by Cullity and Griffin (3). They stated that a sweetish flavor, suggestive of condensed milk, sometimes was noted in the defective butter. After the butter had been held at comparatively high temperatures, this flavor became very objectionable, but the putrid odor seemed to disappear. The authors reported a number of investigations at plants experiencing outbreaks of rabbito, and in each case the defect eventually was traced to the factory water. New water sources eliminated the trouble. During the investigations, many types of bacteria, particularly proteolytic types, were used in experimental churnings. These organisms failed to reproduce the typical defect. Other organisms were isolated from water supplies and, on sterile butter plates, gave an odor resembling surface taint. The cultures were short-lived which suggested that the organisms causing rabbito might have the same characteristic. Apparently, \textit{Achromobacter putrefaciens} was isolated from two water supplies. The authors stated that there appeared to be some variation in the characters of these organisms. An instance was described in which the defect developed after faulty working of the butter.

Cullity and Griffin (3) concluded tentatively that the causative organism was probably water-borne and that foci of contamination were built up in churns and equipment through initial contamination from water. Thorough working and high salt content of the butter, as well as high acidity in the cream, were factors that retarded development of the defect. It was noted that the whole story was not known.

In Canada, an organism that apparently is \textit{Achromobacter putrefaciens} has been isolated from surface taint butter as well as from water supplies of plants experiencing trouble with the defect (12).

\section*{METHODS}

\subsection*{SAMPLES STUDIED}

The samples of commercial putrid butter came from plants in several states in the middle west. With a number of them the original sources were unknown since they were supplied by marketing organizations, often after their return from an eastern customer. Some of the samples were only 4 or 5 days old, but the majority were from several weeks to several months old. The samples showed the putrid defect to varying degrees. Frequently, it was
very marked and typical, while in other cases it was mild or questionable with a definite suggestion of one or more additional defects. Most of the butter was salted.

METHODS OF BACTERIOLOGICAL EXAMINATION.

Commonly, the bacteriological examination of a sample of butter was begun as soon as it was received. When this was impossible it was held at about 5°C and examined as soon as convenient.

Butter was prepared for plating by gently warming a portion in a sterile petri plate over a small flame and stirring with a warmed, sterile pipette. Care was taken to avoid settling of the serum. Dilution blanks were warmed in a 45°C incubator.

Beef infusion agar having a pH of 6.8 to 7.0 was used in all bacteriological examinations. Various other media and modifications were employed from time to time in comparative studies but were discarded as inferior to beef infusion agar. For the purpose of detecting proteolytic and lipolytic organisms on the plates, sterile skimmilk and fat emulsion often were added to the media. The fat emulsion was prepared according to the method of Long and Hamner (7). Five percent of both skimmilk and fat emulsion were used. Plates normally were incubated at room temperature (which varied somewhat) for 5 days, although in some instances duplicate sets were held at 5°C or 10°C for 10 to 14 days. The plates were examined frequently during incubation.

CHURNING METHODS

For making the experimental churnings sweet cream of good flavor was pasteurized at 85°C to 90°C for about 30 minutes. After cooling, the cream was divided among a number of quart jars in each of which about 1 pint of cream could be churned. Defective butter, water samples or cultures of organisms were inoculated into the cream and the jars held at about 10°C overnight for incubation. When inoculating with butter, 5 to 10 grams were used; the cream was warmed and the butter well-mixed with it after which it was again cooled to about 10°C. The cultures or water samples were added to the cream directly in varying amounts.

The churning was done in an experimental churn having compartments for holding the jars and a motor which agitated the cream by rotation of a shaft supporting the compartments. The butter from each jar was washed, worked with hand paddles in enamel bowls and divided into two portions. These portions were placed in small glass jars and held, usually at 21°C and about 5°C. All equipment, wash water and salt (if used) were sterilized by autoclaving.

When more than two samples of butter from one churning were desired, a 1-gallon Dazey churn was used. It was sterilized by filling with a chlorine solution and allowing it to stand overnight. It was rinsed with sterile water before using.
Examinations of experimental butter held at 21°C. were made daily. Butter held at 5°C. was examined at intervals of about 3 to 4 days.

RESULTS

PRELIMINARY STUDIES

PLATING ORIGINAL SAMPLES OF PUTRID BUTTER

Although various investigators have attempted to relate the putrid defect of butter to bacteria developing readily on plates poured with the butter, another attempt along this line was made. Samples of typically putrid butter of different ages were plated on beef infusion agar and the plates incubated at room temperature. Colonies representing the main types were picked into litmus milk. After development, the cultures were inoculated into pasteurized cream and the cream churned. The unsalted butter was held at 21° and 5°C. and examined frequently for appearance of a defect similar to the original. Results regularly were negative, the defects which developed often being slight and never suggesting the typical putrid condition.

Attention was next directed to isolation of organisms which might be expected to cause noticeable changes in butter. Skimmilk and fat emulsion were added to the agar used for plating, and proteolytic and lipolytic types were picked into litmus milk. After development, these were tested for their action on butter. Many of the organisms, when used in pure culture or combinations, produced defects in butter which varied widely. However, none of them gave the typical putrid defect.

ENRICHMENT METHODS

Since the putrid defect develops commercially at comparatively low temperatures, enrichment methods at about 10°C. were used in an attempt to increase the relative numbers of the causative organisms. Pasteurized cream was inoculated with the original defective butter and incubated overnight at about 10°C. It was then churned and the resulting unsalted butter stored at 21° and about 5°C. with frequent examinations for a defect similar to the original. When the butter became putrid it was plated and colonies picked in the manner used with the original commercial samples. In a number of cases the defective experimental butter was inoculated into cream which was then held overnight at about 10°C. and churned. After holding the butter it was plated and colonies picked. In other instances the original butter was inoculated into litmus milk which was incubated at 10°C. for 4 days or at 5°C. for 2 weeks. These cultures were then used in making experimental butter which was plated and colonies picked.

Certain of the isolated cultures were selected for inoculation into
cream to be churned. Particular attention was given to those types which appeared consistently in samples that developed the putrid defect through successive churnings. Some cultures were selected on their ability to cause noticeable changes in litmus milk and also on the frequency with which they occurred in the butter. Early in the work it was believed that cultures might fail to produce an objectionable change in litmus milk and yet cause pronounced flavor defects in butter. Consequently, cultures producing little change in litmus milk were also investigated.

None of the cultures isolated from defective butter with the enrichment procedures produced the typical putrid defect.

Since it appeared that organisms bringing about little change in litmus milk might produce off odors in other media, colonies were picked from plates poured with commercial and experimental putrid butter into cream, casein medium, casein medium plus peptone, and each of the two latter media adjusted to a slightly alkaline reaction. Litmus milk was used as a basis for comparison. No consistent advantage was obtained from the use of these media.

Various solid media were used for plating commercial and experimental putrid butter in an effort to obtain growth of organisms not appearing on ordinary beef infusion agar plates. These included casein agar, casein agar plus peptone, beef infusion agar plus butterfat and casein and beef infusion agar with a pH approximating 7.5. None of these media showed any consistent advantage over beef infusion agar from the standpoint of the colonies developing on it, and several were quite inferior.

USE OF BURRI SMEAR CULTURE TECHNIC IN ISOLATING CAUSATIVE ORGANISMS FROM PUTRID BUTTER

Because of the failure to isolate the causative organisms from commercial or experimental putrid butter by plating, the Burri smear culture technic, which was used on butter by Long and Hammer (8), was employed in the examination of samples. Plates which had been poured with beef infusion agar plus skimmilk and fat emulsion and allowed to solidify were used instead of agar slopes. Attempts were made to avoid the formation of water droplets on the agar, and if such occurred the plates were placed at 37°C until dry. The plates were marked into six sectors, and each sector smeared with a tiny portion of butter. Usually, three plates were smeared with each sample of butter. These were incubated at room temperature for 5 days and were examined daily for different types of colonies. With the plates, the colonies were easily examined and picked, while the addition of skimmilk and fat emulsion to the agar made possible the detection of proteolytic and lipolytic colonies.

The value of the smear technic in demonstrating organisms in butter which are not evident on poured plates was noted by Long and Hammer (8). Similar results were obtained in the examina-
tion of samples of butter having various defects. The technic rather regularly demonstrated types of organisms that did not appear on the poured plates. Frequently, an organism that was absent on the poured plates was obtained in almost pure culture on one or more smeared sectors.

The putrid butter examined with the smear procedure included both commercial and experimental churnings. The following examples illustrate the value of the technic in demonstrating the causative organisms in the abnormal butter and also show the detailed scheme used in studying samples.

**SAMPLE A**

The sample was from a car of butter, most of which had become putrid. The defect was so conspicuous that the butter was practically unsalable and resulted in serious loss to the producing plant. The sample was relatively fresh when received and mildly salted. Since the smear technic was not applied until the later stages of the examination, some of the data comparing it with the plate method are not as complete as with other samples.

The original butter was plated in the usual manner. The bacterial counts and colony types on the plates were as follows:

<table>
<thead>
<tr>
<th>Bacteria per ml.</th>
<th>Colony Types</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>The main types were white, proteolytic or non-proteolytic colonies and pale orange, proteolytic colonies. Neither these nor less common types reproduced the defect.</td>
</tr>
<tr>
<td>Proteolytic</td>
<td>35,000</td>
</tr>
<tr>
<td>Lipolytic</td>
<td>1,000</td>
</tr>
</tbody>
</table>

At the time of plating the original butter, a portion was inoculated into pasteurized cream; this was held overnight at 10°C. and churned. Portions of the butter were held at 21°C and 5°C.

The portion at 21°C. became putrid in 2 days. Plating showed the following bacterial counts and colony types:

<table>
<thead>
<tr>
<th>Bacteria per ml.</th>
<th>Colony Types</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>In general, the same types were present as with the original butter, but a few others were present in small numbers. None produced the defect.</td>
</tr>
<tr>
<td>Proteolytic</td>
<td>2,800,000</td>
</tr>
<tr>
<td>Lipolytic</td>
<td>4,500,000</td>
</tr>
</tbody>
</table>

The portion at 5°C. was putrid in 7 days. Plating showed the following bacterial counts and colony types:

<table>
<thead>
<tr>
<th>Bacteria per ml.</th>
<th>Colony Types</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>Large, moist, lipolytic colonies constituted the main type; they did not reproduce the original defect.</td>
</tr>
<tr>
<td>Proteolytic</td>
<td>1,000</td>
</tr>
<tr>
<td>Lipolytic</td>
<td>3,500,000</td>
</tr>
</tbody>
</table>

When the first experimental churning was made, a small portion of the original butter was inoculated into litmus milk. After incubating 5 days at 10°C., it was added to pasteurized cream and the cream churned. The butter was divided and stored at 21° and 5°C.

The portion at 21°C. became putrid in 1 day. The bacterial counts and colony types shown by plating were as follows:

<table>
<thead>
<tr>
<th>Bacteria per ml.</th>
<th>Colony Types</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>The majority of the colonies were white, non-proteolytic and non-lipolytic. These did not produce the putrid defect.</td>
</tr>
<tr>
<td>Proteolytic</td>
<td>65,000</td>
</tr>
<tr>
<td>Lipolytic</td>
<td>30,000</td>
</tr>
</tbody>
</table>
The portion at 5°C. developed a putrid defect in 7 days. The bacterial counts and colony types on plates were as follows:

<table>
<thead>
<tr>
<th>Bacteria per mL</th>
<th>Colony Types</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>25,000,000</td>
</tr>
<tr>
<td>Proteolytic</td>
<td>Nearly all the colonies were lipolytic and</td>
</tr>
<tr>
<td>Lipolytic</td>
<td>non-proteolytic; they did not produce the</td>
</tr>
<tr>
<td></td>
<td>defect.</td>
</tr>
</tbody>
</table>

At this point the smear method was first applied in attempts to isolate the causative organism. Tiny portions of the butter held at 5°C. were smeared on agar plates, and these were examined frequently for colony types. Those that developed included the types on the poured plates and two additional. One was a small, white, non-proteolytic colony, and the other was typical of *Ach. putrefaciens*. Both of the types were obtained in relatively large numbers, with several smeared sectors showing almost pure cultures of one or the other. When tested, the *Ach. putrefaciens* type produced a putrid defect similar to that of the original butter, while the other type did not.

From the numbers and types of bacteria on plates poured with the original butter and with the various experimental churnings, it appeared that the organisms shown by this method were not related to the defect. With the different lots of putrid butter there was a great variation in both numbers and types of organisms on poured plates, and the situation suggested that species other than those appearing on the plates were concerned with the defect. This idea was supported by the fact that as the examination progressed many cultures and combinations of cultures obtained from poured plates were used to inoculate cream for experimental churnings without reproducing the original defect. Application of the smear method to the experimental butter at once yielded additional bacterial types. *Ach. putrefaciens* was present in considerable numbers and was the only type isolated that was capable of producing the putrid defect.

**SAMPLE B**

The sample represented a shipment of butter which was of high quality when sold to the wholesaler. Shortly afterwards, however, it developed the putrid defect and was returned. The sample, which was lightly salted, was relatively fresh when received.

On plating, the original butter showed the following bacterial counts and colony types:

<table>
<thead>
<tr>
<th>Bacteria per mL</th>
<th>Colony Types</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>9,200,000</td>
</tr>
<tr>
<td>Proteolytic</td>
<td>Most of the colonies were white micrococci</td>
</tr>
<tr>
<td></td>
<td>which produced no defect in butter.</td>
</tr>
</tbody>
</table>

The smear method showed the same colony types as the plate method and, in addition, a number of fluorescent proteolytic colonies which did not reproduce the typical putrid defect.

Pasteurized cream was inoculated with a portion of the original butter, held overnight at 10°C. and churned. The butter was divided and stored at 21°C and 5°C.

The portion at 21°C. became putrid in 2 days. The bacterial counts and colony types shown by the plate method were as follows:

<table>
<thead>
<tr>
<th>Bacteria per mL</th>
<th>Colony Types</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>105,000,000</td>
</tr>
<tr>
<td>Proteolytic</td>
<td>The dominant types were micrococci and grey,</td>
</tr>
<tr>
<td></td>
<td>proteolytic colonies. A few fluorescent,</td>
</tr>
<tr>
<td></td>
<td>non-proteolytic colonies were present. None of</td>
</tr>
<tr>
<td></td>
<td>the types produced the defect.</td>
</tr>
</tbody>
</table>
The smear method showed a large number of the fluorescent, proteolytic colonies in addition to the above types. There was also a number of colonies of the *A. putrefaciens* type; this was the only type which produced the defect in butter.

The portion at 5°C. became putrid in 4 days and when examined by the plate method showed the following bacterial counts and colony types:

**BACTERIA PER ML.**

<table>
<thead>
<tr>
<th>Type</th>
<th>Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>4,500,000</td>
</tr>
<tr>
<td>Proteolytic</td>
<td>1,800,000</td>
</tr>
<tr>
<td>Lipolytic</td>
<td>1,100,000</td>
</tr>
</tbody>
</table>

**COLONY TYPES**

The same types were obtained as with the portion at 21°C.

With the smear method fewer fluorescent colonies were obtained than from the portion at 21°C. Most colonies were dark and proteolytic, and a number were typical of *A. putrefaciens*. When tested, only the *A. putrefaciens* type reproduced the original defect.

The early use of the smear technic in the examination resulted in *A. putrefaciens* being isolated more quickly than with sample A.

**SAMPLE C**

The sample, which was mildly salted, became putrid when held at room temperature to test the keeping quality.

The bacterial counts and colony types obtained by plating were as follows:

**BACTERIA PER ML.**

<table>
<thead>
<tr>
<th>Type</th>
<th>Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>6,800,000</td>
</tr>
<tr>
<td>Proteolytic</td>
<td>1,000</td>
</tr>
<tr>
<td>Lipolytic</td>
<td>1,000</td>
</tr>
</tbody>
</table>

**COLONY TYPES**

All colonies appeared to be micrococi or streptococi; they did not produce the defect.

The smear method showed the same types as the poured plates and in addition a number of dark colonies. None produced the putrid odor in butter.

At the time of plating and smearing, the original butter was inoculated into pasteurized cream; this was held overnight at 10°C. and churned. The butter was stored at 21° and 5°C.

In 2 days the portion at 21°C. became putrid. Results of examination by the plate method were as follows:

**BACTERIA PER ML.**

<table>
<thead>
<tr>
<th>Type</th>
<th>Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>9,100,000</td>
</tr>
<tr>
<td>Proteolytic</td>
<td>35,000</td>
</tr>
<tr>
<td>Lipolytic</td>
<td>1,400,000</td>
</tr>
</tbody>
</table>

**COLONY TYPES**

Most colonies were white, moist and lipolytic. Neither this type nor the less common types produced the defect.

In addition to the types evident on plating, the smear method showed a number of colonies typical of *A. putrefaciens*, as well as some less typical. Only the typical *A. putrefaciens* type reproduced the original defect.

The sample stored at 5°C. developed the putrid defect in 7 days. On plating, the following bacterial counts and colony types were obtained:

**BACTERIA PER ML.**

<table>
<thead>
<tr>
<th>Type</th>
<th>Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>9,700,000</td>
</tr>
<tr>
<td>Proteolytic</td>
<td>300,000</td>
</tr>
<tr>
<td>Lipolytic</td>
<td>2,800,000</td>
</tr>
</tbody>
</table>

**COLONY TYPES**

The types were the same as with the portion at 21°C.

Smears from the portion at 5°C. showed the same types as smears from the portion at 21°C. but contained a larger number of *A. putrefaciens* colonies.
GENERAL OBSERVATIONS ON ISOLATION OF
ACH. PUTREFACIENS FROM PUTRID BUTTER

The results on samples A, B and C are typical of many of those obtained on putrid butter. The smear technic was effective in demonstrating *Ach. putrefaciens* in various samples from which it could not be isolated by plating. While colonies of the organism often were present in small numbers in the smears and scattered among other colony types, they occasionally formed a heavy growth on certain of the sectors.

Figures 1 and 2 show a smeared plate and a poured plate from a sample of putrid butter. On the smeared plate, colonies of *Ach. putrefaciens* are very evident in several of the sectors; the other

---

Fig. 1. Smeared plate from sample of putrid butter; colonies of *Ach. putrefaciens*, some of which are indicated by arrows, are very evident.
colonies are micrococci. On the poured plate no *Ach. putrefaciens* colonies were present, and micrococci constituted nearly the entire flora.

The data on samples A, B and C show that even with the smear technic *Ach. putrefaciens* was not isolated from the original butter, but was obtained from experimental churnings prepared directly or indirectly from the original. In only one of the samples subsequently examined was the organism obtained directly from the commercial sample. The difficulty in isolating the organism may have been due to the age of the butter with the accompanying possibility that many of the organisms had died before examination was begun. Later studies supported this suggestion. Had the
samples been examined when the defect first developed, *Ach. putrefaciens* might have been isolated from them directly.

Apparently the preparation of experimental churnings favored the growth of *Ach. putrefaciens* and resulted in the presence of relatively large numbers in the butter. This may have been due partially to incubation of the inoculated cream at a low temperature before churning. In some of the examinations in which *Ach. putrefaciens* could not be obtained from the first experimental butter, it was isolated when a second generation churning was prepared.

In general, *Ach. putrefaciens* was obtained more readily from the experimental butter held at 5°C than from that held at 21°C. This was very evident with a number of the samples studied, from some of which *Ach. putrefaciens* could not be isolated when the experimental butter was held at 21°C. Presumably, the lower temperature inhibited some types of organisms that grew well at 21°C and allowed *Ach. putrefaciens* to develop with less competition.

In a number of cases smeared plates from putrid experimental samples were incubated at 5°C. Occasionally, these plates showed a greater percentage of *Ach. putrefaciens* colonies than did plates incubated at room temperature, and in one or two cases they were the only plates from a sample on which the organism was found.

With some of the experimental butter, smears were made on successive days after development of the defect. In several instances *Ach. putrefaciens* was obtained on the second or third set of smears (made on the second or third day after the defect developed) when it was not observed on the first set. The various samples were inconsistent in this respect, but the procedure occasionally resulted in isolation of the organism when it was not obtained otherwise.

Frequently *Ach. putrefaciens* was obtained in larger numbers from the interior of a sample of experimental putrid butter than from the surface. While this seldom applied to butter that was only a few days old, it often was true with samples that had been held 1 week or more at 21°C. In the case in which *Ach. putrefaciens* was isolated from the original butter, it was obtained from the interior but not from the surface.

**SUGGESTED PROCEDURE FOR ATTEMPTING ISOLATION OF *ACH. PUTREFACIENS* FROM PUTRID BUTTER**

From the general observations on isolation of *Ach. putrefaciens* from putrid butter, it is apparent that obtaining the organism is often difficult. Various procedures facilitated the isolation but were not always successful, and some samples of putrid butter failed to yield the organism. More information is needed concerning conditions favoring growth of *Ach. putrefaciens* in order to develop an effective method of isolation. The following isolation procedure is suggested as being relatively successful and can be used in whole or in part.
The original butter is smeared on a number of plates, some of which are incubated at 21°C. and some at 5°C. The butter is also inoculated into thoroughly pasteurized cream which is held overnight at 10°C. and churned. The resulting butter is divided and held at 21° and 5°C. After development of the defect, the experimental samples are smeared in a similar manner to the original and are used to inoculate cream for the production of second generation samples; these also are smeared as soon as the defect develops. Even a third successive churning may be advisable in case the organism has not been isolated.

At the time of smearing the original butter, a portion is inoculated into litmus milk which is held at about 5°C. until reduction occurs. This reduced culture is then smeared and the plates incubated at 21° and 5°C. It is also used to inoculate cream for churning, and the resulting butter is held and smeared when the defect appears. All smeared plates held at 21°C. are examined daily for Ach. putrefaciens colonies; those held at 5°C. are examined when the colonies are rather well-developed. The purpose of the whole procedure is to obtain sufficient enrichment of Ach. putrefaciens to permit its growth on some of the plates.

SAMPLES OF COMMERCIAL PUTRID BUTTER YIELDING

ACH. PUTREFACIENS

The suggested procedure was used in more or less detail on various samples of commercial putrid butter. Some of them were not typically putrid and, when inoculated into cream and the cream churned, usually produced rancidity or the cheddar type of cheesiness in the butter. The samples were of different ages and represented churnings made from sour cream as well as from sweet cream. Each sample did not necessarily represent a separate source, since in some instances samples were obtained at intervals from the same plant and represented churnings made at different periods. While certain plants apparently had only an occasional churning of the defective butter, others experienced outbreaks of considerable duration. Undoubtedly there were variations in the conditions under which the butter was produced. Some of the plants were attempting to use adequate sanitary precautions, while with others it is probable that conditions were less satisfactory.

Fifty-eight samples of typical commercial putrid butter were examined. Of these, 41 (70.7 percent) yielded Ach. putrefaciens, six (10.3 percent) contained a type of organism that was similar to Ach. putrefaciens but differed in certain characteristics and was considered a variant, and three (5.2 percent) yielded other organisms capable of producing a putrid defect somewhat like the original; from the remaining eight (13.8 percent), the causative organism could not be obtained.

The 41 samples yielding typical Ach. putrefaciens varied in age
but were generally less than 1 month old, with a number being less than 2 weeks old. All produced a putrid condition in experimental butter when inoculated into cream and the cream churned. The defect occasionally was slight in the first churning but usually became more marked in a second churning made by using the previous experimental butter as inoculating material.

From one sample possessing the defect to a very marked degree, *Ach. putrefaciens* was obtained from the original butter; the age of this butter was not known. Most of the definitely putrid samples yielded the organism in comparatively large numbers from the first experimental churning. With the samples showing the defect to a lesser degree, *Ach. putrefaciens* usually was not obtained until later in the examination and then only in small numbers.

Defective samples from the same source were rather consistent in yielding *Ach. putrefaciens*. For example, 12 of 15 samples from one plant, 3 of 4 samples from a second and 3 of 4 samples from a third yielded the organism.

The six samples from which *Ach. putrefaciens* was not isolated but which yielded a similar type of organism did not differ greatly from the others. In general, the defect was less marked in both the original butter and in the butter made from cream into which it had been inoculated. The organisms were obtained at various stages of the examination and usually in comparatively large numbers. There was no evident connection between this apparent variant form of *Ach. putrefaciens* and the source of the original butter, since the organism was found in samples from different sources.

The three samples yielding organisms which differed from *Ach. putrefaciens* were distinctly putrid. However, when they were inoculated into cream and the cream churned, the resulting butter was not typical of the defect and failed to show the characteristic stages of change. The same general type of defect was produced by the organisms isolated from the samples.

The eight samples from which causative organisms could not be obtained were very definitely putrid. While organisms were isolated from them that were capable of causing off odors in butter, they did not reproduce the original defect and were not considered the causative type.

*Ach. putrefaciens* appeared to be a common cause of the putrid defect in the butter studied. While it could not be obtained from all the samples, it was isolated from most of those that were fresh enough to reproduce the defect in experimental churnings.

**GENERAL CHARACTERS OF ACH. PUTREFACIENS CULTURES ISOLATED**

**COMPARISON WITH ORIGINAL DESCRIPTION**

The *Ach. putrefaciens* cultures isolated from putrid butter con-
formed generally to the description given by Derby and Hammer (4) in morphology, cultural characteristics and most biochemical characteristics, but slight variations occurred in reaction changes in certain bouillons. Nearly all the cultures produced acid from maltose and sucrose, the variability occurring with dextrose, galactose, lactose and levulose. As suggested by Derby and Hammer, the rapid production of ammonia is an interfering factor in fermentation tests and may explain the variations between cultures. The strains isolated from samples of butter from the same source generally showed similar reaction changes.

Some variation from the original description was also noted in growth temperatures, with a number of cultures growing at 37°C. However, the tests were made several months after isolation so that the temperature relationships may have been modified. Thermal resistance of the cultures was low, and freshly isolated strains were destroyed by heating at 60°C for 2 minutes.

One of the *Ach. putrefaciens* cultures grew slowly, both on agar and in litmus milk. Two days at 21°C. were generally required for development of colonies on agar, and 1 to 2 days were required for complete reduction of litmus milk. The strain was probably similar to the slow culture obtained by Derby and Hammer (4); both produced acid from dextrose. Subsequent study indicated that the strain required heavy inoculations for initiation of growth on artificial media. The same characteristic, to a lesser degree, was noted with the *Ach. putrefaciens* cultures generally.

VARIANT FORM

In addition to the cultures considered as typical *Ach. putrefaciens*, several others were obtained which, while differing in a number of respects, were sufficiently like *Ach. putrefaciens* to be regarded as a variant form. This type produced the putrid defect in butter but to a milder degree and at a slower rate than typical *Ach. putrefaciens*. It had the same morphology and produced the same sort of colony, although growth was not as heavy.

The principal difference was in the action in litmus milk. Normal reduction did not occur, but after 6 to 8 days at room temperature the culture was partially proteolyzed and possessed the characteristic amber color of typical *Ach. putrefaciens* cultures of the same age. In general, the type was more fastidious in growth requirements than the usual organism, and occasionally transfers to litmus milk or bouillon failed to develop. Heavy inoculations were required for growth on agar or in liquid media. A significant point was that the variant form was usually isolated from butter showing the putrid defect to a comparatively mild degree and from which typical *Ach. putrefaciens* was seldom obtained.

In general, the observations suggest the possibility of a variant form of *Ach. putrefaciens* being the cause of certain outbreaks of putrid butter.
GROWTH ON MEDIA

Failure of the plate method to demonstrate *Ach. putrefaciens* in putrid butter, from which it was isolated by the smear technic, suggested some peculiarity in the growth requirements. Since development was abundant after once established, it appeared that the peculiarity was in the difficulty of initiating growth on media. When the inoculation at a certain point was heavy, as was more probable with the smear method, the organisms seemed to develop colonies more readily.

Plate counts on milk cultures of *Ach. putrefaciens* further emphasized the peculiarity by showing little correlation between the different dilutions. The plates from one or more dilutions of a series were sometimes crowded, while those from the next higher dilution had very few or no colonies, although on a comparative basis they should have had considerable numbers.

Observations on the growth of *Ach. putrefaciens* in litmus milk further indicated that the organism was slow in establishing itself. When very small colonies were picked into the medium, several days were often required for reduction. With one of the more fastidious strains, the reduction would not occur at all unless large amounts of inoculum were used. However, once growth was well-established, regular loop transfers brought about rapid reduction in succeeding cultures.

Attempts were made to obtain more satisfactory growth of *Ach. putrefaciens* by using different media or modifications of media. Tomato juice, liver infusion and tryptone-dextrose agars were generally inferior to beef infusion agar. The reactions of media were varied by adding different amounts of lactic acid or sodium hydroxide to cover a pH range of approximately 5.0 to 8.5. While the organisms were able to grow over a wide pH range, development was best when the reaction was approximately neutral. Addition of sterile butter serum and sterile butterfat, alone or in combination, to beef infusion agar did not favor growth of *Ach. putrefaciens*.

Various substances were added to beef infusion agar in an attempt to alter the oxidation-reduction potential of the medium and thereby facilitate growth. The materials included reduced iron, sodium thiosulfate, cysteine and potassium permanganate, each of which was sterilized and used separately in the agar. The reduced iron was placed in petri plates or added to the agar before pouring, using about 0.5 gram per plate. The sodium thiosulfate, cysteine and postassium permanganate were added to the agar before pouring in amounts from 0.1 to 1.0 percent, from 0.05 to 0.1 percent and from 0.01 to 0.05 percent, respectively. They were also used in agar blocks according to the method of Allyn and Baldwin (1). In general, the results were disappointing. While some of the modifications, particularly the addition of sodium thiosulfate, resulted
in more abundant development of *Ach. putrefaciens*, they did not alter the irregular results obtained on plating cultures in various dilutions. Furthermore, materials that increased the growth of *Ach. putrefaciens* often stimulated other types and hence were of no advantage in isolation of the organism from defective butter.

**SOURCES OF *ACH. PUTREFACIENS* IN BUTTER**

Since *Ach. putrefaciens* is so easily destroyed by heat, its presence in butter suggests addition to cream or butter following pasteurization of the cream. It could be introduced by the inclusion of raw cream, by its presence in improperly sterilized equipment or by its addition through use of contaminated water for washing the butter. With various plants experiencing outbreaks of the putrid defect, the general conditions apparently are well-controlled and do not suggest contamination from raw cream or equipment.

Olson and Hammer (10), in observations on the influence of the contamination from churns on the keeping quality of butter, noted that cheesiness was the most common defect in unsalted butter from churns that were considered clean. Cullity and Griffin (3) showed that churns and other equipment were an immediate source of organisms causing the putrid defect; however, the water supplies were considered to be the primary source of the organisms. Various investigators (3, 6, 11) have considered that the organisms causing the putrid defect come from contaminated wash water, and instances in which a change of water supply controlled an outbreak have been described.

Because churns and water supplies have been the sources of objectionable organisms in butter so frequently, an attempt was made to isolate *Ach. putrefaciens* from them.

**EXAMINATION OF CHURNS**

In making the examinations, different parts of a churn were cultured with sterile cotton swabs moistened in sterile water. Swabs were made on doors, rolls and ends of churns in 10 plants, none of which had experienced difficulty with spoilage in butter. The swabs were returned to the laboratory the same day in sterile test tubes. On arrival, each swab was placed in a tube of litmus milk and incubated at 10°C until a noticeable change occurred. The cultures were then inoculated into pasteurized cream, the cream churned and the butter held at 21°C and at 5°C.

In most cases the butter developed off odors in 2 to 4 days at 21°C. and in about 10 days at 5°C. While many of the odors were very disagreeable, none was typically putrid. Attempts to isolate *Ach. putrefaciens* by smearing the butter were unsuccessful, although one of the samples that was most suggestive of the putrid condition yielded an organism capable of reproducing the defect.

The results indicated that *Ach. putrefaciens* was not commonly
present in the churns studied, although the difficulty in isolating it could account for failure to obtain the organism.

EXAMINATION OF WATER SUPPLIES

Examinations were made of samples of water from three commercial plants which had experienced difficulty with the putrid defect. When smeared on agar, one sample yielded *Ach. putrefaciens*; the others yielded organisms capable of producing pronounced odors in butter, but these were not of the typical putrid type. In further examination of the samples, one portion of each was used for washing butter from pasteurized cream, and another portion (about 50 ml.) was inoculated into pasteurized cream (about 400 ml.) and the cream churned. With each procedure the butter was held at 21°C and at 10°C.

Each sample of water produced defective butter, either when used for washing butter or when inoculated into the cream. Generally, the defect developed more rapidly in the butter washed with the water. Only the lot of water which yielded *Ach. putrefaciens* in the smears caused the putrid defect. On smearing each lot of butter, only the putrid sample yielded *Ach. putrefaciens*.

The examinations indicated that water from supplies of commercial plants was capable of causing defects when used for washing butter. While only one of the three samples yielded *Ach. putrefaciens*, the data show that water may be a source of the organism.

EFFECTS OF VARIOUS FACTORS ON PRODUCTION OF THE PUTRID DEFECT IN EXPERIMENTAL BUTTER

BY *ACH. PUTREFACIENS*

Since *Ach. putrefaciens* appears to be a common cause of the putrid defect in commercial butter, further information regarding its action in butter was desired.

TIME REQUIRED FOR PRODUCTION OF THE DEFECT

When *Ach. putrefaciens* cultures were inoculated into pasteurized cream and the cream churned, the defect rapidly developed in the unsalted butter. Some variation occurred in the time required, but in most instances the defect developed in 1 day at 21°C, and in 7 days or less at 5°C. The putrid defect passed through different stages of development. At 21°C. the butter had a distinctly putrid odor in 1 or 2 days; the odor greatly decreased during the next 5 or 6 days and became somewhat suggestive of swiss cheese. At 5°C. the changes were of the same general nature but were slower. Trials were carried out in which amounts of a fresh litmus milk culture of *Ach. putrefaciens* varying from 0.01 to 1.0 ml. were added to 400 ml. portions of cream to be churned, and amounts of from 0.001 to 1.0 ml. were added to 400 ml. lots of water used for washing butter from 400 ml. portions of uninoculated cream. At 21°C. the putrid defect developed in all the samples except the
controls in 1 to 2 days. It appeared first in butter made from cream or washed with water having the heavier inoculations, but even with the smallest inoculations in either cream or wash water, sufficient organisms were retained in the butter to produce spoilage very rapidly under favorable growth conditions. The results emphasized the danger in using contaminated water for washing butter.

Because growth of _Ach. putrefaciens_ on media was so irregular, no attempt was made to determine the number of organisms required per gram of butter to produce the defect. Plates smeared with the freshly inoculated cream or wash water showed very few colonies, particularly with the lots having the smaller inoculations, and from these _Ach. putrefaciens_ was regained with difficulty. Undoubtedly more organisms were present than the method of examination indicated, but the trials suggested that the putrid defect might develop in butter made from cream or washed with water containing such small numbers of _Ach. putrefaciens_ that they would be very difficult to detect.

**EFFECT OF pH OF THE CREAM**

The putrid defect in butter has often been attributed to neutralization of the cream to a relatively high pH. Accordingly, pH values were determined on the serums of samples of commercial putrid butter; acid numbers on the fat were also determined on most of them, using the method of Breazeale and Bird (2).

The pH values ranged from 5.8 to 6.8 (table 1) and indicated that the defect is not necessarily confined to butter with a high pH value but develops over the same range as most bacterial defects. None of the pH values indicate over-neutralization. The acid numbers varied from 0.85 to 6.25 and do not appear to be related to the defect.

**TABLE 1. pH VALUES OF SERUM AND ACID NUMBERS ON FAT OF COMMERCIAL PUTRID BUTTER.**

<table>
<thead>
<tr>
<th>Sample no.</th>
<th>pH of serum</th>
<th>Acid number on fat*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6.8</td>
<td>1.35</td>
</tr>
<tr>
<td>2</td>
<td>6.2</td>
<td>1.18</td>
</tr>
<tr>
<td>3</td>
<td>6.4</td>
<td>1.60</td>
</tr>
<tr>
<td>4</td>
<td>6.1</td>
<td>3.45</td>
</tr>
<tr>
<td>5</td>
<td>6.1</td>
<td>6.25</td>
</tr>
<tr>
<td>6</td>
<td>6.4</td>
<td>4.45</td>
</tr>
<tr>
<td>7</td>
<td>6.6</td>
<td>1.40</td>
</tr>
<tr>
<td>8</td>
<td>6.6</td>
<td>1.70</td>
</tr>
<tr>
<td>9</td>
<td>5.8</td>
<td>.85</td>
</tr>
<tr>
<td>10</td>
<td>6.3</td>
<td>1.00</td>
</tr>
<tr>
<td>11</td>
<td>6.5</td>
<td></td>
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<tr>
<td>12</td>
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<td>4.35</td>
</tr>
<tr>
<td>14</td>
<td>6.0</td>
<td>2.60</td>
</tr>
<tr>
<td>15</td>
<td>6.4</td>
<td></td>
</tr>
</tbody>
</table>

* Expressed as ml. of 0.1N KOH per 10 grams fat.
<table>
<thead>
<tr>
<th>Trial no.</th>
<th>Sample no.</th>
<th>pH of cream</th>
<th>1 day at 21°C.</th>
<th>7 days at 5°C.</th>
</tr>
</thead>
<tbody>
<tr>
<td>control*</td>
<td>1</td>
<td>6.5</td>
<td>none</td>
<td>none</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>6.5</td>
<td>marked</td>
<td>moderate</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>6.6</td>
<td>marked</td>
<td>moderate</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>7.0</td>
<td>extreme</td>
<td>marked</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>7.2</td>
<td>extreme</td>
<td>marked</td>
</tr>
<tr>
<td>control</td>
<td>1</td>
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<td>none</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>5.2</td>
<td>very slight</td>
<td>very slight</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>6.0</td>
<td>slight</td>
<td>slight</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>6.7</td>
<td>extreme</td>
<td>marked</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>7.2</td>
<td>extreme</td>
<td>marked</td>
</tr>
<tr>
<td>control</td>
<td>1</td>
<td>4.5</td>
<td>none**</td>
<td>none**</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>6.3</td>
<td>marked</td>
<td>moderate</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>7.1</td>
<td>extreme</td>
<td>marked</td>
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<td></td>
<td>4</td>
<td>7.4</td>
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<td>marked</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>7.8</td>
<td>extreme</td>
<td>marked</td>
</tr>
</tbody>
</table>

*Control—cream not inoculated with *Ach. putrefaciens*.  
**Sample failed to show a defect after 7 days at 21°C., or 20 days at 5°C.

Since considerable variation was noted in the pH values of commercial putrid butter, experiments were carried out to determine the effect of pH of the cream on production of the defect by *Ach. putrefaciens*. In each trial, after removing about 400 ml. of cream to be churned as a control, a lot of pasteurized cream was inoculated with a litmus milk culture of *Ach. putrefaciens* and divided into five portions of about 400 ml. each. The portions were then adjusted to different pH values with sodium bicarbonate or lactic acid.

The putrid defect developed over a wide pH range (table 2), as determined on the cream from which the unsalted butter was churned. In trial 1, where the pH range was from 6.5 to 7.2, the defect was conspicuous in all the butter after 1 day at 21°C. or 7 days at 5°C. In trial 2, where the pH range was from 5.2 to 7.2, the three lots of butter representing pH values of 6.7 to 7.2 developed the defect to a marked degree in 1 day at 21°C. or 7 days at 5°C., whereas the two lots representing pH values of 5.2 and 6.0 were only slightly defective after 1 day at 21°C. or 7 days at 5°C. With trial 3 the pH range in the cream was from 4.5 to 7.8. The butter representing the four pH values from 6.3 to 7.8 was conspicuously putrid in 1 day at 21°C. or 7 days at 5°C. However, the sample from cream having a pH of 4.5 failed to show the defect after 7 days at 21°C. or 20 days at 5°C. The samples showing a slight defect after 1 day at 21°C. or 7 days at 5°C. (made from
cream with a relatively low pH) were usually as strongly putrid after long holding as those which previously had been more noticeable. Furthermore, as all samples became older they tended to assume a swiss cheese odor, the degree of which varied considerably but seemed to bear no definite correlation to the pH value.

The results indicated that *Ach. putrefaciens* was capable of producing the putrid defect in unsalted butter over a wide pH range.

**EFFECT OF SALT AND OF WORKING**

Salt is known to have an inhibitory effect on development of various bacterial defects in butter. Accordingly, the salt contents of samples of commercial putrid butter were determined.

A number of the samples (table 3) had rather low salt contents, several being only slightly more than 1 percent, but four of them had salt contents over 2 percent, which indicates that the presence of considerable salt did not necessarily prevent the development of the defect.

To investigate the effect of salt, and also of working, on development of the putrid defect by *Ach. putrefaciens*, three experimental trials were made. In each, cream was inoculated with a litmus milk culture of *Ach. putrefaciens* and then churned in a 1-gallon Dazey churn. The butter was divided into seven portions. Two percent salt was added to three portions, 1 percent salt was added to three other portions and one portion was unsalted. The three samples with the same salt content were worked to different degrees, designated as little, moderate and thorough. The unsalted sample was worked moderately. The butter was held at 21°C.

In each case (table 4) the unsalted butter, which was moderately worked, became putrid in 1 day. The production of the defect in the salted samples was dependent on the degree of working as well as on the amount of salt. Of the samples having 1 percent salt, those worked little became putrid in 1 or 2 days, those worked moderately were slightly or questionably defective at 2 days, while those worked thoroughly failed to develop the defect after 6 days.
TABLE 4. EFFECT OF SALT AND DEGREE OF WORKING ON DEVELOPMENT OF THE PUTRID DEFECT AT 21°C. IN BUTTER FROM CREAM INOCULATED WITH *Ach. putrefaciens*.

<table>
<thead>
<tr>
<th>Trial no.</th>
<th>Percent salt*</th>
<th>Degree of working</th>
<th>Days to develop</th>
<th>Defect</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>moderate</td>
<td>1</td>
<td>extreme</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>little moderate</td>
<td>2</td>
<td>extreme</td>
</tr>
<tr>
<td></td>
<td></td>
<td>thorough</td>
<td></td>
<td>none at 6 days</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>moderate</td>
<td>1</td>
<td>definite</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>little moderate</td>
<td>2</td>
<td>questionable</td>
</tr>
<tr>
<td></td>
<td></td>
<td>thorough</td>
<td></td>
<td>none at 6 days</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>little moderate</td>
<td>2</td>
<td>definite</td>
</tr>
<tr>
<td></td>
<td></td>
<td>thorough</td>
<td></td>
<td>slight</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>none at 6 days</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>moderate</td>
<td>1</td>
<td>slight</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>little moderate</td>
<td>2</td>
<td>slight</td>
</tr>
<tr>
<td></td>
<td></td>
<td>thorough</td>
<td></td>
<td>none at 6 days</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>little moderate</td>
<td>2</td>
<td>definite</td>
</tr>
<tr>
<td></td>
<td></td>
<td>thorough</td>
<td></td>
<td>none at 6 days</td>
</tr>
</tbody>
</table>

* The percent salt represents the amount added rather than the amount on analysis.

Of the samples having 2 percent salt, those worked little were putrid in 2 days; only one of the samples worked moderately became putrid and none of the thoroughly worked samples developed the defect in 6 days.

The results of the different trials were rather consistent and demonstrated that the amount of working the butter received (9) was an important factor in controlling the putrid defect. Salt, while tending to inhibit the defect, was not completely effective unless combined with thorough working.

EFFECT OF BUTTER CULTURE

Two trials were conducted in a study of the effect of butter culture on development of the putrid defect. Each sample of butter was churned from approximately 400 ml. of cream. The butter culture was added to the pasteurized cream the night before churning, and the cream was held at 10°C. Five hundred ml. of sterile water was used for washing each sample. The butter was unsalted and held at 21°C. In trial 1, *Ach. putrefaciens* (fresh litmus milk culture) was added to the cream immediately before the butter culture. The amount of *Ach. putrefaciens* culture added to each lot of cream was 0.5 ml., while the amount of butter culture
varied from 5 percent to 12 percent. In trial 2, *Ach. putrefaciens* was inoculated into the water used for washing the butter, the amounts used in the water for a sample varying from 0.01 to 1.0 ml. The amount of butter culture used was 10 percent.

In trial 1 (table 5), none of the butter made with butter culture developed the putrid defect in 6 days, while the sample without butter culture became markedly putrid. Five percent of the culture inhibited the defect as effectively as 12 percent. In trial 2, the butter made with butter culture did not show the defect in 6 days, while that made without butter culture developed a strong defect. The butter culture inhibited the defect as effectively when the wash water contained 1.0 ml. of *Ach. putrefaciens* culture as when it contained only 0.01 ml.

The results indicated that butter culture had an inhibiting effect on development of the putrid defect in unsalted butter when *Ach. putrefaciens* gained entrance to the butter either from the cream or from the wash water. The use of butter culture, as suggested by Derby and Hammer (4), appears to be an important measure in attempting to control the putrid defect. However, it is not effective under all conditions since a number of the commercial putrid samples were made from cream with added butter culture.

### Table 5. Effect of Adding Butter Culture to the Cream on Development of the Putrid Defect in Unsalted Butter When *Ach. putrefaciens* Was Inoculated into the Cream or Wash Water.

<table>
<thead>
<tr>
<th>Trial no.</th>
<th>Sample no.</th>
<th>Percent butter culture added</th>
<th>ML. <em>Ach. putrefaciens</em> culture added</th>
<th>Degree of defect after 6 days at 21°C.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0.5</td>
<td>marked</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>5</td>
<td>.5</td>
<td>none</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>8</td>
<td>.5</td>
<td>none</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>10</td>
<td>.5</td>
<td>none</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>12</td>
<td>.5</td>
<td>none</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>0</td>
<td>.0</td>
<td>none</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>0</td>
<td>.5</td>
<td>marked</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>10</td>
<td>.01</td>
<td>none</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>10</td>
<td>.1</td>
<td>none</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>10</td>
<td>.5</td>
<td>none</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>10</td>
<td>1.0</td>
<td>none</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>10</td>
<td>.0</td>
<td>none</td>
</tr>
</tbody>
</table>
EFFECT OF AGE OF EXPERIMENTAL PUTRID BUTTER ON NUMBERS OF *ACH. PUTREFACIENS* PRESENT

The difficulty in isolating *Ach. putrefaciens* from most samples of putrid butter and the failure to obtain it from others suggest that the organism dies rather rapidly after causing the defect. Accordingly, several experiments were conducted to study the effect of age of experimental putrid butter on the numbers present.

Portions of pasteurized cream were inoculated with varying amounts of litmus milk culture of *Ach. putrefaciens*, churned in the usual manner and the butter stored at 21°C. When the putrid defect had developed, and at subsequent intervals, samples were examined with the smear technic to obtain a general idea of the numbers of *Ach. putrefaciens* contained.

In unsalted butter there appeared to be a definite tendency for *Ach. putrefaciens* to die as the storage period increased. Except for a few irregularities, the numbers of colonies on the smears at the last examination were much smaller than at the first. The numbers of colonies obtained from butter 1 day old often were high, and the defect produced was very marked. In general, the numbers of colonies obtained at the end of the examination period were related to the numbers obtained at the first examination, the samples showing the smallest numbers at the final examination generally being those which showed the smallest numbers originally. However, several samples originally showing a large number of colonies failed to yield *Ach. putrefaciens* after 25 days.

A portion of one of the churnings was frozen immediately and held in this condition for 3 days. It was then placed at 21°C. and in 3 days had developed a slight but definite putrid odor. On smearing, *Ach. putrefaciens* colonies were not obtained from the butter. An examination after 8 days likewise failed to reveal colonies.

In examinations of samples of butter that were salted and worked to varying degrees, it again appeared that at 21°C. the organism died rather rapidly. When the defect was present to a slight degree, colonies could not be obtained by smearing after 10 or 20 days, although they had been obtained after 1 and 4 days. In distinction to the unsalted butter, the number of colonies tended to increase during the first few days and then decrease.

The relationship of the amount of salt, the extent of working the butter and the development of the defect to the numbers of colonies obtained on smears was considered. The degree of working not only affected development of the defect but also development of *Ach. putrefaciens*, since fewer colonies were obtained from thoroughly worked samples. In general, the samples containing 2 percent salt showed fewer colonies than the samples containing 1 percent salt. In both cases an increase in the working decreased the number of colonies obtained. With 2 percent salt and thorough
working, the samples did not develop the defect and did not show colonies on smeared plates. The samples made with 1 percent salt and thorough working, although not developing the defect, yielded small numbers of colonies in certain examinations.

The results indicated that, with butter containing Ach. putrefaciens, the organism became more difficult to isolate as the butter aged. The amount of inoculum that had been added to the cream undoubtedly constituted a much greater contamination than would be obtained under plant conditions. Occasionally, even with a large initial inoculation, and particularly with salt present, Ach. putrefaciens was not regained from the defective butter after 20 days; in one instance with a smaller inoculation, it was not regained after 3 days. Under these conditions difficulty would be expected in attempts to isolate the organism from commercial defective butter which has been held for some time, particularly at 21°C. or above.

**DISCUSSION**

*Ach. putrefaciens* appears to be an important cause of the putrid defect in butter. The typical organism or a variant form was isolated from 81.0 percent (70.7 percent and 10.3 percent, respectively) of the commercial putrid butter examined, and this was the only species isolated from any considerable number of samples that produced the putrid defect in experimental butter, including the successive changes in the defect that occur commercially. Its isolation from a number of defective samples from the same source, for example, from 12 of 15 typically putrid samples received at intervals from one plant, further suggests its relationship to the defect.

The inability of *Ach. putrefaciens* to initiate growth readily on media may account for the difficulty in isolating it from putrid butter and also the failure to obtain it from certain samples. Different strains vary in this respect, and the variant form is so fastidious in growth requirements that often it fails to develop when picked into litmus milk.

One of the principal objections to accepting *Ach. putrefaciens* as a cause of the putrid defect is that often it is obtained only in very small numbers from the abnormal butter. Undoubtedly the failure of the organism to grow readily is a factor in this situation, and the numbers of colonies on smeared plates do not accurately represent the numbers of organisms in the butter. The fact that *Ach. putrefaciens* produced a definite putrid odor in butter when added to the cream or wash water in such small numbers that recovery was very difficult or impossible is also significant in this connection. Furthermore, since *Ach. putrefaciens* in experimental putrid butter became increasingly difficult to regain as the butter aged and was more difficult to isolate from old commercial butter than from comparatively fresh butter, the age of the butter may be
an important factor in isolation of the organism.

Undoubtedly *Ach. putrefaciens* is not the only species that can break down the protein in butter and cause a serious defect. Different organisms are involved in protein decomposition in a variety of products, as well as in changes in other constituents. The isolation of *Ach. putrefaciens* from so many lots of commercial putrid butter, even when the growth conditions are only poorly understood, emphasizes its importance.

Various factors influence the growth of *Ach. putrefaciens* in butter. Relatively low pH values in the cream did not prevent the development of the defect by this organism in experimental butter, while the use of butter culture did; this suggests that the presence of butter culture organisms rather than acid was the controlling factor. Since some of the commercial putrid butter was made with culture, other factors apparently are also of significance. Both the salt content and degree of working of the butter definitely influenced growth of the organism, and it appears that the effects of these factors are closely interrelated. The presence of more than 2 percent salt in some of the commercial putrid butter is in agreement with the report of Hood and White (6), who noted that surface taint may occur in butter having a comparatively high salt content. Evidently a thorough distribution of the salt is necessary if growth of *Ach. putrefaciens* is to be controlled. With an irregular distribution the organism could grow sufficiently at the points having a relatively low salt content to produce the defect. Because of the inhibitory effect of salt, low temperatures, etc., *Ach. putrefaciens* may be present in butter which does not become putrid. In one instance a sample of normal fresh butter yielded the organism. This sample kept satisfactorily in keeping quality tests, but when it was inoculated into cream and the cream churned, the resulting unsalted butter developed the putrid defect at 21° or 5°C.

The isolation of *Ach. putrefaciens* from the water supply of a plant having difficulty with the putrid defect indicates one of the sources of the organism and is in agreement with the observations of various investigators. It is probable that the wash water which remains in butter is in relatively large droplets and that gives the organism a better chance for extensive development than it has in comparatively small droplets.
LITERATURE CITED


