Codeword: ASCEND.

With Compliments.
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AN ATTEMPT TO MANUFACTURE CHEDDAR CHEESE CONTAINING ONLY ONE TYPE OF ORGANISM.

T H E S I S

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INTRODUCTION.

There have been many investigations on the effect of various bacteria on the ripening of hard rennet varieties of cheese. Hucker (1922) in a review of the bacteriological aspects of cheese ripening summarizes the position as follows: "As it stands to-day the investigations have clearly demonstrated that the breaking down of the insoluble casein compounds is due to enzymes, either natural or bacterial; while characteristic flavors are produced by the action of certain groups of bacteria (Bacterium casei or coccus group), which depend on the products of B. lactis acidi present in large numbers during the manufacture and early ripening stages." The effect of lactic acid bacteria upon the flavour of Cheddar cheese has been studied by Hastings, Evans and Hart (1912), Evans, Hastings and Hart (1914), Evans (1918), Leitch (1923) and Hucker and Marquardt (1926).

A study of the effect of any organism on the ripening process in cheese is attended by many technical difficulties. The contamination with bacteria which normally takes place at innumerable points during the production of the milk and the manufacture of the cheese complicates any investigation. Even when milk has a low bacterial count after production it contains several species of organisms, some of which may multiply and play a significant role in the ripening of the cheese. For instance, although lactobacilli are usually present in milk only in small numbers, they are capable of multiplying to such an extent that they become the predominant group in the ripened cheese. Again, the fact that certain organisms are present only in small

numbers and are unable to multiply in the cheese, is no proof that they have little or no influence on the product, for the bacterial enzymes liberated after death of the organisms may be capable of producing significant changes. In some investigations that have been recorded, no details of the manufacture of the cheese are given, while in others it is apparent that the method of manufacture was so modified that it was impossible to produce a normal cheese. It is well known that small differences in the details of manufacture, even with milk of high quality, may lead to abnormal characteristics in the product.

A study of the literature has failed to reveal any work on the effect of an organism on the ripening of Cheddar cheese, in which a strict bacteriological control was maintained throughout the production of the milk and the manufacturing process. It was felt that before the effects of specific organisms could be demonstrated such a control was necessary. It was therefore with the object of elaborating a technique whereby the bacteriological aspects could be followed carefully and the number of variable factors reduced to a minimum, that this investigation was undertaken. At the same time it was hoped to gain information on the following points:

(1) The effect of Streptococcus cremoris on the rate of casein breakdown of Cheddar cheese.

(2) The effect of Streptococcus cremoris on the flavour of Cheddar cheese.

(3) The source of the lactobacilli commonly found in mature Cheddar cheese.

MEDIA EMPLOYED.

The medium used most extensively was a tryptic digest of casein. This was prepared by digesting 100 grms.

of sugar-free casein with 20 c.c. of Allen & Hanbury's Liquor Trypsin Co. The casein was mixed with 1000 c.c. of water, the trypsin added and the reaction adjusted to pH 8.1 by addition of 40% NaOH. Chloroform was added to prevent the growth of bacteria. The mixture was incubated for 6 days at 37°C., the bottle being shaken at intervals. The reaction was readjusted to pH 8.1 on the second and fourth days. After six days the liquid was filtered and the filtrate was diluted until it contained 0.5% of nitrogen as determined by the Kjeldahl method. Magnesium sulphate (0.1%) and dipotassium phosphate (0.2%) were then added, the reaction was adjusted to pH 6.8 and the medium was filtered.

The resulting broth formed the basis of the casein agar and sugar media. Casein agar was prepared by adding 1% of dextrose and 2% of agar to the broth. When the agar was to be used for the preservation of stock cultures over long periods the dextrose content was reduced to 0.25%.

For the determination of fermentation reactions 1% of each sugar and 1% of Andrade's indicator were added to the broth.

All the casein digest media were sterilised by three successive steamings.

Other media used were:

Yeast Whey Agar.

Standard Agar.

Nutrient Gelatin.

MacConkey's Broth.

Peptone Water.

Peptone Water Sugars.

The sugar media used for the fermentation tests of the micrococci consisted of peptone water to which 0.5% of each sugar and 1% of Andrade's indicator were added.

BACTERIOLOGICAL METHODS.

Except where it is otherwise stated all cultures were incubated at 30°C.

Determination of Acid and Volatile Acid Production.

200 c.c. of sterilised skim milk (three successive steamings) were inoculated with the organism or mixed culture to be tested and incubated at 22° C. for ten days. The acidity was then determined on 20 grms. of the culture and after subtraction of the control value the result was expressed as percentage of lactic acid.

150 c.c. of the culture were distilled in steam after the addition of 7.5 c.c. 2N sulphuric acid, 5 grams of sodium sulphate and a piece of paraffin wax to reduce frothing. Before commencing distillation the distilled water used in the steam can was allowed to boil for several minutes. One litre of distillate was collected and titrated with N/10 NaOH using phenolphthalein as indicator. The titration given by the control milk was subtracted and the result expressed as a percentage in terms of lactic acid. The amount of volatile acid as a percentage of the total acid was then calculated. Evans (1918) found that after seven days' incubation at 30°C., cultures of lactic acid streptococci showed little or no increase in volatile acid production.

Sugar Fermentations.

The inoculated tubes were incubated at 30°C. for 14 days (28 days in the case of lactobacilli) and were examined at 3 days, 7 days, and 14 days. The degree of fermentation was estimated by the intensity of colour produced through the action of the acid on the indicator. In the Tables the results are indicated by the following symbols:

\- no fermentation.

\+ very slight fermentation.

L slight fermentation.

\+ fermentation.

\+ strong fermentation.

ISOLATION OF THE STARTER ORGANISM.

The difficulty of preparing and maintaining an active "starter" consisting of a pure culture of a lactic streptococcus was realised very early in the work, so an attempt was made to isolate from two commercial "starters" one of the strains most active in producing acid. These two "starters" were being successfully used at the time for the manufacture of Cheddar cheese in the College factory.

The starters were plated out in duplicate on casein agar and incubated at 30°C. for 4 days. By the use of the 1/3" objective, representatives of the several types of colonies were picked off and inoculated into tubes containing 10 c.c. of sterile milk. The rate of acid production of the cultures was determined by titration with N/10 NaOH after incubation for 24 hrs. at 30°C. By this means, five of the most active acid producers (two from starter X and three from starter K) were isolated for further study. For comparative purposes a stock strain of Streptococcus lactis was added to the series.

The six cultures were purified twice by re-isolation from casein agar plates and their characteristics were then determined by repeated observations. Table I gives typical results obtained by sowing one loopful of culture into 10 c.c. of sterile milk, incubating at 30°C. for 24 hours and titrating with N/10 NaOH to phenolphthalein. In each case the control value was subtracted and the result expressed as a percentage in terms of lactic acid.

TABLE I.

Acid Production of Streptococci isolated from Starters

24 hrs. at 30°C.

Culture	Acid Produced expressed as % lactic acid
BX 1	0.43
CX 1	0.59
BK 1	0.46
CK 11	0.31
DK	0.25
Sc. lactis	0.38
Starter X	0.69
Starter K	0.68

It will be observed that none of the isolated strains was as rapid an acid producer as either of the original starters. Of the pure cultures, CX 1 consistently produced the largest amount of acid in 24 hours, although the other cultures gave higher values after several days' incubation, as will be observed in Table II which gives the Total Acid and Volatile Acid results.

TABLE II.

Acid and Volatile Acid Production of Streptococci isolated from Starters.

Culture.	Total Acid Produced as % lactic acid.	Volatile Acid Produced	
		as % lactic acid.	as % Total acid
BX1	0.67	0.011	1.6
CX1	0.55	0.015	2.7
BK1	0.66	0.016	2.4
CK11	0.59	0.015	2.6
DK	0.56	0.010	1.7
Sc. lactis	0.58	0.018	3.0
Starter X	0.78	0.095	12.1
Starter K	0.78	0.079	10.1

The results in Table II show that, whereas all the pure cultures had a low volatile acid production the starters contained organisms capable of producing large amounts of volatile acid.

Table III gives the results of the determination of sugar fermentations.

TABLE III.

Sugar Fermentation Results of Streptococci isolated from Starters X and K.

Culture	Time of Incubation	Glycerin	Xylose	Arabinose	Rhamnose	Sorbite	Mannite	Dulcite	Laevulose	Dextrose	Mannose	Galactose	Saccharose	Maltose	Lactose	Raffinose	Inulin	Dextrin	Starch	Salicin
BX1	3 days	-	-	-	-	-	-	-	+	+	+	+	-	-	+	-	-	-	-	-
CX1		-	-	-	-	-	-	-	+	+	+	+	-	-	+	-	-	-	-	-
BK1		-	-	-	-	-	-	-	+	+	+	+	-	-	+	-	-	-	-	-
CK11		-	-	-	-	-	-	-	+	+	+	+	-	-	+	-	-	-	-	-
DK		-	-	-	-	-	-	-	+	+	+	+	-	-	+	-	-	-	-	-
Sc.lactis		-	-	-	-	-	-	-	+	+	+	+	-	+	+	-	-	L	-	L
BX1	7 days	-	-	-	-	-	-	-	+	+	+	+	-	-	+	-	-	-	-	-
CX1		-	-	-	-	-	-	-	+	+	+	+	-	L	+	-	-	-	-	-
BK1		-	-	-	-	-	-	-	+	+	+	+	-	-	+	-	-	-	-	-
CK11		-	-	-	-	-	-	-	+	+	+	+	-	-	+	-	-	-	-	-
DK		-	-	-	-	-	-	-	+	+	+	+	-	-	+	-	-	-	-	-
Sc.lactis		-	-	-	-	-	-	-	+	+	+	+	-	+	+	-	-	+	L	+
BX1	14 days	-	-	-	-	-	-	-	+	+	+	+	-	-	+	-	-	-	-	-
CX1		-	-	-	-	-	-	-	+	+	+	+	-		+	-	-	-	-	-
BK1		-	-	-	-	-	-	-	+	+	+	+	-	-	+	-	-	-	-	-
CK11		-	-	-	-	-	-	-	+	+	+	+	-	-	+	-	-	-	-	-
DK		-	-	-	-	-	-	-	+	+	+	+	-	-	+	-	-	-	-	-
Sc.lactis		-	-	-	-	-	-	-	+	+	+	+	-	+	+	-	-	+	L	+

From Table III it is evident that none of the isolated pure cultures corresponded with the stock strain of Streptococcus lactis and also that CX1 differed from the other isolated strains by reason of a slow fermentation of maltose. It was concluded that all the five strains isolated from the two starters were types of Streptococcus cremoris according to the classification of Orla Jensen (1919). Strain CX1 was selected as most suitable for use as a starter in the present investigation, because of its more rapid acid production.

CHARACTERS OF THE STARTER ORGANISM.

Morphology.

After 24 hours' growth in milk at 22°C. or at 30°C. the organism appeared as slightly oval Gram-positive cells in pairs and in short chains. Occasionally long chains were formed. Milk cultures incubated at 37°C. showed chiefly diplococci with a small number of single cells, short chains and involution forms. When grown on casein agar at 22°C. or at 30°C. the microscopical appearance of the organism was similar to that seen in the milk cultures at these temperatures. A number of swollen and rod-shaped cells, however, were also present. On occasions, long and very long chains were formed on casein agar plates. At 37°C. the organism tended to form long chains.

The colonies formed on casein agar and yeast whey agar plates were small, spherical, translucent to opaque, with entire margins.

Biochemical Characters.

Growth in milk was rapid and a clot was formed within 24 hours. The rate of acid production was consistently more rapid than was the case with the stock strain of Streptococcus lactis. This may have been due to the fact that CX1 was of recent isolation from a mixed culture, while

the Streptococcus lactis had been growing for several years on artificial media in pure culture. Immediately after isolation the first two transfers of CX1 in milk showed slight gas formation. This property rapidly disappeared, however, and has not been observed since.

It has already been mentioned that CX1 produces only a small proportion of volatile acid.

The sugar fermentation reactions have been given in Table III. They were later determined by the method of Orla-Jensen (1919) in which the amounts of acid formed are measured by titration. The results are given in Table IV and it will be observed that they are in close agreement with those given previously.

TABLE IV.*

Nitrogen Source.	Glycerin	Xylose	Arabinose	Rhamnose	Sorbite	Mannite	Laevulose	Dextrose	Mannose	Galactose
Casein-digest.	1.3	1.1	1.4	1.4	1.4	2.0	6.3	6.1	5.9	4.3

Nitrogen Source.	Saccharose	Maltose	Lactose	Raffinose	Inulin	Dextrin	Starch	Salicin	Milk	Milk + Yeast Extract
Casein-digest	1.6	2.0	5.2	1.3	1.3	1.8	1.4	1.3	5.3	5.1

(results given as grms. of lactic acid per mille)

The proteolytic action of the organism‡ was determined by growth in a trisodium phosphate solution of rennet casein at 22°C. for 14 days. Formol titrations and determinations of the percentage of protein soluble at pH 4 showed that the organism had negligible proteolytic power.

* Figures kindly supplied by Mr.H.R.Whitehead, Dairy Research Institute (N.Z.)

‡ Determined by Mr.H.R.Whitehead, Dairy Research Institute (N.Z.)

A consideration of all the characteristics led to the conclusion that CX1 was most probably a strain of Streptococcus cremoris (Orla-Jensen 1919). This conclusion derives support from a recent paper by Knudsen (1931) who, in discussing Orla-Jensen's classification of the lactic acid bacteria, states: "The deciding factor between the two species (Sc. lactis and Sc. cremoris) is, however, that Streptococcus lactis can ferment maltose and dextrin while Streptococcus cremoris cannot."

For the whole period of the investigation the organism was resown daily in 200 c.c. of sterile milk and incubated at 22°C. During the first month after isolation the rate of acid production decreased until it became necessary to use an inoculum of 1 c.c. daily in order to induce clotting in 24 hours. During the next month the rate of acid production increased until only 4 drops of inoculum were required. The activity of the organism thereafter remained constant and its cultures received the same amount of inoculum as did five cheese "starters" which were being maintained in the laboratory under similar conditions.

Great care was taken to prevent contamination of the starter throughout the whole period. Between the manufacture of Cheese II and Cheese III the starter was examined thoroughly. No contamination could be detected either by plating out on casein and standard agar or by direct microscopical examination. The sugar fermentation reactions proved to be identical with those originally obtained. Determinations of the total and volatile acid production gave values of 0.60% and 0.013% respectively.

It was evident that after several months' cultivation the starter organism had not altered its characteristics in any detail which it was possible to detect.

PREPARATION OF RENNET AND SALT.

The rennet to be used in the manufacture of the cheese was sterilised by passage through a Seitz bacterial filter. Some difficulty was experienced in finding a rennet which would pass sufficiently rapidly through the filter. The rennet finally employed was of Danish manufacture and on account of long storage and passage through the filter was somewhat below standard strength. Not more than the amount needed for one experiment was filtered at the one time. The efficiency of the filtration was tested by sowing large amounts of the filtered rennet on casein agar and standard agar. On no occasion was any growth obtained.

Commercial cheese salt was used. It was sterilised by being heated in a hot-air oven at 160°C. for one hour. No bitterness or other defect could be detected after sterilisation.

PRODUCTION OF THE MILK.

For the purposes of this investigation it was necessary to use milk which, from the bacteriological standpoint, was of the highest possible quality. With this object in view the following procedure was followed.

Approximately 40 gallons of milk were required for each experiment. The milk was obtained from approximately equal numbers of Jersey and Friesian cows together with two Ayrshires. Cheese IV, however, was made from Jersey milk only. No bacteriological examination of the milk from the individual cows had previously been carried out.

The cows were machine milked into pails. The pails, lids, taps and all cans and lids were sterilised in flowing steam for 1½ hours before being used. The

claws, cups and all the rubber parts of the machine were immersed in hypochlorite solution for 3 hours and then thoroughly drained and rinsed in sterile water before use. The udders of the cows were cleaned thoroughly by means of a sponge and running water. The sponge was soaked in a strong hypochlorite solution while not in use. After about one pint of fore-milk had been drawn from the cow by hand, the udder was washed with hypochlorite solution and dried with a cloth wrung out in the same solution. The teat cups were then placed in position taking great care to avoid contamination. As the pails filled, they were replaced by other sterilised pails and carried with lids on to the milk-room where the milk was poured through sterilised muslin strainers into the receiving cans. The cows were stripped by hand and the strippings were rejected. Only the mid-milk obtained by machine as described above was used in the present investigation.

As soon as the requisite quantity of milk had been obtained it was transported to the factory several chains away, weighed in the cans and transferred to a cheese vat which had been thoroughly steamed. The milk was well mixed by stirring with a wooden rake which had been immersed in a copper of boiling water for some time. A sample was then taken with a sterile pipette for bacteriological examination. This sample was labelled "raw milk." On another sample duplicate Babcock fat and Walker casein tests were carried out.

DETAILS OF MANUFACTURE.

Each experiment was uninterrupted from the commencement of milking to the hooping of the curd, the manufacturing process being carried out during the evening. The milk was pasteurised by the holding method at temperatures ranging in the different experiments from 145°F.

to 150°F. for 30 minutes. Heating was effected by passing steam through the water in the jacket of the vat, the milk being stirred continuously with a wooden rake. Care was taken to prevent temperature fluctuation during the process. After the 30 minute holding period the milk was cooled as rapidly as possible to 88°F. A sample was then taken with a sterile pipette for bacteriological examination. This sample was labelled "pasteurised milk."

As far as practicable the entire manufacturing process was performed under strictly sterile conditions. The cheese-maker's clothing, including a cap to prevent contamination from the hair, was autoclaved before use. The cheese bandages and caps were also autoclaved. A large copper of water was kept boiling continuously throughout the period of manufacture. Every utensil which came into contact with the milk or curd was previously immersed in the boiling water for from 30 to 60 minutes. Utensils which were used for a long period or more than once, were sterilised at frequent intervals. Since it was necessary to use the hands on the manipulation of the curd, the hands, nails and arms were cleaned thoroughly and treated with hypochlorite solution. The disinfectant was then washed off in sterile water. This treatment was applied on every occasion before touching the milk or curd.

A canvas cover which had been sterilised in boiling water was placed over the vat to reduce the risk of bacterial contamination from the atmosphere. Throughout the manufacturing process the vat was uncovered only when it was absolutely necessary. The use of two canvas covers made it possible to have one in boiling water while the other was over the vat. The covers were changed frequently.

All samples of milk or whey were taken with sterile pipettes.

As far as was practicable the New Zealand method

of Cheddar cheese manufacture from pasteurised milk was followed. The process was controlled by means of the acidimeter, the condition of the curd and the hot-iron test. No attempt was made to work to a rigid time schedule. Particulars of manufacture are given in Tables V to IX.

During the cooling process after pasteurisation, as soon as the temperature of the milk reached 88°F., 2.5% of the Sc. cremoris starter (CX1) was stirred into the milk. "Ripening" was allowed to proceed until a "rennet test" (Col. Leaflets) of 17 seconds at 88°F. was recorded with standard strength New Zealand rennet. A "rennet test" was then carried out with the sterile Danish rennet. Since this was below normal strength it gave a higher figure. The quantity of Danish rennet to be added was calculated from the results of the rennet tests, assuming that the correct quantity of New Zealand rennet was 4 to 5 ounces per 1000 pounds of milk. The actual quantity to be used in each experiment was decided upon finally by taking into account the firmness of the curd after 25 to 35 minutes in the previous experiment. The rennet was measured in a sterile measuring cylinder and mixed with approximately five times its volume of sterile water. After an acidity test of the milk had been taken the rennet was evenly distributed over the vat and stirred in thoroughly for two minutes. The vat was then covered and left undisturbed until the curd was ready for cutting.

The firmness of the curd was tested by inserting a finger diagonally and raising it gently to the surface. The resistance to the movement of the finger, the type of break and the appearance of the whey in the broken surface were all considered in determining when to cut.

TABLE V.

Cheese I Manufacturing Record.

Milk, Starter, Rennet & Salt.		Particulars of Manufacture.							
Milk: odour	Good	Operation	Time	Temperature	Acidity	Rennet Test.	Time Intervals	hrs.	mins.	Maker's Observations.
flavour	Good	Vat filled	-	-	0.165					At running, curd a little soft; definite "animal" or "cowy" odour. Stirred twice before piling. Cut into 4 blocks. Slow cheddaring; curd carrying a slight excess of moisture. After milling curd broke down rapidly; became firmer; texture slightly short.
Per cent. fat	4.0	Starter added	7.55	88	0.175	N.Z. 16.5 secs.	Ripening period		48	
" " casein	2.53	Renneting	8.43	88	0.18	Danish 30 "	Renneting to cutting		32	
casein/fat ratio	0.63	Cutting	9.15	-	0.12		Cutting to heat on		10	
amount, lb.	374	Heat on	9.25	-	-		Heat on to heat off		45	
pasteurisation temp.	150°F (30 mins)	Heat off	10.10	99	-	Hot-iron Test	Renneting to dry	3	37	
Starter: per cent.	2.5	Running	12.10	-	0.19	¼"	Dry to milling	3	10	
acid	0.70	Dry	12.20	94	0.25	3/8"	Milling to salting.	1	0	
Total milk used, lb.	383	Highest acidity	-	-	0.62	-	Salting to hooping.		35	
Rennet (ozs. standard strength per 1000 lbs.)	4.8	Milling	3.30	83	0.55	1"	Dry to salting	4	10	
Curd, lb. at milling	42.6	Salting	4.30	81	0.55	1⅛"	Duration of pressing	53	0	
Salt, per cent.	2.0	Hooping	5.05	-	-	-				

TABLE VI.

Cheese II Manufacturing Record.

Milk, Starter, Rennet & Salt.		Particulars of Manufacture.						
Milk: odour	Good	Operation	Time	Temperature	Acidity	Rennet Test	Time Intervals	Maker's Observations.
flavour	Good	Vat filled	-	-	0.165		hrs. mins.	
per cent. fat	3.9	Starter added	8.05	88	0.175	N.Z. 17 secs.	Ripening period 48	Acid development slow in whey. A good curd at running; slight "animal" or "cowy" odour. Stirred twice before piling. Cut into 6 blocks. Slow cheddaring and slow acid development. At salting a fair curd although not broken down sufficiently; meaty but not harsh.
" " casein	2.57	Renneting	8.53	88	0.185	Danish 29 "	Renneting to cutting 35	
casein/fat ratio	0.66	Cutting	9.28	-	0.12		Cutting to heat on 10	
amount, lb.	404	Heat on	9.38	-	-		Heat on to heat off 42	
pasteurisation temp. (30 mins)	147°F	Heat off	10.20	100	-	Hot-iron Test	Renneting to dry 3 42	
Starter: per cent.	2.5	Running	12.25	-	0.16	¼"	Dry to milling 2 15	
acid	0.71	Dry	12.35	96	0.25	3/8"	Milling to salting 1 25	
Total milk used, lb.	414						Salting to hooping 30	
Rennet (ozs. standard strength per 1000 lbs.)	4.5	Milling	2.50	92	0.485	7/8"	Dry to salting 3 40	
		Highest acidity.	-	-	0.57	-		
Curd, lb. at milling	44.0	Salting	4.15	84	0.57	1⅛"	Duration of pressing 53 0	
Salt, per cent.	2.0	Hooping	4.45	-	-	-		

TABLE VII.

Cheese III Manufacturing Record.

Milk, Starter, Rennet & Salt		Particulars of Manufacture.						
Milk: odour	Good	Operation	Time	Temperature	Acidity	Rennet Test	Time Intervals	Maker's Observations
flavour	Good	Vat filled	-	-	0.165		hrs. mins.	
per cent. fat	4.0	Starter added	7.30	88	0.175	N.Z. 17 secs.	Ripening period 45	Dipped at an acidity of 0.20% in order to encourage the activity of the starter. Good firm curd; clean odour and flavour; no trace of "animal" odour whatever. Stirred twice before piling. Cut into 6 blocks. Normal cheddaring. Very good curd. At salting nice silky body.
" " casein	2.61	Renneting	8.15	88	0.185	Danish 29 "	Renneting to cutting 35	
casein/fat ratio	0.65	Cutting	8.50	-	0.13		Cutting to heat on 10	
amount, lb.	400	Heat on	9.00	-	-		Heat on to heat off 50	
pasteurisation temp.	145°F (30 mins)	Heat off	9.50	99	-	Hot-iron Test	Renneting to dry 3 18	
Starter: per cent.	2.5	Running	11.20	-	0.20	¼"	Dry to milling 2 12	
acid	0.78	Dry	11.33	97	0.285	½"	Milling to salting 1 0	
Total milk used, lb.	410	Highest acidity.	-	-	0.75	-	Salting to hooping 30	
Rennet (ozs. standard strength per 1000 lbs.)	4.5	Milling	1.45	93	0.72	1¼"	Dry to salting 3 12	
Curd, lb. at milling	45.2	Salting	2.45	90	0.70	1⅝"	Duration of pressing 54 0	
Salt, per cent.	2.0	Hooping	3.15	-	-	-		

TABLE VIII.

Cheese IV Manufacturing Record.

Milk, Starter, Rennet & Salt.		Particulars of Manufacture.						
Milk: odour	Good	Operation	Time	Temperature	Acidity	Rennet Test	Time Intervals	Maker's Observations
flavour	Good	Vat filled	-	-	0.16		hrs. mins.	
per cent. fat	3.5	Starter added	9.05	88	0.175	N.Z. 17 secs.	Ripening period 47	Slightly over-stirred at renneting. Stirred curd gently in the whey.
" " casein	2.89	Renneting	9.52	88	0.185	Danish double strength 8.5 "	Renneting to cutting 33	
casein/fat ratio	0.83	Cutting	10.25	-	0.125		Cutting to heat on 10	Heated slowly to 95°F., kept whey at this temperature for 15 minutes, then ran out most of the water from the jacket of the vat. Fair curd at running. Did not stir before piling. Cut into 2 blocks. Slow cheddaring. Added hot water to jacket of vat to raise the temperature. After milling the escaping whey appeared to be rich in fat. Curd broke down well; slightly harsh & a little tough. Used
amount, lb.	408	Heat on	10.35	-	-		Heat on to heat off 35	
Pasteurisation temp.	145°F. (30 mins)	Heat off	11.10	98	-	Hot-iron Test	Renneting to dry 3 28	
Starter: per cent.	2.5	Running	1.10	-	0.185	3/8"	Dry to milling 2 20	
acid	0.69	Dry	1.20	92	0.32	⅛"	Milling to salting 1 45	
Total milk used, lb.	418	Milling	3.40	87	0.70	1⅛"	Salting to hooping 30	
Rennet (ozs. standard strength per 1000 lbs.)	7.6	Highest acidity	-	-	0.80	-	Dry to salting 4 5	
Curd, lb. at milling	45.6	Salting	5.25	84	0.73	1"	Duration of pressing 36 0	
Salt, per cent.	1.5	Hooping	5.55	-	-	-		

TABLE IX.

Cheese V Manufacturing Record.

Milk, Starter, Rennet & Salt.		Particulars of Manufacture.						
Milk: odour	Good	Operation	Time	Temperature	Acidity	Rennet Test	Time Intervals.	Maker's Observations
flavour	Good	Vat filled	-	-	0.165		hrs. mins.	
per cent. fat	3.75	Starter added	7.25	88	0.18	N.Z. 17 secs.	Ripening period 1 0	A good curd at running; odour
" " casein	2.61	Renneting	8.25	88	0.195	Danish 27 "	Renneting to cutting 45	and flavour clean; no trace of
casein/fat ratio	0.70	Cutting	9.10	-	0.14		Cutting to heat on 10	"animal" odour. Stirred twice.
amount, lb.	411	Heat on	9.20	-	-		Heat on to heat off 45	Cut into 6 blocks.
pasteurisation temp.	145°F. (30 mins)	Heat off	10.05	99	-	Hot-iron Test	Renneting to dry 3 15	Curd a little tender during cheddaring; after milling broke down
Starter: per cent.	2.5	Running	11.33	-	0.21	3/8"	Dry to milling 2 0	well; at salting a good silky
acid	0.73	Dry	11.40	97	0.35	½"	Milling to salting 1 0	body.
Total milk used, lb.	421	Milling	1.40	93	0.84	1¼"	Salting to hooping 35	
Rennet (ozs. standard strength per 1000 lbs.)	4.7	Highest acidity.	-	-	0.84	-	Dry to salting 3 0	
Curd, lb. at milling	45.5	Salting	2.40	89	0.81	-	Duration of pressing 54 0	
Salt, per cent.	2.2	Hooping	3.15	-	-	-		

When the curd was considered firm enough it was cut into half inch cubes by means of sterile curd knives. The curd which remained clinging to the sides and bottom of the vat was removed by hand. Stirring was carried out gently for 10 minutes before the application of heat. Then the temperature was raised in 40 to 50 minutes to the desired "cooking" or "scalding" temperature, namely 99°F. to 100°F. Stirring was continued throughout the cooking period.

The time of "running" was determined by the firmness of the curd and the acidity of the whey. In order to encourage the development of acidity in the curd after drying the whey was run off at an acidity 0.01 to 0.02% higher than usual.

The curd was stirred twice before being piled at the end of the vat away from the tap. The pile was trimmed twice and the trimmings were placed on top. The curd was allowed to "mat" for 20 to 30 minutes and then cut into 4 or 6 pieces depending upon the moisture content. The blocks were then turned over at intervals of 15 minutes. In order to assist the cheddaring process the blocks were piled two and three deep after the second and third turnings respectively.

The time of "milling" was determined by examination of the curd texture, by the acidity of the whey and by the length of "threads" on the hot iron. The curd was weighed in a sterile metal tray, cut into smaller blocks and passed through a sterile curd mill which cut the curd into rectangular pieces approximately ½" × ½" × 4 to 6" in size. The milled curd was stirred and spread over the bottom of the vat in a layer 6 to 8 inches deep. Stirring was repeated at 15 minute intervals.

When the curd had "broken down" into a smooth plastic mass with a characteristic "silky" or "meaty" texture and the mechanical holes had disappeared, the salt

was applied. At this stage also the hot-iron test and the acidity of the whey were recorded. The amount of salt to be added was determined by the condition of the curd. Half the salt was spread evenly over the curd, the other half being applied after turning. A thorough stirring was then given and this was repeated 15 minutes later, the curd being piled in one corner of the vat in the meantime.

When the salt had dissolved the curd was distributed into four hoops, which had previously been sterilised and lined with sterile cheese cloth. The hoops were packed firmly, sterile caps and lids were put into place and a steady pressure was applied. After 30 to 45 minutes the cheeses were removed from the press and "dressed" i.e. the ragged edges were removed and the ends of the bandages adjusted. They were then replaced in the press and a greater pressure was applied and maintained for 53 to 54 hours, after which time the cheeses were removed, marked, and rubbed over with unsalted lard to prevent excessive loss of moisture. The lard, which had previously been treated with formalin, was heated to 100°C. for several minutes, cooled until near the solidification point, and then applied.

The cheeses were allowed to ripen at 60°F. in a room maintained at this temperature by a heater and thermostat. The relative humidity of the room varied from 70 to 90%.

EXAMINATION OF THE SAMPLES OF RAW AND PASTEURISED MILK.

Estimation of Number of bacteria.

The numbers of bacteria in the milk samples were estimated by dilution and plating on casein agar and yeast whey agar at 30°C. On every occasion the plates were prepared within two hours of the removal of the sample from

the vat. The samples were tested for the presence of B. coli by inoculation of 1 c.c. into tubes of MacConkey's broth. In the case of the samples taken during the manufacture of Cheeses III and V, 10 c.c. quantities were inoculated into MacConkey Tubes. On no occasion was a positive result obtained.

The plates were counted after incubation for 48 hours by means of a lens giving a magnification of 10 diameters. The results are presented in Table X.

TABLE X.

Raw and Pasteurised Milk Counts.

Cheese	Medium	Raw Milk Plate Count per c.c.	Raw Milk B.coli.	Pasteurised Milk Plate Count per c.c.	Pasteurised Milk B.coli
I	Casein agar	4,380	None in 1 c.c.	92	None in 1 c.c.
	Yeast whey agar	4,500		29	
II	Casein agar	1,440	None in 1 c.c.	3	None in 1 c.c.
	Yeast Whey agar	1,530		5	
III	Casein agar	1,720	None in 10 c.c.	458	None in 10 c.c.
	Yeast Whey agar	1,770		466	
IV	Casein agar	17,800	None in 1 c c.	243	None in 1 c.c.
	Yeast whey agar	20,400		689	
V	Casein agar	3,440	None in 10 c c.	252	None in 10 c.c.
	Yeast whey agar	4,140		-	

The counts of the raw milk samples ranged from 1,440 to 4,380 per c.c. on casein agar and from 1,530 to 4,500 on yeast whey agar except in experiment IV where much higher counts were recorded. This exception is probably accounted for by the fact that this was the first experiment and that there were probably errors in the technique in the milking shed. The counts of the pasteurised samples ranged from 3 to 438 per c.c. on casein agar and from 5 to 466 on yeast

whey agar. In every case the yeast whey agar gave a slightly higher count than the casein agar.

At this point it is convenient to make a short digression to discuss the question of bacteria in freshly drawn milk. Numerous investigators have studied the bacterial flora of milk drawn under aseptic conditions and have determined the number of organisms present. The following table (Dorner 1930) is a summary of the findings of various workers.

TABLE XI.

Author	Average count per c.c.	Number of Cows.
Schulz (1892)	2,330	-
Russell (1894)	330	-
Marshall (1900)	295	-
von Freudeureich (1902)	295	-
Lux (1903)	1,391	10
Harding & Wilson (1913)	428	78
Burri & Hohl (1917)	347	16
Copeland & Olson (1926)	1,546	40
Alice F. Breed (1928)	964	12

According to Gorini (1902) the number varies from zero to 300,000 per c.c. Sherman (1915) found that 13.4% of the samples he examined contained over 10,000 per c.c. and 28.8% more than 5,000 per c.c. Dorner (1930) examined 993 samples of milk drawn under aseptic conditions from 132 cows of 6 herds in New York State. The examinations were carried out by the standard agar plate method and the Burri quantitative smear technique. From the results, the probable bacterial count of the milk from each herd was calculated. The lowest herd count calculated from the Burri slant results was 3,965 per c.c., the highest was 9,635 per c.c. and the average was 7,475 per c.c. The herd counts calculated from the standard agar plate results ranged from 530 per c.c. to 4,390 per c.c., the average being 2,775 per c.c. Dorner considered that the large difference between the counts obtained by the two methods was due to the failure of the most common organism (rods related to Bacterium lipolyticus (Evans)) to develop on standard agar plates. Later at Liebefeld, Switzerland, Dorner examined 944 samples from 241 cows of 22 herds by the Burri method and obtained a calculated average herd count of 3,099 per c.c. Incidentally organisms of the Bacterium lipolyticus type were fewest in number .

It is interesting to record that in the present investigation rods similar to Bacterium lipolyticus (Evans) were found in several samples of raw and pasteurised milk.

Taking into account the media used and the temperature of incubation, it will be seen that the counts obtained with the raw milk in this investigation compared

very favourably with those obtained by other investigators with aseptically drawn milk.

Determination of types of bacteria.

The plates used for the estimation of the total number of bacteria were incubated for a further period of 5 days. Streak cultures of the raw and pasteurised samples were also made on casein agar and standard agar plates. These were incubated for 7 days at 30°C. The colonies were examined by the naked eye and under the 1/3" objective and from 20 to 50 colonies were picked from the pasteurised milk plates. Smears were made on slides and stained by Gram's method. Except in experiment V the examination of the raw milk samples was not so thorough.

The organisms found were classified as accurately as possible after consideration of size, shape, opacity, colour and other colony characteristics and also morphology and staining reactions. At the same time the percentage of each type present was estimated as accurately as possible. The results are given in Table XII. It will be observed that there is good agreement in the types and to a lesser extent in their relative frequency of occurrence in all the raw and pasteurised milk samples.

TABLE XII.

Percentage Distribution of Types of Bacteria found in the Raw & Pasteurised Milk Samples.

Cheese	Raw Milk.		Pasteurised Milk.	
I	"Yellow" micrococci	75 per cent.	"Yellow" micrococci	80 per cent
	"White" "	25 "	"White" "	20 "
	"Orange" "	< 1 "	"Orange" "	Absent
II	"Yellow" micrococci	80 per cent.	"Yellow" micrococci(S)	25 per cent
	"White" "	20 "	"White" "	38 "
			"Yellow" " (P)	12 "
			Bacterium X	25 "
III	"Yellow" micrococci	50 per cent.	"Yellow" micrococci	98 per cent
	"White" "	28 "	"White" "	< 1 "
	"Orange" "	15 "	"Orange" "	Absent
	Bacterium X	7 "	Bacterium X	< 1 "
IV	"Yellow" micrococci	75 per cent.	"Yellow" micrococci	95 per cent
	"White" "	24 "	"White" "	5 "
	"Orange" "	1 "	"Orange" "	Absent.
V	"Yellow" micrococci	40 per cent.	"Yellow" micrococci	70 per cent
	"White" "	35 "	"White" "	20 "
	"White" "(R)	15 "	"White" " (R)	10 "
	"Orange" "	10 "	"Orange" "	Absent.
	Bacterium X	< 1 "	Bacterium X	"

Representatives of each type were subcultured into milk and on to casein agar and standard agar slopes so that the morphology, cultural characteristics and biochemical reactions could be studied in greater detail. The results are given in Tables XIII to XVII.

TABLE XIII.

Characters of Organisms isolated from Pasteurised Milk I.

Yellow Micrococcus.

Morphology: Gram-positive coccus approximately 1 μ in diameter. Cells spherical.

Casein agar: Luxuriant growth; large, lemon-yellow, opaque, slightly raised colony with entire margin. Pigmentation not developed until colonies 4 to 6 days old.

Standard agar: Good growth.

Litmus milk: Acid developed slowly; clot at 14 days. A lemon coloured deposit formed at bottom of tube. At 4 weeks the lower half of the litmus decolorized. No digestion.

TABLE XIII (Continued)

Gelatin: Slow liquefaction.

Indole Negative

Fermentation of Sugars:

	1 day	2 days	3 days	7 days	14 days
Dulcite	-	-	-	-	-
Dextrose	+	+	+	+	+
Lactose	+	+	+	+	+
Saccharose	+	+	+	+	+

White Micrococcus

Morphology: Gram-positive coccus 1 μ in diameter, occurring chiefly in pairs; cells spherical.

Casein agar: Luxuriant growth; colony smaller than that of the "Yellow" micrococcus, translucent to opaque, whitish, with entire margin.

Standard agar: Good growth.

Litmus milk: Acid developed slowly; clot at 6 days; at 8 days decolorization commenced from bottom. At 4 weeks lower half decolorized. No digestion.

Gelatin: Slow liquefaction.

Indole: Negative

Fermentation of Sugars:

	1 day	2 days	3 days	7 days	14 days
Dulcite	-	-	-	-	-
Dextrose	+	+	+	+	+
Lactose	+	+	+	+	+
Saccharose	+	+	+	+	+

TABLE XIV.

Characters of Organisms isolated from Pasteurised Milk II.

Yellow Micrococcus (S).

Morphology: Gram-positive coccus about 1 μ in diameter.

Casein agar: Luxuriant growth; colony large, yellow, opaque, with entire margin.

Litmus milk: At 4 days slightly acid with yellow deposit at bottom. Acid and clot in 9 days. At 12 days litmus decolorized leaving only a red ring at top. No digestion in 21 days.

Gelatin: Slow liquefaction.

Fermentation of Sugars:

	1 day	2 days	3 days	7 days	14 days
Dulcite	-	-	-	-	-
Dextrose	+	+	+	+	+
Lactose	L	L	L	+	+
Saccharose	-	-	L	L	+

Yellow Micrococcus (P).

Morphology: Gram-positive coccus 1 to 1.5 μ in diameter, occurring chiefly as tetrads.

Casein agar: Luxuriant growth; colony large, yellow, opaque, with entire margin.

Litmus milk: Good growth but no visible action in 21 days.

Gelatin: Comparatively rapid liquefaction. Very good liquefaction in 8 days.

Fermentation of Sugars:

Good growth in dulcite, dextrose, lactose and saccharose, but no fermentation in 21 days.

White Micrococcus.

Morphology: Gram-positive coccus 0.5 μ in diameter.

Casein agar: Good growth; colony smaller than either S or P, whitish, opaque, with entire margin.

Litmus milk: Acid developed very slowly; no clot in 21 days. No digestion.

Gelatin: Slow liquefaction.

TABLE XIV. (Continued)

Fermentation of Sugars:

	1 day	2 days	3 days	7 days	14 days.
Dulcite	-	-	-	-	-
Dextrose	l	L	+	+	+
Lactose	-	-	L	+	+
Saccharose	-	-	-	+	+

Bacillus X.

Morphology: Short, thin Gram-positive rod; non-motile; non-sporing. From casein agar approximately 0.5 to 0.7 μ by 1 to 2 μ . In milk usually 0.5 to 0.7 μ by 2 to 4 μ . Rods straight, a few slightly curved. No chains. Characteristic arrangement as shown by "diphtheroid organisms" is common; rods often lying parallel to one another in groups of 2 or 3; often in pairs forming various angles; sometimes rods crossed at ends, ; occasionally branching forms found, Metachromatic staining common; bipolar staining occasionally present.

Casein agar: Good growth in 3 to 5 days. Colony very small, transparent to translucent, slightly glistening surface, spherical with entire margin. Microscopic examination (1/3" objective) reveals a characteristic, very small, dense portion in centre of colony.
Optimum temperature 30°C. At 37°C. growth is almost as rapid.

Litmus milk: Poor growth; often difficult to obtain multiplication. No visible effect other than occasional slight acid formation.

Gelatin: Poor growth; colonies very small; no liquefaction.

Peptone Solution: Slow growth; no indole formed.

Acidity in milk (21 days): 0.04 per cent.

Volatile Acidity(21 days): 0.007 per cent.

Fermentation of Sugars (casein digest medium):

Time of Incubation.	Glycerin	Xylose	Arabinose	Rhamnose	Sorbite	Mannite	Dulcite	Laevulose	Dextrose	Mannose	Galactose	Saccharose	Maltose	Lactose	Raffinose	Inulin	Dextrin	Starch	Salicin
3 days	-	-	-	-	-	-	-	-	l	-	-	-	-	-	-	-	-	-	-
7 "	-	-	-	-	-	l	-	L	+	L	l	L	L	L	-	-	l	l	l
14 "	-	-	-	-	-	+	-	+	+	L	l	+	+	+	-	-	L	L	L
21 "	-	-	-	-	-	+	-	+	+	+	L	+	+	+	-	-	+	+	+
28 "	-	-	-	-	-	+	-	+	+	+	L	+	+	+	-	-	+	+	+

TABLE XV.

Characters of Organisms isolated from Pasteurised Milk III.

No study was made of the "Yellow" and the "White" micrococci found in this sample. A thorough investigation, however, was carried out on the Bacillus X type isolated. It was found to be identical with the Bacillus X type of pasteurised milk II.

TABLE XVI.

Characters of Organisms isolated from Pasteurised Milk IV.

Yellow Micrococcus.

Morphology: Gram-positive coccus 1 μ in diameter.

Casein agar: Luxuriant growth; large, yellow, slightly raised, opaque, glistening colony; spherical with entire margin.

Standard agar: Good growth.

Litmus milk: Acid developed slowly; clot at 14 days. At 3 days a yellow deposit formed at bottom of tube. Decolorization from bottom. No digestion.

Gelatin: Slow liquefaction.

Indole: Negative.

Fermentation of Sugars:

	1 day	2 days	3 days	7 days	14 days
Dulcite	-	-	-	-	-
Dextrose	-	+	+	+	+
Lactose	-	+	+	+	+
Saccharose	-	L	+	+	+

White Micrococcus.

Morphology: Gram-positive coccus 1 μ in diameter, occurring chiefly in pairs.

Casein agar: Good growth. Colony white, opaque, raised, somewhat tough.

Litmus milk: Acid developed slowly; clot at 14 days. Decolorization from bottom. No digestion.

Gelatin: Slow liquefaction.

Indole: Negative.

TABLE XVI. (Continued)

Fermentation of Sugars:

	1 day	2 days	3 days	7 days	14 days
Dulcite	-	-	-	-	-
Dextrose	-	+	+	+	+
Lactose	-	+	+	+	+
Saccharose	-	+	+	+	+

TABLE XVII.

Characters of Organisms isolated from Pasteurised Milk V.

White Micrococcus (R)

Morphology: Gram-negative coccus 1.3μ in diameter, occurring chiefly in pairs; cells slightly oval.

Casein agar: Good growth; colony white, translucent, glistening, with entire margin.

Litmus milk: At 10 days slightly alkaline; well-defined at 21 days; deeply alkaline at 28 days.

Gelatin: No liquefaction in 28 days.

Indole: Negative.

Fermentation of Sugars:

	1 day	2 days	3 days	14 days	28 days.
Dulcite	-	-	-	-	-
Dextrose	-	-	-	-	-
Lactose	-	-	1	1	+
Saccharose	-	-	1	1	+

It is evident that two distinct types of bacteria were found in the raw and pasteurised milk samples, namely, micrococci and rods similar to Bacterium lipolyticus (Evans). (For want of a better name these rods have been designated Bacterium X).

Although a thorough search was made in the plates from the pasteurised milk samples for streptococci and lactobacilli, none were found. It was concluded that these organisms were not present in the milk to the extent of one per cubic centimetre. One apparent streptococcal colony was found on one of the raw milk plates in Expt.III. The organism, however, failed to grow when picked into milk and the possibility of confirmation was lost.

With the object of encouraging the multiplication of any lactobacilli which may have been present in the milks in very small numbers, all the milk samples were incubated at 30°C. for several weeks. Unfortunately there was no opportunity later for the examination of the samples.

It will be convenient here to review briefly the present position of knowledge with regard to types of bacteria found in milk drawn under aseptic conditions. Owing to the conditions prevailing in the normal udder, the number of types of bacteria found there is strictly limited. Three main types have been recognised, namely, micrococci, streptococci and rod forms related to Brucella abortus (Meyer & Shaw) and to Bacterium lipolyticus (Evans).

Micrococci.

The group usually constitutes the greatest proportion of the udder organisms. Gorini found a preponderance of these organisms in aseptically drawn milk. Dorner (1930) summarized the results of other workers as follows:-

TABLE XVIII.

Author.		Percentage of micrococci among cultures isolated.
von Freudenreich	(1902)	nearly 100
Lux	(1903)	90 - 95
Esten & Mason	(1908)	95
Harding & Wilson	(1913)	75
Evans	(1916)	58.8 *
Burri & Hohl	(1917)	82.5 *

* These numbers are percentages of micrococci in the total number of samples examined.

Dorner (1930) in a study of 993 samples of aseptically drawn milk from 132 cows in New York State, found the micrococci to be the most common type on standard agar plates but the least common type on Burri slants. Later, in Switzerland, a study of 944 samples by the Burri method showed the micrococci to be the most abundant type.

Table XII of the present investigation shows that the micrococci undoubtedly predominated in both raw and pasteurised milk samples. They comprised from 93 to 100 per cent. of the flora of the raw milk samples and from 75 to 100 per cent. of the flora of the pasteurised milk samples. Three types of micrococci were observed, "White," "Yellow" and "Orange." In almost every instance the "Yellow" micrococci were present in greater numbers than the "White". The "Orange" micrococci were present in numbers varying from 1 to 10 per cent in 60% of the raw milk samples, but were absent in the pasteurised milk samples. It appeared therefore that the "Orange" organisms were unable to withstand pasteurisation although their absence in the pasteurised milk may have been only apparent and have been due not to their destruction but to an alteration in their pigment-producing properties. The "Yellow" micrococci appeared to have greater powers of heat resistance than the "White." No more detailed examination of these organisms was made mainly on account of the fact that there is no satisfactory classification at present.

Streptococci.

Sherman & Hastings (1915) found streptococci in 38.6% of the samples from 88 cows of 4 herds. Evans (1916) found streptococci in 15.1% of 192 samples from 161 cows. Dorner (1930) examined 993 samples from 132 cows of 6 herds and obtained for streptococci an average frequency of 15.8% on Burri slants and 12.1% on standard agar plates.

Streptococcus lactis has been found only in a few exceptional cases. Evans concluded that "Streptococcus lactis does not localize and multiply in the udder." In many cases it is doubtful whether steptococci isolated from

apparently normal udders were parasitic or potentially pathogenic. When found they were usually present in large numbers.

As has been stated before, no streptococci were isolated from the samples examined in the present work. This is not surprising for it must be remembered that all the cows in the herd had been under constant veterinary observation for several years and that direct microscopic examination of the herd milk was made daily. There was no difficulty, therefore, in excluding from the group of cows used for this work, any animals which on any occasion had shown symptoms of udder disease or abnormal secretion.

Rod Forms.

Evans (1918a) described a number of Gram-positive abortus-like organisms to which she gave the name Bacterium lipolyticus. They were present in 23.4% of samples from 161 cows. The presence of similar bacteria in milk was later reported by Steck (1921). Dorner (1930) found that his samples contained rods of this type in 34.8% of cases when examined by the Burri slant method and in 8.8% of cases by the standard agar plate method. In his later work in Switzerland he found the rods in 15.4% of the samples examined by the Burri method. In the present work rods of this type were found in numbers varying from 1 to 25% in 40% of the raw and pasteurised milk samples. It is apparent that the rods were able to withstand pasteurisation at 145°F. for 30 mins. Detailed characteristics of the organisms are given in Table XIV.

BACTERIOLOGICAL EXAMINATION OF THE CHEESE.

Sampling.

A pair of forceps, a scalpel and a cheese "trier" were sterilised in boiling water for five minutes. The lard was removed from a small area on the side of the cheese

and the rind treated with a strong solution of hypochlorite. By means of the scalpel and forceps a portion of the rind about 1/8" in thickness was raised and the cooled trier inserted. A plug was drawn, the outer half inch was discarded and the remainder was placed in a sterile sample bottle. The plug hole was filled with melted paraffin wax and the rind was replaced. Subsequent plugs were taken at intervals round the circumference of the cheese about one third of the distance from either end. In order to prevent contamination, the plugs taken for bacteriological examination were spaced as widely apart as possible and always drawn before those used for chemical analysis. For the same reason, not more than two bacteriological examinations were performed on any one cheese; other cheeses in the same series were used for further examinations. For most of the bacteriological examinations two plugs representing a total of 20 - 25 grms. were taken for each examination. In the later examinations only one plug was used. The "trier" used reached to the centre of the cheese.

Bacteriological examination.

The cheeses were examined at 2 days, 6 days, 16 days, 1 month and 2 months. The sample plugs in each case were thoroughly ground in a sterile mortar with 100 c.c. of sterile water at approximately 30°C., care being taken to avoid contamination. The emulsion so obtained was transferred to a sterile rubber stoppered bottle. Total and volatile acid production of the mixture of organisms present in the cheese were determined by inoculation of 1 c.c. of the emulsion into 200 c.c. sterile milk, incubation at 22°C. and subsequent examination of the milk as previously described. "Sugar" tubes were also inoculated from the cheese emulsion. The results of both these examinations are given in Tables XIX to XXII.

TABLE XIX.

Sugar Fermentation, Acid and Volatile Acid Results of Cheese Emulsion I.

Cheese	Age	Incubation Period	Glycerin	Xylose	Arabinose	Rhamnose	Sorbite	Mannite	Dulcite	Laevulose	Dextrose	Mannose	Galactose	Saccharose	Maltose	Lactose	Raffinose	Inulin	Dextrin	Starch	Salicin	Total acid produced as % lactic acid.	Volatile acid produced as % lactic acid.	Volatile acid produced as % total acid.
1-15-11	2 days																					0.50	0.0057	1.1
	6 days																					0.57	0.0150	2.6
2-15-11	16 days	3 days	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	-	-	-	+	0.65	0.0162	2.5
		7 "	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	-	-	-	+			
		14 "	-	-	L	-	L	+	-	+	+	+	+	+	+	+	+	-	-	-	+			
	1 month	3 days	-	-	-	-	1	1	-	+	+	+	1	+	+	+	+	-	-	-	+	0.65	0.0414	6.4
		7 "	-	-	-	-	L	L	-	+	+	+	L	+	+	+	+	-	-	-	+			
		14 "	-	-	-	-	+	+	-	+	+	+	+	+	+	+	+	-	-	-	+			
3-15-11	2 months	3 days	-	-	-	-	L	L	-	+	+	+	L	+	+	+	+	-	-	-	+	0.89	0.1080	12.1
		7 "	-	-	-	-	+	+	-	+	+	+	+	+	+	+	+	-	-	-	+			
		14 "	-	-	-	-	+	+	-	+	+	+	+	+	+	+	+	-	-	-	+			

TABLE XX.

Sugar Fermentation, Acid and Volatile Acid Results of Cheese Emulsion II.

Cheese	Age	Incubation Period	Glycerin	Xylose	Arabinose	Rhamnose	Sorbite	Mannite	Dulcite	Laevulose	Dextrose	Mannose	Galactose	Saccharose	Maltose	Lactose	Raffinose	Inulin	Dextrin	Starch	Salicin	Total acid produced as % lactic acid.	Volatile acid produced as % lactic acid.	Volatile acid produced as % total acid.
Pasteurised Milk	..	....								...				...				...				0.38	0.0060	1.6
1-29-11	2 days	3 days	-	-	-	-	-	-	-	+	+	+	+	-	-	+	-	-	-	-	-			
		7 "	-	-	-	-	-	-	-	+	+	+	+	-	L	+	-	-	-	-	+	0.67	0.0168	2.5
		14 "	-	-	-	-	-	1	-	+	+	+	+	-	+	+	-	-	-	-	+			
	6 days	3 "	-	-	-	-	-	-	-	L	L	L	L	L	L	L	-	-	-	-	-			
		7 "	-	-	-	-	+		-	+	+	+	+	+	+	+	-	-	-	-		0.69	0.0174	2.5
		14 "	-	-	-	-	+	+	-	+	+	+	+	+	+	+	+	+	L	-	+			
2-29-11	16 days	3 "	-	-	-	-	-	-	-	L	L	L	1	1	L	L	-	-	-	-	L			
		7 "	-	-	-	-	-	1	-	+	+	+	L	L	+	+	-	-	-	-	+	0.66	0.0372	5.6
		14 "	-	-	-	-	L	+	-	+	+	+	+	+	+	+	+	+	+	-	+			
	1½ mths	3 "	-	-	-	-	+	+	-	+	+	+	+	+	+	+	-	+	-	-	+			
		7 "	-	-	-	-	+	+	-	+	+	+	+	+	+	+	-	+	-	-	+	0.70	0.1068	15.3
		14 "	-	-	-	-	+	+	-	+	+	+	+	+	+	+	-	+	-	-	+			
3-29-11	2 mths	3 "	-	-	-	-	-	L	-	+	+	+	L	+	+	+	-	-	-	-	+			
		7 "	-	-	-	-	-	+	-	+	+	+	L	+	+	+	-	-	-	-	+	0.88	0.1113	12.7
		14 "	-	-	-	-	1	+	-	+	+	+	+	+	+	+	-	-	-	-	+			
	2½ mths	3 "	-	-	-	-	+	+	-	+	+	+	L	+	+	+	-	+	-	-	-			
		7 "	-	-	-	-	+	+	-	+	+	+	L	+	+	+	-	+	-	-	L	0.90	0.0978	10.9
		14 "	-	-	-	-	+	+	-	+	+	+	+	+	+	+	-	+	-	-	L			

TABLE XXI.

Sugar Fermentation, Acid and Volatile Acid Results of Cheese Emulsion III.

Cheese	Age	Incubation Period	Glycerin	Xylose	Arabinose	Rhamnose	Sorbite	Mannite	Dulcite	Laevulose	Dextrose	Mannose	Galactose	Saccharose	Maltose	Lactose	Raffinose	Inulin	Dextrin	Starch	Salicin	Total acid produced as % lactic acid.	Volatile acid produced as % lactic acid.	Volatile acid produced as % total acid.
Pasteurised Milk	..				....					...				...				...				0.18	0.0126	7.0
1-10-1	2 days	3 days	-	-	-	-	-	L	-	+	+	+	+	-	L	+	-	-	-	-	-			
		7 "	-	-	-	-	-	+	-	+	+	+	+	L	+	+	-	-	-	-	1	0.71	0.0420	5.9
		14 "	-	-	-	-	-	+	-	+	+	+	+	+	+	+	-	-	-	-	1			
	6 days	3 "	-	-	-	-	L	L	-	+	+	+	L	+	+	+	-	-	-	-	+			
		7 "	-	-	-	-	+	+	-	+	+	+	+	+	+	+	-	-	-	-	+	0.79	0.0366	4.6
		14 "	-	-	-	-	+	+	+	+	+	+	+	+	+	+	-	-	-	-	+			
	16 days	3 "	-	-	-	-	-	L	-	+	+	+	L	+	+	+	-	-	-	-	+			
		7 "	-	-	-	-	1	L	-	+	+	+	L	+	+	+	-	-	-	-	+	0.78	0.1156	14.9
		14 "	-	-	-	-	+	+	-	+	+	+	+	+	+	+	-	-	-	-	+			
2-10-1	1 mth.	3 "	-	-	-	-	-	L	-	+	+	+	L	+	+	+	-	-	-	-	+			
		7 "	-	-	-	-	-	L	-	+	+	+	L	+	+	+	-	-	-	-	+	0.79	0.0825	10.4
		14 "	-	-	-	-	-	+	-	+	+	+	L	+	+	+	-	-	-	-	+			

TABLE XXII.

Sugar Fermentation, Acid and Volatile Acid Results of Cheese Emulsion IV.

Cheese	Age	Incubation Period	Glycerin	Xylose	Arabinose	Rhamnose	Sorbite	Mannite	Dulcite	Laevulose	Dextrose	Mannose	Galactose	Saccharose	Maltose	Lactose	Raffinose	Inulin	Dextrin	Starch	Salicin	Total acid produced as % lactic acid.	Volatile acid, produced as % lactic acid.	Volatile acid produced as % total acid.
Raw Milk		..	....							...				...				...				0.64	0.0144	2.3
1-i-11	2 days																					0.16	0.0300	16.7
	6 days																							
2-i-11	16 days																					0.69	0.0705	10.2
	1 month																					0.80	0.0732	9.2
3-i-11	2½ mths.	3 days	-	-	-	-	+	+	-	+	+	+	⌐	+	+	+	-	-	-	-	+	0.86	0.1101	12.8
		7 "	-	-	-	-	+	+	-	+	+	+	+	+	+	+	-	⌐	-	-	+			
		14 "	-	-	-	-	+	+	-	+	+	+	+	+	+	+	-	+	+	-	+			

A large loopful of the cheese emulsion was streaked on both casein agar and standard agar plates. The plates were incubated for 6 days at 30°C. and the types of organisms present were then determined by the same methods as were used in the examination of the milk plates. The numbers of each type were also estimated as before. The percentages of the various types present are given in Table XXIII.

TABLE XXIII.

Percentage Distribution of Types of Bacteria present in the Cheeses.

Cheese Group	Cheese	Age	Distribution	per cent.
I	1-15-11	2 days	Streptococci	100
			Micrococci ("White")	< 1
		9 days	Streptococci	85
			Lactobacilli	15
			Micrococci ("White")	< 1
	2-15-11	16 days	Streptococci	30
			Lactobacilli	70
			Micrococci ("White")	< 1
		1 month	Lactobacilli	100
II	1-29-11	2 days	Streptococci	95
			Micrococci ("White")	5
		6 days	Streptococci	80
			Lactobacilli	20
	2-29-11	16 days	Streptococci	10
			Lactobacilli	90
		1½ mths.	Lactobacilli	100
	3-29-11	2 mths.	Streptococci	15
			Lactobacilli	85
		2½ mths.	Lactobacilli	100
III	1-10-1	2 days	Streptococci	100
			Lactobacilli	< 1
		16 days	Streptococci	90
			Lactobacilli	10
	2-10-1	1 month	Streptococci	80
			Lactobacilli	20

TABLE XXIII (Continued).

Cheese Group	Cheese	Age	Distribution	per cent.
IV	1-1-11	2 days	Streptococci	60
			Micrococci ("White")	38
			Micrococci ("Yellow")	2
		6 days	Streptococci	98
			Micrococci ("White")	2
			Micrococci ("Yellow")	<1
	2-1-11	16 days	Streptococci	35
			Micrococci ("White")	35
			Lactobacilli	30
		1 month	Streptococci	10
			Micrococci ("White")	<1
			Lactobacilli	90
	3-1-11	2½ mths.	Lactobacilli	100
V	1-31-1	2 days	Streptococci	100
		6 days	Streptococci	100

As before, representatives of each type were picked off and sown into milk and on to casein agar and standard agar slopes, so that morphology, cultural characteristics and biochemical reactions could be studied in detail. The results are summarized in Tables XXIV to XXVIII.

TABLE XXIV.

Characters of Organisms isolated from Cheese I.

Organism A.

Cheese: 2-15-11 Age: 14 days.

Morphology: (a) Casein agar.

Rods approximately 2 to 3 μ by 0.8 to 1 μ ; straight and slightly curved. Majority in medium, long and very long chains; chains well twisted.

(b) Milk.

Rods 2 to 6 μ by 0.8 to 1 μ ; straight and slightly twisted; many in chains of 2, 3 and 4.

Casein agar: Good growth in 2 days. At 5 days whitish, opaque, spherical colony with entire margin.

TABLE XXIV (Continued)

Milk: Smooth clot in 3 days.

Acid produced: 0.60 per cent.

Volatile acid produced: 0.139 " "

Percentage vol.A/A: 23.1 " "

Fermentation of Sugars:

Time of Incubation	Glycerin	Xylose	Arabinose	Rhamnose	Sorbite	Mannite	Dulcite	Laevulose	Dextrose	Mannose	Galactose	Saccharose	Maltose	Lactose	Raffinose	Inulin	Dextrin	Starch	Salicin
3 days	-	-	-	-	-	+	-	+	+	+	L	+	+	L	-	-	-	-	+
7 " "	-	-	-	-	-	+	-	+	+	+	L	+	+	L	-	-	-	-	+
14 " "	-	-	-	-	-	+	-	+	+	+	+	+	+	+	-	-	-	-	+
21 " "	-	-	-	-	-	+	-	+	+	+	+	+	+	+	-	-	-	-	+
28 " "	-	-	-	-	-	+	-	+	+	+	+	+	+	+	-	-	-	-	+

Organism B.

Cheese: 2-15-11. Age: 1 month.

Morphology: (a) Casein agar.

Rods 1.5 to 2.5 μ by 1 μ ; straight. A large number of chains of 3 and 4; chains slightly twisted.

(b) Milk.

Rods 2 to 2.5 μ by 1 μ ; straight and slightly curved; occasional rods 4 in length. Majority occur singly; a number of chains of 2 and 3.

Casein agar: Good growth in 2 days. Colony translucent to opaque, spherical with entire margin.

Milk: Clot in 6 days.

Acid produced: 0.47 per cent.

Volatile acid produced: 0.035 " "

Percentage vol.A/A: 7.4 " "

TABLE XXIV (Continued)

Fermentation of Sugars:

Time of Incubation	Glycerin	Xylose	Arabinose	Rhamnose	Sorbite	Mannite	Dulcite	Laevulose	Dextrose	Mannose	Galactose	Saccharose	Maltose	Lactose	Raffinose	Inulin	Dextrin	Starch	Salicin
3 days	-	-	-	-	-	+	-	+	+	+	+	-	+	+	-	-	+	+	+
7	-	-	-	-	-	+	-	+	+	+	+	-	+	+	-	-	+	+	+
14	-	-	-	-	-	+	-	+	+	+	+	-	+	+	-	-	+	+	+
21	-	-	-	-	-	+	-	+	+	+	+	-	+	+	-	-	+	+	+
28	-	-	-	-	-	+	-	+	+	+	+	-	+	+	-	-	+	+	+

TABLE XXV.

Characters of Organisms isolated from Cheese II.

Organism C.

Cheese: 2-29-11. Age: 1½ months.

Morphology: (a) Casein agar.

Rods 2.5 to 3.5 μ by 1 μ . Majority in medium and long chains; chains slightly twisted. Cultures sometimes show short stout rods with well rounded ends.

(b) Milk.

Rods 4 to 6 μ by 0.8 to 1 μ . A small number of twisted chains of 2 and 3.

Casein agar: Good growth in 2 days. At 5 days colony opaque, spherical, with entire margin.

Milk: Clot in 3 days.

Acid produced: 0.73 per cent.

Volatile acid produced: 0.123 " "

Percentage vol. A/A: 16.9 " "

Fermentation of sugars:

Time of Incubation	Glycerin	Xylose	Arabinose	Rhamnose	Sorbite	Mannite	Dulcite	Laevulose	Dextrose	Mannose	Galactose	Saccharose	Maltose	Lactose	Raffinose	Inulin	Dextrin	Starch	Salicin
3 days	-	-	-	-	1	1	-	+	+	+	L	+	+	+	-	-	-	-	+
7 "	-	-	-	-	L	L	-	+	+	+	L	+	+	+	-	-	-	-	+
14 "	-	-	-	-	+	+	-	+	+	+	+	+	+	+	-	-	-	-	+
21 "	-	-	-	-	+	+	-	+	+	+	+	+	+	+	-	-	-	-	+
28 "	-	-	-	-	+	+	-	+	+	+	+	+	+	+	-	-	-	-	+

Organism D.

Cheese: 3-29-11. Age: 2 months.

Morphology: (a) Casein agar.

Rods 2.5 to 3.5 μ by 0.8 to 1 μ ; many twisted. Long twisted chains.

(b) Milk.

Rods 3 μ by 0.5 to 0.7 μ . Numerous long chains.

TABLE XXV (Continued)

Casein agar: Comparatively poor growth; not visible in two days. Characteristic colony; at 6 days small, translucent, with undulate to ragged margin; never opaque even on prolonged incubation.

Milk: Clot in 2 days.

Acid produced: 0.96 per cent.

Volatile acid produced: 0.016 " "

Percentage vol.A/A: 1.6 " "

Fermentation of Sugars:

Time of Incubation	Glycerin	Xylose	Arabinose	Rhamnose	Sorbite	Mannite	Dulcite	Laevulose	Dextrose	Mannose	Galactose	Saccharose	Maltose	Lactose	Raffinose	Inulin	Dextrin	Starch	Salicin
3 days	-	-	-	-	-	-	-	L	+	+	L	-	1	1	-	L	-	-	-
7 "	-	-	-	-	-	-	-	+	+	+	+	1	+	L	-	+	-	-	-
14 "	-	-	-	-	-	1	-	+	+	+	+	L	+	L	-	+	-	-	1
21 "	-	-	-	-	-	L	-	+	+	+	+	+	+	+	-	+	-	-	
28 "	-	-	-	-	1	+	-	+	+	+	+	+	+	+	-	+	-	-	+

Organism E.

Cheese: 3-29-11- Age: 2 months.

Morphology: (a) Casein agar.

Rods 2.5 to 3.5 μ by 0.8 to 1 μ ; straight. No chains.

(b) Milk.

Rods 2.5 to 3.5 μ by 0.8 to 1 μ ; straight. Majority occur singly; a small number of straight chains of 2 and 3.

Casein agar: Good growth in 2 days. At 5 days small, translucent spherical colony with entire margin; older colonies translucent to opaque.

Milk: Clot in 8 days.

Acid produced: 0.33 per cent.

Volatile acid produced: 0.035 " "

Percentage vol.A/A: 10.5 " "

TABLE XXV. (Continued)

Fermentation of Sugars:

Time of Incubation	Glycerin	Xylose	Arabinose	Rhamnose	Sorbite	Mannite	Dulcite	Laevulose	Dextrose	Mannose	Galactose	Saccharose	Maltose	Lactose	Raffinose	Inulin	Dextrin	Starch	Salicin
3 days	-	-	-	-	-	⊥	-	⊥	⊥	L	L	+	+	1	-	-	-	-	L
7 "	-	-	-	-	-	+	-	+	+	+	⊥	+	+	1	-	-	-	-	+
14 "	-	-	-	-	-	+	-	+	+	+	+	+	+	L	-	-	-	-	+
21 "	-	-	-	-	-	+	-	+	+	+	+	+	+	+	-	-	-	-	+
28 "	-	-	-	-	-	+	-	+	+	+	+	+	+	+	-	-	-	-	+

Organism F.

Cheese: 3-29-11. Age: 2½ months.

Morphology: (a) Casein agar.

Rods 2.5 to 3.5 μ by 0.8 μ ; straight and slightly curved. Majority present in long twisted chains.

(b) Milk.

Rods 2.5 to 3.5 μ by 0.5 to 0.8 μ . Numerous long chains.

Casein agar: Poor growth in two days; at 5 days colony translucent to opaque, spherical with entire margin; colony opaque at centre but always translucent to transparent at margin.

Milk: Clot in 3 days.

Acid produced: 0.71 per cent.

Volatile acid produced: 0.022 " "

Percentage vol. A/A: 3.0 " "

Fermentation of Sugars:

Time of Incubation	Glycerin	Xylose	Arabinose	Rhamnose	Sorbite	Mannite	Dulcite	Laevulose	Dextrose	Mannose	Galactose	Saccharose	Maltose	Lactose	Raffinose	Inulin	Dextrin	Starch	Salicin
3 days	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-
7 days	-	-	-	-	-	L	-	L	1	1	L	-	-	+	-	-	-	-	-
14 "	-	-	-	-	-	+	-	+	+	+	+	-	1	+	-	-	-	-	-
21 "	-	-	-	-	-	+	-	+	+	+	+	-	+	+	-	-	-	-	L
28 "	-	-	-	-	-	+	-	+	+	+	+	-	+	+	-	-	-	-	+

TABLE XXVI.

Characters of Organisms isolated from Cheese III.

Organism G.

Cheese: 1-10-1 Age: 2 days.

Morphology: (a) Casein agar.

Rods 1 to 2 μ by 1 μ ; a number appearing like oval cocci. Numerous twisted chains.

(b) Milk.

Rods 2 to 6 μ by 1 μ ; straight and twisted. A number of chains of 2 and 3; many twisted.

Casein agar: Good growth in 3 days. At 5 days opaque, spherical colony with entire margin.

Milk: Clot in 4 days.

Fermentation of Sugars.

Time of Incubation	Glycerin	Xylose	Arabinose	Rhamnose	Sorbite	Mannite	Dulcite	Laevulose	Dextrose	Mannose	Galactose	Saccharose	Maltose	Lactose	Raffinose	Inulin	Dextrin	Starch	Salicin
3 days	-	-	-	-	1	L	-	+	+	+	1	+	+	+	-	-	-	-	L
7 "	-	-	-	-	+	+	-	+	+	+	L	+	+	+	-	-	-	-	+
14 "	-	-	-	-	+	+	-	+	+	+	L	+	+	+	-	-	-	-	+
21 "	-	-	-	-	+	+	-	+	+	+	+	+	+	+	-	-	-	-	+
28 "	-	-	-	-	+	+	-	+	+	+	+	+	+	+	-	-	-	-	+

Organism H.

Cheese: 1-10-1 Age: 16 days.

Morphology: (a) Casein agar.

Rods 2 to 3 μ by 0.8 to 1 μ ; occurring chiefly singly; a few short and long chains; chains straight and twisted.

(b) Milk.

Rods 2 to 6 μ by 0.8 to 1 μ ; numerous twisted chains of 2, 3 and 4.

Casein agar: Good growth in 2 days. At 5 days whitish, opaque, spherical colony with entire margin.

Milk: Clot in 4 days.

TABLE XXVI (Continued)

Acid produced: 0.53 per cent.

Volatile acid produced: 0.099 " "

Percentage vol.A/A: 18.7 " "

Fermentation of Sugars:

Time of Incubation	Glycerin	Xylose	Arabinose	Rhamnose	Sorbite	Mannite	Dulcite	Laevulose	Dextrose	Mannose	Galactose	Saccharose	Maltose	Lactose	Raffinose	Inulin	Dextrin	Starch	Salicin
3 days	-	-	-	-	-	+	-	+	+	+	-	+	+	+	-	-	-	-	+
7 "	-	-	-	-	-	+	-	+	+	+	-	+	+	+	-	-	-	-	+
14 "	-	-	-	-	-	+	-	+	+	+	-	+	+	+	-	-	-	-	+
21 "	-	-	-	-	-	+	-	+	+	+	+	+	+	+	-	-	-	-	+
28 "	-	-	-	-	-	+	-	+	+	+	+	+	+	+	-	-	-	-	+

Streptococci.

A number of streptococci were isolated and studied fully. All were found to be identical with the starter organism in morphological, cultural and biochemical characters. The sugar fermentation and acid and volatile acid results of one of the cultures are given below.

Acid produced: 0.56 per cent.
Volatile acid produced: 0.008 " "
Percentage vol.A/A: 1.4 " "

Fermentation of Sugars:

Time of Incubation	Glycerin	Xylose	Arabinose	Rhamnose	Sorbite	Mannite	Dulcite	Laevulose	Dextrose	Mannose	Galactose	Saccharose	Maltose	Lactose	Raffinose	Inulin	Dextrin	Starch	Salicin
3 days	-	-	-	-	-	-	-	-	+	+	-	-	-	+	-	-	-	-	-
7 "	-	-	-	-	-	-	-	+	+	+	-	-	-	+	-	-	-	-	-
14 "	-	-	-	-	-	-	-	+	+	+	+	-	-	+	-	-	-	-	-

TABLE XXVII.

Characters of Organisms Isolated from Cheese IV.

Organism M.

Cheese 1-1-11- Age: 2½ months.

Morphology: (a) Casein agar.

Rods 1.5 to 2.5 μ by 1 to 1.3 μ ; straight. Numerous twisted chains of 3 and 4; a small number of long twisted chains.

(b) Milk.

Rods 2 to 6 μ by 1 μ ; straight and slightly curved. A small number of chains of 2 and 3.

Casein agar: Good growth in 2 days. At 5 days opaque, spherical colony with entire margin.

Milk: Clot in 3 days.

Acid produced: 0.53 per cent.

Volatile acid produced: 0.044 " "

Percentage vol.A/A: 8.2 " "

Fermentation of Sugars:

Time of Incubation	Glycerin	Xylose	Arabinose	Rhamnose	Sorbite	Mannite	Dulcite	Laevulose	Dextrose	Mannose	Galactose	Saccharose	Maltose	Lactose	Raffinose	Inulin	Dextrin	Starch	Salicin
3 days	-	-	-	-	L	1	-	⊥	+	⊥	1	+	+	+	-	-	-	-	+
7 "	-	-	-	-	+	+	-	+	+	+	⊥	+	+	+	-	-	-	-	+
14 "	-	-	-	-	+	+	-	+	+	+	+	+	+	+	1	-	-	-	+
21 "	-	-	-	-	+	+	-	+	+	+	+	+	+	+	L	-	-	-	+
28 "	-	-	-	-	+	+	-	+	+	+	+	+	+	+	+	-	-	-	+

White Micrococcus.

Morphology: Gram-positive coccus 1 μ in diameter.

Casein agar: Luxuriant growth; colony large, white, opaque, glistening with entire margin.

Litmus milk: Slow growth; no change in 14 days.

Gelatine: Slow liquefaction.

Indole: Positive.

TABLE XXVII (Continued)

Fermentation of Sugars:

Dulcite, dextrin, lactose and saccharose were not attacked although rapid growth occurred.

Yellow Micrococcus.

The Yellow Micrococcus exhibited similar characters to the White, the only difference being the size of the cells (0.6 μ).

TABLE XXVIII.

Characters of Organisms isolated from Cheese V.

Streptococci.

A number of streptococci were isolated and studied fully. All were found to be identical with the starter organism in morphological, cultural and biochemical characters. The sugar fermentation and acid and volatile acid results of one of the cultures are given below.

Acid produced: 0.64 per cent.

Volatile acid produced: 0.017 " "

Percentage vol. A/A: 2.7 " "

Fermentation of Sugars:

Time of Incubation	Glycerin	Xylose	Arabinose	Rhamnose	Sorbite	Mannite	Dulcite	Laevulose	Dextrose	Mannose	Galactose	Saccharose	Maltose	Lactose	Raffinose	Inulin	Dextrin	Starch	Salicin
3 days	-	-	-	-	-	-	-	L	+	+	-	-	-	+	-	-	-	-	-
7 "	-	-	-	-	-	-	-	+	+	+	1	-	-	+	-	-	-	-	-
14 "	-	-	-	-	-	-	-	+	+	+	+	-	1	+	-	-	-	-	-

At first it was intended to differentiate the lactobacilli found in the cheese into various species according to morphology and colony characteristics. This however, was found to be impossible for there was a decided inconstancy in the morphological characters of the bacilli. Further, in the absence of important data such as the type of lactic acid produced and the amount of soluble and amino nitrogen formed in milk it was not possible to separate the organisms into species depending on their biochemical characteristics. All that can be said of the cheese lactobacilli, therefore, is that they all belonged to the genus Streptobacterium (Orla-Jensen 1919). This classification was made on the basis of the optimum growth temperature which was lower than that of the Thermobacteria and on the fermentation of salicin and absence of gas production which differentiated them from the Betabacteria. The results for each cheese will now be reviewed in detail.

Cheese I.

The pasteurised milk used in the manufacture of this group of cheeses gave a count of 29 to 92 bacteria per cubic centimetre, approximately 80% of which were "Yellow" and 20% were "White" micrococci. After two days the flora of the cheese was composed of streptococci together with less than 1% of "White" micrococci After nine days lactobacilli were present to the extent of 15%. This observation was confirmed by the increase in the total and volatile acid figures in the milk culture of the cheese emulsion made at this time, for it was afterwards found that most of the lactobacilli present in the cheeses gave high volatile acid figures, while all the streptococci gave low figures. The streptococci still predominated in the cheese and only one colony of micrococci was found. After 16 days "White" micrococci still persisted in very small numbers while lactobacilli constituted 70% of the flora, an apparently very rapid increase.

It should, however, be borne in mind that the streptococci at this period were dying and that the total number of colonies was diminishing. This factor accounted partly for the increase in percentage of lactobacilli. In all the experiments it was found that the numbers of colonies on the plates decreased progressively during the first two or three weeks after the manufacture of the cheese. Thereafter the numbers increased steadily throughout the period of examination (2½ months). The method of enumerating colonies by smearing loopfuls on plates is of course somewhat rough, but it is an adaptation of the Burri method which seems to have proved satisfactory in some investigations.

After one month the flora consisted entirely of lactobacilli. This group of cheeses thus showed the most rapid disappearance of the streptococci which was observed during the work. The increase in the proportion of lactobacilli was corroborated at each stage by an increase in total acid and volatile acid figures in milk cultures made from the cheese emulsion.

After two months the flora still consisted entirely of lactobacilli and the total and volatile acid production in the milk culture showed a further increase.

The sugar fermentations given by the cheese emulsions are given in Table XIX. Sugars were not sown from the earlier emulsions, but it will be noticed that the organisms in the 14 day emulsion fermented saccharose and salicin readily and sorbite and mannite more slowly. Later emulsions from this group of cheeses gave a more rapid fermentation of sorbite and mannite. It will be remembered that the original starter organism was unable to attack sorbite, mannite, saccharose and salicin. If therefore the presence of micrococci is neglected (only one colony was found on the plates) the sugar fermentations may be taken as indicating the presence and gradual multiplication of lactobacilli. It was found, in fact, that the sugar reactions of the lactobacilli isolated from this cheese in pure culture accounted for the fermentation of all the sugars except sorbite.

Two varieties of lactobacilli (A and B) were isolated and their characteristics studied in detail. The

results are given in Table XXIV. Both the organisms were classified as Streptobacteria.

Organism A fermented saccharose, maltose, lactose, mannite and salicin as well as all the hexoses. Of the four hexoses, galactose was the least readily fermented. This latter fact applies to all the lactic acid bacteria tested and is in agreement with Orla-Jensen's observation (1919) that of the four hexoses, galactose is, as a rule, the least fermented by the lactic acid bacteria. Organism A was the highest volatile acid producer isolated. Its total acid production in milk was also rapid and comparatively high. Organism A is probably identical with organism H isolated from Cheese III.

Organism B fermented dextrin and starch only slowly and almost failed to attack saccharose. It was not so active an acid producer in milk and took six days at 30°C. to form a clot. Its volatile acid production was comparatively low.

The only discrepancy to be observed when the fermentation reactions of organisms A and B are compared with the reactions of the cheese emulsion is in the matter of sorbite. This may be due to failure to isolate some sorbite fermenting organism or to a symbiotic fermentation of sorbite by the mixture of organisms in the cheese emulsion. The former explanation would seem the more probable since in all the other groups of cheese sorbite-fermenting lactobacilli were found.

Cheese II.

The pasteurised milk used in the manufacture of this group of cheeses contained from 3 to 5 organisms per cubic centimetre consisting of approximately 37% of "Yellow" micrococci, 38% of "White" micrococci, and 25% of Bacterium X.

After two days the flora of the cheese was composed

of streptococci 95% and "White" micrococci 5%. After six days lactobacilli were present to the extent of 20%. The micrococci had disappeared, the remaining 80% being streptococci. The total and volatile acid figures of the milk culture showed an increase. After 16 days lactobacilli constituted 90% of the flora and there was a coincident increase in the volatile acid figure of the milk culture. After 1½ months the flora consisted solely of lactobacilli and the volatile acid production in the milk culture reached 0.113%.

Another cheese of the same group examined when 2 months old gave 15% of streptococci but at 2½ months no streptococci were found in the same cheese.

Although Bacterium X was present in the pasteurised milk it was never isolated from any of the cheeses of this group or indeed from any cheese whatever made during the course of the work. This is not altogether surprising when the characteristics of the organism are considered (Table XIV). It grew very poorly in milk and sometimes failed to grow altogether. Even at 22°C. its growth was very slow (its optimum temperature was 30 - 37°C.). so that at 15.5°C., the ripening temperature of the cheese, growth in the not very favourable medium would probably fail very soon.

The sugar fermentations of the cheese emulsions and the total and volatile acid production in milk cultures of the cheese emulsions, again fitted in with the gradual rise in numbers of lactobacilli.

Four strains of lactobacilli C, D, E and F were isolated and studied in detail. Organism C was characterized by its fermentation of sorbite and by high acid and volatile acid production in milk. It was probably identical with organism G isolated from Cheese III.

Organism D differed markedly from all the other lactobacilli which were isolated. It was characterized

by the nature of its colony on casein agar, its morphology in milk cultures, its fermentation of inulin and its low volatile acid production. It was the most active total acid producer of all the lactobacilli. It preferred maltose and lactose and attacked saccharose only slowly.

Organism E clotted milk only after 8 days at 30°C. or 21 days at 22°C. Its volatile acid production was relatively high. The organism showed a preference for saccharose and maltose and fermented lactose only slowly. It did not attack sorbite.

Organism F was characterized by rapid fermentation of lactose, slow fermentation of maltose and refusal to attack saccharose. It was a high acid producer but formed only small amounts of volatile acid.

It will be observed that the earlier cheese emulsions fermented raffinose, dextrin and starch, whereas no organisms capable of fermenting these sugars were ever isolated. No bacteria, however, were isolated until the cheese was 1½ months old. It appears probable therefore that the organisms capable of fermenting these three sugars died out in the cheese or became so few in number that they escaped observation.

Cheese III.

The pasteurised milk contained approximately 458 organisms per cubic centimetre consisting of 98% "Yellow" micrococci, less than 1% "White" micrococci and less than 1% Bacterium X.

After two days the cheese flora consisted almost entirely of streptococci with less than 1% of lactobacilli. The presence of the lactobacilli presumably accounted for the fermentation of mannite and saccharose by the cheese emulsion and the production of a large proportion of volatile acid in the milk culture.

After 16 days the lactobacilli had increased to

10% of the total flora and the total and volatile acid production showed an increase. After one month the flora consisted of 20% lactobacilli and 80% streptococci. Micrococci and Bacterium X were not found in any examination of this group of cheeses. The streptococci persisted in greater numbers and presumably for a longer period than in any of the other groups of cheeses. It is interesting to note that in all the groups of cheese there is correlation between the rate of disappearance of the streptococci and the activity of the starter in the vat and also between the rate of disappearance and the moisture content of the cheese.

The total and volatile acid figures and the sugar fermentations corresponded with the presence and gradual increase of lactobacilli in the cheese. After two days mannite and saccharose were fermented slowly while the fermentation of salicin was indicated. After 6 days the rate of fermentation of mannite and saccharose had increased and sorbite and salicin were definitely fermented.

Two strains of lactobacilli (G and H) were isolated and studied in detail. Both of these strains appear to be identical with strains isolated from Cheese II - organism G was identical with organism C and organism H with E.

The sugar fermentations of the cheese emulsions were completely accounted for by the fermentations produced by the organisms isolated.

Cheese IV.

The pasteurised milk contained approximately 690 organisms per cubic centimetre, consisting of 95% "Yellow" micrococci and 5% "White" micrococci.

After two days the flora of the cheese consisted of 60% streptococci, 2% "Yellow" micrococci" and 38% "White"

micrococci many of which developed a faint tinge of yellow. After six days 97% of streptococci, 2% of "White" and less than 1% of "Yellow" micrococci were found.

After 16 days lactobacilli were present to the extent of 30%. There was a coincident increase in the volatile acid figure in the milk culture.

After 1 month the lactobacilli constituted 90% of the flora while after 2½ months lactobacilli were present in pure culture.

This group of cheeses was the first made and unfortunately the sugar reactions were not carried out until the cheese was 2½ months old. Only one culture, M, was isolated. The organism was characterized by very slow fermentation of raffinose.

Cheese V.

This cheese was the last made. It was not completely examined because of lack of time and the results are too meagre to be included in the Tables.

The pasteurised milk contained 252 organisms per cubic centimetre consisting of 70% "Yellow" and 30% "White" micrococci.

After 2 days and after 6 days the flora of the cheese consisted entirely of streptococci.

CHEMICAL METHODS.

Sampling.

Two plugs reaching to the centre of the cheese were drawn at points on the circumference one-third the height from either end and were placed in an air-tight sample bottle. The plugs were rapidly ground to a paste in a dry mortar, cut into small pieces by means of a palette knife and transferred immediately to the sample bottle. Analysis was carried out immediately. All determinations were done in duplicate. When unsatisfactory

duplicates were obtained, the determinations were repeated.

Moisture.

For the determination of moisture the vacuum method of the New Zealand Dairy Science Association was employed.

The sample (3 or 5 grams) was accurately weighed into a flat-bottomed 50 ml. flask. The upper portion of the neck of the flask was carefully wiped before the second weighing. The flask was placed in a steam oven for 2 hours, a current of air being blown through several times during this period. It was then attached to a vacuum pump and heated for a further 3 hours. The flask was allowed to cool in a desiccator and weighed. The percentage moisture content was calculated from the loss of weight and the weight of cheese taken.

Fat.

The sample (about 1.5 grams) was accurately weighed into an extraction tube. Five c.c. of distilled water, 10 c.c. of concentrated hydrochloric acid and a small piece of tile were added. The tube was heated gently over a sand-bath until all the cheese had dissolved, producing a clear pink or light brown aqueous layer. The mixture was cooled and the fat was extracted according to the Werner-Schmid procedure.

Total Nitrogen.

The percentage of total nitrogen was determined by the Kjeldahl method on a portion (about 1 gram) of the prepared sample, mercurous iodide being used as the catalyst. The percentage of protein was calculated from the total nitrogen content, using the factor 6.38.

Soluble Nitrogen.

From a weighing bottle the prepared sample (about 5 grams) was weighed accurately into a clean dry mortar. The mortar was placed in a water-bath maintained

at 60° C. By means of a pipette 25 c.c. of distilled water at 60°C. were added and the cheese was ground very thoroughly until complete mixing was obtained. Another 25 c.c. of distilled water were added and the grinding repeated. 50 c.c. of water were added with further grinding. The resultant mixture was filtered twice through dry filter-paper and 20 c.c. of the filtrate were used for each Kjeldahl determination. The percentage of total nitrogen present as soluble nitrogen was calculated from the total nitrogen and soluble nitrogen figures.

Salt.

The salt content was determined by the method described by McDowall and Whelan (1931).

CHEMICAL EXAMINATION OF THE CHEESE.

An attempt was made to follow by chemical analysis the progress of ripening of the cheeses. Although a more detailed analysis of the protein changes would have been desirable, an opportunity of doing this did not present itself. The cheeses, when five days old, were analysed for content of moisture, fat, salt, total nitrogen and soluble nitrogen. The percentage of protein, the soluble nitrogen expressed as a percentage of the total nitrogen, and the percentage of fat in the water-free solids were calculated from these figures.

Further estimations of the soluble nitrogen and total nitrogen contents of the cheese were carried out, at periods of 19 days, 33 days, and 2 months after manufacture. Moisture, soluble nitrogen, and total nitrogen determinations were again made when the cheeses were 6 or 8 months old.

The results of the chemical examinations are presented in Tables XXIX to XXXIII. The soluble nitrogen/

total nitrogen figures may be compared with figures for a normal Cheddar cheese* (see end of Table XXIX).

Chemical Composition.

The chemical composition of all groups of cheeses except Cheese IV is normal in comparison with the average composition of New Zealand Cheddar cheese. Cheese IV, however, was not only prepared from a milk with a high casein/fat ratio (0.83) but was also slightly overstirred at renneting. If the figures for this cheese are neglected, it will be seen that the fat in the water-free solids varied from 52.3 to 54.0 per cent. The percentage of protein ranged from 25.3 to 26.4, while the percentage of salt varied from 1.45 to 1.82.

Soluble Nitrogen.

The relative activity of the starter in the vats is evident from the manufacturing records cited in Tables V to IX. It is obvious that the highest acidity reached in the vat constitutes a reliable index of the rate of acid development. For convenience the highest vat acidities, and the quantities of rennet used have been included in the Tables of results of chemical analysis.

The soluble nitrogen results show that the rate of casein breakdown of all the groups of cheeses except Cheese IV is normal. Cheese IV, however, displayed the most rapid rate of increase in soluble nitrogen, due no doubt to the large quantity of rennet used. It is also evident that the rate of casein breakdown is determined by the combined influence of the activity of the starter in the vat and the quantity of rennet used.

It has been shown that the starter organism was non-proteolytic. In spite of this, comparatively normal rates of casein breakdown were obtained. Furthermore, with equal quantities of rennet the rate of breakdown was

* Figures kindly supplied by Dr. F.H.McDowall, Dairy Research Institute (N.Z.)

greatly influenced by the activity of the starter. There is, therefore, an indication that the more rapid rate of casein breakdown usually obtained with starters containing a mixed flora is due to the high acidity produced rather than to the presence of proteolytic types.

TABLE XXIX.

CHEMICAL ANALYSIS OF CHEESE I.

Analysis at 5 days.

	per cent.	per cent.	mean.
Moisture	34.54	34.95	34.8
Fat	34.62	-	34.6
Fat w.-f. s.*			53.1
Total nitrogen	3.96	4.01	3.99
Soluble nitrogen	0.43	0.43	0.43
Percentage Sol.N./total N.			10.8
Protein			25.5
Salt	1.80	1.84	1.82

Analysis at 8 months.

Moisture	29.99	30.08	30.0
Loss of moisture			4.8

Soluble Nitrogen Results.

Cheese	Age	Total Nitrogen per cent.	per cent.	mean.	Soluble Nitrogen per cent.	per cent.	mean	Percentage sol.N./ total N.
1-15-11	5 days	3.96	4.01	3.99	0.43	0.43	0.43	10.8
2-15-11	19 "	3.95	3.93	3.94	0.71	0.62	0.67	17.0
2-15-11	33 "	3.95	4.02	3.99	0.84	0.81	0.83	20.8
2-15-11	2 mths.	4.01	4.01	4.01	1.11	1.12	1.12	27.9
3-15-11	8 "	4.80	4.89	4.85	1.62	1.63	1.63	33.6

Highest Vat Acidity: 0.62%. Rennet (fl.ozs.per 1000 lbs.):4.8

Soluble Nitrogen Figures for a Normal Cheese.

Age	Percentage sol.N./total N.
5 days	9.5 - 10.0
19 "	16.0 - 17.0
33 "	20.0 - 21.5
54 "	25.0 - 26.0
4 months	28.0 - 29.0

* Percentage of fat in the water-free solids.

TABLE XXX.

CHEMICAL ANALYSIS OF CHEESE II.

Analysis at 5 days.

	per cent.	per cent.	mean.
Moisture	33.36	33.59	33.5
Fat	35.14	35.59	35.4
Fat w.-f. s.*			53.2
Total nitrogen	4.17	4.11	4.14
Soluble nitrogen	0.27	0.32	0.30
Percentage sol.N./total N.			7.3
Protein			26.4
Salt	1.58	1.60	1.59

Analysis at 7½ months.

Moisture	28.86	28.83	28.8
Loss of moisture			4.7

Soluble Nitrogen Results.

Cheese	Age	Total Nitrogen per cent.	mean.	Soluble Nitrogen per cent.	mean.	Percentage sol.N/total N
1-29-11	5 days	4.17) 4.11)	4.14	0.27) 0.32)	0.30	7.3
2-29-11	19 days	4.16) 4.25)	4.21	0.50) 0.50)	0.50	11.9
2-29-11	45 days	4.20) -)	4.20	0.84) 0.84)	0.84	20.0
3-29-11	7½ mths.	4.42) 4.43)	4.43	1.39) 1.37)	1.38	31.2

Highest Vat Acidity: 0.57%. Rennet (fl.ozs.per 1000 lbs.):4.5

* Percentage of fat in the water-free solids.

TABLE XXXI.

CHEMICAL ANALYSIS OF CHEESE III.

Analysis at 5 days.

	per cent.	per cent.	mean.
Moisture	35.30	35.33	35.3
Fat	35.04	34.77	34.9
Fat w.-f. s.*			54.0
Total nitrogen	3.95	3.98	3.97
Soluble nitrogen	0.39	0.38	0.39
Percentage sol N/total N.			9.6
Protein			25.3
Salt	1.47	1.43	1.45

Analysis at 6 months.

Moisture	30.98	31.03	31.0
Loss of moisture			4.3

Soluble Nitrogen Results.

Cheese	Age	Total Nitrogen per cent.	mean	Soluble Nitrogen per cent.	mean.	Percentage sol.N/total N
1-10-1	5 days	3.95 3.98	3.97	0.39 0.38	0.39	9.6
2-10-1	45 days	3.98 -	3.98	0.98 0.97	0.98	24.6
3-10-1	6 mths.	4.21 4.21	4.21	1.33 1.39	1.36	32.3

Highest Vat Acidity: 0.75%. Rennet (fl.ozs.per 1000 lbs.): 4.5

*Percentage of fat in the water-free solids.

TABLE XXXII.

CHEMICAL ANALYSIS OF CHEESE IV.

Analysis at 5 days.

	per cent.	per cent.	mean.
Moisture	37.80	37.69	37.7
Fat	28.44	28.30	28.4
Fat w.-f. s.*			45.9
Total nitrogen	4.45	4.43	4.44
Soluble nitrogen	0.55	0.56	0.56
Percentage sol N/total N.			12.4
Protein			28.3
Salt	1.31	1.30	1.31

Analysis at 8½ months.

Moisture	31.14	31.19	31.2
Loss of moisture			6.5

Soluble Nitrogen Results.

Cheese	Age	Total Nitrogen per cent.	mean	Soluble Nitrogen per cent.	mean.	Percentage sol.N/total
1-1-11	5 days	4.45 4.43	4.44	0.55 0.56	0.56	12.4
2-1-11	19 days	4.45 4.45	4.45	1.04 1.05	1.05	23.6
2-1-11	33 days	4.48 4.50	4.49	1.23 1.26	1.25	27.8
2-1-11	2½ mths	4.54 4.54	4.54	1.58 1.58	1.58	34.8
3-1-11	8½ mths	4.95 4.96	4.96	2.05 2.09	2.07	41.7

Highest Vat Acidity: 0.80%. Rennet (fl.ozs. per 1000 lbs.): 7.6

* Percentage of fat in the water-free solids.

TABLE XXXIII.

CHEMICAL ANALYSIS OF CHEESE V.

Analysis at 9 days.

	per cent.	per cent.	mean.
Moisture	36.34	36.39	36.4
Fat	33.29	-	33.3
Fat w.-f. s.*			52.3
Total nitrogen	4.04	4.05	4.05
Soluble nitrogen	0.53	0.53	0.53
Percentage sol.N/total N.			13.1
Protein			25.8
Salt	1.69	1.69	1.69

Analysis at 5½ months.

Moisture	33.43	33.33	33.4
Loss of moisture			3.0

Soluble Nitrogen Results.

Cheese	Age	Total Nitrogen per cent.	mean.	Soluble Nitrogen per cent.	mean.	Percentage sol.N/total N
1-31-1	9 days	4.04 4.05	4.05	0.53 0.53	0.53	13.1
1-31-1	24 days	3.98 4.04	4.01	0.79 0.87	0.83	20.7
3-31-1	5½ mths.	4.20 4.16	4.18	1.27 1.27	1.27	30.4

Highest Vat Acidity: 0.84%. Rennet (fl.ozs.per 1000 lbs.): 4.7.

*Percentage of fat in the water-free solids.

GRADING RESULTS.

The cheeses were examined by two experienced graders after 16 days, 1 month, 2 months, 3 months, 5 to 6 months and in some cases 8 months. Particular attention was paid to flavour, body and texture. The results are summarized in Table XXXIV. As soon as the final examinations had been made each cheese was cut in two and the cut surface photographed in order to obtain a permanent record of the texture. The cut cheeses were allowed to stand at room temperature for two days after which time the cut surfaces were again photographed. The most significant feature to be observed was the comparatively close texture of all the cheeses. The main points of each group of cheeses as disclosed by the grading examinations are considered below.

Cheese I.

Two distinct flavours were noticed in cheeses of this group. Firstly, there was a "caramel" or "cooked" flavour which was detected at several of the grading examinations. This flavour may have been due to the rather high temperature of pasteurisation (150°F (65.5°C.) for 30 minutes.) Secondly, a peculiar "cowy" or "animal-like" odour was evident on several occasions. This flavour had been noticed in the vat during the manufacturing process and it persisted in the cheese even after 8 months although it was not detected at every examination. None of the cheeses in the group ever developed the characteristic Cheddar flavour.

The rate of ripening as determined by the "body" of the cheese was slow during the first fortnight after manufacture but thereafter was considered normal. The cheese was slightly "sweet" as was to be expected from the lack of activity of the starter during the process of manufacture.

The texture of the cheese as disclosed by the plugs drawn at various times was very "close." When cheese No. 3 of the group was cut in two at an age of 8 months three "sweet-holes" were observed. A bacteriological examination was made but no organisms except lactobacilli could be detected. No yeasts had ever been found in the cheeses. The cause of the holes is therefore obscure.

Cheese II.

Only on one occasion during examinations of this group of cheeses was a "caramel" flavour detected. The pasteurisation temperature had been somewhat lower in this experiment (147°F. (63.9°C.) for 30 minutes). Although the "cowy" flavour had been observed during manufacture it was evidently not so marked in the cheese as it was in the previous experiment, for it was only noticed by the graders on two occasions. On one occasion only, when the cheese was 5 months old a slight Cheddar flavour was detected.

The rate of ripening as determined by "body" was more rapid than Cheese I during the first fortnight and thereafter rather slower. The chemical results, however, showed that Cheese II was slower in ripening than Cheese I throughout the whole period.

The cheese was somewhat "sweet" but very close in texture.

Cheese III.

The milk used in this experiment had been pasteurised at 145°F. (62.8°C.) for 30 mins. A slight "cooked" or "caramel" flavour was detected in the cheese at 16 days but it subsequently disappeared. During a later period in the ripening process a bitter flavour developed but this also tended to disappear as the cheese became older. On one or two occasions the graders

detected a flavour which they described as "phenol" flavour in the cheese. No disinfectant except a hypochlorite solution had been used during the process of manufacture so that there was no possibility that phenol or any phenol derivative had been incorporated in the curd. It is well known that some spore-forming organisms are capable of producing p-cresol or phenol in milk, but no similar organisms had been isolated in the present case. It is most probable therefore that the flavour was not actually due to phenol but merely bore some resemblance to it.

The most significant feature in these cheese was the development of a Cheddar flavour which was quite distinct after 6 months.

The rate of ripening as judged by the "body" was normal.

The texture was very close on the whole but one or two "sweet" holes were revealed when the cheese was cut in two. Again it was difficult to account for these apparent gas holes for no organisms capable of forming gas in these quantities had been found in the cheese.

Cheese IV.

The milk used in this experiment had been pasteurised at 145°F. (62.8°C.) for 30 minutes. A "cooked" or "scorched" flavour was noticed in the cheese practically throughout the whole period of grading. A bitter flavour was also present at one stage of the ripening period but it tended to disappear.

No development of Cheddar flavour was noticed until the cheese was 8½ months old.

The rate of ripening as judged by the "body" was normal.

The cheese was inclined to be sweet and its texture was very close.

Cheese V.

The milk used in this experiment had been pasteurised at 145°F. (62.8°C.) for 30 minutes. No trace of "cooked" or "caramel" flavour was detected in the cheese. Again, a bitter flavour was present during one stage of the ripening process but disappeared as the cheese grew older.

A slight Cheddar flavour developed in the cheese.

The rate of ripening was normal as judged by the body.

The texture was close at first but a few "slit" openings developed later.

TABLE XXXIV.

CHEESE GRADING RESULTS.

Cheese I.

Cheese: 1-15-11.	Age: 16 days.
Flavour	Clean; characteristic flavour as noted in curd during manufacture. No Cheddar flavour.
Body	Curdy; very little breakdown; slightly "fatty."
Texture	A few slit openings; no mechanical openness.
Colour	High colour; perhaps indicating insufficient acid.

Cheese: 2-15-11.	Age: 1 month.
Flavour	Strong "caramel" flavour.
Body	Breaking down well; a little crumbly; not "fatty."
Texture	Very close; slightly marbled.
Colour	Somewhat amber in colour; appears to be slightly "sweet."

Cheese: 2-15-11.	Age: 2 months.
Flavour	Clean; very faint "cooked" flavour. No Cheddar flavour.
Body	Very good body; ripening well.
Texture	Very close.

Cheese: 3-15-11.	Age: 3 months.
Flavour	Poor flavour; rather "strong."
Body	Tender; a little "doughy."
Texture	Close.
Colour	Highly coloured.

Cheese: 3-15-11.	Age: 5½ months.
Flavour	"Unclean." No Cheddar flavour.
Body	Smooth.
Texture	Numerous "sweet-holes."

TABLE XXXIV (Continued)

Cheese: 3-15-11.	Age: 8 months.
Flavour	"Caramel" combined with distinct "animal" flavour. No Cheddar flavour.
Body	A little pasty.
Texture	Cut surface showing three "sweet-holes. Close.
Colour	Too yellow; "sweet-like."

CHEESE II.

Cheese: 1-29-11.	Age: 16 days.
Flavour	Clean curdy flavour. No Cheddar flavour.
Body	Apparently a little "dry." Breakdown fair; much better than Cheese I at same age.
Texture	Very close; slightly marbled.
Colour	A little too highly coloured.

Cheese: 2-29-11.	Age: 1 month.
Flavour	Curdy; not cheesy.
Body	Gritty and tough.
Texture	Very close.

Cheese: 3-29-11.	Age: 2½ months.
Flavour	Slight "animal" flavour. No Cheddar flavour.
Body	Curdy, short; lacking sufficient acid.
Texture:	Close; a number of "sweet-holes."
Colour	"Sweet" amber colour.

Cheese: 2-29-11.	Age: 5 months.
Flavour	Trace of Cheddar flavour.
Body	Harsh.
Texture	Close.

TABLE XXXIV (Continued)

Cheese: 3-29-11.	Age: 7½ months.
Flavour	"Animal," with slight "caramel" flavour.
Body	Normal; a little "sandy."
Texture	Cut surface showing two small mechanical holes; otherwise very close.

Cheese III.

Cheese: 1-10-1.	Age: 16 days.
Flavour	Curdy. No Cheddar flavour. Semblance of "cooked" flavour; also semblance of "phenol" flavour.
Body	Very good. Breaking down normally.
Texture	Close. One slit opening.
Colour	Normal.

Cheese: 2-10-1.	Age: 1 month.
Flavour	Bitter. Also Cheddar flavour.
Body	Very "nice" body.
Texture	Very close.

Cheese: 2-10-1.	Age: 3½ months.
Flavour	Very bitter.
Body	Sticky.
Texture	Two very small slit openings.

Cheese: 3-10-1.	Age: 6 months.
Flavour	Distinct Cheddar flavour. Also slightly bitter; slight "phenol" flavour.
Body	Slightly sticky. Well broken down.
Texture	Cut surface showing a number of slit openings. "Slits" with characteristic direction of this type of openness. Also two small "sweet-holes."
Colour	Normal.

TABLE XXXIV (Continued)

Cheese IV.

Cheese: 1-1-11.	Age: 16 days.
Flavour	Clean; insipid. No Cheddar flavour.
Body	"Leanish," probably due to low fat-content. Breaking down normally.
Texture	Very close; slightly marbled.

Cheese: 1-1-11.	Age: 1 month.
Flavour	Slight "cooked" odour; bitter flavour, perhaps due to temperature of pasteurisation.
Body	Good; a little sandy.
Texture	Close.

Cheese: 2-1-11.	Age: 2½ months.
Flavour	"Cooked" flavour and odour; very bitter.
Body	Pasty and sandy. Well broken down.
Texture	Very close.

Cheese: 2-1-11.	Age: 3½ months.
Flavour	Very bitter.
Body	Pasty and weak.
Texture	Very close. Slightly "sweet."

Cheese: 3-1-11.	Age: 6 months.
Flavour	Scorched. No Cheddar flavour.
Body	Pasty and sandy.
Texture	Close; one small slit opening.

Cheese: 3-1-11.	Age: 8½ months.
Flavour	The Cheddar flavour now developing. Slight "caramel" flavour. Bitterness disappearing rapidly.
Body	Pasty and sandy.

TABLE XXXIV. (Continued)

Texture	Cut surface close; appearance sandy. The "break" is Cheshire rather than Cheddar.
Colour	Too yellow; "sweet-like."

Cheese V.

Cheese: 1-31-1. Age: 16 days.

Flavour	Normal Cheddar flavour. Also trace of "phenol" flavour.
Body	A little tender. Breaking down normally.
Texture	Very close.
Colour	Normal.

Cheese: 2-31-1. Age: 3 months.

Flavour	Very bitter.
Body	Very nice body.
Texture	Close.

Cheese: 3-31-1. Age: 5½ months.

Flavour	Clean acid flavour with semblance of Cheddar flavour. Cheddar "bouquet" lacking. Slight bitterness.
Body	Very good body. Normal breakdown.
Texture	Cut surface showing four slit openings.
Colour	Normal.

SUMMARY AND CONCLUSIONS.

With the object of elaborating a technique whereby the effect of any given organism added as a starter could be determined, an attempt was made to manufacture Cheddar cheese under conditions in which extraneous organisms could be excluded as far as possible. The bacteriological flora of the milk was reduced to a minimum and there was good reason for supposing that the greater portion of the small number of bacteria present came from the udder. A starter consisting of a single strain of lactic streptococci was used. The organisms found in the cheese immediately after manufacture were exclusively those udder types present originally in the milk, together with the starter strain. As the ripening progressed, these organisms disappeared and there was a comparatively rapid development of lactobacilli. The presence of the latter can be accounted for only in two ways:

(a) Contamination during manufacture.

(b) The presence of lactobacilli in the original milk in such small numbers that they remained undetected.

It is considered that the latter explanation is the more likely.

The milk used in the manufacture of the cheese was aseptically drawn and was pasteurised by the holding method. Only two groups of bacteria were found in both the raw and pasteurised milk samples, namely, micrococci and rods related to Bacterium lipolyticus (Evans). The micrococci were undoubtedly predominant. They comprised from 95 to 100 per cent. of the flora of the raw milk samples and from 75 to 100 per cent. of the flora of the pasteurised milk samples. They were found in small numbers during the early stages of ripening of some of the cheeses,

the "White" type greatly outnumbering the "Yellow." Apparently they were unable to multiply in the cheese for they rapidly died out.

The rods related to Bacterium lipolyticus (Evans) were able to withstand the pasteurisation temperatures used. They were, however, never isolated from any of the cheeses. It was concluded that they had little or no influence on the ripening process.

No streptococci were found in the raw and the pasteurised milk samples. The starter organism (Sc.cremoris) was present in large numbers immediately after manufacture but it rapidly died out. The rate of disappearance was correlated with the activity of the starter in the vat and the moisture content of the cheese.

The most significant bacteriological feature was the appearance and rapid development of lactobacilli in all of the cheeses. No lactobacilli were found in one c.c. of the raw and pasteurised milk samples. There was little likelihood of contamination during manufacture. It was therefore concluded that lactobacilli were present in the milk to the extent of less than one per c.c. and that they were able to multiply rapidly in the cheese. All the lactobacilli were members of the genus Streptobacterium (Orla-Jensen).

The chemical composition of the cheeses was normal in comparison with the average composition of New Zealand Cheddar cheese. Although the starter organism was non-proteolytic, the rate of casein breakdown as judged by the soluble nitrogen content was normal.

The characteristic Cheddar flavour failed to develop to any extent in any of the cheeses.

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