then were inoculated with various dilutions of cheese emulsion and incubated at 21.0° C. When growth occurred, it usually was visible after 4 or 5 days. Pure cultures of organisms capable of growing in the Ayres medium were isolated by streaking material from tubes showing growth on plates of tomato juice agar (20) and picking colonies after 24 hours at 21.0° C.

Procedure Used in Manufacture of Cheddar Cheese

The milk used for making experimental cheese was considered to be of good quality. It contained about 3.5 per cent fat and had a desirable flavor and odor. When pasteurized milk was used for cheese making, it was pasteurized at 62.8° C. (145° F.) for 30 minutes.

Most of the cheese was made in a vat constructed in such a way that it contained five compartments (19). Each of the compartments was independent of the others and had a capacity of 50 lb. of milk. Thus, simultaneously five lots of cheese were made under essentially identical conditions. Additional lots of cheese were made in sterilized "shotgun" cans and in cheese vats each having a capacity of 300 lb. of milk.

Except for the size of the curd knives used, the cheese was made according to the method of Lane and Hammer (26). Each lot of milk was inoculated with 1.5 to 2.0 per cent commercial cheese culture containing 0.8 to 0.9 per cent acid, calculated as lactic. The milk was ripened about 20 minutes at approximately 26.7° C. (80° F.) after which the temperature was adjusted to 30.0° C. (86° F.). Commercial cheese color and rennet were added at the rate of 1 and 3 ounces per 1000 lb. of milk, respectively. After about 25 minutes the curd was cut into 1-inch cubes with a curd knife and then cooked slowly to 40.0° C. (104° F.) The curd was held at this temperature until the acidity of the whey reached 0.15 to 0.16 per cent and the desired firmness of the curd was obtained. The curd then was dipped and cheddared until 0.5 to 0.6 per cent acidity in the whey was reached. After milling, the curd was forked for about 30 minutes, and 3.0 per cent salt was added. Not less than 45 minutes were required to dissolve the salt, after which the curd was rinsed with scalding water and placed in the hoops. The cheese was pressed under continuous pressure for about 18 hours, put in a drying room at 7.2° C. (45° F.) for 48 hours, paraffined, and then placed in a curing room at 10.0° C. (50° F.).

EXPERIMENTAL

Bacteria in Cheddar Cheese with Reference to Their Lipolytic Activity and Ability to Utilize Sodium Butyrate

A possible explanation for the disappearance of the rancidity that develops in cheddar cheese made with certain procedures is that butyric and other lower fatty acids responsible for the con-

dition are destroyed by bacteria in the product. In order to determine the presence of such organisms, the microflora of a number of cheddar cheese samples was investigated. The lipolytic organisms were given special attention in this connection since it generally is believed that some organisms which hydrolyze fat also attack the products of fat hydrolysis. Fouts (11) found that various lipolytic organisms destroyed sodium butyrate in a synthetic medium in which the butyrate was the sole source of carbon.

The numbers of total bacteria and of lipolytic bacteria were determined on 27 samples of cheddar cheese from various sources. Most of the samples were considered to be good cheese. Of the samples examined, 16 were made in Iowa, 7 in Wisconsin, and 4 were samples of rancid cheese selected in Canada. The ages of the Iowa and Wisconsin samples varied from 2 weeks to 6 months; data on the ages of the Canadian samples were not available. Smeared plates were made in duplicate on meat infusion agar containing 0.5 per cent natural fat emulsion, using dilutions of 1 to 100, 1 to 1000, 1 to 10,000, 1 to 100,000, 1 to 1,000,000 and 1 to 10,000,000. The data are presented in Table 1.

Table I. Numbers of Total Bacteria and of Lipolytic Bacteria in Cheddar Cheese from Various Sources

Counts made on smeared plates of meat infusion agar containing 0.5 per cent natural fat emulsion and incubated 7 days at 21.0° C.

Sample No.	Source of cheese	Age of cheese	Quality of cheese	Bacteria per gram cheese	
				Total	Lipolytic
1	Wisconsin	3 mo.	good	132,000,000	400
2	"	3 "	"	84,000,000	300
3	"	3 "	"	180,000,000	400
4	Iowa	6 "	sl. rancid	480,000	600
5	"	6 "	good	140,000	100
6	"	6 "	fair	130,000	300
7	Wisconsin	3 "	poor	18,000,000	<100
8	Iowa	4 "	good	600,000	400
9	"	5 "	sl. rancid	80,000	100
10	Wisconsin	4 "	good	15,000,000	<100
11	"	4 "	good	35,000,000	<100
12	"	4 "	good	16,000,000	300
13	Iowa	2 wks.	"	320,000	<100
14	"	2 "	"	620,000	100
15	"	2 "	"	140,000	200
16	"	1 mo.	"	900,000	200
17	"	1 "	"	13,000,000	<100
18	"	1 "	"	560,000	100
19	Canada	unknown	rancid	40,000,000	300
20	"	"	"	30,000,000	200
21	"	"	"	12,000,000	100
22	"	"	"	700,000	200
23	Iowa	3 mo.	good	400,000	400
24	"	3 "	"	460,000	100
25	"	3 "	"	280,000	<100
26	"	3 "	"	390,000	<100
27	"	3 "	"	430,000	200

The total numbers of bacteria per gram of cheese, as determined by the smeared plate method, varied from 80,000 to 180,000,000. Most of the colonies appeared to be well developed and were easily counted when the counts were made after 7 days incubation at 21.0° C. Only plates having between 30 and 300 colonies were used. Generally, the counts on the Iowa cheese were considerably lower than those on the cheese from other sources. No information is available that would explain these lower counts. The numbers of lipolytic bacteria per gram of cheese, as determined by the smeared plate method, were always low and varied from less than 100 to 600. There seemed to be no relationship between the numbers of lipolytic organisms in the cheese and the presence of rancidity; the numbers of lipolytic organisms in the rancid samples were no larger than in some of the samples which were not rancid. There seemed to be no relationship between the total bacterial counts and the numbers of lipolytic organisms present.

In order to determine whether any of the 68 representative lipolytic and non-lipolytic organisms isolated from 27 cheese could utilize sodium butyrate as a sole source of carbon, a loop of litmus milk culture of each organism was transferred to a tube of the Ayres medium. No growth was visible in any of the tubes after incubating 14 days at 21.0° C., and it was assumed that the butyrate was not utilized. Similar inoculations were made into tubes of modified Ayres medium containing, in addition to the usual ingredients, 0.1 per cent glycine and also into tubes of the Ayres medium modified by the addition of 0.1 per cent peptone. No visible growth occurred during 7 days at 21.0° C. It appeared that none of the organisms isolated from cheddar cheese was capable of growing in the Ayres medium, even with the modifications used.

Organisms Obtained from the Ayres Medium Inoculated with Cheddar Cheese Emulsion

The failure of organisms isolated from cheddar cheese by the smeared plate method to utilize sodium butyrate suggested the inoculation of small quantities of cheese into the Ayres medium to determine whether uncultured species, or combinations, in the cheese might be capable of butyrate utilization.

Samples of cheese from a number of sources were emulsified in the usual manner. After grinding each sample, 0.1 ml. of the emulsion was inoculated directly into tubes of the Ayres medium. This quantity was the equivalent of 0.01 gram of cheese. Other tubes of the Ayres medium were inoculated with greater dilutions of cheese emulsion but the amount of material inoculated was always 0.1 ml. All tubes were incubated 4 or 5 days at 21.0° C. Growth regularly was observed when 0.01 gram of cheese had been added. Frequently, turbidity developed in the medium when 0.001 gram of cheese had been used for inoculation. After 5 days' incubation, a loopful of medium from each tube showing growth was transferred to tomato juice agar plates which were incubated 24 to 48 hours at 21.0° C.

The colonies developing on the tomato juice agar were predominantly of one type. They were smooth, slightly raised, glistening, and well developed. When observed with a hand lens, the colonies had a rather characteristic granular appearance. It seemed logical to conclude that the organisms were capable of utilizing butyrate as a source of carbon since other organisms ordinarily present in cheese did not seem to grow in the Ayres medium. Furthermore, a slight turbidity developed in the Ayres medium when it was inoculated with material from well-isolated colonies. Microscopically, the organisms were large rods (0.8 to 1.3 by 4.0 to 8.0 microns) that appeared to be in pure culture. The cells were variable to the gram stain. However, when stained with carbol fuchsin an unsual structure often was noted. Each cell suggested that two or more cocci had merged together, forming a large rod. Hereafter in this report, this type of large rod will be referred to as an X type organism.

The X type organism regularly was obtained when more than 200 samples of cheddar cheese from various sources were inoculated into the Ayres medium. Most of the cheese studied were produced in Iowa and Idaho, the balance being manufactured in Wisconsin, Illinois, Oregon, New York, Washington, Nebraska, and Canada; the sources of three of the samples were unknown. The age of the cheese was not known in all cases but most of the samples were 3 to 6 months old.

Frequently, plates of meat infusion agar containing natural fat emulsion were smeared with 0.1 ml. of an emulsion of a cheese sample at the time the Ayres medium was inoculated. This was done to observe the types of organisms present in the samples. Very few colonies similar to those produced by the X type organism developed on these plates.

All strains of the X type organism isolated from the Ayres medium grew well on tomato juice agar and yielded abundant growth in 24 to 48 hours at 21.0° C. Plates poured with this medium were smeared with six different strains of the organism and placed in the cheese curing room, which was held at 10.0° C., to determine the effect of relatively low temperatures on their development. Fair growth was observed on most of the plates after 7 days and it seemed evident that the organism can grow at temperatures used for ripening cheese.

The lipolytic activities of the X type organism were tested by making transfers to plates of meat infusion agar containing 0.5 per cent natural fat emulsion, and also to plates of nile-blue sulphate agar, the latter being prepared according to the method of Hammer and Collins (14); butter fat was used in both types of media. None of the 28 strains of the organisms studied showed lipolytic activities on the media.

Cultures of the X type organism in litmus milk produced gas and became acid, but did not coagulate when incubated at 21.0° C. Gas formation could be detected in young litmus milk cultures

but was not apparent in old cultures. The organism apparently is well suited to growth in milk because it was viable for several weeks in litmus milk cultures held at 21.0° C. Repeated transferring of a culture from litmus milk to litmus milk continued to yield the same cultural characteristics.

Further biochemical and cultural studies of the X type organism revealed that it produced a metallic sheen on Levine's eosin methylene blue agar, acid and gas in lactose broth, and an unexplainable transition to a gram-negative rod conforming to the description of *Escherichia coli,* as recorded in Bergey's Manual of Determinative Bacteriology (3).

Disappearance of Rancidity Produced in Cheddar Cheese by Addition of Butyric Acid to the Milk

As previously stated, rancidity in cheddar cheese that results from certain types of manufacturing procedures often disappears as the product ripens. Presumably, the rancid flavor and odor are due to an accumulation of butyric and other lower fatty acids which are set free by lipolytic enzymes acting on the milk fat. A study was made to determine whether rancidity produced in cheddar cheese by addition of butyric acid would disappear during the ripening of the cheese.

Eight series of cheese were made. Each series consisted of two lots of cheese, one being prepared from raw milk and one from pasteurized milk. Two to 5 ml. of butyric acid was added to each 50 lb. lot of milk just before the cheese culture was added. The milk was agitated vigorously while the butyric acid was being added to avoid coagulation of the milk at the point of contact with the acid and to insure even distribution of the acid. The treated milk was then made into cheese in the usual manner. A definite odor of butyric acid was noted in all lots of milk throughout the manufacturing process. During the first 14 days of the ripening, each cheese was examined several times to detect any disappearance of the rancid flavor and odor. Table II gives the results.

The first examination was made when the cheese was removed from the press (1 day), and a very rancid flavor and odor were noted in both the raw and pasteurized milk cheese. After the product had ripened 3 or 4 days there was some reduction in the rancid flavor and odor. In series 1, 5, 7, and 8 the rancidity had disappeared completely from the cheese made with pasteurized milk. After the experimental cheese had ripened 14 days, a slight flavor and odor of butyric acid could be detected in some of the raw milk cheese, but in two raw milk cheese and in all of the pasteurized milk cheese there was no evidence of rancidity. In general the rancidity disappeared from the pasteurized milk cheese before it did from the raw milk cheese.

At various times smeared plates were made with meat infusion agar containing natural fat emulsion to determine the numbers and

types of organisms developing in each cheese. All the plates were incubated 5 days at 21.0° C. The first bacterial counts made showed large numbers of typical *S. lactis* colonies. Usually, no other types of organisms were observed. However, counts on the cheese after ripening 3 to 4 days showed large numbers of colonies that appear-

Table II. Flavor of Cheddar Cheese at Various Ages When Made from Raw and Pasteurized Milk Containing Added Butyric Acid

Two to 5 ml. of butyric acid was added to the 50 lb. of milk used in making each lot of cheese.

Series No.	Milk used	Degree of rancidity in cheese after 1 day	3 or 4 days	14 days
1	raw	Very rancid	rancid	sl. rancid
	past.	" "	not rancid	not rancid
2	raw	" "	rancid	sl. rancid
	past.	" "	sl. rancid	not rancid
3	raw	" "	rancid	sl. rancid
	past.	" "	sl. rancid	not rancid
4	raw	" "	rancid	sl. rancid
	past.	" "	sl. rancid	not rancid
5	raw	" "	rancid	not rancid
	past.	" "	not rancid	not rancid
6	raw	" "	rancid	sl. rancid
	past.	" "	sl. rancid	not rancid
7	raw	" "	sl. rancid	not rancid
	past.	" "	not rancid	not rancid
8	raw	" "	rancid	sl. rancid
	past.	" "	not rancid	not rancid

ed to be of the X type; comparatively few *S. lactis* colonies could be detected. With a complete disappearance of rancidity in both raw and pasteurized milk cheese there was an almost complete disappearance of the X type organisms. Replacing these organisms was a more or less typical cheddar cheese flora. Similar results were obtained when eight lots of cheddar cheese were made in sterile "shotgun" cans and pure cultures of *S. lactis* were used as a culture.

Effect of Rancidity on the Bacterial Flora of Cheddar Cheese Made from Raw and from Pasteurized Milk

Bacterial flora of cheddar cheese made from raw and from pasteurized milk containing added butyric acid

In order to study in more detail the bacterial flora of cheddar cheese made from milk containing added butyric acid, six series of cheese were made. Each series consisted of two lots of cheese, one made from raw milk and one from pasteurized milk. From 3 to 5 ml. of butryic acid were added to each 50 lb. lot of milk used in the manufacture and resulted in a rancid flavor and odor in the fresh cheese.

Care was taken throughout the manufacture to eliminate as

completely as possible all sources of contamination. The vats were washed carefully and treated with boiling water and live steam just before the milk was received. Only milk of the best quality available was used. The raw milk employed in making the cheese usually had a bacterial count of less than 10,000 per ml., and the pasteurized milk frequently had a count of less than 500; the counts were made on tryptone glucose extract agar with the plates incubated 48 hours at 37.0° C. Butyric acid was added to each lot of milk with a sterile pipette just before addition of the cheese culture. The cheese were made in the usual manner.

At the time the cheese were removed from the press (1 day), bacterial counts were made on each lot, using smeared plates of meat infusion agar containing 0.5 per cent natural fat emulsion; the plates were incubated 5 days at 21.0° C. The counts showed that each cheese contained between 200 and 500 million organisms per gram. All the colonies observed in dilutions that could be counted were typical of those produced by *S. lactis*. Microscopically, the organisms in the colonies were gram-positive, short chain streptococci. When some of the colonies were transferred to litmus milk, a typical *S. lactis* reaction regularly resulted.

When the cheese were 2 days old, samples were again smeared on plates of the usual medium and the plates incubated 5 days at 21.0° C. The total numbers of colonies that developed were essentially the same as when the cheese were removed from the press, but the type of colonies had changed somewhat. In most cases the colonies were much larger than those observed when the first examination was made. Frequently, the colonies observed on plates were 2 to 3 mm. in diameter. The colonies also developed much more rapidly on the agar than those produced by typical *S. lactis*. Often good growth was observed after 48 hours at 21.0° C. Except for size, the colonies were similar to those produced by *S. lactis*. Microscopic examination of smears made from a number of the colonies showed gram-positive, short chain streptococci, but their appearance was not typical of *S. lactis*. Chains of two or more cells seemed to have merged together to form large rods. In some cases, single cocci seemed to have elongated until they appeared rod-shaped. A number of the colonies that were well isolated were transferred to litmus milk. After incubating 48 hours at 21.0° C., a typical *S. lactis* reaction was observed in most of the tubes, but in several cases gas was formed in the litmus milk.

The next set of samples was taken when the cheese were 4 days old. Smeared plates again were made on the usual medium and incubated 5 days at 21.0° C. Table III presents data showing the numbers of the X type organism developing on the plates after incubating 48 hours. No other types of colony developed even when the plates were incubated 5 days. In some cases the total counts were not as large as when the cheese were taken from the press, but the numbers were not materially reduced. Smears made from well-isolated colonies showed large rod-shaped organisms that appeared to be the X type organism. When a number of the col-

onies were transferred to tubes of litmus milk, a reaction similar to that produced by the X type organism was observed. All the lots of cheese were still somewhat rancid after 4 days, but those made from pasteurized milk were less rancid than those made

Table III. Numbers of the X Type Organism in Cheddar Cheese Made from Milk Containing Added Butyric Acid

Counts made on smeared plates of meat infusion agar containing 0.5 per cent natural fat emulsion and incubated 48 hours at 21.0° C.

Series No.	Butyric acid per 50 lb. of milk	Milk used	Numbers of the X type organism per gram of cheese after	
			4 days	14 days
1	3 ml.	raw	190,000,000	<100
	3 ml.	past.	290,000	4,200
2	3 ml.	raw	126,000,000	1,200
	3 ml.	past.	160,000	22,000
3	5 ml.	raw	400,000,000	1,400
	5 ml.	past.	230,000	18,000
4	3 ml.	raw	18,000,000	4,000
	3 ml.	past.	90,000	1,500
5	5 ml.	raw	500,000,000	2,100
	5 ml.	past.	180,000	13,000
6	4 ml.	raw	48,000,000	23,000
	4 ml.	past.	14,000,000	26,000

from raw milk. Larger numbers of the X type organism were present in the raw milk cheese than in the pasteurized milk cheese. With the 4-day-old cheese, the counts on the raw milk cheese varied from 18,000,000 to 500,000,000 per gram while the counts on the pasteurized milk cheese varied from 90,000 to 14,000,000 per gram.

The next series of plates was made when the cheese were 14 days old. Table III shows the numbers of the X type organism developing on smeared plates made with the usual medium and incubated 48 hours at 21.0° C. Comparatively few of the X type colonies had developed on any of the plates and no additional X type colonies were present after incubating 5 days. The X type colonies were replaced by a number of colonies that seemed to be more or less typical of the normal cheddar cheese flora. The total numbers of colonies developing were considerably less than the number observed when the cheese were first made. Very little rancidity could be detected in any of the samples; the flavor and odor of butyric acid had disappeared more rapidly from the pasteurized milk cheese than from the raw milk cheese. The numbers of the X type organism in the raw milk cheese and in the pasteurized milk cheese were essentially the same. In the raw milk cheese the counts varied from less than 100 to 23,000 per gram, while in the pasteurized milk cheese the counts varied from 1,500 to 26,000 per gram.

Young cheddar cheese made without butyric acid always con-

tained large numbers of *S. lactis*. Few organisms of the X type were ever observed on smeared plates made from young normal cheese. Since the colonies produced by the X type organism are similar to those produced by *S. lactis* on meat infusion agar containing natural fat emulsion, except for size, the X type organism may be a variant of *S. lactis*.

Bacterial flora of cheddar cheese made from homogenized milk

Homogenization of raw milk greatly increases the surface of the fat globules and gives the natural lipase of milk a better opportunity to hydrolyze the fat. Milk treated in this manner develops a rancid flavor and odor, presumably because of the freeing of some of the lower fatty acids, such as butyric and caproic. In order to study further the effect of rancidity on the bacterial flora, cheddar cheese were made from homogenized milk. Twelve lots of cheese were made in stainless steel vats. The milk used in the experiments was of good quality and was homogenized at a pressure of 1,000 lb. per square inch and a temperature of 43.3° C. (110° F.). Two hundred pounds of milk were used for each lot of cheese. Eight lots were made from homogenized raw milk and four lots from milk that had been pasteurized at 62.8° C. (145° F.) before homogenization.

Bacterial examinations were made of the lots of cheese when they were 2 days old. Smeared plates prepared in the usual manner showed that the cheese contained between 300 and 500 million organisms per gram. All the colonies appeared to be typical *S. lactis*. When well-isolated colonies were transferred to litmus milk a typical *S. lactis* reaction resulted after 24 hours at 21.0° C. Microscopic examination of smears made from colonies showed gram-positive, short chain streptococci, but the cells seemed somewhat elongated.

Table IV gives additional data on the cheese. After the cheese had ripened 7 days, the eight lots made from homogenized raw milk contained large numbers of the X type organism that were evident on the plates within 48 hours. The counts varied from 22 to 230 million per gram and the X type colonies seemed to be present in very nearly pure culture. The morphological and cultural characteristics of these organisms appeared to be identical with those observed previously. On the smeared plates the four lots of cheese made from homogenized pasteurized milk showed only typical *S. lactis* colonies. No X type colonies, or types other than *S. lactis*, were observed even when the plates were incubated 10 days at 21.0° C.

After 4 months all the lots of cheese showed comparatively few organisms on the smeared plates. However, from 6 to 24 thousand organisms of the X type per gram of cheese were still noted on plates prepared from some of the lots of cheese made from homogenized raw milk. No organisms of the X type were noted on plates

prepared from two lots of cheese made from homogenized pasteurized milk.

During the manufacturing process a rancid flavor and odor were detected in the cheese made from the homogenized raw milk. However, only a very slight rancid flavor and odor could be detected in the cheese made from the homogenized pasteurized milk. Presumably, the absence of a rancid flavor and odor in the cheese made from homogenized pasteurized milk was due to the destruction of the milk lipase by pasteurization.

Table IV. Numbers of the X Type Organism in Cheddar Cheese Made from Homogenized Raw Milk and Homogenized Pasteurized Milk.

Counts made on smeared plates of meat infusion agar containing 0.5 per cent natural fat emulsion and incubated 48 hours at 21.0° C.

Lot No.	Treatment of the raw milk	Numbers of the X type organism per gram of cheese after 7 days	4 months
1	homogenized	46,000,000	
2	homogenized	84,000,000	20,000
3	homogenized	22,000,000	
4	homogenized	230,000,000	6,000
5	homogenized	55,000,000	24,000
6	homogenized	88,000,000	
7	homogenized	66,000,000	
8	homogenized	121,000,000	14,000
9	pasteurized homogenized	<100	<100
10	pasteurized homogenized	<100	<100
11	pasteurized homogenized	<100	
12	pasteurized homogenized	<100	

Seven-day-old cheese made from homogenized raw milk had a definite rancid flavor and odor; that made from homogenized pasteurized milk, however, had no rancid flavor or odor. When the cheese had ripened 4 months, that made from the homogenized raw milk was still somewhat rancid and the cheese made from homogenized pasteurized milk had become bitter.

Effect of Adding the X Type Organism to Milk Used in Making Cheddar Cheese

Effect of the X type organism in cheddar cheese made from pasteurized milk containing added butyric acid

In view of the observations made, it seemed that the X type organism might be instrumental in bringing about the disappearance of rancidity in cheddar cheese. Accordingly, a study was made to determine whether the inoculation of a pure culture of the X type organism into milk, containing added butyric acid, that

was used in making cheddar cheese might be a means of eliminating rancidity.

Table V presents data on four series of cheese, each cheese being made from 50 lb. of pasteurized milk. In series 1, 2 and 3 the amounts of butyric acid used per 50 lb. of milk were 4 ml., 3 ml., and 4 ml., respectively; series 4 contained no butyric acid and served as a control. Each series included two lots of milk that were inoculated with 25 ml. of a milk culture of the X type organism isolated from cheddar cheese, and series 1, 2 and 3 also included one lot of milk not so inoculated. The X type organism and the cheese culture were added to the milk at the same time. The cheese were made in the usual manner.

Bacterial counts were made on the cheese using the usual medium and incubation conditions. The 1-day-old cheese made from milk inoculated with the X type organism contained from 2 to 9 million X type organisms per gram. When the cheese were 1 month old, the lots made with the X type organism and butyric acid added contained from 6,000 to 88,000 of these organisms per gram; the lots not inoculated with the X type organism showed from 5,000 to 33,000 per gram. Without butyric acid added to the milk, the counts of the X type organism were 14,000 and 30,000 per gram. After the cheese had ripened 2 months, the lots made with the X type organism and butyric acid added contained from 300 to 22,000 per gram, while those made without the X type organism contained from 3,000 to 6,000 per gram. The lots made without added butyric acid contained 3,000 and 16,000 per gram.

After 1 day all lots of cheese made with butyric acid added to the milk were very rancid, but at the end of 1 month the rancidity had disappeared. As would be expected, after ripening 2 months and 4 months, the cheese also were free of rancidity. After ripening 4 months the cheese had the flavor of well cured cheese. The cheese made without added butyric acid were not rancid at any age.

The observations show that after 1 month ripening all the lots of cheese were free of rancidity. There appeared to be little or no advantage in adding the X type organism to cheddar cheese made rancid by adding butyric acid to the milk used in the manufacture. The disappearance of rancidity in the cheese made from milk with butyric acid added but not inoculated with the X type organism may be explained by the bacterial counts which were obtained occasionally on some of the lots of cheese during the ripening process. Counts made after the lots of cheese had ripened 3 or 4 days frequently showed large numbers of the X type organism. The added butyric acid may have stimulated the development of the organism. With the disappearance of the rancidity in the cheese, the X type organism diminished in numbers.

Table V. Effect of the X Type Organism on Cheddar Cheese Made Rancid by Adding Butyric Acid to the Pasteurized Milk.

One-day-old cheese made from milk inoculated with the X type organism contained from 2 to 9 million of these organisms per gram.

Series No.	Butyric acid added per 50 lb. of milk	Strain of the X type organism added; 25 ml. milk culture per 50 lb. of milk	Numbers of the X type organism and degree of rancidity after			
			1 month		2 months	
			Organisms per gram	Rancidity	Organisms per gram	Rancidity
1	4 ml.	none	5,000	none	3,000	none
		M	8,000	"	5,000	"
		U	88,000	"	18,000	"
2	3 ml.	none	33,000	none	6,000	none
		L	40,000	"	22,000	"
		K	6,000	"	2,200	"
3	4 ml.	none	7,000	none	5,000	none
		J	12,000	"	18,000	"
		M	16,000	"	300	"
4	none (control)	L	30,000	none	16,000	none
		M	14,000	"	3,000	"

Effect of the X type organism in cheddar cheese made from homogenized raw milk

In order to study the effect of added X type organisms on cheddar cheese made rancid by homogenizing the raw milk, four series of cheese were made. The milk was homogenized at 2,000 lb. pressure and 32.2° C. (90° F.). In each series, four 50 lb. lots of milk were inoculated with 25 ml. of a milk culture of the X type organism isolated from cheddar cheese and one lot was not inoculated and served as a control. The X type organism and the cheese culture were added to the milk at the same time and the cheese were made in the normal manner.

Bacterial counts on the cheese were made in the usual way. The 1-day-old cheese made from milk inoculated with the X type organism contained from 2 to 11 million X type organisms per gram. When the cheese were 1 month old, those with the X type organism added contained from 4,000 to 4,000,000 of these organisms per gram; the cheese without the X type organism added contained from 5,700 to 20,000 per gram. After ripening 2 months, the cheese with the X type organism added contained from 200 to 214,000 of the organisms per gram, whereas the cheese without the X type organism added contained from 3,000 to 5,600 per gram.

After 1 day all the lots of cheese were rancid and at the end of 1 month they were still rancid. After ripening 2 months all the lots of cheese were slightly rancid, and after ripening 4 months they were very slightly rancid.

The observations indicate that rancidity in cheddar cheese due to the homogenization of the milk disappeared slowly. There seemed to be little or no advantage in adding the X type organism. Presumably, the X type organism developed in the cheese to which it was not added in much the same manner that it did in cheese made from milk containing added butyric acid. With the disappearance of the rancidity, the organism again diminished in numbers.

Effect of the X type organism in cheddar cheese made from pasteurized milk containing added pancreatin

Lane and Hammer (30) observed that a rancid condition in cheddar cheese could be produced by adding pancreatin to the milk used in the manufacture. A study was made to determine whether rancidity in cheddar cheese, produced by adding pancreatin to the milk, would disappear during ripening with the addition of the X type organism to the milk.

Four series of cheese were made from 50 lb. lots of pasteurized milk. The amounts of pancreatin used per 50 lb. of milk in series 1, 2, 3, and 4 were 1.0 gram, 0.1 gram, 0.5 gram, and 0.5 gram, respectively. In each series, four out of the five 50 lb. lots of milk were inoculated with 25 ml. of a milk culture of the X type organism isolated from cheddar cheese; one lot of milk was not inocu-

lated and served as a control. The X type organism and the cheese culture were added to the milk at the same time. The cheese were made in the usual way.

Bacterial counts on the cheese were obtained in the customary manner. The 1-day-old cheese made from milk inoculated with the X type organism contained from 2 to 7 million X type organisms per gram. When the cheese were 1 month old, the lots with the X type organism added contained from less than 100 to 7,700 of these organisms per gram and the lots without the X type organism added contained from less than 100 to 1,100 per gram. After the cheese had ripened 2 months, the lots with the X type organism added showed from less than 100 to 22,000 X type organisms per gram; the lots without the X type organism added showed from less than 100 to 900 per gram.

After 1 day all the lots of cheese were very rancid. At the end of 1 month, 2 months, and 4 months, all the lots were too rancid to judge.

The observations indicate that rancidity in cheddar cheese due to the addition of pancreatin to the milk does not disappear during the ripening. There seemed to be no advantage in adding the X type organism. Apparently, the pancreatin produced rancidity so actively that the X type organisms were unable to cause its disappearance; toxicity of the acids set free may have been a factor in this. Total counts showed that comparatively few organisms of any type existed in the cheese after ripening 1, 2 and 4 months; those that did survive were yellow micrococci and spore-forming rods.

Ability of the X Type Organism to Utilize Sodium Butyrate

Ability of the X type organism to utilize sodium butyrate in the Ayres medium and in enriched Ayres medium

In an attempt to determine whether the X type organisms isolated from cheddar cheese were capable of utilizing sodium butyrate, 50 ml. portions of the Ayres medium were placed in bottles with screw caps and sterilized. Two bottles of the medium were inoculated with each of the organisms studied and incubated at 21.0° C. After 14 and 30 days, the contents of a bottle inoculated with each organism were placed in a Kjeldahl flask. The bottle was rinsed with 15 ml. of boiled distilled water and this was added to the flask. Two ml. of 5 N sulfuric acid was added to each flask to free any remaining fatty acid from the salt. The flask was placed on a distilling apparatus and heated until 50 ml. of distillate was obtained. The distillate was titrated with N/10 sodium hydroxide, using phenolphthalein as an indicator. Fifty ml. bottles of medium that had not been inoculated were treated in the same manner as the inoculated bottles and served as controls. It was assumed that a decrease in yield of volatile acid would be due to utilization of the butyrate by the organism that had been growing in the medium.

The X type organisms obtained from the Ayres medium inoculated with cheddar cheese emulsions did not grow well when transferred to flasks of the medium and the distillation gave no evidence of butyrate utilization.

In order to obtain better growth of the X type organisms, 50 ml. bottles of the Ayres medium were enriched by adding 0.1 per cent lactose, 0.1 per cent lactose and 0.1 per cent peptone, 0.1 per cent peptone, 0.2 per cent peptone, 0.5 per cent peptone, and 1.0 per cent cheddar cheese emulsion; unmodified medium served as a control. Five strains of the X type organism, designated as J, K, L, M, and N, were tested in each medium and uninoculated bottles of the media were included as controls. Little growth was observed in the unmodified Ayres medium until after 4 or 5 days; even then the growth was not abundant. However, turbidity developed in 2 days in all of the inoculated bottles containing lactose and/or peptone.

After incubating 14 days at 21.0° C., the contents of each bottle were acidified and distilled and the volatile acidity was determined. Table VI shows that no appreciable amounts of butyrate were utilized by the organisms in any of the trials. Apparently, the turbidity that developed in the enriched medium was the result of lactose and/or peptone utilization rather than butyrate utilization. The natural turbidity of the cheese emulsion made it difficult to observe any growth of the organisms in the bottles containing it, but the titrations indicate that very little or none of the butyrate was utilized. All inoculated bottles of the unmodified Ayres medium contained living organisms after 14 days since transfers from the bottles to tomato juice agar plates yielded good growth. This indicates that the organisms are able to live in the medium.

Since none of the organisms seemed to utilize sodium butyrate with the procedure used in the first trials, additional tests were made. A number of strains of the X type organism isolated from cheddar cheese were inoculated into bottles of the Ayres medium containing 0.125 per cent sodium butyrate as well as into bottles with the usual 0.5 per cent. This was done because preliminary work had shown that smaller amounts of butyrate were very satisfactory for the isolation of the X type organism from cheddar cheese. It was believed that smaller amounts of butyrate might be less toxic to the organism. Inoculations also were made into bottles of the Ayres medium which had been acidified with lactic acid until the pH was 5.3, a reaction somewhat similar to that of cheddar cheese. Strains of the organism likewise were inoculated into the Ayres medium containing 0.5 per cent sodium butyrate and 1.0 per cent cheddar cheese emulsion, and into the Ayres medium containing 0.125 per cent sodium butyrate and 1.0 per cent cheddar cheese emulsion. All the bottles were incubated 30 days at 21.0° C. and then distilled in the usual manner. Uninoculated bottles of the media were used as controls.

Table VI. Action of the X Type Organism on Sodium Butyrate in the Ayres Medium and in Enriched Ayres Media

The values represent the ml. of N/10 sodium hydroxide required to neutralize the acid in 50 ml. of distillate obtained from 50 ml. of acidified medium

Incubation 14 days at 21.0° C.

MEDIUM	Strain of the X type organism inoculated					Uninoculated
	J	K	L	M	N	
Ayres	21.2	20.3	19.6	20.7	20.8	20.1
Ayres + 0.1% lactose	21.3	20.0	21.3	20.8	21.1	21.3
Ayres + 0.1% lactose and 0.1% peptone	20.8	21.4	18.2	21.1	20.4	20.5
Ayres +0.1% peptone	21.0	21.5	18.4	20.4	21.1	20.5
Ayres + 0.2% peptone	20.5	21.3	20.8	20.5	20.4	20.5
Ayres +0.5% peptone	20.3	20.8	20.7	20.5	20.2	20.7
Ayres + 1.0% cheddar cheese emulsion	20.5	21.3	21.3	21.2	20.7	20.9

Little, if any, of the sodium butyrate was utilized by any of the strains tested. However, a slight turbidity developed in all the inoculated bottles of the Ayres medium with the exception of those which had been acidified with lactic acid. Presumably, the acidified medium was so toxic that the X type organism was unable to grow. Since sodium butyrate was the sole source of carbon in the bottles showing visible growth, the organism presumably utilized the butyrate to a slight extent. All bottles containing 1.0 per cent cheese emulsion were so turbid that the growth of organisms would not be evident.

Ability of 48-hour yeast extract broth cultures of the X type organism to utilize added sodium butyrate

The apparent inability of the X type organism to utilize sodium butyrate in the Ayres medium or in enriched Ayres medium does not eliminate the possibility of its destroying butyric acid in cheddar cheese. Various conditions and nutrients are present in cheddar cheese that are absent in the Ayres medium. It would seem that if large numbers of the X type organism could be grown in a suitable medium until most of the nutrients had been utilized, it might use added sodium butyrate as a source of carbon. In an effort to find more favorable conditions for butyrate utilization, yeast extract broth cultures were employed.

Large numbers of the X type organism were obtained by inoculating it into 2-liter flasks containing 1000 ml. of 0.1 per cent or 0.5 per cent yeast extract broth. After incubating 48 hours at 21.0° C., marked turbidity was observed in all the inoculated flasks.

Then, 10 ml. of sterile 10.0 per cent sodium butyrate solution was added aseptically to each of two 2-liter flasks containing 1000 ml. of 0.1 per cent yeast extract broth culture and also to an uninoculated 1000 ml. quantity of 0.1 per cent yeast extract broth which served as a control. In the case of the 2-liter flask containing 1000 ml. of 0.5 per cent yeast extract broth culture, 20 ml. of sterile 10.0 per cent sodium butyrate solution was added, and 20 ml. of the butyrate solution also was added to an uninoculated 1000 ml. quantity of 0.5 per cent yeast extract broth. All the flasks were incubated at 21.0° C. After 3, 7, 14, and 21 days, 100 ml. quantities of material were removed aseptically from the flasks, acidified, and distilled. When 100 ml. quantities of distillate had accumulated, they were titrated.

The data given in Table VII show that after 3 and 7 days at 21.0° C. very little of the added sodium butyrate had been utilized by the X type organism. However, after 14 days considerable of the added sodium butyrate apparently had been utilized. The largest butyrate utilization occurred in the 0.1 per cent yeast extract broth cultures containing approximately 0.1 per cent sodium butyrate, but a relatively large amount of butyrate also was utilized in the case of the 0.5 per cent yeast extract broth culture containing approximately 0.2 per cent added sodium butyrate. At the end of 21 days the titration values for the 0.1 per cent yeast extract broth cultures were about the same as after 14 days. Perhaps the butyrate was completely utilized after 14 days and the small titration values were caused by some compound other than liberated butyric acid. With the 0.5 per cent yeast extract broth culture, more butyrate utilization was evident after 21 days than after 14 days.

The ability of the X type organism to utilize sodium butyrate, when it was added to dilute yeast extract broth cultures, suggested that this organism may be capable of bringing about the disappearance of rancidity in cheddar cheese. It appears that several environmental factors influence the ability of the X type organism to destroy sodium butyrate. The age and number of organisms present, the absence of readily available food supply, and the presence of metabolic products of organisms may be of importance. It seems that somewhat similar conditions in cheddar cheese favor the utilization of butyric acid by the X type organism.

Relationship of the X Type Organism to *S. lactis*

Comparative numbers of S. lactis and the X type organism in cheddar cheese made from raw milk containing added butyric acid

From the results of various investigators it is evident that plates poured in studying young cheddar cheese manufactured in the usual way commonly show large numbers of *S. lactis* colonies. Often the *S. lactis* colonies occur on the plates in nearly pure culture. The numbers of these colonies vary somewhat with different lots of young cheese, but frequently the numbers developing on meat

Table VII. Action of the X Type Organism on Sodium Butyrate in 48-hour Yeast Extract Broth Cultures

The values represent the ml. of N/10 sodium hydroxide required to neutralize the acid in 100 ml. of distillate obtained from 100 ml. of acidified medium

Medium	Inoculated with the X type organism				Uninoculated			
	After incubating at 21.0° C. for				After incubating at 21.0° C. for			
	3 days	7 days	14 days	21 days	3 days	7 days	14 days	21 days
1,000 ml. of 0.1% yeast extract broth + 10 ml. sterile 10% sodium butyrate after 48 hours	7.8	7.3	1.0	1.1	7.8	7.8	7.7	7.7
1,000 ml. of 0.1 % yeast extract broth + 10 ml. sterile 10% sodium butyrate after 48 hours	7.7	7.4	1.7	0.9				
1,000 ml. of 0.5% yeast extract broth + 20 ml. sterile 10% sodium butyrate after 48 hours	15.0	12.0	6.5	4.0	14.8	14.6	14.7	14.4

infusion agar containing 0.5 per cent natural fat emulsion total 500,000,000, or more, per gram of cheese. Although the numbers diminish somewhat during ripening, *S. lactis* colonies continue to be predominant for rather extended periods.

Table VIII presents data on ten cheddar cheese made from 50 lb. lots of milk containing from 2 to 5 ml. of added butyric acid. When the various lots of rancid cheese were 1 day old, smeared plates were made on meat infusion agar containing natural fat emulsion, and after the plates had been held 5 days at 21.0° C. the numbers and types of colonies were determined. Each cheese had a bacterial count of 200 to 500 million typical *S. lactis* colonies

Table VIII. Comparative Numbers of *S. lactis* and the X Type Organism,* After 1 Day and 3 or 4 Days, in Cheddar Cheese Made from Raw Milk Containing Added Butyric Acid

Counts made on smeared plates of meat infusion agar containing 0.5 per cent natural fat emulsion and incubated at 21.0° C. for 5 days.

Bacteria per gram of Cheese (dilution 1 to MM)

Cheese No.	Butyric acid per 50 lb. of raw milk	After ripening 1 day		After ripening 3 or 4 days	
		S. lactis (estimated)	Other organisms	*S. lactis*	The X type organisms
1	3 ml.	200,000,000 to 500,000,000	none	none	190,000,000
2	3 ml.	200,000,000 to 500,000,000	none	none	126,000,000
3	5 ml.	200,000,000 to 500,000,000	none	none	400,000,000
4	3 ml.	200,000,000 to 500,000,000	none	none	18,000,000
5	5 ml.	200,000,000 to 500,000,000	none	none	500,000,000
6	4 ml.	200,000,000 to 500,000,000	none	none	48,000,000
7	2 ml.	200,000,000 to 500,000,000	none	none	52,000,000
8	3 ml.	200,000,000 to 500,000,000	none	none	106,000,000
9	5 ml.	200,000,000 to 500,000,000	none	none	321,000,000
10	3 ml.	200,000,000 to 500,000,000	none	none	148,000,000

*Some of these counts have been used in a previous table.

per gram. In the dilutions used, all the colonies developing appeared to be *S. lactis*. After the same cheese had ripened 3 or 4 days, smeared plates were made again, and the numbers and types of colonies noted. A complete change in the bacterial flora was evident. The typical *S. lactis* colonies could no longer be observed, and replacing them were the X type colonies. Counts of these colonies for the several lots of cheese varied from 18 to 500 million per gram.

Several cheese made from milk to which butyric acid had been added were examined daily for some days to observe, in more detail, the changes in the bacterial flora. When the cheese had ripened 1 day, the colonies present on smeared plates of meat infusion agar, containing natural fat emulsion, after 5 days at 21.0° C. appeared to be typical *S. lactis* colonies. After ripening 2 days, the cheese yielded two types of colonies; one type suggested *S. lactis* and the other suggested the X type organism. Microscopic examinations of the organisms in each type of colony showed coccus forms that seemed to be elongating and in some cases merging together. This condition was most commonly observed in the colonies suggestive of the X type. After the cheese had ripened 3 or 4 days, all the colonies that developed were of the X type, no typical *S. lactis* colonies being discernible.

As mentioned previously, small amounts of cheddar cheese emulsion inoculated into tubes of the Ayres medium always yielded the X type organism. Frequently, the quantity of cheese inoculated into the Ayres medium was as little as 0.001 gram. In such a quantity of cheese the only organism that ordinarily would be present in appreciable numbers is *S. lactis,* which also indicates that the X type organism developing from the cheese might be a variant of *S. lactis*. This general idea was strengthened when a number of normal cheese samples failed to show the X type colonies on smeared plates, while the same cheese inoculated into the Ayres medium yielded the X type organism.

Attempts to produce the X type organism from S. lactis

Since small quantities of normal cheddar cheese inoculated into the Ayres medium always yielded the X type organism and since the X type organism was observed in large numbers in young cheddar cheese (3 or 4 days old) made from raw milk containing added butyric acid, it seemed probable that the organism might be obtained by inoculating *S. lactis* into a medium containing butyric acid or sodium butyrate.

Nine strains of *S. lactis* were obtained by plating nine different butter cultures on tomato juice agar, incubating the plates 5 days at 21.0° C. and inoculating well-isolated colonies of the *S. lactis* type into tubes of litmus milk. After these tubes had been incubated 48 hours at 21.0° C., reactions typical of those produced by *S. lactis* were observed. The cultures again were plated on tomato juice agar, and well-isolated colonies transferred to tubes of litmus

milk. In some instances the plating procedure was repeated five times. Before using the cultures they were transferred several times in litmus milk so that they would be active; they finally were used after 48 hours in litmus milk. Microscopic examinations indicated that each culture was pure.

One-tenth ml. of each of the nine 48-hour litmus milk cultures of *S. lactis* isolated from butter cultures was inoculated into tubes of the Ayres medium containing 0.1 per cent peptone. Three stock cultures of *S. lactis* grown in litmus milk 48 hours also were inoculated into the medium. After incubating 7 days at 21.0° C., a slight turbidity was observed in several of the tubes. Transfers from some of the tubes to tomato juice agar yielded a number of typical X type colonies. Not all cultures of *S. lactis,* however, showed the X type organism at the same time. Sometimes transferring 0.1 ml. of the culture in Ayres medium containing 0.1 per cent peptone to fresh tubes of the same medium aided in the development of the X type organism. Certain strains, however, were so stable that a number of transfers were necessary before the X type organism could be demonstrated. Better growth always seemed to take place in the Ayres medium containing 0.1 per cent peptone than in the Ayres medium alone.

Additional attempts were made to produce the X type organism by inoculating *S. lactis* into a number of different culture media containing varying amounts of butyric acid or sodium butyrate. Media studied included plain broth, lactose broth, yeast extract broth, heart infusion, peptonized milk, and litmus milk. In certain cases 2.5 per cent sodium chloride was added to the media to produce concentrations somewhat comparable to those found in cheddar cheese. The amount of inoculation used with each medium usually was 0.1 ml. of a litmus milk culture of *S. lactis.* In a number of cases, however, tubes of the medium also were inoculated with a loop of the litmus milk culture. All the tubes were incubated several days at 21.0° C., and were examined daily to note any change in the morphology of *S. lactis.* None of the media was as satisfactory as the Ayres medium from the standpoint of bringing about a variation in *S. lactis.*

Different investigators have reported that variations in organisms can be brought about by adding such compounds as lithium chloride or phenol to culture media used for growing the organisms. Various attempts were made to stimulate the formation of rod-shaped organisms from *S. lactis* by adding small quantities of these compounds to culture media containing butyric acid or sodium butyrate. The addition of the compounds, however, did not seem to be of value in bringing about a change in the morphology of *S. lactis.* From the work reported, it appears that the formation of the X type organism from *S. lactis* is dependent on the proper combination of environmental factors which, as yet, has not been fully evaluated.

Observations of several investigators on bacterial variation

As already noted, the X type organism seems to be a variant of *S. lactis*. Bacterial life cycles and mechanisms of variability have been extensively studied and Hadley (12), Braun (4), and other investigators have reviewed the literature on microbic dissociation in detail. Certain references seem to be of special importance from the standpoint of the results obtained with *S. lactis*.

Löhnis and Smith (27) studied the life cycle of a number of bacteria. They believed that each life cycle is composed of several sub-cycles showing wide morphological and physiological differences. Hadley (12) considered that microorganisms are capable of responding to a changing environment by alterations in body state, both morphological and biochemical, in order to generate another and more stable type.

Evans (9) reported that "a *Streptococcus*, a filterable form, and an aerobic spore-bearing rod are phases in the life cycle of an organism cultivated from cases of epidemic encephalitis and from the so-called herpetic and encephalitic viruses." The metamorphosis was observed many times. Tunnicliff (37) observed bacillary forms in cultures from convalescing scarlet fever patients. These forms reverted to typical streptococci in sub-cultures.

The observations cited suggest that bacterial variation is rather common, and should be expected under certain conditions. It seems likely that the X type organism develops from *S. lactis* in cheddar cheese made rancid by various manufacturing procedures because of the unfavorable environment. With the disappearance of the butyric and other lower fatty acids in the cheese, *S. lactis* presumably returns to its original form.

DISCUSSION

The data presented confirm the idea that rancidity may be produced in cheddar cheese by certain procedures. The addition of butyric acid to the pasteurized milk or homogenization of the raw milk, used in the manufacture of the cheese, regularly resulted in a rancid product. As the cheese ripened, the odor and flavor of rancidity disappeared and usually a desirable product resulted. The large numbers of bacteria in cheese suggest that the utilization of butyric and other lower fatty acids by organisms may account for the disappearance of the rancidity.

It seemed reasonable to assume that lipolytic organisms might be instrumental in bringing about the disappearance of rancidity since it generally is believed that organisms which hydrolyze fat also attack one or more of the products of fat hydrolysis. However, only small numbers of lipolytic organisms were found in cheese, and from the results obtained it appears that they are not related to the disappearance of rancidity.

The data presented show that cheese, made rancid by certain procedures, regularly yielded large numbers of rod-shaped organ-

isms during the early days of ripening. Frequently, these organisms, which were designated the X type, were present in practically pure culture. Presumably, they were related to the disappearance of rancidity since they were not observed in non-rancid cheese. This idea was strengthened when the X type organisms were recovered from Ayres medium inoculated with small amounts of normal cheese emulsified in sodium citrate solution. The ability of the X type organism to grow in a medium in which sodium butyrate was the sole source of carbon suggested that this organism probably was capable of destroying butyric acid in cheese.

Further evidence of the relationship of the X type organism to the disappearance of rancidity in cheddar cheese was obtained when periodic bacterial counts were made on young rancid cheese. One-day-old cheese, made rancid by certain procedures, always contained from 200 to 500 million *S. lactis* per gram, but as the cheese ripened the X type organism replaced *S. lactis*. With the disappearance of rancidity, the X type organisms diminished in numbers and were replaced by a more or less typical cheddar cheese flora. On the other hand, cheddar cheese made from pasteurized milk that was homogenized failed to become rancid, and the X type organism was not obtained from it. Similar observations were made when pure cultures of *S. lactis* were used to make the cheese, and it appears that the X type organisms are variants of *S. lactis*. The fact that the X type organism has the cultural and biochemical characteristics of *E. coli* is unexplainable at this time. It seems, however, that the transition of *S. lactis* to the X type organism is due to the environment produced in cheese by butyric and perhaps other fatty acids present. This hypothesis is in conformity with Hadley's (12) statements.

Since the X type organism appeared to be associated with the disappearance of rancidity in cheddar cheese, it seemed that pure cultures of the organisms might be a means of eliminating rancidity in cheese. However, inoculation of the X type organisms into cheese made rancid by certain procedures, did not accelerate the rate at which the rancidity disappeared. Presumably, the organisms normally present in cheese are capable of modifying their characteristics so that they can destroy small amounts of butyric, and perhaps other fatty acids. Under certain environmental conditions, *S. lactis* seems to be capable of changing its morphological and cultural characteristics in order to better utilize some of the products of fat hydrolysis.

The data presented show that the X type organism did not grow well when inoculated into Ayres medium. However, butyrate utilization was demonstrated by distillations and titrations of the distillates when sterile sodium butyrate solution was added to actively growing dilute yeast extract broth cultures of the X type organism. It appears that several environmental factors influence the ability of this organism to destroy sodium butyrate. The age and number of organisms present, the absence of a readily available food supply, and the presence of metabolic products of organ-

isms may be of importance. Apparently environmental conditions somewhat similar to those present in rancid cheddar cheese are favorable for butyrate utilization.

Attempts to produce the X type organisms from pure cultures of *S. lactis* in the laboratory were not uniformly successful, even though a number of media containing varying amounts of sodium butyrate and butyric acid were used. Best results were obtained when tubes of Ayres medium, containing 0.1 per cent peptone, were inoculated with 0.1 ml. of a vigorous 48-hour litmus milk culture of *S. lactis*. Frequently, it was necessary to incubate the enriched Ayres medium culture for 1 week or more at 21.0° C. before the X type organism developed under these conditions. However, the X type organism was obtained from several cultures of *S. lactis* by inoculating into the butyrate medium. The lack of uniform results may be ascribed to the inability to produce the conditions existing in cheese.

It seems that certain conditions which are factors in the formation of the X type organism are present in cheese. Such conditions may not be present in culture media, or, if originally present, may be altered by sterilization. Furthermore, the *S. lactis* in cheese may be conditioned by its environment. Very little carbohydrate is available as a source of carbon and energy. Other food materials may be in such forms that the organisms cannot utilize them readily. These, and probably other factors, may be of importance in the development of the X type organism.

The data presented indicate that the disappearance of rancidity produced in cheddar cheese in various ways is due to the activity of microorganisms. In young cheddar cheese large numbers of *S. lactis* are normally present, but the readily available food supply is limited. Under certain conditions these organisms may utilize butyric and perhaps other lower fatty acids as a source of carbon. In order to utilize these acids under the environmental conditions present in cheddar cheese, *S. lactis* may be forced to change its morphological and biochemical properties to those of the X type organism. With the disappearance of rancidity in cheddar cheese, the X type organism decreases in numbers and is replaced with a more typical cheddar cheese flora. Presumably, the same general mechanism is operative in cheese made in the usual way, and may explain why raw milk cheese commonly does not develop rancidity even when the acidity of the fat increases considerably.

SUMMARY AND CONCLUSIONS

The work reported involved a study of the relationship of microorganisms to the disappearance of rancidity produced in cheddar cheese with various procedures. The following observations were made:

1. There was no relationship between the total bacterial counts and the numbers of lipolytic organisms in the cheddar cheese

studied, the numbers of lipolytic organisms regularly being very low.

2. When a loop of litmus milk culture of various organisms isolated from cheddar cheese was inoculated into the Ayres medium, no growth was visible after 14 days at 21.0° C.

3. The Ayres medium, inoculated with emulsified cheddar cheese and incubated 4 or 5 days at 21.0° C., regularly yielded a large rod-shaped organism which was designated the X type organism. The organism had characteristics conforming to the accepted description of *Es. coli.*

4. The X type organism developed regularly after 3 or 4 days in cheddar cheese made rancid by adding butyric acid to the milk used in the manufacture. As the cheese ripened the rancidity disappeared, and the X type organism diminished in numbers. The disappearance of rancidity was more rapid in pasteurized milk cheese than in raw milk cheese.

5. Periodic bacterial examinations of young cheddar cheese made rancid by adding butyric acid to the raw milk used in the manufacture showed the X type organism often replaced *S. lactis* when the cheese had ripened 4 days. Microscopic studies of the colonies developing from 2-day-old cheese frequently suggested that two or more cells of *S. lactis* had merged to form large rods. When the cheese were 14 days old, bacterial examination showed that the X type organism had been replaced by organisms more or less typical of the normal cheddar cheese flora.

6. The X type organism developed regularly after 7 days in cheddar cheese made rancid by homogenizing the raw milk used in the manufacture. As the cheese ripened the rancidity slowly disappeared and the X type organism diminished in numbers. Cheddar cheese made from pasteurized milk that was homogenized failed to become rancid, and the X type organism was not obtained from it.

7. Frequently, the X type organism completely replaced *S. lactis* in rancid cheese made from raw milk containing added butyric acid and in rancid cheese made from homogenized raw milk.

8. The X type organism appears to be a variant of *S. lactis.*

9. The rate at which rancidity disappeared in cheddar cheese made from milk containing added butyric acid or from homogenized raw milk was not accelerated by adding pure cultures of the X type organism to the milk.

10. Rancidity did not disappear in cheese made from milk containing pancreatin, and inoculation of pure cultures of the X type organism into the milk did not result in a disappearance of the rancidity in the cheese.

11. Inoculation of pure cultures of the X type organism into

the Ayres medium and into enriched Ayres medium failed to show utilization of the sodium butyrate after 14 or 30 days at 21.0° C.

12. Sodium butyrate was utilized when added to 48-hour yeast extract broth cultures of the X type organism. Utilization of the butyrate was evident after 14 days at 21.0° C. and was more complete after 21 days.

13. Tubes of the Ayres medium, containing 0.1 per cent peptone, that were inoculated with 0.1 ml. of a 48-hour litmus milk culture of *S. lactis* occasionally yielded the X type organism after 7 days at 21.0° C.

14. The disappearance of rancidity produced in cheddar cheese with various procedures appears to be due to the activity of microorganisms normally present in the cheese. Under the conditions that develop, certain of the organisms normally in cheese appear to change their morphological and biochemical characteristics.

LITERATURE CITED

(1) Ayres, Henry S., Rupp, Philip, and Johnson, Wm. T., Jr. A study of the alkali-forming bacteria found in milk. U. S. Dept. Agr. Bull. 782. 1919.

(2) Babel, F. J., and Hammer, B. W. Fat degradation in cheddar cheese made from pasteurized milk, without and with added lipase. Jour. Dairy Sci. 28: 201-208. 1945.

(3) Bergey, David H., and others. Bergey's manual of determinative bacteriology. Ed. 5, 389-394. Williams and Wilkins Co., Baltimore. 1939.

(4) Braun, Werner. Bacterial dissociation. Bact. Rev. 2: 75-114. 1947.

(5) Browne, C. A., Jr. A contribution to the chemistry of butterfat. Jour. Amer. Chem. Soc. 21: 975-994. 1899.

(6) Bryant, H. W. The effect of *Penicillium roqueforti* on some lower fatty acids. B. W. Hammer panegyric, 119-122. Collegiate Press, Inc. Ames, Iowa. 1937.

(7) Coolhaas, C. Zur Kenntnis der Dissimilation fettsaurer Salze und Kohlenhydrate durch thermophile Bakterien. Centr. fur Bakt. Abt. 2. 75: 161-170. 1928.

(8) Dorner, W., and Widmer, A. Homogenization and milk rancidity. Milk Plant Monthly 21: 50-57, 86, 88. 1932.

(9) Evans, Alice C. The metamorphosis of streptococci into spore-bearing rods and into filterable forms. Pub. Health Rep. 47: 1723-1738. 1932.

(10) Fouts, E. L. Effect of lactic acid on the hydrolysis of fat in cream by pure cultures of lipolytic microorganisms. Jour. Dairy Sci. 23: 303-306. 1940.

(11) Fouts, E. L. Relation of volatile acidity of butterfat to rancidity. Jour. Dairy Sci. 23: 307-314. 1940.

(12) Hadley, Philip. Microbic dissociation—The instability of bacterial species with special reference to active dissociation and transmissible autolysis. Jour. Infect. Diseases 40: 5-312. 1927.

(13) Hammer, B. W. Dairy bacteriology. Ed. 3. 460-461. John Wiley and Sons, Inc. New York. 1948.

(14) Hammer, B. W., and Collins, M. A. The numbers of lipolytic bacteria in various dairy products, as determined with nile-blue sulphate. Iowa Agr. Exp. Sta. Res. Bul. 169. 1934.

(15) Hlynka, I., Hood, E. G., and Gibson, C. A. Agitation and temperature of cheese milk and the development of rancid and unclean flavors in cheddar cheese. Jour. Dairy Sci. 26: 1111-1119. 1943.

(16) Hood, E. G., and White, A. H. Rancid flavor in cheddar cheese. Canada Dept. Agr. Bul. 146 (new series) 1-16. 1931.

(17) Krukovsky, V. N., and Sharp, Paul F. Effect of the properties of the fat and of the fat globule surface on lipolytic activity in milk. Jour. Dairy Sci. 23: 1109-1118. 1940.

(18) Koser, S. A. Utilization of salts of organic acids by the colon-aerogenes group. Jour. Bact. 8: 493-520. 1923.

(19) Lane, C. B. A vat for experimental cheese. National Butter and Cheese Jour. 30: No. 2. Feb.: 32. 1939.

(20) Lane, C. B., and Hammer, B. W. Bacteriology of cheese. II. Effect of *Lactobacillus casei* on the nitrogenous decomposition and flavor development in cheddar cheese made from pasteurized milk. Iowa Agr. Exp. Sta. Res. Bul. 190. 1935.

(21) Lane, C. B., and Hammer, B. W. Studies on the fat of cheddar cheese. Iowa State College Jour. Sci. 13: 149-155. 1939.

(22) Lane, C. B., and Hammer, B. W. Application of the smear culture technique to the examination of cheddar cheese. Abst. Jour. Dairy Sci. 22: 432. 1939.

(23) Lane, C. B., and Hammer, B. W. Relationship of fat hydrolysis to flavor development in cheddar cheese. Abst. Jour. Dairy Sci. 22: 432. 1939.

(24) Lane, C. B., and Hammer, B. W. Effect of lipolytic enzymes on the ripening of cheddar cheese. Abst. Jour. Dairy Sci. 23: 519. 1940.

(25) Laxa, O. Uber die Spaltung des Butterfettes durch Mikroorganismen. Arch. Hyg. 41: 119-151. 1901-1902.

(26) Lea, C. H. Rancidity in edible fats. 1-230. Chem. Pub. Co. New York. 1939.

(27) Lohnis, F., and Smith, N. R. Life cycles of the bacteria. Jour. Agr. Res. 6: 675-702. 1916.

(28) Long, H. F., and Hammer, B. W. Methods for the detection of lipolysis by microorganisms. Iowa State College Jour. Sci. 11: 343-351. 1937.

(29) Mattick, E. C. V., and Kay, H. D. A lipase (tributyrinase) of cow's milk. 1. Occurrence, method of estimation and relationship to lactation cycle. Jour. Dairy Res. 9: 58-71. 1938.

(30) Orla-Jensen, S. Studien uber des Ranzigwerden der Butter. Centbl. Bakt. Abt. 2, 8: 11-409. 1902.

(31) Palmer, Leroy S. Bitter milk of advanced lactation. Jour. Dairy Sci. 5: 201-211. 1922.

(32) Peterson, Merlin H., Johnson, Marvin J., and Price, Walter V. Lipase activity during making and ripening of cheddar cheese. Jour. Dairy Sci. 31: 39-46. 1948.

(33) Rice, Frank E., and Markley, Alton L. Proof of the presence of lipase in milk and a new method for the detection of the enzyme. Jour. Dairy Sci. 5: 64-82. 1922.

(34) Schreiber, Karl. Fettzersetzung durch Mikroorganismen. Arch. Hyg. 41: 328-347. 1901-1902.

(35) Triebold, Howard O. Rancidity. Cereal Chemistry 8: 518-532. 1931.

(36) Trout, G. M., Halloran, C. P., and Gould, I. The effect of homogenization on some of the physical and chemical properties of milk. Mich. Agr. Exp. Sta. Tech. Bul. 145. 1935.

(37) Tunnicliff, Ruth. The various colonies of hemolytic streptococci in scarlet fever. Jour. Infect. Diseases 49: 357-365. 1931.

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