UNIVERSITY OF EDINBURGH THESIS

Author (surname, initials): ABDEL - MALEK Degree: Ph.D. Year: 1942

This thesis is an unpublished typescript and the copyright is held by the author. *All persons consulting this thesis, or having copies made, must sign the Copyright Declaration below.*

Copying regulations:

(1) This thesis may be copied in whole or in part for the use of individuals and for libraries wishing to add this thesis to their stock. *Copying must be done by Edinburgh University Library.*

(2) This thesis must not be copied, either in whole or in part, without the applicant obtaining the author's written permission. *If permission is granted, then copying must be done by Edinburgh University Library.* (The bibliography/list of works consulted may be copied without the author's permission, provided the copying is done by Edinburgh University Library.)

(3) This thesis may be copied only in so far as the copying does not contravene the 1956 Copyright Act. *Copying must be done by Edinburgh University Library.*

Copyright Declaration: I undertake fully to observe the author's copyright in this thesis, not to publish the whole or any part of it without the author's written permission, and not to allow any other person to use any copy made for me.

Date	Name and Address (BLOCK CAPITALS)	Signature	Pages copied (if any)

UNIVERSITY OF EDINBURGH

THE EFFECT OF LOW-TEMPERATURE PASTEURISATION ON THE BACTERIAL FLORA OF MILK

BY

YOUSEF Y. ABDEL-MALEK, B.Sc.(EGYPT), B.Sc.(HONS.) EDIN.

THESIS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

JUNE 1942

C O N T E N T S

SOURCES/

CONTENTS

(Continued)

EXPERIMENTAL METHODS

The following methods have been used throughout this work:-

Sampling and Plating.

In sampling, making of the dilution series and plating of the milk, the recommendations of Wilson (72) were followed. Sterile water was used in making the dilution series. This was thought to be quite suitable because the whole process of making the dilutions and plating did not take more than 15 minutes to be completed.

Pasteurisation.

Pasteurisation was carried out at 63°C. ± 0.2°C. in sterile stoppered tubes using a water bath for heating. The milk (about 10 ml.) was allowed to stay in the bath for 35 minutes; 30 minutes being the pasteurisation exposure and the extra 5 minutes were allowed in order to heat up the bulk of the milk to the pasteurisation temperature. When the time of exposure was up, the tubes containing the pasteurised milk were immersed immediately in cold running water for 10 minutes.

Action on Litmus Milk.

Litmus milk cultures were incubated for 14 days at 30°C. after which the final results were recorded as:-

1. Acid-forming
2. Acid-peptonizing
3. Peptonizing
4. Alkali-forming
5. Inert types.

The acid forming group included types which curdled the milk as well as those which did not. Ayers and Johnston (2) who introduced the litmus milk tube technique for the grouping of/

of the milk bacteria divided the acid-forming types into acid-forming and acid-coagulating. Since then, almost all workers who employed this method of grouping, accepted this division although in some instances, it was pointed out that the coagulating character is not a stable one (61). Throughout this investigation the coagulating character was found to be unstable, especially with the long incubation period employed. For this reason this division was ignored.

Ability to Digest Casein.

During the early stage of this investigation agar plates, to which 2 ml. of sterile skimmed milk was added prior to pouring, were used. Before recording the results, the plates were flooded with 10 per cent hydrochloric acid. This method was later substituted by the pure casein agar plates as recommended in the British standard methods for the microbial examination of butter (12). Surface inoculations were carried out and the plates incubated at 30°C. for 5 days.

Ability to Hydrolyse Butter Fat.

The copper soap formation method described by Berry (7) was employed. Plates were incubated at 30°C. for 5 days.

Ability to Liquefy Gelatin.

The gelatin stab cultures were incubated at 22°C. for 30 days except in the case of some of the micrococci strains which were incubated for a period as long as 6 months. The medium employed contained:-

Gelatin	120 gm.
Meat extract	5 gm.
Peptone	5 gm.
Water	1000 ml.
Adjust reaction to pH	7.4

Diastase Production./

Diastase Production

Surface inoculation on 0.2 per cent starch agar plates was carried out and the plates incubated at 30°C. for 5 days. The plates were then flooded with iodine. A clear area around the growth indicated the production of diastase.

Catalase Production.

Hydrogen peroxide (1%) solution was poured over the surface of agar slope cultures and evolution of gas indicated the production of catalase.

Ammonia Nitrogen Assimilation.

The ability of the organisms to use ammonium phosphate as the only source of nitrogen was carried out according to Hucker's method (34).

Haemolysis of Blood.

Haemolysis was determined in 5 per cent defribrinated ox blood agar. Poured plates of 24 hours dextrose broth cultures were prepared in the case of the streptococci, while surface inoculation was used for the micrococci. The plates were incubated at 37°C. for 48 hours.

Production of Ammonia from Peptone.

4 per cent Bacto-peptone solution was used. Incubation was for 6 days at 30°C. Presence of ammonia in the medium was determined by means of Nessler's reagent.

Reduction of Nitrate.

The ability to reduce nitrate was determined in 0.1 per cent nitrate-peptone water media after 7 days' incubation at 30°C. The sulphanillic acid and d-naphthylamine solutions were used as reagents for testing the presence of nitrites.

Fermentation of Sugars.

Sugar fermentation was determined in a 1 per cent peptone/

peptone water containing 0.5 per cent of the desired sugar and 0.4 per cent of a saturated alcoholic solution of brom-cresol-purple. Growth and acid production were observed after 7 days' incubation at 30°C.

Voges-Proskauer Test (Barritt's Modification).

To 1 ml. dextrose broth culture 0.5 ml. 0.6% α-naphthol in alcohol and 0.5 ml. 16% sodium hydroxide solution were added. The mixture was shaken until it frothed. The tube was then laid in a sloping position so that the mixture was exposed to aeration. A red colour is a positive reaction. Final recording of the results was made after two hours.

Citrate Utilisation.

Sodium citrate	0.3 per cent
$NH_4H_2PO_4$	0.1 per cent
K_2HPO_4	0.1 per cent
Agar	1.5 per cent
Brom-thymol blue	½ per cent of 1.6 per cent alcoholic solution

pH 6.8

Used as slopes. Utilisation of citrate is indicated by the production of alkalinity.

Heat-resistance Tests.

0.5 ml. of a 24 hours broth culture was pipetted carefully in the bottom of a sterile tube, care being taken not to contaminate the wall of the tube. 5 ml. of sterile milk was pipetted carefully in the tube which was afterwards subjected to the heat treatment in a water bath. When the time of exposure was up, the tube was cooled in cold water and then the contents were mixed well and 0.5 ml. was plated. The plates were incubated at 30°C. for 5 days.

Test for Gas Production by the Members of the Genus Leuconostoc/

Test for Gas Production by the Members of the Genus Leuconostoc

A litmus milk tube which contained 5 per cent dextrose, 0.25 per cent yeastrel and 4 per cent tomato juice was inoculated from a quick growing culture of the organism under examination. 1 ml. of melted agar which had been cooled to 50°C. was blown in the milk and another 1.5 ml. was run carefully on the wall of the tube so that it covered the surface of the milk and acted as a seal. These quantities of agar were for 5" x $\frac{1}{2}$" tubes. Double these quantities should be used in the case of 6" x $\frac{5}{8}$" tubes. The results were recorded after 3 and 7 days incubation at 30°C. In the case of Leuconostoc strains the CO_2 produced forces the agar seal upwards and the cultures possess a blown appearance. (see photograph page 73).

THE EFFECT OF PASTEURISATION ON THE BACTERIAL FLORA OF MILK.

For the determination of the effect of pasteurisation on the bacterial Flora of milk three types of milk were used:-

1. Aseptically-drawn samples which were examined before proliferation was likely to occur.
2. Certified milk at the stage of delivery to consumers.
3. The bulked milk of numerous producers obtained from the storage tanks of a pasteurising plant.

This choice was expected to provide examples of the more distinctive types of bacterial flora.

The samples, before and after pasteurisation at 63°C. for 30 minutes, were plated in duplicate on meat infusion agar containing 1 per cent trytone peptone and 0.25 per cent dextrose. Immediately before pouring plates which were to receive less than 1ml. of inoculum, o.5 ml. of sterile milk was added to each tube of agar. Plates were incubated for 5 to 6 days at 30°C. This combination of medium and incubation was chosen with the object of securing the growth of as many as possible of the bacteria in the samples. After incubation every colony - the number varied from 22 to 59 - on a suitably diluted plate or on duplicate plates or on opposite sectors of a plate, was transferred to a tube of litmus milk containing 0.25 per cent yeastrel and 0.25 per cent dextrose. The milk cultures were incubated at 30°C. and/

and then transferred to other media for characterisation.

ASEPTICALLY-DRAWN MILK

Samples taken aseptically from 18 cows were examined. These cows were from herds tested regularly for mastitis infection. Three cows were infected with Streptococcus agalactiae at the time when the samples were taken; three others had been infected with Streptococcus agalactiae but were free from infection at the time of sampling. The rest of the samples were from healthy animals. The plate counts tended to be high in the infected samples, being 4350 per ml. for one sample and more than 300,000 per ml. for the other two. The plate counts for the rest of the samples varied from less than 100 to 2570 per ml.

The predominant flora in nine of these samples was examined and the results are shown in table 1. The dominant bacteria in the majority of the samples were micrococci while the diphtheroids, mainly of the Corynebacterium bovis type predominated in one sample and were about equal to the micrococci in another. This is in agreement with the results of Evans(22) Steck (67) and Dorner (17) who reported that the frequency with which these diphtheroids occur in eseptically-drawn milk/

milk vary from one animal to another.

TABLE 1. - Predominant microflora of Aseptically-drawn milk.

Sample	Plate Count per ml.	Percentage	
		Micrococci	Corynebacteria
1	470	100.0	-
2	110	100.0	-
3	1300	1.7	98.3
*4	190	100.0	-
ø5	300,000	100.0	-
6	1025	100.0	-
7	140	64.0	36.0
*8	4350	87.8	12.2
9	140	52.2	47.8

*From a cow infected with Streptococcus agalactiae

øFrom a cow that had been infected with Streptococcus agalactiae but was free from infection when sample was collected.

While all the colonies picked from sample 5, which revealed an exceptionally high plate count, were Micrococcus aureus, micrococci in the other samples belonged to several species. Table 2 gives the result of the characterisation of the 192 strains of micrococci isolated from the nine samples. It is worth noting that 40 out of the 47 strains of M. aureus were isolated from the samples which were, or had been, infected with Streptococcus agalactiae

TABLE 2 - Species of micrococci isolated from aseptically-drawn milk.

Specific Name	No.of Strains	Percentage
Micrococcus aureus	47	24.5
Micrococcus aurantiacus	5	2.6
Micrococcus albus	79	41.2
Micrococcus citreus	4	2.1
Micrococcus candicans	51	26.5
Micrococcus caseolyticus	3	1.6
Micrococcus flavescens	1	0.5
Sarcina sp.	2	1.0
Total	192	100.0

The frequency with which micrococci have been reported in pasteurised milk, and the common belief that organisms of this group occurring in milk are largely derived from the ducts of the udder, might be held to explain the low efficiency of pasteurisation frequently observed in the case of milks containing small numbers of bacteria. It was somewhat surprising, therefore, that when the 18 udder samples were examined not a single colony could be detected on the plates inoculated with 1 ml. of the pasteurised milk. These udder samples were apparently sterilised by pasteurisation. In the case of nine of them the heated milk was incubated at 22°C. for 24, 36 or 48 hours and still failed to yield colonies from 1 ml. Three were incubated in the raw state for 24 hours at 22°C. and then pasteurised, but no growth was obtained after heating. It appears, therefore, that the micrococci of pasteurised milk are derived from other sources. These results also indicate that if milk were produced without contamination it would be practically sterilised by low-temperature pasteurisation.

In contrast to these results Hileman et. al. (31) found that 140 cultures of micrococci which survived pasteurisation in milk belonged to species which had previously been isolated by other workers from the cow's udder. Their findings led them to believe that the udder is the ultimate source of thermoduric micrococci in milk. The discrepancy in the conclusions reached in the two investigations may be due in part to the employment of different methods of pasteurisation. It may also be associated with the fact that certain species of micrococci which have been recorded as occurring in the udder have so far not been detected in aseptically-procured milk in this investigation.

As/

As will be shown in a later section, Hucker's classification of the micrococci, which was employed in the previous studies, might also be responsible for this discrepancy.

There is no information in the literature concerning the ~~proportion~~ distribution of the bacterial groups in milk as it leaves the udder with regard to their action on milk. The recording of such groups in the samples examined also seemed desirable, for the sake of noting changes taking place after organisms, other than those present in the udder, gain access to the milk during the various conditions of production and handling. Table 3 shows the percentages of the bacterial groups in the aseptically-drawn milk samples according to the action of the organisms on litmus milk, and Table I in the appendix shows the percentages of the bacterial groups according to the action on both lactose and casein.

TABLE 3 - The bacterial groups in aseptically-drawn milk according to the action on litmus milk.

Sample	Percentage				
	Acid-Producing	Acid-Peptonizing	Peptonizing	Alkali-Forming	Inert
1	100.0	-	-	-	-
2	100.0	-	-	-	-
3	1.7	-	-	-	98.3
*4	95.0	-	5.0	-	-
Ø5	-	100.0	-	-	-
6	48.0	52.0	-	-	-
7	54.5	-	-	-	45.5
*8	26.8	61.0	-	-	12.2
9	4.4	30.4	-	13.0	52.2

* See foot-note table 1.
Ø " " " " "

The tables agree in the main. The slight difference between the two is caused by the failure of some of the acid-peptonizing strains to show visible peptonization in litmus milk and by some/

some strains which produce acid from lactose but do not change the reaction of litmus milk during the 14 days' incubation at 30°C.

As might be expected, variation in the proportion of the different bacterial groups are present among the samples. Some points, however, are worth noting: the complete absence of the peptonizing group from all but one sample, where it appeared to the extent of a small percentage, seems to be significant. The same could be noted about the alkali-forming group. On the other hand, the acid-peptonizing group formed a high proportion of the flora present in 4 samples which included those from udders showing infection with Streptococcus agalactiae where proteolytic staphylococci were encountered in large numbers. The acid-producing group showed high percentages in most samples with the exception of those which showed predominance of the inert group of Corynebacterium bovis among their flora. (Compare tables 1 and 3)

CERTIFIED MILK

Thirteen certified milk samples were examined. They were found to fall into two distinct groups. On pasteurisation, the first group showed little or no survival and, therefore, will be referred to as "clean certified milk". The second group, on the other hand, contained a comparatively large number of organisms resisting the heat treatment and will be referred to as "certified milk".

Clean Certified Milk.

The clean certified milk comprised 5 samples. The results of their examination are presented in table 4. The months/

months in which the samples were taken were: No. 1 in March 1940, No. 2 in September 1940, No. 3 in January 1941, No. 4 in March 1941 and No. 5 in September 1941. Nos. 1 and 5 were produced on one farm, and Nos 2, 3 and 4 on three others

TABLE 4 - The effect of pasteurisation on the microflora of clean certified milk.

Sample and Treatment	Plate count per ml	Percentage			
		Strepto-cocci	Micro-cocci	Coryne-bacteria	Other bacteria
1 Raw	2900	21.4	60.7	17.9	-
Pasteurised	1	-	-	-	(100)
2 Raw	4450	17.5	82.5	-	-
Pasteurised	0	-	-	-	-
3 Raw	3850	6.1	81.8	9.1	3.0
Pasteurised	0	-	-	-	-
4 Raw	24200	6.1	12.1	18.1	63.7 Pseudomonas
Pasteurised	11	-	-	-	100.0
5 Raw	4700	18.2	13.6	27.3	40.9
Pasteurised	0	-	-	-	-

The microflora of the raw milks consisted largely of three groups: streptococci, micrococci and corynebacteria. Organisms of other groups were detected in significant numbers in samples No. 4 and No. 5. In sample No. 4 they were mainly Pseudomonas fluorescens with a smaller number of organisms which belong to the genus Alcaligenes. In sample No. 5 they were mainly Alcaligenes viscosus var. dissimilis Hammer.

The first three samples in the table show a similar bacterial flora characterised by the presence of a high percentage of micrococci - not unlike udder milk. Samples No. 4 and No. 5 differ from the other three samples by containing a small percentage of micrococci and a high percentage of gram-negative rod/

rod shaped bacteria, capable of active growth at low temperature. This variation may have been the result of the proliferation of the organisms in the milk during storage. The high plate count in sample No. 4 is in accord with this view.

The micrococci group was represented by 100 strains distributed as follows: M. aureus - 41; M. albus - 17; M. citreus - 9; M. aurantiactus - 13; M. candicans - 14; M. freudenreichii - 1 and M. lipolyticus - 5. The streptococci comprised 26 strains identified as: 17 mastitis streptococci; 3 S. lactis; 5 Leuconostoc sp. and 1 unidentified (see page 77). Of the mastitis streptococci 9 strains proved to be S. agalactiae. The corynebacteria were a miscellaneous collection which had little resistance to heat.

The results of the pasteurisation test are interesting. Only in samples No. 1 and No. 4 did any colonies appear on the plates inoculated with 1 ml. of the pasteurised milk. These were mainly spore-formers (Bacillus licheniformis and Bacillus pumilus) along with a few Actinomyces. In all samples the streptococci, micrococci and corynebacteria were not heat-resistant.

The group percentages of the bacterial flora in the clean certified milk samples are presented in table 5 and table II in the appendix. Table 5 shows the group percentages of the bacteria according to their action on litmus milk and table II in the appendix shows the grouping of the same organisms according to their action on lactose and casein. Samples 1,

2/

2 and 3 showed a similar microflora not unlike that of udder milk.

TABLE 5 - The effect of pasteurisation on the bacterial groups in clean certified milk according to the action on litmus milk.

	Sample and Treatment	Percentage				
		Acid-Producing	Acid-Peptonizing	Peptonizing	Alkali-Forming	Inert
1	Raw	43.0	10.7	3.6	10.7	32.1
	Pasteurised	-	-	(100)	-	-
2	Raw	50.0	50.0	-	-	-
	Pasteurised	-	-	-	-	-
3	Raw	48.5	33.3	-	6.1	12.1
	Pasteurised	-	-	-	-	-
4	Raw	18.2	-	39.4	15.2	27.2
	Pasteurised	-	-	(100)	-	-
5	Raw	18.2	2.3	-	45.5	34.0
	Pasteurised	-	-	-	-	-

The acid-producing bacteria formed the largest group, the acid-peptonizing group was next while the peptonizers and alkali-formers were in the minority. In samples 4 and 5, the relative proportions of the groups were quite different. In sample 4, where Pseudomonas fluorescens was present in large numbers, the peptonizers formed the largest group, while in sample 5, where Alcaligenes types were dominant, the alkali-forming group was the largest. The acid-producing group was small in both samples and the acid-peptonizing group was in the minority.

After pasteurisation, the few colonies which appeared on the plates of samples 1 and 4 belonged to the peptonizing group. This indicates the possibility that when such clean milk is pasteurised, it may become putrid on standing, instead of/

sour.

Certified Milk.

The predominant microflora before and after pasteurisation of the second group of the certified milk samples, which showed a significant number of organisms capable of surviving the heat treatment, is presented in table 6. The months in which they were taken were: No. 1 in December 1939, No. 2 and 3 in April 1940, No. 4 in October 1940, No. 5 in April 1941, No. 6 in June, No. 7 in August, No. 8 in November 1941. Nos. 1 and 7 were produced on one farm. Nos. 2, 4 and 5 on another, Nos. 3 and 8 on a third and No. 6 on a fourth.

TABLE 6 - The effect of pasteurisation on the microflora of certified milk.

	Sample and Treatment	Plate count per ml.	Percentage			
			Strepto-cocci	Micro-cocci	Coryne-bacteria	Other bacteria
1	Raw	9400	56.5	41.0	2.5	-
	Pasteurised	210	-	-	95.0	5.0
2	Raw	11000	6.4	19.1	49.0	25.5
	Pasteurised	6950	-	-	100.0	-
3	Raw	17100	9.0	45.5	18.2	27.3
	Pasteurised	235	-	21.3	78.7	-
4	Raw	17000	2.5	60.0	32.5	5.0
	Pasteurised	11000	-	4.3	95.7	-
5	Raw	8700	5.1	30.8	59.0	5.1
	Pasteurised	5400	-	17.3	82.7	-
6	Raw	10200	25.0	5.0	30.0	40.0
	Pasteurised	2145	-	-	100.0	-
7	Raw	840000	-	-	-	100.0
	Pasteurised	5000	-	-	100.0	-
8	Raw	239000	6.9	3.4	13.8	75.9
	Pasteurised	3400	-	-	100.0	-

The/

The bacterial counts of the raw milks were low except in samples 7 and 8 which showed plate counts of 840,000 and 239,000 organisms per ml. respectively. When these were pasteurised the bacterial counts were low, not unlike those obtained from the other samples. This suggests that the high plate count in the raw milks was mainly due to bacterial multiplication.

Again, the results show that the microflora of the raw milks consisted largely of three groups - streptococci, micrococci and corynebacteria. Organisms of other groups were detected in significant numbers in five samples and were gram-negative non-sporing rods which belonged to the genera: Pseudomonas, Alcaligenes, Achromobacter and Flavobacterium. Pseudomonas fluorescens was the main organism in samples 2 and 3. In sample 7 all the colonies picked from the raw milk were Alcaligenes viscosus var. dissimilis. In sample 8 the gram-negative bacteria in the raw milk were mainly Pseudomonas fluorescens and Alcaligenes viscosus type. It is interesting to note that in samples 7 and 8, where proliferation of the organisms seems to have occurred for some time in the raw milk prior to examination, the dominant organisms in the raw milks were gram-negative bacteria as in samples 4 and 5 of the clean certified milk (table 4).

The identity and frequency of encounter of the members of the streptococci and micrococci groups in the raw milk are shown in table 7.

TABLE/

TABLE 7. - Species of streptococci and micrococci isolated from certified milk.

Streptococci			Micrococci		
Specific name	No.of strains	Percentage	Specific name	No.of strains	Percentage
*Mastitis streptococci	23	54.8	M. aureus	4	5.4
S. lactis	9	21.4	M. aurantiacus	3	4.1
S. cremoris	1	2.4	M.albus	10	13.5
S. faecalis	3	7.1	M. caseolyticus	4	5.4
Leuconostoc sp.	6	14.3	M. candicans	12	16.2
			M. epidermidis	4	5.4
			M. citreus	3	4.1
			M. luteus group	20	27.0
			M. lipolyticus	11	14.9
			Sarcina sp.	3	4.1
Total	42	100.0		74	100.1

*Of these, 7 representative strains proved to be S.agalactiae

The prevalence of the mastitis streptococci in certified milk as a whole, is in agreement with the findings of Jones (42). The presence of the heat resistant Micrococcus luteus group among the predominant microflora of the above samples is significant because of the absence of this type from the two previous types of milk.

The corynebacteria were different from those encountered in the aseptically-drawn and clean certified samples in that they were mainly heat resistant types. Thus, of the 89 strains isolated, 59 were C. liquefaciens and 26 were C. lacticum. The remaining 4 strains were similar to the types encountered in the clean certified samples.

As a result of pasteurisation, streptococci in all samples disappeared from among the predominant organisms, a result associated with the fact that species other than mastitis types were only encountered to the extent of a small percentage. The effect

of/

of the heat treatment on micrococci indicates that only samples 3, 4 and 5 were infected with appreciable numbers of thermoduric organisms of this type from sources outside the udder. These uniformly belonged to the M. luteus group. The most significant feature of the results of pasteurisation is the preponderance in the surviving microflora of corynebacteria. They comprised 282 strains which were characterised as: C. lacticum, 77 strains and C. liquefaciens, 205 strains.

The bacterial groups in certified milk before and after pasteurisation. Table 8 shows the percentages of the bacterial groups in the certified milk samples before and after pasteurisation according to the action on litmus milk and table III in the Appendix shows the bacterial groups according to the action on lactose and casein.

TABLE 8. - The effect of pasteurisation on the bacterial groups in certified milk according to the action on litmus milk.

	Sample and Treatment	Percentage				
		Acid-Producing	Acid-Peptonizing	Peptonizing	Alkali-Forming	Inert
1	Raw	64.0	15.4	-	5.1	5.1
	Pasteurised	95.0	-	2.5	2.5	-
2	Raw	57.4	12.6	25.5	-	4.4
	Pasteurised	100.0	-	-	-	-
3	Raw	63.5	-	23.0	4.5	9.0
	Pasteurised	83.0	-	-	-	17.0
4	Raw	47.5	5.0	5.0	20.0	22.5
	Pasteurised	100.0	-	-	-	-
5	Raw	79.5	5.1	5.1	5.1	5.1
	Pasteurised	100.0	-	-	-	-
6	Raw	57.5	-	15.0	22.5	5.0
	Pasteurised	100.0	-	-	-	-
7	Raw	-	-	-	100.0	-
	Pasteurised	81.2	-	-	-	18.8
8	Raw	24.1	-	44.8	31.0	-
	Pasteurised	100.0	-	-	-	-

The results obtained from the raw milks show that the acid-producing group was the dominant one in all the samples with the exception of samples 7 and 8 which showed exceptionally high plate counts, most probably due to the multiplication of the organisms in the milk prior to examination. In sample 7, where Alcaligenes viscosus var. dissimilis was predominant all the organisms isolated were alkali-formers. Samples 2, 3 and 8 revealed high percentages of peptonizing bacteria which were mainly Pseudomonas fluorescens.

The effect of pasteurisation on the bacterial groups is particularly interesting, because the findings of previous investigators, on the relative proportions of the bacterial groups after the pasteurisation of low count milk, are contradictory. Thus, Ayers and Johnston (2) found that pasteurisation increased the percentage of the total acid group from 40.9 in the raw milk to 73.1 in the pasteurised product while the percentage of the other groups decreased. Thurston and Olson (70) noted that the total percentages of the acid-producing group remained unchanged in low count milk after pasteurisation at 145°C. for 30 minutes; the percentages being 66.18 and 66.86 respectively in the raw and pasteurised milk. The changes in the proportion of the other groups were not significant. In both the above investigations the litmus milk tube technique was employed in grouping the organisms. On the other hand, Black et al. (10) and Prouty (53) employed a Bacto-caseinate agar medium containing brom-cresol purple as indicator, for making differential bacterial counts on both raw and pasteurised low count milk. The effect of pasteurisation on the bacterial groups was found to decrease the acid-producing group from 26.17 to 14.6 per cent in the first investi-

gation/

investigation and from 29.4 to 14.6 per cent in the second. In both investigations the major proportion of the dominant bacteria in either the raw or the pasteurised milk was made up of the alkali-forming and inert group. In the present study, the grouping of the organisms was carried out by the litmus milk tube technique and by the action of the organisms on both lactose and casein. The results obtained from both methods, show that in all the samples examined, the acid-producing group was the largest group in the predominating flora of the pasteurised milk. Other groups were encountered only occasionally in some of the samples to the extent of a small percentage.

Although the findings reported here are in agreement with the conclusion of Ayers and Johnston that pasteurisation results in an increase in the percentage of the total acid-producing group, the percentages obtained in this study were considerably higher than those reported by them. This may be due to the employment in this investigation of a method which ensured that all the organisms inoculated into litmus milk did grow, thus avoiding the inclusion in the inert group of an appreciable proportion of organisms which did not happen to grow. That such was the case in Ayers and Johnston's results is admitted by them. No comment could be made on Thurston and Olson's report as their original paper was unobtainable for detailed inspection.

The discrepancy in the findings of this investigation, and those reported by Black et al. and Prouty, may be due to their employment of the agar plate technique for grouping the organisms. This method is not accurate. The difference may have been also due to the employment by Black et al. of a lower pasteurisation temperature (61.4°C. as compared with 63°C.) and the incubation

of/

of the plates at 37°C. instead of 30°C. as employed here. Prouty, on the other hand, utilised 145°C. for pasteurisation and incubated the plates at 20°C. for 4 days. The milk, however, was pasteurised in a 200-gallon pasteuriser, unlike the controlled laboratory pasteurisation.

TANK MILK

Ten specimens of the bulked milk of numerous producers were examined, with the results shown in table 9.

TABLE 9. - The effect of pasteurisation on the microflora of tank milk.

	Sample and Treatment	Plate count per ml.	Percentage			
			Strepto-cocci	Micro-cocci	Coryne-bacteria	Other bacteria
1	Raw	345,000	44.8	27.6	17.2	10.4
	Pasteurised	59,000	49.2	20.3	30.5	-
2	Raw	870,000	63.6	3.0	6.1	27.3
	Pasteurised	57,500	72.0	12.0	12.0	4.0
3	Raw	3,650,000	62.9	25.7	-	11.4
	Pasteurised	58,000	60.0	8.0	32.0	-
4	Raw	920,000	81.5	3.5	-	15.0
	Pasteurised	45,000	80.0	2.5	17.5	-
5	Raw	1,130,000	60.5	11.6	2.3	25.6
	Pasteurised	50,000	11.1	22.2	66.7	-
6	Raw	355,000	66.7	5.6	-	27.7
	Pasteurised	33,000	43.8	6.2	50.0	-
7	Raw	770,000	18.0	2.6	2.6	76.9
	Pasteurised	126,500	56.3	6.3	37.5	-
8	Raw	39,000,000	86.8	2.6	-	10.5
	Pasteurised	174,000	58.3	13.9	27.8	-
9	Raw	6,800,000	78.6	10.7	-	10.7
	Pasteurised	154,000	40.5	2.7	56.8	-
10	Raw	380,000	53.1	18.8	-	28.1
	Pasteurised	6,900	2.9	5.9	91.2	-

The/

The months of production were: No. 1 in December 1939, No. 2 in February 1940, No. 3 in March, No. 4 in April, No. 5 in November, No. 6 in February 1941, No. 7 in June, No. 8 in August, No. 9 in September, and No. 10 in October. The plate counts indicate that much of the milk was produced without regard to bacteriological purity.

Streptococci constituted the dominant group in almost all the raw milks, the most prevalent forms being S. lactis and Leuconostoc types. Otherwise the microflora of these milks did not differ greatly in composition from that of certified samples. As might be expected, the organisms under the heading "other bacteria" were a miscellaneous collection consisting mainly of gram-negative, non-sporing rods which belong to the genera: Pseudomonas, Achromobacter, Alcaligenes and Flavobacterium.

Pasteurisation revealed that the tank milk was distinguished from purer milk by its content of the thermoduric streptococci. S. thermophilus and S. bovis were the species most frequently encountered. Streptococci formed the largest group of organisms encountered in the pasteurised milks of 6 samples, while corynebacteria were the dominant group in the other 4 samples. Since the publication of a preliminary report on some of these results (24), the presence of large numbers of corynebacteria in pasteurised milk has been confirmed by Rowlands and Provan (61). The occurrence of a small percentage of streptococci in the pasteurised milk reported by these workers might have been due to the incubation of the plates at 22°C. as compared with 30°C. in this investigation.

The streptococci, micrococci and corynebacteria which were encountered in the raw and pasteurised tank milk are illustrated in more detail in table 10.

TABLE 10/

TABLE 10 - Species of streptococci, micrococci and corynebacteria isolated from tank milk before and after pasteurisation.

Streptococci					Micrococci					Corynebacteria				
Specific name	Raw		Pasteurised		Specific name	Raw		Pasteurised		Specific name	Raw		Pasteurised	
	No. of strains	Percentage	No. of strains	Percentage		No. of strains	Percentage	No. of strains	Percentage		No. of strains	Percentage	No. of strains	Percentage
S. lactis	87	41.8	-	-	M. aureus	1	2.7	-	-	C. liquefaciens	3	27.3	106	64.6
S. cremoris	15	7.2	-	-	M. albus	3	8.1	-	-	C. lacticum	3	27.3	58	35.4
*Mastitis streptococci	28	13.5	-	-	M. caseolyticus	4	10.8	-	-	Corynebacterium sp.	5	45.4	-	-
S. faecalis	8	3.8	9	4.9	M.epidermidis	1	2.7	-	-					
S. liquefaciens	2	1.0	-	-	M. candicans	3	8.1	-	-					
S. bovis	2	1.0	70	38.0	M. lipolyticus	19	51.4	-	-					
S. thermophilus	6	2.9	102	55.4	M. luteus	5	13.5	40	97.6					
Leuconostoc sp.	60	28.8	3	1.6	Sarcina sp.	1	2.7	1	2.4					
Total	208	100.0	184	99.9		37	100.0	41	100.0		11	100.0	164	100.0

* 11 representative strains proved to be S. agalactiae

The common occurrence of the Leuconostoc types in the raw milk was reported by Ayers, Johnston and Mudge (3). As in the case of certified milk, Micrococcus luteus group was the commonest type of micrococcus in the pasteurised milk.

The bacterial groups in tank milk before and after pasteurisation. Table 11 shows the percentage of the bacterial groups in the samples according to the action on litmus milk and table IV in the appendix shows the percentage of the bacterial groups according to the action on lactose and casein.

TABLE 11. - The percentage of the bacterial groups in tank milk before and after pasteurisation according to the action on litmus milk.

Sample and Treatment		Percentage				
		Acid-Producing	Acid-Peptonizing	Peptonizing	Alkali-Forming	Inert
1	Raw	72.5	6.9	10.3	6.9	3.4
	Pasteurised	100.0	-	-	-	-
2	Raw	57.6	9.0	27.3	-	6.1
	Pasteurised	92.0	-	-	4.0	4.0
3	Raw	68.6	8.6	11.4	-	11.4
	Pasteurised	100.0	-	-	-	-
4	Raw	81.5	3.5	15.0	-	-
	Pasteurised	100.0	-	-	-	-
5	Raw	62.8	4.7	13.9	9.3	9.3
	Pasteurised	95.6	-	-	2.2	2.2
6	Raw	69.4	5.6	19.4	2.8	2.8
	Pasteurised	100.0	-	-	-	-
7	Raw	25.6	-	46.2	7.7	20.5
	Pasteurised	87.5	3.1	-	-	9.4
8	Raw	86.8	-	-	10.5	2.6
	Pasteurised	100.0	-	-	-	-
9	Raw	71.4	-	3.6	14.3	10.7
	Pasteurised	100.0	-	-	-	-
10	Raw	59.4	3.1	3.1	21.9	12.5
	Pasteurised	97.1	-	2.9	-	-

A/

A comparison of the results in tables 9, 11 and table IV in the appendix will show that in the raw milk, the relative proportions of the acid-producing group on one hand and the peptonizing alkali-forming and inert groups on the other, appear to depend on the ratio of streptococci to the gram-negative low temperature growing types present in the samples. Thus, a high streptococci percentage was usually associated with a low percentage of the psychrophilic types and consequently large acid-producing groups and small peptonizing, alkali-forming and inert groups were found. The relative proportions of the streptococci and the low-temperature growing types are naturally affected to a great extent by the temperature at which the milk is handled prior to examination. Low temperatures, for example, are likely to favour the quick multiplication of the psychrophilic organisms while the growth of the lactic streptococci is slow. This may be the explanation of the predominant flora encountered in the raw milk of sample 7 (table 9) and the corresponding high percentages of the peptonizing and inert groups (table 11 and table IV of the appendix). On the other hand, when milk is handled at a temperature high enough to allow quick multiplication of the lactic acid bacteria, the percentage of the streptococci in the milk is likely to be high. Such might have been the case in the raw milk of sample 8 which showed a high plate count indicating active multiplication of the organisms in the milk prior to examination. The above discussion aims to point out that great variations in the relative proportions of the bacterial groups in the raw milks are to be expected because of the different conditions to which the individual samples are exposed before being examined. Another aim is to suggest some of the factors which might have influenced the distribution of the

different/

different bacterial groups encountered in the raw milk samples examined.

After pasteurisation the bacterial groups, as determined by the action on litmus milk and the action on lactose and casein, show similar relative proportions to those encountered in the pasteurised certified milk samples. The acid-producing group uniformly formed the largest group of the total flora while the other groups were encountered only occasionally in a few samples to the extent of a small percentage. These results are in agreement with those of previous investigators, (2, 31, 61),

A COMPARISON OF THE PREDOMINANT BACTERIAL FLORA BEFORE AND AFTER PASTEURISATION IN THE FOUR GRADES OF MILK EXAMINED

Before any attempt is made to compare the microflora of the different grades of milk, it should be stressed that the relative proportions of the predominant bacterial groups in raw milk vary a great deal according to the different conditions under which the milk is handled before being examined, e.g. degree of contamination, temperature of the milk during handling and the age of the milk. The average of the proportions of the different bacterial groups present in random samples may, however, give an indication of what might be expected when milk of the same category is examined. It must be admitted that the number of samples examined here is rather small to allow of any definite statement being made from such average comparison. On the other hand, the average results obtained from the freshly pasteurised product would be expected to yield more to such comparison, because only the organisms capable of surviving the heating will appear according to their degree of preponderance and also because there/

there is no variation due to the multiplication of the organisms prior to examination c.f. raw milk.

Raw Milk.

Table 12 shows the average percentages of the different groups which form the predominant microflora of the four types of raw milk examined.

TABLE 12. - The average predominant microflora in the different grades of raw milk.

	Percentage			
	Strepto-cocci	Micro-cocci	Coryne-bacteria	Other bacteria
Aseptically-drawn	-	78.4	21.6	-
Clean certified	13.8	50.1	14.5	21.5
Certified	13.9	25.6	25.6	34.9
Tank milk	61.7	11.2	2.8	24.4

The figures in the table point to certain trends of variation. While the streptococcus group was absent in the predominant flora of the aseptically-drawn milk, it formed the largest group in tank milk and appeared to a smaller extent in both types of certified samples, i.e. the more contaminated the milk ~~becomes~~, the more likely the streptococci ~~would~~ be numerous. The micrococci, on the other hand, ~~appeared in a decreasing order among the predominant flora of the various milks being~~ largest in the udder milk samples and smallest in the most contaminated milk. Bacteria included under the heading "Other bacteria" have obviously gained access to the milk after leaving the udder. As mentioned before, they were mainly a miscellaneous collection of gram-negative rod shaped organisms of types well recognised as being commonly found/

found in water.

Table 13 presents the average percentages of the bacterial groups in the raw milks according to the action on litmus milk, and table V in the appendix shows the average percentages according to the action of lactose and casein.

TABLE 13. - The average percentages of the predominant bacterial groups in the different grades of raw milk according to the action on litmus milk.

	Percentage				
	Acid-Producing	Acid-Peptonizing	Peptonizing	Alkali Forming	Inert
Aseptically-drawn	47.8	27.1	0.6	1.4	23.1
Clean certified	35.6	19.2	8.6	15.5	21.1
Certified	50.5	4.8	14.8	23.5	6.4
Tank milk	65.6	4.1	15.0	7.3	7.9

From both tables it could be seen that the acid-peptonizing group showed a similar trend of variation to that of the micrococci. As will be shown later, this group was made up mainly of micrococci. The same could be observed about the peptonizing and alkali-forming groups and the organisms included under the heading "Other bacteria".

Pasteurised Milk.

Table 14 compares the predominant microflora of the pasteurised milks and table 15 and table VI in the appendix give the average proportions of the bacterial groups according to/

to the action on litmus milk and on lactose and casein respectively.

TABLE 14. - The average predominant microflora in the different grades of milk after pasteurisation.

	Percentage			
	Strepto-cocci	Micro-cocci	Coryne-bacteria	Other bacteria
Aseptically-drawn	-	-	-	-
Clean certified	-	-	-	(100)
Certified	-	5.4	94.0	0.5
Tank milk	52.0	8.8	38.9	0.3

TABLE 15.- The average percentages of the predominant bacterial groups according to the action on litmus milk as they appeared after the pasteurisation of the different grades of raw milk.

	Percentage				
	Acid-Producing	Acid-Peptonizing	Peptonizing	Alkali-Forming	Inert
Aseptically-drawn	-	-	-	-	-
Clean certified	-	-	(100)	-	-
Certified	94.9	-	0.3	0.3	4.5
Tank milk	97.7	0.3	0.2	0.5	1.3

While a great similarity between the relative proportions of the bacterial groups in both pasteurised-certified and pasteurised-tank milk appears in table 15 and table VI of the appendix, a glance at table 9 will reveal a significant difference. Although the average percentages of the acid-producing group in both milks were approximately the same, the bacteria forming this group in/

in both differed. The acid-producing group in the certified samples was made up mainly of corynebacteria, while in tank milk it was made up mainly of streptococci and corynebacteria.

DISCUSSION

The study described above is restricted to a limited number of samples on account of the time-consuming nature of this type of incestigation, but it nevertheless includes most seasons of the year and has yielded new information on the bacteriology of pasteurisation. Certain tentative conclusions concerning the effect of the low-temperature process on the bacterial flora of raw milk may be drawn from the results:

1. Carefully produced milk can be practically sterilised.

2. Predominant flora of pasteurised, moderately contaminated milk and milk produced without care are essentially acid-forming types.

3. In moderately contaminated milk, the dominant organisms after the heating, may be expected to be corynebacteria and, in certain samples, a smaller number of micrococci.

4. In milk produced without care the principal organisms which survive the treatment are likely to be streptococci, corynebacteria and micrococci in that order.

5. Organisms of other groups are probably of little importance in the freshly processed milk unless they were introduced in the plant. They are detected on pasteurised milk plates in this work only to the extent of a few aerobic spore-formers, a few actinomyces and one *Alcaligenes* strain.

The occurrence of large numbers of corynebacteria in pasteurised milk has received little attention in the past. It is likely that they have frequently been overlooked as a result of incubating plates at 37°C. The thermoduric species which occur in milk produce little or no growth at that temperature.

CHANGES WHICH OCCUR IN THE PREDOMINANT FLORA OF RAW MILK AFTER INCUBATION AT DIFFERENT TEMPERATURES AND THE EFFECT OF SUCH INCUBATION ON THE FLORA OF THE PASTEURISED PRODUCT

When raw milk is left to stand for some time at different temperatures the relative proportions of the bacterial flora undergo some changes. These changes undoubtedly depend on the original flora and on the temperature and length of time at which the milk is kept. That such changes in the incubated raw milk may result in a change in the pasteurised milk flora is quite possible. To observe the nature of such changes, raw milk samples of the different milk grades were divided into three portions: one portion was examined immediately before and after pasteurisation; another portion was heated to 22°C. ~~in water~~ and then kept ~~in~~ at 22°C. ~~incubator~~ while still immersed in the water; the third portion was cooled by immersing in cold water and kept in a cool place, the temperature of which did not show much fluctuation - varying between 9°-11°C. in winter and 10°-14°C. in summer. The length of incubation time at 22°C. was originally planned to be 6 hours for all the samples examined but it was found necessary to increase the time to 24 hours in the case of certified milk samples. This alteration in the incubation time was found necessary because the results obtained from the 6 hours' period of incubation showed little or no change in the bacterial population of the clean certified milk samples. At the lower temperature of incubation all milk samples were held for 24 hours. The incubated milks were examined in the usual way immediately before and after pasteurisation. The temperatures at which the samples were held were chosen in order to represent summer and winter conditions.

Clean/

TABLE 16 - Effect of storage at 22°C. for 6 hours and at 9°-11°C. prior to pasteurisation on the microflora of clean certified milk

Sample:		1						2					
Treatment:		Ø 0		6 hours at 22°C.		24 hours - 9°-11°C.		0		6 hours at 22°C.		24 hours - 9°-11°C.	
Raw	Plate count per ml.	3850	%	1605	%	6400	%	24200	%	17600	%	90000	%
	Streptococci " "	235	6.1	143	8.9	-	-	1476	6.1	2517	14.3	2070	2.3
	Micrococci " "	3149	81.1	1320	82.3	2400	37.5	2928	12.1	5034	28.6	4230	4.7
	Corynebacteria "	350	9.1	-	-	397	6.2	4380	18.1	1513	8.6	10440	11.6
	Other bacteria "	116	3.0	143	8.9	3603	56.3	15415	63.7	8536	48.5	73260	81.4
Pasteurised	Plate count per ml.	-	-	2		2		11		8		12	
	Streptococci " "	-		-		-		-		-		-	
	Micrococci " "	-		-		-		-		-		-	
	Corynebacteria "	-		-		-		-		-		-	
	Other bacteria "	-		2	(100)	2	(100)	11	(100)	8	(100)	12	(100)

Ø 0 = Fresh sample.

Clean Certified Milk.

The effect of 6 hours' incubation at 22°C. for 24 hours at 9°-11°C. on the predominant microflora of two clean certified milk samples before and after pasteurisation is shown in table 16. The months in which the samples were taken were: No. 1 in January and No. 2 in March. The samples were produced on two different farms. Plate counts obtained from the two samples after 6 hours' incubation at 22°C. showed a slight decrease. The reduction in the plate counts might have been due to one or more of three possibilities: no multiplication of the organisms took place (the reduction was within the probable error of the plate count); a result of the bacteriostatic effect of the milk; and/or organisms were in the lag phase. The relative proportions of the bacterial groups after the 6 hours' incubation at 22°C. consequently did not show any significant changes.

The effect of the 24 hours' incubation at 9°-11°C. is interesting. The plate counts showed practically the same increase as that shown by the organisms included under the heading "Other bacteria". These were mainly psychrophilic types of which Pseudomonas fluorescens was the most encountered and, therefore, their big increase in the raw milk held at low temperatures is quite understandable.

Holding the clean certified milk samples at the high and low temperatures had no noticeable effect on the pasteurised product.

The changes in the predominant bacterial groups according to the action on litmus milk are shown in table 17, and to the action on lactose and casein, in table VII of the appendix. The main changes which occurred correspond to the changes in the microflora/

TABLE 17. - Effect of storage at 22°C. for 6 hours and at 9°-11°C. for 24 hours prior to pasteurisation on the bacterial groups of clean certified milk according to the action on litmus milk.

Sample	Treatment	Percentage									
		Raw					Pasteurised				
		Acid-Producing	Acid-Peptonizing	Peptonizing	Alkali-Forming	Inert	Acid-Producing	Acid-Peptonizing	Peptonizing	Alkali-Forming	Inert
1	0	48.5	33.3	-	6.1	12.1	-	-	-	-	-
	6 hrs. at 22°C.	73.5	20.6	-	-	5.9	-	-	(100)	-	-
	24 hrs. at 9-11°C.	21.9	15.6	34.3	18.8	9.4	-	-	(100)	-	-
2	0	18.2	-	39.4	15.2	27.2	-	-	(100)	-	-
	6 hrs. at 22°C.	22.9	22.9	25.7	11.4	17.1	-	-	(100)	-	-
	24 hrs. at 9-11°C.	2.3	11.6	65.1	11.6	9.3	-	-	(100)	-	-

microflora. Thus, the changes were mainly an increase of the peptonizing group corresponding to the increase in the number of the psychrophilic bacteria when the samples were held at 9°-11°C. for 24 hours.

Certified Samples.

Four samples were employed for determining the effect of incubation on the predominant flora in the raw milk and in their pasteurised product. All four samples were incubated at 22°C. for 24 hours, and only three of the samples were incubated at lower temperatures as well. Of these samples, one was held at 10°-13°C., another at 12°-14°C. and the third at 8°-10°C. The effect of the incubation on the microflora is shown in table 18. The months in which the samples were taken were: No. 1 in October, No. 2 in April, No. 3 in June and No. 4 in November.

The plate counts of the incubated raw milk showed active multiplication of the organisms. Before discussing the results in the table it should be mentioned that changes in the percentages of the bacterial groups indicate changes in their relative proportions and not in their numbers. Thus, in sample 3, for example, the drop in the percentage of the streptococci in the raw milk held at 12°-14°C. does not mean a decrease in their numbers but a decrease in their relative proportion due to the greater rate of growth shown by the organisms included under the heading "Other bacteria".

The results show that the corynebacteria in the raw milk were over-grown by the other groups of bacteria when the milk was held at the different temperatures. These corynebacteria were mainly of the Corynebacterium lacticum type.

Holding the samples at 22°C. favoured the growth of streptococci/

TABLE 18. - Effect of 24 hours' storage at 22°C. and at 8°-14°C. prior to pasteurisation on the microflora of certified milk.

Sample	Raw milk held for 24 hours at:	Raw					Pasteurised				
		Plate Count per ml.	Percentage				Plate count per ml.	Percentage			
			Strepto-cocci	Micro-cocci	Coryne-bacteria	Other bacteria		Strepto-cocci	Micro-cocci	Coryne-bacteria	Other bacteria
1	-	17,000	2.5	60.0	32.5	5.0	11,000	-	4.3	95.7	-
	22°C.	7,600,000	25.7	34.3	-	40.0	97,000	92.3	2.6	5.1	-
2	-	8,700	5.1	30.8	59.0	5.1	5,400	-	17.3	82.7	-
	22°C.	29,600,000	21.7	4.3	-	73.9	14,200	56.6	3.8	39.6	-
	10°-13°C.	1,880,000	-	10.3	-	89.7	6,200	-	16.1	83.9	-
3	-	10,200	22.5	5.0	30.0	42.5	2,150	-	-	100.0	-
	22°C.	51,000,000	50.0	6.3	-	43.7	12,000	20.0	-	77.1	2.9
	12°-14°C.	18,000,000	14.3	5.7	-	80.0	16,000	6.9	-	13.8	79.3
4	-	239,000	6.9	3.4	13.8	75.9	34,000	-	-	100.0	-
	22°C.	82,000,000	52.6	-	-	47.4	35,000	-	-	97.2	2.8
	8°-10°C.	315,000	16.0	-	12.0	72.0	47,000	-	-	100.0	-

some of the results obtained by previous workers who reported large alkali-forming groups in the pasteurised milk.

TABLE 19. - Effect of 24 hours' storage at 22°C. and at 8°-14°C. prior to pasteurisation on the predominant species of streptococci in certified milk.

	Specific name	Percentage		
		Fresh Milk	Raw milk held for 24 hrs. at	
			22°C.	8°-14°C.
Raw	(S. lactis	30.0	38.8	10.0
	(S. cremoris	5.0	10.4	20.0
	(Mastitis streptococci	35.0	20.9	-
	(Leuconostoc sp.	30.0	20.9	70.0
Pasteurised	(S. bovis	-	8.8	-
	(S. thermophilus	-	1.5	-
	(S. paracitrovorus	-	89.7	(100)

Table 19 illustrates the changes which occurred among the predominant streptococci in the raw milk held at the two different temperatures and the subsequent changes which resulted among the streptococci group in the pasteurised product. Incubation of the raw milk at 22°C. revealed small changes in the relative proportions in favour of the lactic streptococci group. On the other hand, incubation at the low temperature favoured the leuconostoc types and caused the mastitis streptococci to disappear from among the predominant flora. This is not surprising as the latter possess a minimum growth temperature above that at which the milk was held.

The effect of storing certified milk at the high and low temperatures, prior to pasteurisation, on the predominant bacterial groups in the raw and pasteurised milk, is presented in table 20 and table VIII in the appendix. Table 20 shows the grouping of/

TABLE 20. - Effect of 24 hours' storage at 22°C. and at 8°-14°C. prior to pasteurisation on the bacterial groups in certified milk according to the action on litmus milk.

Sample	Raw Milk Held for 24 hrs.at:	Percentage									
		Raw					Pasteurised				
		Acid-Forming	Acid-Peptonizing	Peptonizing	Alkali-Forming	Inert	Acid-Forming	Acid-Peptonizing	Peptonizing	Alkali-Forming	Inert
1	-	47.5	5.0	5.0	20.0	22.5	100.0	-	-	-	-
	22°C.	28.6	31.4	-	22.9	17.1	100.0	-	-	-	-
2	-	79.5	5.1	5.1	5.1	5.1	100.0	-	-	-	-
	22°C.	26.1	-	13.0	26.1	34.8	100.0	-	-	-	-
	10°-13°C.	6.9	-	-	48.3	44.8	100.0	-	-	-	-
3	-	57.5	-	15.0	22.5	5.0	100.0	-	-	-	-
	22°C.	58.3	-	18.8	16.7	6.3	97.1	-	-	2.9	-
	12°-14°C.	17.1	-	-	42.9	40.0	20.7	-	-	79.3	-
4	-	24.1	-	44.8	31.0	-	100.0	-	-	-	-
	22°C.	52.6	-	2.6	44.7	-	97.2	-	-	2.8	-
	8°-10°C.	28.0	-	32.0	40.0	-	100.0	-	-	-	-

of the organisms according to the action on litmus milk and table VIII of the appendix shows the grouping according to the action on lactose and casein. The tables show changes in harmony with those which appeared in the predominant microflora (table 18).

Tank Milk.

Table 21 presents data obtained concerning the predominant microflora of tank milk after storage at 22°C. for 6 hours and at 10°-14°C. for 24 hours prior to pasteurisation. Table 22 and table IX in the appendix show the corresponding relative proportions of the bacterial groups according to the action on litmus milk and on lactose and casein respectively. The months in which the samples were taken were: No. 1 in November, No. 2 in February, No. 3 in May and No. 4 in October.

Although the raw milk plate counts showed a great increase after the holding of the samples, there was practically no change in the numbers of bacteria surviving pasteurisation. The growth of the thermoduric bacteria in the raw milk during the holding periods must have been slight and/or their heat resistance might have been decreased due to the passage from the resting stage to the logarathmic growth phase. Robertson (59) reported that cultures of Microbacterium lacticum, Sarcina lutea and Streptococcus thermophilus were more susceptible to heat in the accelerative growth stage than in the resting stage.

The great increase in the bacterial numbers in the raw milk after storage, with no corresponding significant change in the post pasteurisation counts, may afford an explanation to many observations made by previous workers. For instance, Macy (45) observed that the bactericidal efficiency of pasteurisation is greater in summer than in winter. Hussong and

Hammer/

TABLE 21 - Effect of storage at 22°C. for 6 hours and at 10°-14°C. for 24 hours prior to pasteurisation on the microflora of tank milk.

Sample	Raw milk held at	Raw					Pasteurised				
		Plate count per ml.	Percentage				Plate count per ml.	Percentage			
			Streptococci	Micrococci	Corynebacteria	Other bacteria		Streptococci	Micrococci	Corynebacteria	Other bacteria
1	-	1,130,000	60.5	11.5	2.3	25.6	50,000	11.1	22.2	66.7	-
	11°-13°C. for 24 hrs.	26,500,000	59.3	3.7	-	37.0	42,000	21.2	6.1	72.7	-
2	-	355,000	66.7	5.6	-	27.7	33,000	43.8	6.2	50.0	-
	22°C. for 6 hrs.	1,075,000	46.5.	2.3	-	51.2	31,000	57.1	10.7	32.1	-
	10°-11°C. for 24 hrs.	7,250,000	32.4	-	-	67.6	30,000	57.5	15.2	27.3	-
3	-	770,000	18.0	2.6	2.6	76.9	126,500	56.3	6.3	37.5	-
	22°C. for 6 hrs.	11,500,000	42.9	-	-	57.1	116,500	82.7	3.5	13.8	-
	12°-13°C. for 24 hrs.	39,000,000	35.5	-	-	64.5	132,000	73.8	3.3	23.0	-
4	-	380,000	53.1	18.8	-	28.1	6,900	2.9	5.9	91.2	-
	22°C. for 6 hrs.	36,000,000	37.5	12.5	-	50.0	6,700	11.4	8.6	71.4	8.6
	12°-14°C. for 24 hrs.	270,000,000	72.2	2.8	-	25.0	9,550	5.1	2.6	79.5	12.8

TABLE 22. - Effect of storage at 22°C. for 6 hours and at 10°-14°C. for 24 hours prior to pasteurisation on the bacterial flora of tank milk according to the action on litmus milk.

Sample	Raw Milk Held at:	Percentage									
		Raw					Pasteurised				
		Acid-Forming	Acid-Peptonizing	Peptonizing	Alkali-Forming	Inert	Acid-Forming	Acid-Peptonizing	Peptonizing	Alkali-Forming	Inert
1	-	62.8	4.7	13.9	9.3	9.3	95.6	-	-	2.2	2.2
	11°-13°C. for 24 hrs.	63.0	-	7.4	11.1	18.5	100.0	-	-	-	-
2	-	69.4	5.6	19.4	2.8	2.8	100.0	-	-	-	-
	22°C. for 6 hours	48.8	2.3	34.9	4.7	9.3	100.0	-	-	-	-
	10°-11°C. for 24 hrs.	32.4	-	27.0	2.7	37.8	100.0	-	-	-	-
3	-	25.6	-	46.2	7.7	20.5	87.5	3.1	-	-	9.4
	22°C. for 6 hrs.	57.1	-	2.4	16.7	23.8	100.0	-	-	-	-
	12°-13°C. for 24 hrs.	57.6	3.0	9.1	18.2	12.1	100.0	-	-	-	-
4	-	59.4	3.1	3.1	21.9	12.5	97.1	-	2.9	-	-
	22°C. for 6 hrs.	43.8	-	-	46.9	9.4	88.6	-	-	11.4	-
	12°-14°C. for 24 hrs.	72.2	-	-	25.0	2.8	87.2	-	-	12.8	-

Hammer (39), Anderson and Meanwell (1) and Scott and Wright (64) found that the plate counts of raw milk do not bear any relation to the plate counts after pasteurisation and thus, assessing the quality of the raw milk from the point of view of pasteurisation on its plate count, is futile and misleading.

Changes in the relative proportions of the bacterial groups in the raw milk induced by the multiplication of the organisms during the holding periods show that when streptococci (table 21) or the acid-forming group (tables 22 and IX) decreased in proportion, the "Other bacteria" group or the alkali-formers and the peptonizers uniformly increased and vice versa.

TABLE 23 - Effect of storage prior to pasteurisation on the predominant species of streptococci in tank milk.

	Specific Name	Percentage		
			Raw Milk Held at:	
		0	22°C. for 6 hrs.	10°-14°C. for 24 hrs.
Raw	(Mastitis streptococci	6.7	1.5	-
	(S. lactis	56.0	46.3	60.4
	(S. cremoris	6.7	31.4	7.5
	(S. faecalis	2.6	-	-
	(S. bovis	2.7	-	-
	(S. thermophilus	1.3	-	-
	(Leuconostoc sp.	24.0	20.9	32.1
Pasteurised	(S. bovis	53.8	70.5	68.5
	(S. thermophilus	46.2	29.5	31.5

The effect of holding the raw milk at 22°C. for 6 hours and at 10°-14°C. for 24 hours on the predominant species of streptococci encountered before and after pasteurisation, is illustrated in table 23. Hucker (35) found that holding raw milk at 20°C. as compared with 10°C. for 4 hours prior to pasteurisation/

pasteurisation caused the percentage of S. thermophilus in the pasteurised milk to increase at the expense of the other species of cocci. This finding could not be confirmed in this study.

SUMMARY

1. Storage at 22°C. usually favoured the development of the lactic streptococci in the raw milk. 6 hours' storage of badly contaminated milk greatly increased the bacterial population but had no effect on the post pasteurisation count or on the surviving microflora. On the other hand, 24 hours' storage of moderately contaminated milk resulted in an increase in the post pasteurisation count on account of the enrichment of thermoduric streptococci. These were largely heterofermentative types.

2. 24 hours' storage at 8°-14°C. generally favoured the development in the raw milk of gram-negative psychrophilic rods. The post pasteurisation count increased in two samples due to the enrichment of a heat resistant Alcaligenes type. Otherwise there was practically no apparent change in the microflora surviving the heat treatment.

THE BACTERIAL DEVELOPMENT IN MILK PASTEURISED IN THE LABORATORY AND HELD AT DIFFERENT TEMPERATURES

After having determined the groups of bacteria which survived pasteurisation, their subsequent development was determined in some of the samples. To start with, the development of the bacterial flora in the pasteurised samples was traced only after a short period of holding at a comparatively high and a low temperautre. Later, however, the development of the bacteria in the pasteurised samples was followed up for a long period, usually until spoilage occurred.

Bacterial development in pasteurised milk held at 22°C. and at 10°-14°C. for 24 hours.

The pasteurised samples were divided immediately after the process of pasteurisation into 3 portions: the first portion was plated for the purpose of determining the initial predominant flora; the second was warmed to 22°C. and then held in a 22°C. incubator,(the milk was kept immersed in water while in the incubator so that any slight fluctuation in the temperature would have a negligible effect on the temperature of the milk); the third portion was placed in cold water and held in a steady cool place, the temperature of which changed according to the weather conditions and season between 8°and 14°C. At the end of the 24 hours' holding period the predominant flora in the incubated portions were determined in the usual manner as described before.

Eight samples in all - 2 clean certified, 3 certified and 3 tank milk samples - were used in this study. The changes in the predominant microflora after the incubation of the pasteurised samples are presented in table 23 and the corresponding changes in the predominant bacterial groups, according to

action/

TABLE 23. - The effect of 24 hours' holding at 22°C. and at 8-14°C. on the microflora of pasteurised milk.

Ø Sample	Held 24 hrs. at:	Plate count per ml.	Percentage				
			Strepto-cocci	Micro-cocci	Coryne-bacteria	Alcali-genes	Other bacteria
Cl.C.1	-	0	-	-	-	-	-
	22°C.	1	-	-	-	-	(100)
	10-12°C.	1	-	-	-	-	(100)
Cl.C.2	-	11	-	-	-	-	(100)
	22°C.	30	-	20.0	13.3	-	66.7
	10-12°C.	20	-	-	-	-	100.0
C.1	-	5,400	-	17.3	82.7	-	-
	22°C.	46,000	-	2.2	15.2	82.6	-
	10-13°C.	5,700	-	13.9	83.3	2.8	-
C.2	-	2,145	-	-	100.0	-	-
	22°C.	281,000	-	-	50.0	50.0	-
	12-14°C.	3,300	-	-	97.1	2.9	-
C.3	-	34,000	-	-	100.0	-	-
	22°C.	3,200,000	-	-	3.2	96.8	-
	10°C.	41,000	-	-	91.9	8.1	-
T.1	-	33,000	43.8	6.2	50.0	-	-
	*22°C.	40,000	68.5	14.2	17.2	-	-
	10°C.	37,000	61.9	9.5	28.6	-	-
T.2	-	126,500	56.3	6.3	37.5	-	-
	22°C.	965,000	100.0	-	-	-	-
	12-13°C.	107,000	64.3	7.1	28.6	-	-
T.3	-	6,900	2.9	5.9	91.2	-	-
	22°C.	360,000	-	3.3	3.3	93.3	-
	12-14°C.	11,700	4.3	-	43.5	52.2	-

*This sample was held for 6 hours only.

Ø Cl.C. = Pasteurised clean certified sample; C. = Pasteurised certified milk; T. = Pasteurised tank milk.

TABLE 24. - The effect of 24 hours' holding at 22°C. and at 8-14°C. on the predominant bacterial groups in pasteurised milk according to the action on litmus milk.

Ø Sample	Held 24 hrs. at:	Percentage				
		Acid-forming	Acid-peptonizing	Peptonizing	Alkali-forming	Inert
	-	-	-	-	-	-
Cl.C.1	22°C.	-	-	(100)	-	-
	10-12°C.	-	-	(100)	-	-
	-	-	-	100.0	-	-
Cl.C.2	22°C.	26.7	-	73.3	-	-
	10-12°C.	-	-	85.7	-	14.3
	-	100.0	-	-	-	-
C.1	22°C.	17.4	-	-	82.6	-
	10-13°C.	97.2	-	-	2.8	-
	-	100.0	-	-	-	-
C.2	22°C.	50.0	-	-	50.0	-
	12-14°C.	97.1	-	-	2.9	-
	-	100.0	-	-	-	-
C.3	22°C.	3.2	-	-	96.8	-
	8-10°C.	91.9	-	-	8.1	-
	-	100.0	-	-	-	-
T.1	*22°C.	97.1	2.9	-	-	-
	10-11°C.	100.0	-	-	-	-
	-	87.5	3.1	-	-	9.4
T.2	22°C.	100.0	-	-	-	-
	12-13°C.	100.0	-	-	-	-
	-	97.1	-	2.9	-	-
T.3	22°C.	6.7	-	-	93.3	-
	12-14°C.	47.8	-	-	52.2	-

*This sample was held for 6 hours only.

ØCl.C. = Pasteurised clean certified samples; C. = Pasteurised certified sample; T. = Pasteurised tank milk sample.

action on litmus milk, are shown in table 24 and according to the action on lactose and casein in table X in the appendix.

In the pasteurised clean certified sample 1, the appearance of one colony (Bacillus subtilis) on each of the duplicate 1 ml. plates of both incubated portions, was the only change in the flora. This result supports the finding that the udder flora do not survive the pasteurisation treatment. Clean certified sample 2, however, showed some increase in the number of colonies appearing on the 1 ml. plates. This increase was accompanied only in the portion incubated at 22°C. by the appearance of a few micrococci and a few corynebacteria in addition to the original flora which consisted of spore-formers and actinomyces. The micrococci, which were identified as M. epidermidis, when tested for their heat resistance showed a major death point below 63°C. Their appearance in the incubated milk was, therefore, due to the development of a few cells surviving the pasteurisation treatment.

Pasteurised certified milk samples held at 22°C. for 24 hours showed interesting changes in their bacterial flora. The corynebacteria and micrococci, which were the main bacterial groups in the freshly pasteurised samples, were uniformly overgrown by Alcaligenes viscosus b., and consequently the acid-producing group diminished and the alkali-forming group greatly increased. When Alcaligenes viscosus b. was tested for its heat resistance in milk, some strains survived 63°C. for 30 minutes in reduced numbers; but when these heated milks were incubated at 22°C. all the strains produced growth.

Similar changes in the bacterial flora took place in the portions held at 10°-14°C. but the changes were small because

of/

of the slight bacterial development which occurred.

When the pasteurised tank milk was held at the two different temperatures the bacterial flora showed two distinct changes depending on the predominant microflora in the freshly pasteurised samples. Thus, sample 3 which contained after pasteurisation a predominant flora not unlike that of the pasteurised certified samples, on being held at both 22°C and 13°C. for 24 hours showed a great increase in the proportion of the alkaliformers due to the rapid development of Alcaligenes viscosus b. On the other hand, Samples 1 and 2 which were distinguished by the presence of a high percentage of streptococci, showed different bacterial changes when held at 22°C. The streptococci uniformly tended to overgrow the other groups. This change in the microflora has no effect on the relative proportions of the bacterial groups according to the action on litmus milk (table 24), the method most frequently used for recording the changes in the bacterial flora of pasteurised milk. When the latter two samples were held at 10° - 13°C there was practically no change either in the plate counts or the bacterial groups. This may become understandable when it is noted that the streptococci encountered in both samples were either S. thermophilus or S. bovis. both possessing a minimum growth temperature above that at which the milk was held. Also the corynebacteria, as was seen in the pasteurised certified milk, grow slowly in milk, especially at the low temperatures.

Bacterial/

Bacterial development in pasteurised milk held at 22°C and at 10° - 14°C until spoilage.

The qualitative and quantitative changes in the bacterial flora of pasteurised milk when held at various temperatures seem to have attracted the attention of only a few investigators. Ayers and Johnston (2) reported that when different grades of milk are pasteurised at 63°C for 30 minutes and held at 21.1° - 23.9°C the bacterial flora may undergo three distinct changes. First when a fair quality milk is pasteurised, the acid group may develop at once and overgrow all the other groups. Second, when a poor quality milk is pasteurised the peptonizing group may grow rapidly at first along with the acid group which later overgrows it. Third, when a good grade of milk is pasteurised the peptonizing bacteria may overgrow the acid group of organisms. These same grades of milk, when held at 10°C showed entirely different changes in their bacterial contents. The growth of the peptonizing group is restrained so that they are of little importance. The percentage of the acid group remain about the same through a long period. Occasionally the percentage of the alkali group increases after five days, but eventually the acid group forms the major group. These conclusions were based on the result of studying three samples of milk which had been graded according to the raw milk plate count irrespective of the post-pasteurisation count. Thus, it is not astonishing to find that what was considered poor quality milk gave a pasteurised milk which contained 8400 bacterial clumps per ml. after being held for 24 hours at 21.1°-23.9°C while the fair quality milk revealed a post-pasteurisation count/

count of 21,700 per ml. Prouty (53) noted that when pasteurised low count milk was held at 21.1°C. (70°F.) for 24 hours, the acid forming bacteria decreased, but after 48 hours they increased rapidly so that they formed the major group. Rowlands and Provan (61) noted the changes in the bacterial flora of milk pasteurised by the "in bottle" process, during storage at 15.5°C. and found that the acid-producing organisms were superseded by alkali-forming and peptonizing types which formed only a relatively small proportion of the total flora of fresh samples. After 72 hours organisms producing acid predominated. Study of the acid-forming types isolated after 72 hours' storage showed that they differed from those isolated from earlier platings. Whereas the organisms isolated from fresh samples produced acid very slowly in litmus milk at 30°C., the majority of those isolated after 72 hours appeared to be typical Streptococcus lactis.

The above review of literature shows the present scanty knowledge of the bacterial development in pasteurised milk. In the earlier section of this report, the study of the effect of pasteurisation on the bacterial flora of milk produced under varying hygienic conditions have shown that in moderately contaminated milk (certified milk) the dominant organisms after heating were corynebacteria, and, in certain samples, a smaller number of micrococci, while in milk produced without care (tank milk) the principal organisms which survived the treatment were streptococci, corynebacteria and micrococci in that order. The corynebacteria were shown to develop slowly when pasteurised milk was held at either 22°C. or 10°-14°C. and were uniformly overgrown by the alkali-former Alcaligenes viscosus b. On the other hand, when

streptococci/

streptococci formed the dominant group they tended to overgrow the other groups present when the pasteurised milk was held at 22°C. while no significant change in either plate count or in the bacterial groups was noted when the milk was held at 10-14°C. These streptococci, as mentioned before, were almost exclusively S. thermophilus and S. bovis. Both types have a minimum growth temperature of about 20°C. and therefore, their inability to grow in pasteurised milk held at the low temperatures seemed understandable. Pasteurised milk, however, is often known to sour when held at temperatures much lower than 20°C. The nature and identity of the organisms which cause this souring still appear to be obscure. As an example, the following statement is taken from a leading dairy text book (60): "There are other streptococci found in milk, particularly in pasteurised milk, which are more heat resistant than the typical Streptococcus lactis and which have an optimum temperature around 37°C. These bacteria have not been so carefully studied in their physiological actions but they appear to be closely related to the typical lactic streptococcus and perhaps represent modified strains. From their growth in pasteurised milk held at 10°C. it appears that they have a wide growth range". It is apparent, therefore, that any information regarding the bacterial development which occurs in pasteurised milk and the significance of the bacterial groups which predominate in the milk immediately after the heat treatment and the subsequent development of the organisms, would be of great interest/

interest. With that in mind, the changes in the bacterial flora of four pasteurised milk samples held at both 22°C. and 10-14°C . were studied and the results obtained from each sample are given below. The milk samples were pasteurised in stoppered test tubes. Immediately after pasteurisation some tubes were heated up to 22°C. and held in the 22°C. incubator, several other tubes were cooled in cold water and held at 10-14°C. while one of the tubes was used for immediate plating. At various intervals one of each of the two sets of the incubated milk tubes was used for plating. Plates were incubated and colonies were picked and characterised in the usual way as described before.

Sample I.

This was a pasteurised certified sample produced in the month of November. The changes in the predominant microflora during holding at 22°C. and 10-11°C. and the corresponding changes in the bacterial groups according to the action on litmus milk are given in tables XI and XII in the appendix. In order to show the changes in the groups more plainly, the results in the tables have been plotted in Figs. 1, 2, 3 and 4. At 22°C. the milk was sweet with a slight fruity flavour on the 7th day and showed an acid curd with slight separation of whey on the 9th day. At 10-11°C. a slight "old flavour" was noted on the 15th day and a decidedly acid flavour was present on the 21st day.

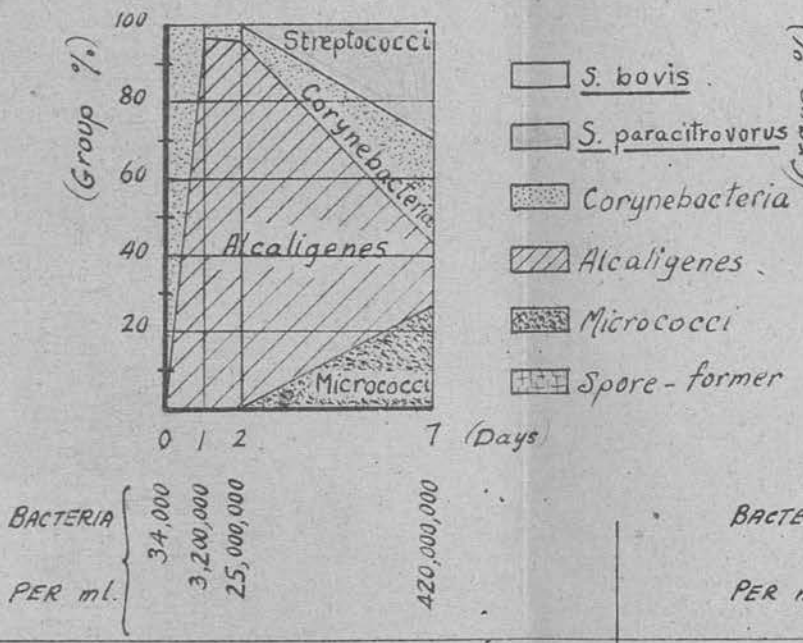
When freshly pasteurised, the sample showed a predominant flora which consisted of acid-forming corynebacteria. One day's storage at 22°C. produced a different picture. The acid-forming group decreased from 100 per cent to 3.2 per cent on account of the quick development of the alkali-formers which appeared to the extent of 96.8 per cent of the flora. Although the plate count

showed/

CHANGES OF BACTERIAL FLORA IN PASTEURISED

SAMPLE I

Fig. 1 – DURING STORAGE AT 22° C.



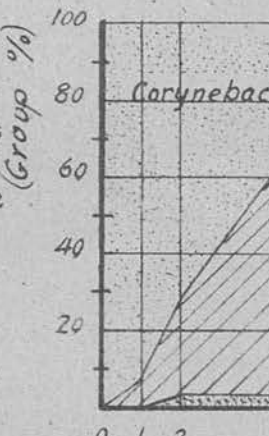
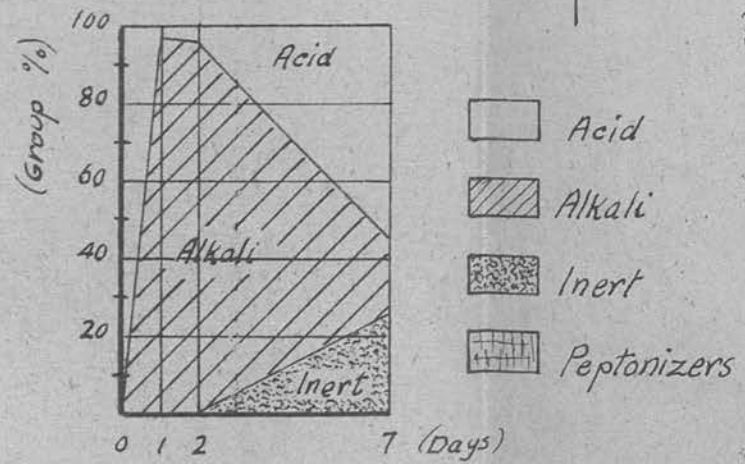


Fig. 2 – DURING STORAGE AT 10°–11° C.



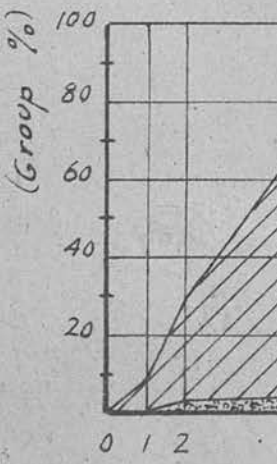


Fig. 3 – DURING STORAGE AT 22° C.

(ACCORDING TO ACTION ON LITMUS MILK)

Fig. 4 – DURING STORAGE AT 10°–11° C.

showed an approximately eight fold increase on the second day of storage, yet, with the exception of a slight increase in the relative proportion of the acid-formers, there was no change in the bacterial flora. On the seventh day, however, the alkali-formers greatly diminished in proportion, due to a big increase in the acid-forming group and the appearance of an inert type of micrococcus to the extent of 26.5 per cent. The acid formers differed from those encountered up to the second day by including a big proportion of streptococci.

Storage at 10°-11°C. produced, on the whole, similar changes to those which appeared at 22°C. The changes, however, were slow in taking place because of the slow development of the organisms as could be seen from the small rate of increase in the plate counts. Thus, the acid-forming corynebacteria decreased uniformly in proportion until the seventh day when they formed only 4.2 per cent of the flora. At the same time, the alkali-formers increased to the extent of 91.6 per cent. On the 15th day, there was an increase in the relative proportion of the acid-producers caused by the appearance of streptococci, to the extent of 38.9 per cent. The alkali-formers on the other hand decreased. An inert group of micrococci, which appeared to the extent of a small percentage on the second day, kept to the same proportion until the 15th day. On the 21st day, peptonizers were encountered to the extent of 22.2 per cent; otherwise there was no significant change except for the disappearance of the inert micrococci from among the predominant flora.

The corynebacteria were of the C. lacticum type; the Alcaligenes group were exclusively of the Alcaligenes viscosus b. variety with ropy and non-ropy strains present; and the micro-

cocci/

micrococci belonged to the M.candicans group and had a major death point below 60°C. for 30 minutes which indicates that their appearance was due to the enrichment of a few cells surviving the pasteurisation treatment. The appearance of the streptococci during the holding time is interesting, especially as they belonged to the heterofermentative S. paracitrovorus Hammer. The peptonizing group which, appeared in the pasteurised milk held at the low temperature, was formed of Bacillus cereus Frankland.

Sample II

This was a pasteurised tank milk sample examined in the month of October. It showed immediately after pasteurisation a predominant flora not unlike that encountered in the previous sample. Tables XIII and XIV in the appendix and Figs.5, 6, 7 and 8 show the changes in the predominant microflora and the corresponding changes in the bacterial groups, according to the action on litmus milk, when the milk was held at 22°C. and at 12°-14°C. This milk showed an acid curd with slight separation of whey after four days' holding at 22°C. and 17 days' holding at 12° - 14°C.

The changes in the flora during storage were somewhat similar to those which appeared in Sample I. The acid-forming group which consisted mainly of corynebacteria, was rapidly over-grown by the alkali-formers but on further incubation it increased again, mainly on account of the development of streptococci. At the low storage temperatures, the peptonizing spore-formers developed to the extent of 56.7 per cent on the third day, but were later suppressed by the increase in the acid forming group.

The qualitative changes in the streptococci are interesting. The freshly pasteurised milk contained streptococci among the predominant flora to the extent of 2.9 per cent. These were exclusively S. bovis. When the milk was held at 22°C. the streptococci/

CHANGES OF BACTERIAL FLORA IN PASTEURISED

SAMPLE II

Fig. 5 - DURING STORAGE AT 22° C.

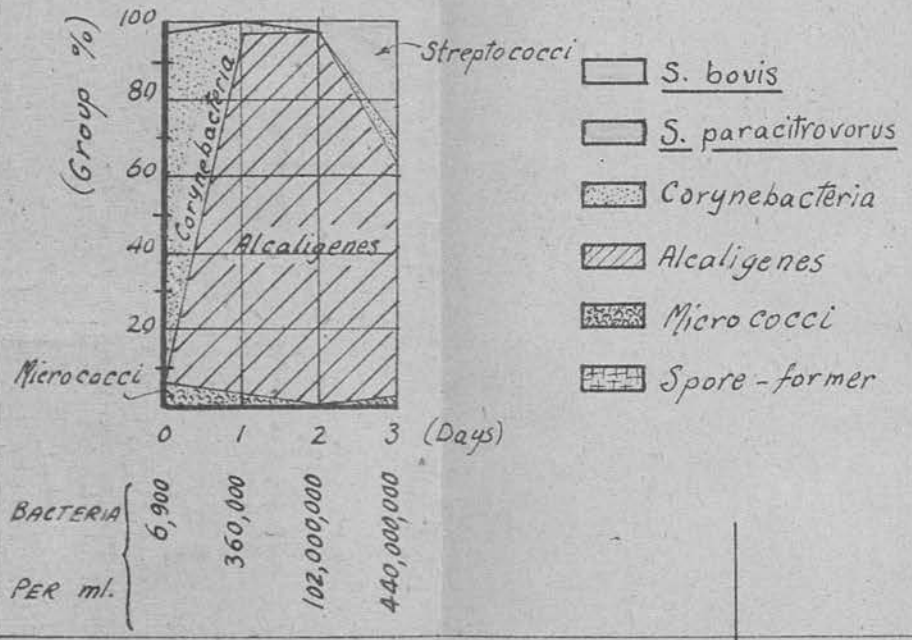
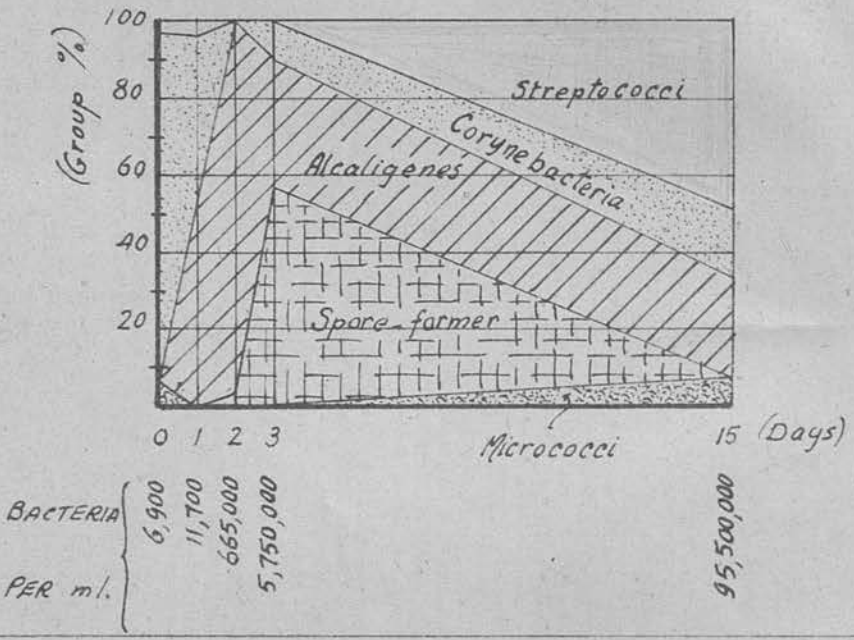


Fig. 6 - DURING STORAGE AT 12°-14° C.



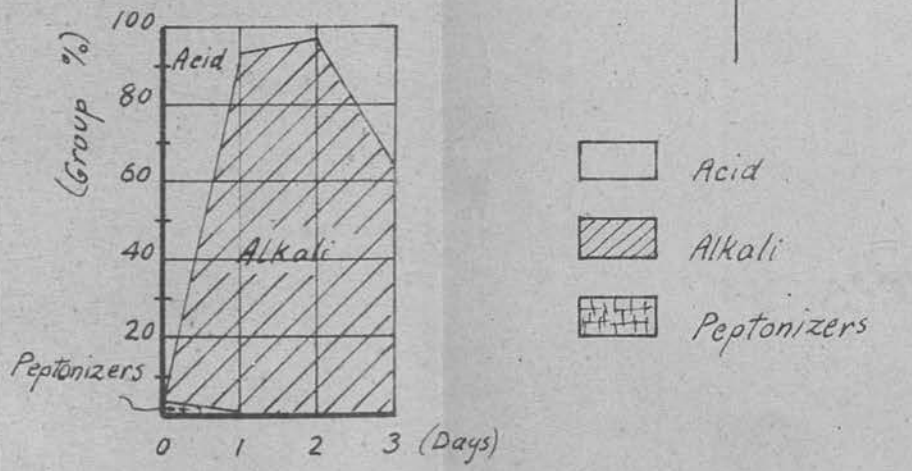


Fig. 7 - DURING STORAGE AT 22° C

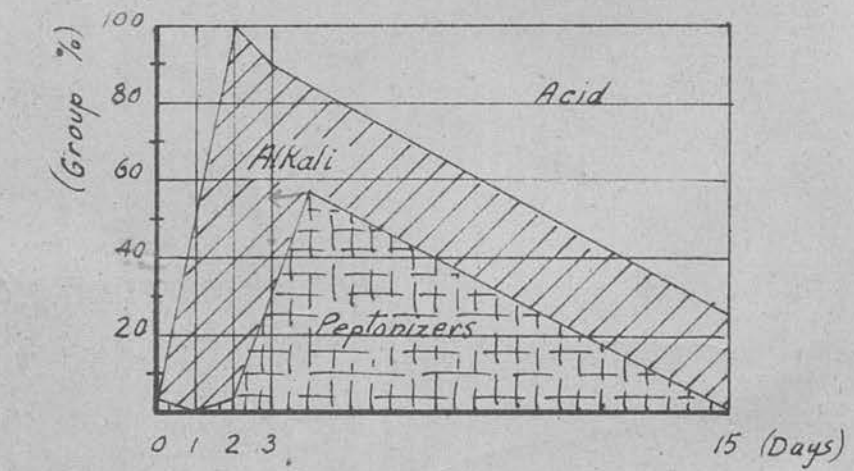


Fig. 8 - DURING STORAGE AT 12°-14° C.

(ACCORDING TO ACTION ON LITMUS MILK)

streptococci which appeared on the third day to the extent of 30.6 per cent, consisted of 19.5 per cent S. bovis and 11.1 per cent S. paracitrovorus. At 12-14°C., however, S. bovis was not encountered among the streptococci which made an appearance on the 15th day. These consisted entirely of S. paracitrovorus.

The corynebacteria belonged to the C. lacticum type and the alkali-forming group was formed exclusively by Alcaligenes viscosus b. Bacillus cereus again formed the peptonizing group.

Sample III

This was a pasteurised tank milk sample examined in the month of March. The predominant microflora which appeared immediately after pasteurisation was different from the previous samples in consisting largely of streptococci. The changes in the flora during holding at 22°C. and 9°-11°C. appear in tables XV and XVI of the appendix and in Figs. 9, 10, 11 and 12. At 22°C. the milk showed acid curdling on the 9th day, while at 9-11°C. 4 out of the 5 incubated tubes showed pronounced casein digestion on the 15th day, the 5th portion showed a soft acid curd with separation of whey on the 21st day. The data obtained during the first days of holding at 9-11°C. were from one of the portions which showed digestion of casein on the 15th day, while those obtained on the 15th and 20th days were from the odd portion which soured. The changes in the bacterial flora at either temperature of holding are extremely interesting. The plate counts of the milk held at 22°C. showed a continuous increase starting from the first day. The acid-forming group was on the whole predominant during the holding period. The alkali-forming group showed a great increase on the seventh day but declined again/

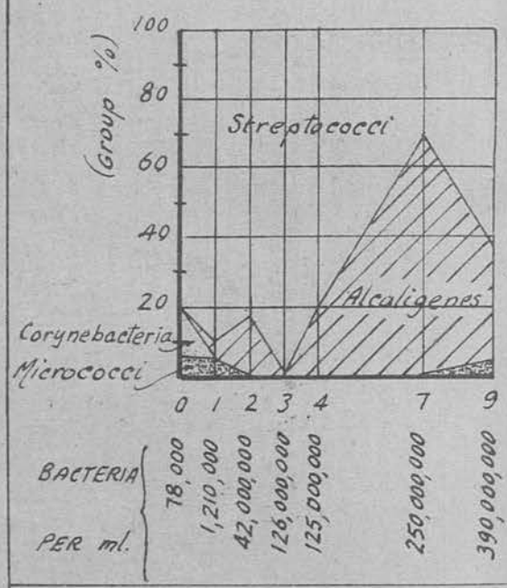
again on the ninth day. This is similar to the changes reported by Ayers and Johnson (2) for their fair quality milk sample. The acid formers were mainly Streptococcus bovis and, to a much smaller extent, Streptococcus thermophilus. These streptococci were the only types encountered. The alkali-formers were exclusively Alcaligenes viscosus b.

In contrast to these changes, the milk portions held at 9°-11°C. showed no increase in the plate counts until the seventh day when all the predominant flora was made up of Bacillus cereus. During the apparent stationary period, i.e. the first four days - apart from the appearance of a small percentage of Alcaligenes viscosus b. and the disappearance of the small Micrococcus luteus group and the Corynebacterium lacticum group, there was no qualitative change in the bacterial groups. The streptococci were S. bovis and S. thermophilus. The study of the predominant flora which appeared in the ~~odd~~ which was sour by th portion gave a different picture. The main flora was formed on the 15th day of Alcaligenes viscosus b., Streptococcus paracitrovorus and a few Corynebacterium lacticum types, and on the 20th day, was almost exclusively S. paracitrovorus. The disappearance of S. bovis and S. thermophilus and the appearance of S. paracitrovorus instead, shows clearly that the first two streptococci, although they form the predominant flora of the freshly pasteurised milk, do not multiply in the milk when it is held at low temperatures and that spoilage of pasteurised milk stored at low temperatures is caused by the subsequent development of other organisms present in small numbers after the pasteurisation process. A support to this finding can be found among the data given by Ayers and Johnson (2) in which the plate counts of the pasteurised milk samples/

CHANGES OF BACTERIAL FLORA IN PASTEURISED

SAMPLE III

Fig. 9 - DURING STORAGE AT 22° C



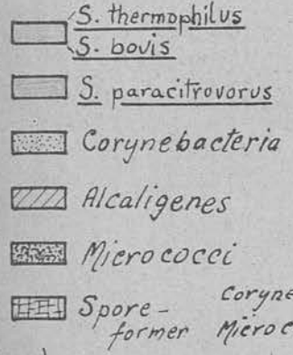
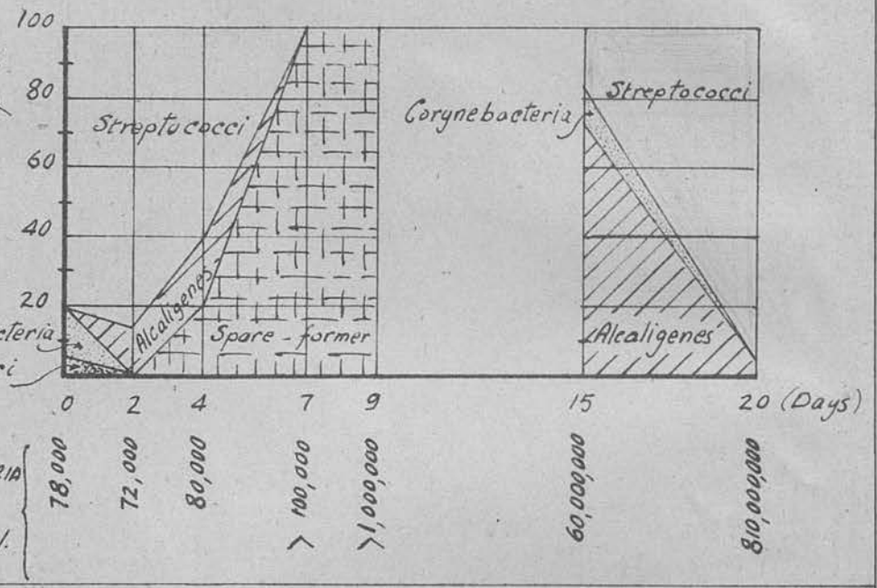
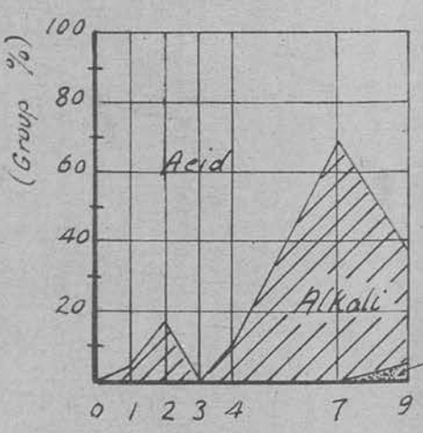
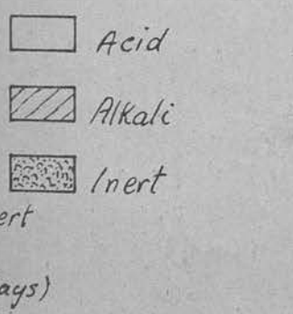


Fig. 10 - DURING STORAGE AT 9° - 11° C.







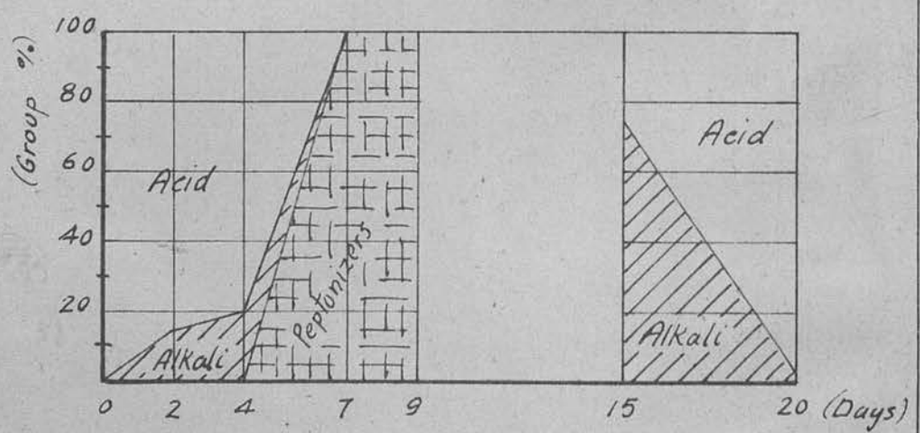


Fig. 11 - DURING STORAGE AT 22° C.

Fig. 12° - DURING STORAGE AT 9° - 11° C.

(ACCORDING TO ACTION ON LITMUS MILK)

samples held at 10°C. kept stationary until the 7th day.

Sample IV

This was a pasteurised tank milk examined in March. The predominant microflora after pasteurisation was very similar to that of sample III. The changes which occurred in the predominant flora during holding at 22°C. and 9-11°C. are given in tables XVII and XVIII of the appendix and Figs. 13, 14, 15 and 16. At 22°C. the milk produced a soft acid curd on the 9th day and at 9-11°C. it curdled on the 20th day. The changes in the bacterial flora which took place at 22°C. were, on the whole, similar to those which occurred in sample III except that Alcaligenes viscosus b. did not develop to the extent encountered in the latter sample. Streptococcus bovis and Streptococcus thermophilus were the only streptococci predominant during the whole period of holding.

The changes which occurred at 9-11°C. did not show the great increase in the peptonizing group that was encountered in sample III. The plate count, however, kept stationary during the first four days of holding and when it did show increase on the 7th day, the predominant flora changed, in that Streptococcus bovis and Streptococcus thermophilus were overgrown by S. paracitrovorus which developed quickly to form the dominant group of bacteria during the rest of the holding period until spoilage occurred. These changes are in agreement with the discussion put forward for sample III.

The common development of the peptonizing spore-formers in pasteurised milk stored at the low temperatures and not at the high temperatures employed in this study, may be due to the slow growth/

CHANGES OF BACTERIAL FLORA IN PASTEURISED

SAMPLE IV

Fig. 13 – DURING STORAGE AT 22° C.

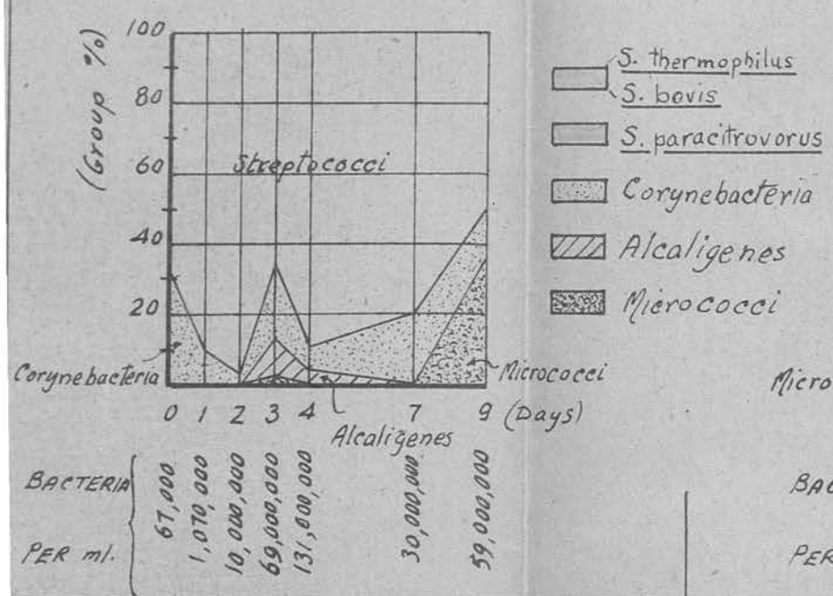
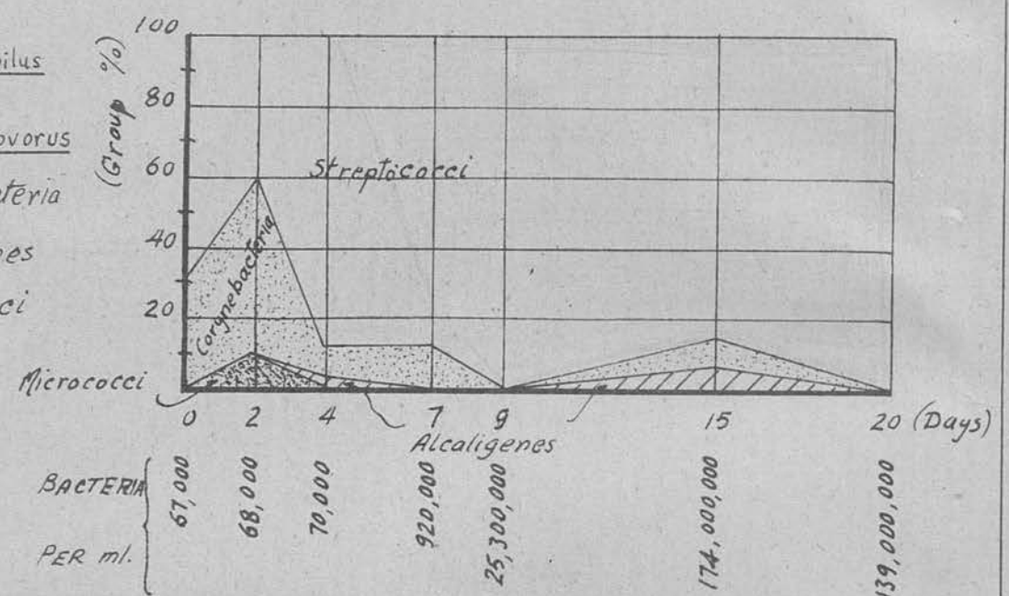


Fig. 14 – DURING STORAGE AT 9°–11° C.



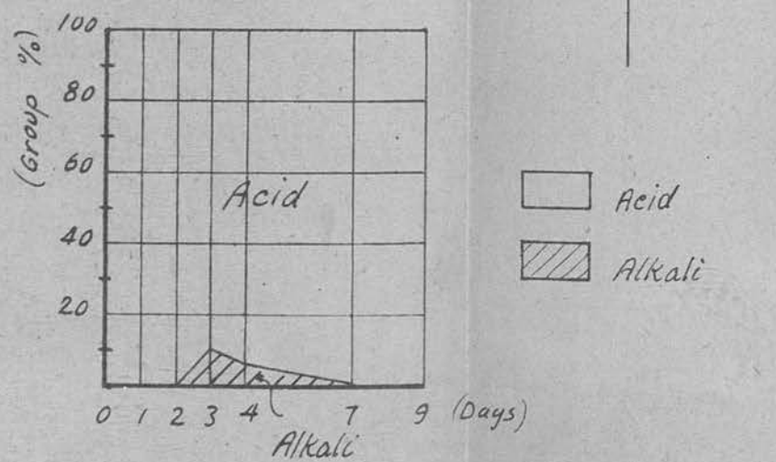


Fig. 15 – DURING STORAGE AT 22° C.

Fig. 16 – DURING STORAGE AT 9°–11° C.

(ACCORDING TO ACTION ON LITMUS MILK)

rate of the other types, especially the acid-formers.

In these four samples the milk, when held at the two different temperatures, kept in a liquid condition for a comparatively long period, contrary to what is experienced in commercially pasteurised milk. This long keeping quality of the laboratory pasteurised milk, which was also experienced by Ayers and Johnston (2), may be due to several reasons: the milk is pasteurised in small quantities which ensures uniform heating; pasteurisation is carried out under conditions which make reinfection impossible, It is also quite possible that when milk is pasteurised in bulk, it may show on holding, the development of organisms that have not been encountered in this investigation because of the survival of a few cells in the big bulk treated, e.g., few cells per gallon. However, it appears quite certain that the thermoduric Streptococcus thermophilus and Streptococcus bovis, which generally form the dominant group in commercially pasteurised milk, do not develop if the milk is held at low temperatures such as those used in this study.

SUMMARY

The predominant bacterial flora of freshly pasteurised milk appeared to play little part in the subsequent spoilage during storage, except when milk with a dominant flora of streptococci was stored at as high a temperature as 22°C.

Pasteurised milk souring at low temperatures was found to be uniformly caused by the development of heterofermentative streptococci, while at temperatures as high as 22°C. homofermentative streptococci, if present, might participate as well.

At/

At low temperatures, when the acid-forming types failed to develop, spore-formers played a part in the spoilage.

QUALITATIVE STUDY OF THE PREDOMINANT BACTERIAL GROUPS IN MILK BEFORE AND AFTER PASTEURISATION

All the organisms isolated from the raw and pasteurised milks were subjected to some extensive examination and identified as far as possible. In this section all the organisms which formed the different bacterial groups according to the action on litmus milk are presented. These organisms include those isolated from the raw and pasteurised milk, incubated raw milk and their freshly pasteurised product. In all, 1741 organisms were isolated from the raw milks and 1293 organisms from their pasteurised product.

BACTERIAL GROUPS IN THE RAW MILKS

The acid-forming group of bacteria.

This group was represented by 794 organisms. Table 25 illustrates their frequent appearance among the four grades of raw milk examined. Each grade of milk revealed the predominance of certain types of organisms. Thus, in the aseptically-drawn milk, the group consisted mainly of micrococci, in clean certified milk, of micrococci and a smaller number of streptococci, in certified milk, of streptococci, corynebacteria and micrococci in that order, and in tank milk of streptococci. Other groups of bacteria were encountered in such small numbers as to make them of little importance.

The acid-peptonizing group of bacteria

The acid-peptonizing group of organisms comprised 173 strains. The identity and distribution of the strains are shown in table 26. Most of the group was mainly made up of micrococci, the/

TABLE 25. - Acid-producing organisms isolated from raw milk.

Specific Name	Aseptically-drawn		Clean certified		Certified		Tank milk	
	No.of Strains	%	No.of Strains	%	No.of Strains	%	No.of Strains	%
Streptococci	-	-	34	33.3	108	47.4	321	91.2
S. lactis	-	-	7	-	29	-	149	-
S. cremoris	-	-	-	-	14	-	40	-
S. faecalis	-	-	-	-	3	-	8	-
S. bovis	-	-	-	-	-	-	3	-
S. thermophilus	-	-	-	-	-	-	6	-
Mastitis streptococci	-	-	20	-	30	-	31	-
Leuconostoc sp.	-	-	7	-	32	-	84	-
Micrococci	112	100.0	61	59.8	37	16.2	9	2.6
M. aureus	3	-	9	-	-	-	-	-
M. albus	54	-	13	-	13	-	3	-
M. citreus	4	-	13		3	-	-	-
M. aurantiacus	5	-	11	-	1	-	-	-
M. candicans	46	-	13	-	9	-	1	-
M. epidermidis	-	-	-	-	-	-	3	-
M. luteus	-	-	-	-	8	-	1	-
Sarcina sp.	-	-	2	-	3	-	1	-
Corynebacteria	-	-	-	-	78	34.2	4	1.1
C. lacticum	-	-	-	-	26	-	3	-
C. liquefaciens	-	-	-	-	52	-	1	-
"Other bacteria"	-	-	7	6.9	5	2.2	18	5.1
Coliforms	-	-	1		2	-	16	-
Lactobacillus casei	-	-	6	-	1	-	1	-
Achromobacter sp.	-	-		-	2	-	1	-
Total	112		102		228		352	

TABLE 26 - Organisms constituting the acid-peptonizing group in raw milk.

Specific Name	Aseptically-drawn		Clean certified		Certified		Tank milk	
	No.of Strains	%	No.of Strains	%	No.of Strains	%	No.of Strains	%
Streptococci	-	-	-	-	-	-	2	15.4
S. liquefaciens	-	-	-	-	-	-	2	-
Micrococci	74	100.0	56	94.9	27	100.0	7	53.8
M. aureus	44	-	42	-	4	-	1	-
M. luteus	-	-	-	-	8	-	3	-
M. caseolyticus	3	-	3	-	6	-	2	-
M. albus	25	-	8	-	9	-	1	-
Sarcina sp.	2	-	3	-	-	-	-	-
Corynebacteria	-	-	1	1.7	-	-	2	15.4
C. erythrogenes	-	-	1	-	-	-	-	-
Corynebacterium sp.	-	-	-	-	-	-	2	-
"Other bacteria"	-	-	2	3.4	-	-	2	15.4
Coliforms	-	-	-	-	-	-	1	-
Achromobacter aromafaciens	-	-	2	-	-	-	-	-
Flavobacterium desidiosum	-	-	-	-	-	-	1	-
Total	74		59		27		13	

TABLE 27 - Organisms constituting the peptonizing group in raw milk

Specific Name	Aseptically-drawn		Clean certified		Certified		Tank milk	
	No.of Strains	%	No.of Strains	%	No.of Strains	%	No.of Strains	%
Micrococci	1	100.0	3	4.7	3	4.9	3	3.5
M. albus	-	-	-	-	2	-	-	-
M. caseolyticus	-	-	1	-	1	-	3	-
M. flavescens	1	-	-	-	-	-	-	-
Sarcina sp.	-	-	2	-	-	-	-	-
Corynebacteria	-	-	3	3.7	-	-	-	-
Corynebacterium sp.	-	-	3	-	-	-	-	-
"Other bacteria"	-	-	57	90.5	58	95.1	83	96.5
Pseudomonas fluorescens	-	-	36	-	34	-	45	-
Achromobacter lipolyticum	-	-	14	-	5	-	16	-
Alcaligenes bookerii	-	-	-	-	1	-	2	-
Alcaligenes albus	-	-	-	-	7	-	-	-
Achromobacter sp.	-	-	-	-	8	-	-	-
Flavobacterium sp.	-	-	7	-	3	-	19	-
Bacillus licheniformis	-	-	-	-	-	-	1	-
Total	1		63		61		86	

the majority of which are udder types.

The peptonizing group of bacteria.

Table 27 presents 211 organisms which formed the peptonizing group in the raw milk according to the action on litmus milk. The table shows that the most prevalent organisms of the group belong to Gram-negative rod types, well recognised as being commonly found in water.

The alkali-forming group of bacteria.

Table 28 illustrates the identity and distribution of 258 organisms which constituted the alkali-forming group in the raw milk according to the action on litmus milk. The most prevalent bacteria in the group belonged to the Alcaligenes viscosus type of which only a small proportion produced ropiness in milk. The non-ropy strains corresponded to Alcaligenes viscosus var. dissimilis Hammer.

The inert group of bacteria.

The 304 strains which constituted this group were a heterogenous collection of organisms which either had no action on lactose and casein, or had acted on either or both but produced no apparent change in litmus milk during the 14 days' incubation at 30°C. It is probable that many of the organisms might have produced a change in litmus milk if the tests were repeated. In table 29 which presents the identity and distribution of the organisms, Leuconostoc sp., Micrococcus candicans, Micrococcus aurantiacus, Micrococcus luteus, Corynebacterium liquefaciens and Lactobacillus plantarum type fermented lactose with the production of acid. Micrococcus lipolyticus and Alcaligenes viscosus might have produced an alkaline reaction

on/

TABLE 28. - Organisms constituting the alkali-forming group in raw milk.

Specific Name	Aseptically-drawn		Clean certified		Certified		Tank milk	
	No.of Strains	%	No.of Strains	%	No.of Strains	%	No.of Strains	%
Micrococci	-	-	4	8.9	9	6.3	14	21.5
M. epidermidis	-	-	-	-	4	-	-	-
M. albus	-	-	-	-	1	-	-	-
M. lipolyticus	-	-	4	-	4	-	14	-
Corynebacteria	3	100	4	8.9	2	1.3	1	1.6
Corynebacterium sp.	3	-	4	-	2	-	1	-
"Other bacteria"	-	-	37	82.2	134	92.4	50	76.9
Alcaligenes viscosus a.	-	-	31	-	128	-	48	-
Alcaligenes viscosus b.	-	-	-	-	5	-	2	-
Alcaligenes sp.	-	-	6	-	-	-	-	-
Bacillus brevis	-	-	-	-	1	-	-	-
Total	3		45		145		65	

TABLE 29. - Organisms constituting the inert group in raw milk.

Specific Name	Aseptically-drawn		Clean certified		Certified		Tank milk	
	No.of Strains	%	No.of Strains	%	No.of Strains	%	No.of Strains	%
Streptococci	-	-	2	3.8	-	-	5	7.3
Streptococcus sp.	-	-	1	-	-	-	-	-
Leuconostoc sp	-	-	1	-	-	-	5	-
Micrococci	5	3.4	13	25.0	20	55.6	15	22.1
M. candicans	5	-	3	-	3	-	-	-
M. freudenreichii	-	-	1	-	-	-	-	-
M. aurantiacus	-	-	2	-	2	-	-	-
M. luteus	-	-	-	-	4	-	-	-
M. lipolyticus	-	-	5	-	11	-	15	-
Sarcina sp.	-	-	2	-	-	-	-	-
Corynebacteria	144	96.6	28	53.8	3	8.3	3	4.4
C. bovis	121	-	4	-	-	-	-	-
C. liquefaciens	-	-	-	-	1	-	-	-
Corynebacterium sp.	23	-	24	-	2	-	3	-
"Other bacteria"	-	-	9	17.3	13	36.1	45	66.2
Lactobacillus plantarum	-	-	1	-	-	-	-	-
Achromobacter sp.	-	-	2	-	-	-	29	-
Flavobacterium sp.	-	-	6	-	-	-	-	-
Proteus morganii	-	-	-	-	1	-	-	-
Alcaligenes viscosus a.	-	-	-	-	12	-	15	-
Actinomyces sp.	-	-	-	-	-	-	1	-
Total	149		52		36		68	

in litmus milk if incubated at room temperature or if the test had been repeated.

BACTERIAL GROUPS IN FRESHLY PASTEURISED MILK

The acid-forming group.

This group was represented by 1163 organisms. Their identity and frequent occurrence among the different grades of milk when pasteurised are shown in table 30. All the

TABLE 30. - Organisms constituting the acid-forming group in pasteurised milk.

Specific Name	Certified milk		Tank milk	
	No.of Strains	%	No.of Strains	%
Streptococci	75	14.1	301	47.8
S. thermophilus	1	-	137	-
S. bovis	6	-	152	-
S. faecalis	-	-	9	-
S. paracitrovorus	68	-	3	-
Micrococci	20	3.8	53	8.4
M. luteus	19	-	53	-
Sarcina sp.	1	-	-	-
Corynebacteria	438	82.1	276	43.8
C. lacticum	122	-	82	-
C. liquefaciens	316	-	194	-
Total	533		630	

streptococci which appeared in the pasteurised certified milk were isolated from the samples which were incubated prior to pasteurisation. S. thermophilus and S. bovis formed a big proportion of the acid-forming group in the pasteurised tank milk. They were encountered in the pasteurised certified milk only to the extent of a few colonies which appeared on the plates of/

of a pasteurised incubated sample. The more carefully the milk is produced the less thermoduric streptococci are likely to appear in the pasteurised milk. Corynebacteria were the most prevalent type among the organisms comprising the acid forming group in the pasteurised certified samples.

The acid-peptonizing group.

Only one strain of a yellow micrococci represented this group. This strain belonged to the Micrococcus luteus group.

The peptonizing group.

68 organisms formed this group. Their identity and distribution are shown in table 31. With the exception of one

TABLE 31. - Organisms constituting the peptonizing group in pasteurised milk

Specific Name	Number of Strains		
	Clean Certified	Certified	Tank Milk
Micrococcus luteus	-	-	1
Bacillus carotarum	-	1	-
Bacillus subtilis	6	-	-
Bacillus licheniformis	36	-	-
Bacillus pumilus	13	-	-
Actinomyces sp.	11	-	-
Total	66	1	1

micrococcus, all the organisms were spore-formers which were mainly isolated from the pasteurised clean certified milk.

The alkali-forming group.

Table 32 presents the identity and distribution of the 38 organisms isolated from the freshly pasteurised milks.

Alcaligenes/

Alcaligenes viscosus b. was the prevalent organism in the group.

TABLE 32. - Organisms constituting the alkali-forming group in pasteurised milk.

Specific Name	Number of Strains	
	Certified Milk	Tank milk
Micrococcus subflavescens	-	1
Sarcina sp.	-	1
Alcaligenes viscosus b.	25	10
Bacillus brevis	1	-
Total	26	12

The inert group.

This group was composed of 23 organisms, which, although they produced acid from lactose, did not show any change in litmus milk. All the organisms were isolated from pasteurised certified milk and were identified as follows: Micrococcus luteus, 5, Corynebacterium liquefaciens, 8, and Corynebacterium lacticum, 1.

SUMMARY

The acid-forming group in carefully produced milk consisted mainly of micrococci; in moderately contaminated milk, streptococci, corynebacteria and micrococci were the most important types and in milk produced without care, streptococci were predominant. After pasteurisation, corynebacteria were the most encountered acid-formers in moderately contaminated milk while streptococci and corynebacteria were the main types in milk produced without care. Micrococci appeared in both grades of milk to the extent of a small percentage only.

The acid-peptonizing group in all the grades of raw milk/

milk examined was made up mainly of micrococci, which were mostly udder types. After pasteurisation, the group was only encountered to the extent of one micrococcus.

Peptonizers in moderately contaminated milk and milk produced without care were principally gram-negative rods. Spore-formers and a few actinomyces constituted the main types surviving the heat treatment.

Alcaligenes types were the most common alkali-formers in both raw and pasteurised milk. Those encountered in the processed milk were of a type which could be distinguished by its relatively high heat resistance.

The inert group was made up of a miscellaneous collection of organisms, a proportion of which fermented lactose but had no action on litmus milk.

CLASSIFICATION OF THE ORGANISMS STUDIED

STREPTOCOCCI

The characterisation of all streptococci isolated in this study was carried out according to the classification of Sherman (65). This classification, however, holds only for the homofermentative types. It became, therefore, obvious from the start, that a quick means of differentiating the heterofermentative streptococci, which belong to the genus Leuconostoc, from the homofermentative ones was most needed. The usual methods of differentiation based on the accurate determination of the ratio of volatile to non-volatile acids, the amount of CO_2 and the isometric type of lactic acid produced during the fermentation of sugars, are laborious and time-consuming and, therefore, cannot be utilised in any work of this nature. A reliable and quick method of separating the Leuconostoc types was looked for.

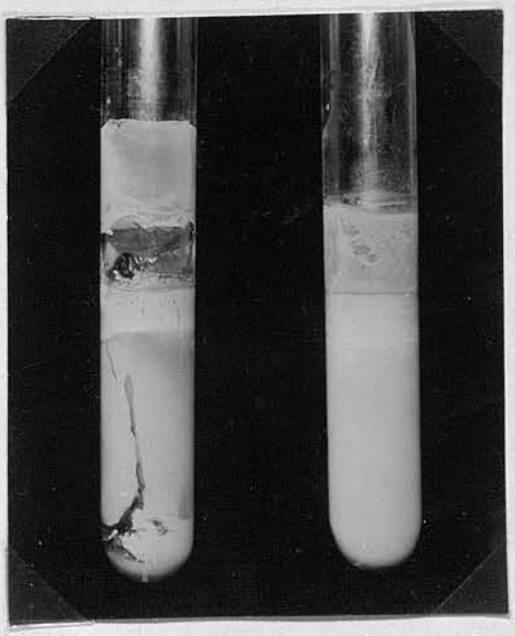
In a study of 41 representative strains of the genus Leuconostoc, Hucker and Pederson (37), found that they were uniformly active gas producers. On the basis of the amount of sugar fermented, all the strains of Leuconostoc produced more than 12% CO_2 while the majority formed 20-25%. This amount of gas was in striking contrast to the amount produced by the homofermentative streptococci isolated from the same sources. These organisms generally produced approximately 5% or less of gas and no strain of this type producing more than 6% of CO_2 was encountered. This small amount of gas is apparently produced from the peptone and not from the carbohydrate. Such results

and/

and others (4, 14) show that the production of gas should offer a suitable method for quick recognition of the heterofermentative streptococci, if the gas formation could be readily demonstrated.

The presence of gas bubbles in some Leuconostoc milk cultures is a common observation which was utilised by some workers to distinguish some Leuconostoc types. However, the presence of gas bubbles in milk cultures is not infrequently observed in some homofermentative streptococci strains. Such a characteristic, therefore, cannot be used for the separation of the Leuconostoc types

After many trials in various directions a quick and reliable method for the demonstration of the CO_2 produced during the fermentation of sugars by the Leuconostoc strains was devised. The test is described in detail under "Methods". It is based on the large evolution of gas which can be demonstrated when heterofermentative types are grown in milk containing 5% dextrose and o.25% yeastrel and 4% tomato juice with an agar seal on top. The photograph illustrates typical results of this test.



Typical gas production by heterofermentative streptococci with S.lactis as control.

The dextrose, yeastrel and tomato juice ingredients can either be added to the litmus milk tubes just before inoculation or steamed in the media during the making without affecting the results. Tomato juice seems to hasten the appearance of gas although in its absence good results were obtained with the majority of the strains tested. Its presence, however, was found necessary in the case of some slow growing heterofermentative lactobacilli isolated from silage.

During the development of the above test some interesting observations were made. Only in a few instances was gas production noticeable when litmus milk or litmus milk plus 0.25% yeastrel was employed without the addition of dextrose. Gas production however, increased markedly by the addition of dextrose and the amount of gas produced appeared to be in proportion to the amount of dextrose added. This was somewhat surprising as litmus milk contains about 5% lactose. A similar observation was made by Hassouna and Allen (29) who, using a capillary tube method for determining gas production by a strain of Leuconostoc mesenteroides, found that no gas was evolved from separated milk, while considerable quantities were produced from milk containing 1% dextrose and 0.3% yeastrel. These observations point to the likelihood of slow and incomplete fermentation of lactose by the Leuconostoc strains.

It is worth mentioning that all attempts to show visible gas formation in liquid media other than milk were unsuccessful. The use of highly buffered media containing up to 5% peptone and 5% dextrose at a pH range of 6.2-7.2 did not yield visible gas in Durham fermentation tubes or when the agar seal technique was employed. The successful production of gas in Durham fermentation tubes/

by the heterofermentative streptococci reported by Davis and Thiel (16) could not, therefore, be confirmed.

Gas production in solid media (shake and stab cultures) was only observed in the case of a few cultures which showed slight cracks in 5% sucrose gelatin stabs (c.f. Orla-Jensen, 49).

Some of the factors that are likely to affect the results of the above test were investigated. Litmus milk contains less CO_2 in solution when freshly steamed than after storage. Such a variation in the degree of CO_2 saturation in the medium is likely to affect the amount of visible gas produced. No noticeable difference could be seen, however, when the test was carried out in both freshly steamed and 35 days old milk.

The technique of adding the ager to the inoculated fortified milk cultures was also investigated. Five alternative methods were tried. These were:-

1. 1 ml. of liquid agar mixed throughout the milk.
2. Same as (1) but using 2 ml. of liquid agar.
3. Same as (1) with the addition of an extra 1 ml. of liquid agar to cover the surface of the milk.
4. 2 ml. of liquid agar were used only to cover the surface of the milk.
5. No addition of agar.

Visible gas production ranked best in treatment No. 3 followed by treatment No. 4.

As all this work was carried out in 5" x $\frac{1}{2}$" tubes, the determination of the effect of the width of the tube and the quantity of the media in it seemed desirable because of the variations in these two factors in different laboratories. 6" x $\frac{5}{8}$" tubes a quarter, half and three-quarters full were used and the results showed that, although gas production was

obvious/

obvious in the three treatments, the amount of gas was biggest in tubes three-quarters full and decreased with the decrease in the quantity of the media in the tubes.

The test was carried out on 217 heterofermentative strains and 70 homofermentative strains. In all cases the test proved reliable. The heterofermentative strains included 1 slime-producing Leuconostoc strain isolated from butter, 3 Leuconostoc and 4 heterofermentative lactobacilli strains isolated from silage by Dr. A. Cunningham of the Edinburgh and East of Scotland College of Agriculture, and 4 Leuconostoc strains obtained from the Lister Institute. The remainder of the cultures were isolated in this study. The 70 homofermentative strains tested were distributed as follows: 13 S. lactis, 7 S. cremoris, 1 S. faecalis, 9 S. thermophilus, 8 S. bovis, 5 S. agalactiae, 25 mastitis streptococci, 1 Lactobacillus bulgaricus and 1 Lactobacillus casei. Although bubbles appeared in one S. cremoris, one S. thermophilus and one L. bulgaricus litmus milk cultures, no visible gas could be seen when the agar seal method was employed. Three strains of the heterofermentative lactobacilli which produced the smallest amount of CO_2 among those isolated from silage by Cunningham and Smith (14, table 2 group V strains i,e and l) required the presence of tomato juice before visible gas could be recorded by this test.

For the identification of the homofermentative streptococci only a limited number of tests were chosen from those recommended by Sherman (65) and were applied as routine tests for all the strains isolated. This was decided upon in order to cut down the amount of work involved and time consumed. The tests/

tests chosen were: Catalase production, ability to grow at 10°-14°C. and 50°C., ability to grow in the presence of 0.1 per cent methylene blue, production of ammonia in 4 per cent bacto-peptone, liquefaction of gelatin, fermentation of dextrose and lactose, action on litmus milk, the ability to reduce litmus before curdling, ability to haemolyse ox blood and, in doubtful cases, the ability to survive heating in milk at 60°C. for 30 minutes. Streptococci which grew at 50°C. were tested for the ability to ferment maltose before they were considered S. thermophilus. All members of the mastitis group were tested for their ability to grow in the presence of 0.01 per cent methylene blue and only some representative strains were tested for the ability to hydrolyse sodium hippurate, split aesculin and to ferment mannite, raffinose, salicin and inulin.

With the exception of 5 strains of S. cremoris which failed to grow in the presence of 0.1 per cent methylene blue, and 2 S. faecalis strains, which failed to grow at 45°C., this scheme of routine tests proved to be a quick and reliable means for the identification of the great number of strains isolated. One unidentified streptococcus, which was isolated from a clean certified sample (see page 13) was a poor growing strain which was very similar to S. agalactiae in morphology. No acid production could be demonstrated, however, from dextrose, lactose, maltose, mannite, raffinose or inulin. Sodium hippurate was not hydrolysed and aesculin was not split. The test for the ability to ferment dextrose was carried out in both 1 per cent dextrose tryptone infusion broth and 1 per cent dextrose tryptone infusion stabs to which Brom-cresol purple or Brom-thymol blue had been added as an indicator. No change could be/

be observed in either indicator after 14 days' incubation at 30°C. or 7 days at 37°C. Gelatin was not liquefied. Partial haemolysis was produced on blood agar only after 3 days' incubation at 37°C. The strain, however, died out suddenly before any more observations could be made. The inability of this strain to produce acid from dextrose is interesting since such a characteristic is uncommon among the streptococci. It is quite possible that this strain belongs to the S. acidominimus group and the apparent failure to ferment sugars is in accordance with Smith and Sherman's statement (66): "Because of the extremely feeble acid-producing power of these organisms, perhaps the most unique characteristic of the group, it is frequently difficult to determine exactly whether or not fermentation has taken place".

The members of the genus Leuconostoc formed a distinct group in connection with the characters employed for the identification of the strains belonging to the genus Streptococcus. They were catalase negative, did not liquefy gelatin, grew slowly at 10°C., produced no growth at 45°C., 50°C. nor in the presence of 0.1 per cent methylene blue and failed to produce ammonia from 4 per cent bacto-peptone. Only the Leuconostoc strains isolated from the freshly pasteurised or incubated pasteurised milk samples, were examined in some detail. Table 33 gives the four different combinations of reactions exhibited by the strains examined as compared with those shown by 4 strains received from the Lister Institute, London under the following names:

Leuconostoc citrovorum (Hammer) Hucker & Pederson, No. 3739
Leuconostoc mesenteroides (Cienkowski) v. Tiegh No. 3351
Leuconostoc dextranicum (Beig) Hucker & Pederson No. 3354 a Hucker strain
Leuconostoc dextranicum No. 3740 a Hammer strain of Streptococcus paracitrovorus

None/

TABLE 33. - Cultural reactions of the heterofermentative streptococci isolated from pasteurised milk as compared with the type cultures of Leuconostoc strains obtained from the Lister Institute.

Source	Survive 30 min. at		Growth at		Ø Action on Milk	Sodium Hippurate	Split	Acid production from									
	60°C.	63°C.	10-11°C.	45°C.				Dextrose	Lactose	Sucrose	Maltose	Mannitol	Raffinose	Inulin	Salicin	Starch	Xylose
Freshly pasteurised certified milk	+	+	sl	-	A or ACpR	-	-	+	+	+	+	-	-	-	-	-	-
Incubated pasteurised tank milk	+	+	sl	-	A or ACpR	-	-	+	+	+	+	+	+			+	-
Incubated pasteurised certified milk	+	sl	sl	-	A or ACpR	-	-	+	+	+	+	-	+	-	-	-	-
	+	-	sl	-	ACpR	-	-	+	+	+	+	-	+	-	-	-	-
	+	+	sl	-	ACpR	-	-	+	+	+	+	-	+			-	-
* Leuconostoc citrovorum	-	-	sl	-	Na			+	+	+	+	-	-	-	-	-	-
* Leuconostoc mesenteroides	-	-	sl	-	Na			+	-	+	+	-	-	-	-	-	-
* Leuconostoc dextranicum	-	-	sl	-	Na			+	-	+	+	-	+	-	+	+	+
* Streptococcus paracitrovorus	-	-	sl	-	A			+	+	+	+	-	+	-	+	+	+

*Obtained from the Lister Collection.

Ø A = acid AC = acid and clot pR = partial reduction Na = no action
Sl = slight

None of these type cultures survived the pasteurisation treatment and as the only reference to the occurrence of heat-resistant strains was made by Hammer and Baker (27) in their study of Streptococcus paracitrovorus, it was decided to refer to the heat resistant strains isolated here as Streptococcus paracitrovorus Hammer.

MICROCOCCI

At the start of this investigation it was decided to employ the classification proposed by Hucker (36) for the identification of the members of the genus Micrococcus. This decision was teken with the idea of obtaining results comparable with those of earlier workers who seem to have favoured this classification. However, during the progress of this study, pasteurisation tests indicated that the micrococci in the aseptically-drawn and the clean certified milk samples were destroyed by heating at 63°C. for 30 minutes, while those in milk of indifferent quality frequently survived the treatment. The examination of the results showed that, although the prevalent types of micrococci in pasteruised milk have not been encountered among those isolated from the uncontaminated milk, a small number of micrococci would be considered according to Hucker's classification similar to those of the udder types. In addition, it was noticed that organisms with heterogenous characters, especially in connection with the heat resistance, would be named together while others which showed great similarity would be grouped differently simply because of the few tests employed. This classification was, therefore, abandoned.

Hucker's/

Hucker's classification of the micrococci is based on Chromogenesis, gelatin-liquefaction, nitrate reduction and ability to utilise ammonium salts as the only source of nitrogen. Pigment formation cannot be considered a satisfactory criterion for the purposes of differentiation as it is determined by several factors such as temperature, light, nature of the medium, length of cultivation and others. Also, the discovery that pigment production was a variable characteristic of a given strain, was made by Neumann(48), who stated that from a single strain of an orange staphylococcus he produced yellow, white and flesh coloured variants. Similar observations have been made by Dudgeon (19), Mellon and Caldwell (47), Biggar, Boland and O'Meara (8), Pinner and Voldrich (51), and Hoffstadt and Youmans (32 and 33). In spite of this, Hucker separated the larger groups of micrococci according to the production of pigment on ordinary agar slopes. An example of what this might lead to can be found in the case of M. caseolyticus Evans. Hucker considered this organism non-pigmented. Evans (21) who was the first to describe and name this type found white, ivory-yellow, buff, yellow and ochre-orange strains. This was experienced by Frazier and Rupp (52), Cunningham (74) and others. Again, Evans in her description of this group gave the ability to reduce nitrate as variable while Hucker considered it to be nitrate reducing. Such variations in M. caseolyticus, if present, and they seem to be present, will place these variants according to Hucker's classification with M. conglomeratus, M. flavus M. citreus, M. aureus and M. freudenreichii. Another example of the futility of this classification can be demonstrated by one of Hucker's own strains of M. varians which was obtained from Nat. Coll Type Cul., Lister Institute, London. This strain/

strain appears to have lost its power to produce the yellow pigment and therefore would be considered by Hucker now as M.epidermidis ! Another character to which Hucker has attached great importance in the differentiation of the micrococci species, is the ability to utilise ammonium salts as the only source of nitrogen. According to Hucker and Rettger (38) the more strictly parasitic types such as M.aureus M.albus and M.citreus, in all cases failed to utilise ammonium phosphate as the only source of nitrogen. He utilised this character as a basis for the separation of M.citreus from M. conglomeratus and M.albus from M.caseolyticus. However, Breed (11) in a study of the udder micrococci in which she strictly adhered to the key devised by Hucker, reported 7 out of the 33 strains of M.aureus and 13 out of the 21 strains of M.albus as being able to utilise ammonium phosphate. If strains of M. aureus and M. albus are capable of utilizing ammonium phosphate, one would expect the more saprophitic M.citreus to envelop strains capable of utilizing this salt. That the presence of such strains have not been reported by those who employed Hucker's classification is obviously because Hucker separates M.citreus from M.conglomeratus solely on basis of the utilization of ammonium phosphate. It should be mentioned at this point that throughout this study, the test for the ability to utilize ammonium salts as recommended by Hucker, proved to be somewhat variable because it seems to be affected by many factors. Breed states, when discussing her M.flavus strains: "These cultures were somewhat atypical in that only one strain utilized ammonium phosphate. Upon the repetition of the test/

test five strains utilized this salt". Also in connection with the M.aureus and M.albus strains which were able to utilise ammonium phosphate, she states "This, however, may have been due to the fact that organic material was carried over in the inoculation from ordinary nutrient agar slants. Such organic material might be sufficient to permit growth on the ammonium phosphate medium".

The previous discussion points out the futility of the classification proposed for the genus Micrococcus by Hucker. Indeed, one may feel inclined to go as far as to question the significance of the names attached to the various micrococci reported by earlier workers. Such a state of affairs might, at least in part, explain the conclusion arrived at by Hileman (30) and Hileman et al.(31), that the udder is the ultimate source of the thermoduric micrococci in milk, contrary to the results reported here.

In an attempt to find a satisfactory way of grouping the members of the genus micrococcus, a study of some representative strains isolated from a variety of sources was carried out. Unfortunately, this step was taken at a late stage during this investigation and, therefore, a smaller number of strains were examined than would have been possible at an earlier period. However, the results obtained from the examination of 245 strains isolated from udder samples, raw, freshly pasteurised and incubated pasteurised milks, pasteurised dairy utensil swabs, hands, contaminants on various plates, 1 Staphylocuccus aureus and 1 Staphyloccus citreus strain (obtained from the bacteriology department, Edinburgh University) and 11 type cultures from the Lister Institute, London, pointed to the importance of some characteristics/

characteristics in the grouping of the micrococci strains. The following key to the various groups of micrococci examined will help in illustrating the results obtained:

A. Acid formed in dextrose broth

1. V.P. -

a Disastase +; survive 60°C for 30 minutes..M.luteus group
aa Diastase - ; do not survive 60°C for 30 minutes
b Gelatin liquefied rapidly.....M.caseolyticus group.
bb Gelatin not liquefied or only very slowly.....M.candicans group.

2. V.P. +

a Gelatin liquefied
b. Orange pigment produced..... .M.aureus group
bb. Yellow pigment produced..... M.citreus group
bbb. No pigment produced..... M.albus group
aa Gelatin not liquefied
b. Orange pigment produced..... M.aurantiacus group
bb. No pigment produced..... M.epidermidis group.

B. No acid formed in glucose broth

1. Gelatin liquefied

a. Yellow pigment produced
b. survive 60°C for 30 minutes..... M.subflavescens Bergey etal.
bb. do not survive 60°C for 30 minutes... M.flavescens Henrici
aa Red pigment produced.....
aaa. No pigment produced..... M.freudenreichii group

2. Gelatin not liquefied

a. Gram positive, citrate not utilized as the only source of carbon.....
aa. Gram negative, citrate utilized as the only source of carbon..... M.lipolyticus ~~Henr~~.. Stark & Schleb.

In the above scheme use was made of production of acid in glucose broth, Barritt's modification of the Voges-Proskauer test, diastase production, ability to survive heating in milk at

60°/

60°C for 30 minutes, gelatin liquefaction, chromogenesis, and ability to utilize sodium citrate as the only source of carbon, as diagnostic criteria for the grouping of the members of the genus Micrococcus. With the exception of liquefaction of gelatin and pigment production, these characters appear to be employed for the first time in connection with the differentiation of the micrococci. Any information regarding the stability of these characters is, therefore, of interest.

In the studies carried out on the dissociation of the staphylococci group (19, 47, 51, 32, 33) the ability to produce acid from glucose has always been stable in all the recorded variants. Unfortunately, similar studies have not been carried out on other types of the genus Micrococcus and, in consequence, no information is available concerning the constancy of this character when applied to the genus as a whole. However, until any contradictory results are reported, it seems feasible to assume the stability of this character.

Barritt's modification of the Voges-Proskauer test has not been used in connection with the members of the genus Micrococcus. The correlation of this test with the types whose habitat is usually regarded as skin, mucus membranes and infection - i.e. animal sources - is, therefore, interesting.

The diastase test appears to have been tried by some previous workers (34, 28) in connection with the micrococci, but its correlation with acid production from dextrose and heat resistance is quite new. Of the strains which formed acid in glucose broth only those which produced diastase survived heating at 60°C for 30 minutes. This correlation was obtained with 110 strains isolated from various sources and, therefore, appears to/

to be significant.

Regarding the heat resistance tests, attention should be drawn to the method employed for the determination of this character (see methods) Many previous workers have formed their conclusions about this property from the mere isolation of the strains from pasteurised milk plates, or from recording the appearance of growth in the heated cultures after a period of incubation. Both these methods do not indicate the ability of the organisms to survive the heat treatment in sufficient numbers to justify the conclusions drawn.

Chromogenesis, as has been pointed out before, shows some variation. Nevertheless, pigment production may have a certain taxomic value, and, provided not too much weight is placed upon it, it can be used for subsidiary classification. As pointed out by Dudgeon (19), the pigment of cultures obtained directly from tissues is a useful character in identifying the albus and aureus types of staphylococci. During the present study an investigation for the best conditions with regard to chromogenesis was undertaken. It was found that while potato always gave good results in this aspect, it possessed a serious disadvantage in that some strains failed to grow and others produced growth on one batch of potato and not on another. This variation in supporting growth made the utilisation of potato for the recording of pigment production difficult. On the other hand, agar slopes, to which 20 per cent sterile milk was added prior to sloping, gave just as good results as potato, with the difference in supporting luxuriant growth for all the strains studied. Best results were obtained from milk agar slopes which had been left to dry for some time at room temperature after sloping, then inoculated by a middle streak/

streak and incubated at 22°C. for 5 days followed by 5 days at room temperature exposed to light. This method was superior to ordinary agar slopes as, apart from providing a white background which facilitated the recording, pigment production was enhanced in a number of yellow and orange strains which appeared non-pigmented on ordinary agar slopes.

M. luteus (Schröter)Cohn group.

This group comprised 110 strains. They were the commonest types of micrococci encountered in pasteurised milk and pasteurised dairy utensil swabs. Table 34 shows the general characters of the group. The strains are gathered into several groups in order to show some of the variation encountered. Members of the group as a whole showed a tendency to grow in tetrads, to have an optimum temperature at 30°C. and to produce little or no growth at 37°C. Gelatin liquefaction, when present, was very slow, appearing after 20 days' incubation at 22°C. with the exception of strain '1' and 2 strains of 'f' which showed liquefaction in 5 days. Growth on agar slopes was pale lemon-yellow, crumbly in the gelatin non liquefying strains, 'a' and 'b', and deep lemon-yellow generally somewhat mesenteric in the rest of the chromogenic strains. The white strains showed soft growth not unlike that of the yellow gelatin non-liquefiers. Nitrate reducing strains did not show any significant difference from the non-reducing ones. The case was the same with those which utilised ammonium phosphate as the only source of nitrogen. It was quite clear, therefore, that the setting up of different species based on these two characters was very unjustified. If Hucker's classification was to be applied to this group, the result would prove interesting. Strains 'a' would be M. luteus, 'b'/

TABLE 34. - The Cultural Reactions of 110 strains of M. luteus group.

Ref. No.	Total No. of strains	Source					Survive 30 min. heating at			Acid from dextrose	V.P. +	Diastase +	Gelatin liquefaction	Chromogenesis	Gram reaction	Nitrate reduction	NH_4 utilisation	NH_3 from 4% peptone	Citrate utilisation	Casein digestion	Fat hydrolysis	Acid from lactose	Acid from mannite	Ø Action on Milk
		Udder	Raw milk	Past. milk	Incubated past. milk	Dairy Utensils	60°C.	63°C.	65°C.															
a	48	0	3	2	19	24	48	48	48	48	0	48	-	lmY	48+	0	12	0	0	0	0	48	0	48A
b	36	0	1	22	0	13	36	36	36	36	0	36	-	lmY	36+	36	13	0	0	0	0	36	0	36A
c	4	0	1	2	0	1	4	4	4	4	0	4	-	W	4+	4	-	0	0	0	0	4	0	4A
d	7	0	0	6	0	1	7	7	7	7	0	7	7	lmY	7+	0	6	0	0	0	0	7	0	7AC
e	7	0	0	5	0	2	7	7	7	7	0	7	7	lmY	7+	7	3	0	0	0	0	7	0	7AC
f	4	0	2	2	0	0	4	4	0	4	0	4	4	*lmY	4+	4	4	0	3	0	0	4	0	4ACP
g	1						1	1	0	1	0	1	1	lmY	1+	1	1	0	0	0	0	1	0	1A
h	1						1	1	0	1	0	1	-	W	1+	1	1	0	0	0	1	1	0	1K
i	1						1	1	0	1	0	1	1	lmY	1+	1	1	0	0	0	0	1	0	1Na
l	1	0	0	1	0	0	1	1	1	1	0	1	1	W	1+	0	0	0	0	1	0	0	0	1KCP
Total	110	0	7	40	19	41	110	110	103	110	0	110	21		110	54	41	0	3	5	1	109	0	

Ø A = acid, C = curd, P = peptonization, K = alkaline Na = no action.

* lmY = lemon yellow, W = white.

* 2 strains produced a long standing brown followed by violet coloured rings which spread from the margin inwards.

'b', M. varians, 'c' and 'h' M. epidermidis, 'd', M. flavus, 'e' M. citreus (4 strains) and M. conglomeratus (3 strains), 'f', 'g' and 'i', M. conglomeratus and 'l' M. freudenreichii. This will not only show the futility of such a classification but will also throw doubt on the significance of the names recorded by previous workers according to Hucker's methods. Strains 'g', 'h' and 'i', which were obtained from the Lister Institute for the sake of comparison had the following names:

'g'; M. sulphureus Zimm Cul.,No. 1631 - typed by Hucker (36) as M. varians.
'h'; M. varians Migula Cul., No. 2685 - a Hucker strain.
'i'; M. conglomeratus Migula Cul., No. 2677 - a Hucker strain.

M. caseolyticus Evans group.

This group is made up of only 7 strains. This number is too small to allow of any definite conclusion. The general characters of these strains are shown in table (35). The ability to digest casein which is considered by previous workers to be one of the main characters attached to this name, was not unifromly shown by the members of the group. This character was not found, however, to be constant for any particular group. For this reason, it seems that the name M. caseolyticus may not hold. However, until more strains are studied to justify the use of another name, it was decided to use the above title for the group in the meantime. This group can be easily distinguished from the M. albus group by the failure to give a V.P. positive test and by possessing a low maximum growth temperature of about 37°C. as compared with that of M. albus which is above 40°C. Two strains belonging to this group produced a yellowish-orange pigment with a brownish tinge but were in all other respects similar to the non-pigmented strains. This group grows well on ordinary agar slopes with a smooth thin growth On potato, the white strains produce a greyish-white thin/

TABLE 35. - Cultural reactions of 7 strains of M. caseolyticus group.

Ref. No.	No. of strains	Sources		Survive 30 Min. Heating at:			Acid from dextrose	V.P. +	Diastase +	Gelatin liquefied	#Chromogenesis	Gram reaction	Nitrate reduction	NH_4 utilisation	NH_3 from 4% peptone	Citrate utilisation	Casein digestion	Fat hydrolysis	Acid from lactose	Action on Litmus Milk: Acid	Action on Litmus Milk: Acid curd	Action on Litmus Milk: Acid peptonization
		Udder	Raw milk	Unknown	60°C.	63°C.	65°C.															
a	2	0	2	0	0	0	0	2	0	0	2	YOr	2+	2	0	2	0	1	2	0	1	1
b	5	3	1	1	0	0	0	5	0	0	5	W	5+	4	2	0	0	4	1	4	3	1	1
Total	7	3	3	1	0	0	0	7	0	0	7		7+	6	2	2	0	5	2	6	3	2	2

#YOr = yellowish-orange, W = white.

thin growth, and the pigmented strains produce yellowish-brown raised growth. The potato is uniformly decolorised. Gelatin is liquefied quickly. Optimum temperature is about 30°C.

M.candicans Flugge group.

Table 36 illustrates the general characters of this group. The main proportion of the strains were isolated from aseptically-drawn milk samples. Some of the cultures were obtained from pasteurised milk held at 10°-14°C. for 15 days indicating the ability of a few cells to survive the heat treatment. Two strains showed very slow gelatin liquefaction which appeared after 40 days' incubation at 22°C. Milk was acidified slowly by 7 strains. Growth on artificial media was uniformly slower and scantier than in the case of other types and took place at 37°C. but not at 40°C. Again the number of strains studied in this group is too small to allow of any conclusion.

V.P. Positive Groups.

Strains which gave a V.P. positive test possess many properties in common which permit their discussion together. The pathogenic property of some members of this group makes the separation of the group from the other saprophitic types of importance. Table 37 shows the results of studying 89 strains belonging to this group. The group was subdivided on the basis of gelatin liquefaction and pigment production although the subgroups were obviously related to each other. Thus all the 89 strains showed a relatively high optimum and maximum growth temperature. (37°C. and above 40°C. respectively), did not survive heating at 60°C. for 30 minutes, did not produce diastase or utilise ammonium phosphate as the only source of nitrogen and, in general were able to reduce nitrates, produce ammonia

from/

TABLE 36. - Cultural reactions of 12 strains of M. candicans group.

Ref. No.	No. of strains	Source: Udder	Source: Raw milk	Source: Incubated past. milk	Source: Contamination	Survive 30 Min. Heating at: 60°C.	Survive 30 Min. Heating at: 63°C.	Survive 30 Min. Heating at: 65°C.	Acid from dextrose	V.P. +	Diastase +	Gelatin liquefied	#Chromogenesis	Gram reaction	Nitrate reduction	NH_4 utilisation	NH_3 from 4% peptone	Citrate utilisation	Casein digestion	Fat hydrolysis	Acid from lactose	Action on Litmus Milk: Acid	Action on Litmus Milk: No action
c	2	0	2	0	0	0	0	0	2	0	0	2	W	2+	0	0	0	0	0	0	1	1	0
d	10	7	0	2	1	0	0	0	10	0	0	0	W	10+	0	0	0	0	0	0	7	6	4
Total	12	7	2	2	1	0	0	0	12	0	0	2		12+	0	0	0	0	0	0	8	7	4

\# W = white

TABLE 37 - Cultural reactions of 89 strains of the V.P. + groups

Ref. No.	No. of strains	Sources						Survive 30 min. at 60°C.	Acid from dextrose	V.P. +	Diastase +	Gelatin liquefied	*Chromogenesis	Gram reaction	Nitrate reduction	NH_4^+ utilisation	NH_3 from 4% peptone	Citrate utilisation	Casein digestion	Fat hydrolysis	Acid from lactose	Action on litmus milk					
		Udder	Raw milk	Incubated past. milk	Pus	Hands	Contaminant															Acid	Acid curd	Acid peptonization	Peptonized	Alkaline	No action
													M. albus group														
a	28	12	5	0	0	0	11	0	28	28	0	28	W	28+	26	0	26	0	26	26	10	0	5	5	16	2	0
b	7	0	0	0	3	4	0	0	7	7	0	7	W	7+	6	0	7	0	4	7	6	0	3	3	1	0	0
c	2							0	2	2	0	2	W	2+	2	0	2	0	2	0	2	0	0	2	0	0	0
Total	37	12	5	0	3	4	11	0	37	37	0	37		37+	34	0	35	0	32	33	18	0	8	10	17	2	0
													M. aureus group														
d	12	6	4	0	1	1	0	0	12	12	0	12	Or	12+	12	0	12	0	6	8	11	0	9	2	1	0	0
e	1							0	1	1	0	1	Or	1+	1	0	1	0	1	1	1	0	0	1	0	0	0
Total	13	6	4	0	1	1	0	0	13	13	0	13		13+	13	0	13	0	7	9	12	0	9	3	1	0	0
													M. citreus group														
f	12	0	0	0	0	12	0	0	12	12	0	12	Y	12+	12	0		0	0	12	1	1	0	0	0	11	0
g	1							0	1	1	0	1	Y	1+	1	0	1	0	0	1	1	0	1	0	0	0	0
Total	13	0	0	0	0	12	0	0	13	13	0	13		13+	13	0	1	0	0	13	2	1	1	0	0	11	0
													M. aurantiacus group														
h	3	1	1	0	0	1	0	0	3	3	0	0	Or	3+	3	0	3	0	0	1	1	0	0	0	0	0	3
													M. epidermidis group														
k	23	0	4	4	0	10	5	0	23	23	0	0	W	23+	22	0	15	0	0	23	9	10	0	0	0	0	13

* W = white, Or = Orange, Y = yellow.

from peptone and hydrolyse fat. Among the strains which liquefied gelatin two orange strains started the liquefaction after 34 and 30 days' incubation in contrast to the rest which did so within 6 days. The presence of such slow liquefying strains appears to link the quick-liquefying with non-liquefying orange strains. This statement finds support in Pinner and Voldrich (51) dissociation studies on Staphylococcus aureus in which they reported differences in the ability to liquefy gelatin among the variants secured. Whether or not all the members of this group belong to one species, and the difference in chromogenesis and gelatin liquefaction are normal variations among the whole group, cannot be discussed at the present stage.

The ability to digest casein is of common occurrence among the M. albus group and, therefore, many of the strains considered as M. caseolyticus Evans by previous workers may fall in this group. 'c' strains, for example, which were obtained from the Lister Institute under the following names:-

Micrococcus caseolyticus Evans, No.4161 - Originally
M. casei-liquefaciens Orla-Jensen
Staphylococcus cremoris-viscosi Hammer & Cordes, No.963

fell in this group.

'e' and 'g' strains were obtained from the Medical Bacteriology department of Edinburgh University under the names Staphyloccus aureus and Staphylococcus citreus respectively.

Micrococci forming no acid in dextrose.

With the exception of M. lipolyticus ~~H.&S.~~ Stark and Scheib, the number of strains studied is so small that it would be sufficient to present the different groups of characteristics exhibited by the strains examined. This is done in table 38. Of the yellow gelatin-liquefying strains, group 'a' appears to agree/

TABLE 38. - Cultural reactions of 27 strains forming no acid from dextrose.

Reference group no	Source	No. of strains	Survive 30 Min. heating at: 60 C.	63 C.	65 C.	Acid from dextrose	V. P. +	Diastase +	Gelatin liquefied	Chromogenesis*	Gram-negative: Positive	Variable	Negative	Nitrate reduction	NH_4 utilisation	NH_3 from 4% peptone	Citrate utilisation	Casein digestion	Fat hydrolysis	Acid from lactose	Action on Litmus milk: Peptonized	Alkaline	Inert
a	Pasteurised milk	1	1	1	1	0	0	1	1	Y	1	0	0	0	0	0	0	1	0	0	0	1	0
	Lister Coll. No. 2680	1	1	0	0	0	0	1	1	Y	1	0	0	0	1	1	0	1	1	0	0	0	1
b	Contaminant	1	0	0	0	0	0	0	1	Y	1	0	0	0	0	0	0	1	0	0	0	0	1
	Lister Coll. No. 2678	1	0	0	0	0	0	1	1	Y	1	0	0	0	1	0	0	1	0	0	0	0	1
c	Contaminant	1	0	0	0	0	0	1	1	R	1	0	0	0	0	0	0	1	1	0	1	0	0
d	Raw milk	1	0	0	0	0	0	0	1	W	0	1	0	0	0	0	0	1	0	0	0	0	1
	Lister Coll. No. 2679	1	0	0	0	0	0	0	1	W	0	1	0	0	0	1	0	1	0	0	1	0	0
e	Contaminant	1	0	0	0	0	0	0	0	W	1	0	0	1	1	0	0	0	1	0	0	0	1
	Lister Coll. No. 1657	1	0	0	0	0	0	0	0	W	1	0	0	1	0	1	0	0	0	0	0	0	1
	Lister Coll. No. 2684	1	0	0	0	0	0	0	0	W	1	0	0	1	1	1	0	1	1	0	0	0	1
f	Raw milk	17	0	0	0	0	0	0	0	W	0	0	17	0	1	0	17	0	17	0	0	15	2

* Y = yellow, R = red, W = white.

agree with the description of M. subflavescens Bergey et al.(6). One of these strains was obtained from the Lister Institute under the name M. luteus (Schrot.) Cohn, No. 2680. Group 'b' agrees with Bergey's description of M. flavescens Henrici. This group included a Hucker's strain of M. flavus (Flügge) Lehm. & Neum., No. 2678, Nat. Coll. type cul. Lister Institute. The non-pigmented gelatin liquefying group 'd' included a Hucker's strain of M. freudenreichii Guillebeau, No. 2679, Nat. Coll. type cul. Lister Institute.

The gelatin non-liquefying non-pigmented, gram-positive group 'e' consisted of three cultures, two of which were obtained from the Lister Institute under the following names:-

M. ureae Cohn, No. 2684 - A Hucker strain
M. candicans Flügge, No. 1657.

The strains of M. lipolyticus ~~Heiz~~. (group 'f') formed a uniform group which agreed well with the description given by Stark and Scheib (68). These authors, however, did not mention the presence of strains that produced ropiness in milk or broth. Such strains were encountered in this study. This group possesses a striking similarity to Alcaligenes viscosus a which is discussed in a later section. The only difference is the shape of the cells. It is quite possible that this group should be classified with Alcaligenes viscosus a. in spite of the morphological difference.

The work is being continued with the objects of testing and exploring this preliminary classification. In the meantime, however, all the members of the genus Micrococcus encountered in this investigation were typed accordingly. The strains which were isolated in the early stage before the start of this par-

ticular/

particular study, were also identified as far as their tested characteristics agreed with the above groups.

CORYNEBACTERIA

The corynebacterium group of organisms in milk have received comparatively little attention considering the surprisingly large numbers in which they occur in raw and pasteurised milk.

Some attention has been paid to the diphtheroids which appear in aseptically-drawn milk. The first mention of them seems to have been by Bergey (5) under the name of Bacillus pseudodiphtheria. Fourteen years later Evans (22) described them under the name of Bacillus abortus var. lipolyticus, the variety name having been suggested by the most conspicuous biochemical property of the organism. In a later publication (23) a further description was given under the name Bacterium lipolyticum. The examination of the strains isolated in this work, and a type culture of Bacterium lipolyticum Evans, obtained from t he Lister Institute, made it clear that the organism is a corynebacterium not unlike C. bovis Bergey et al. During 1941 Black (9) reported the probable identity of diphtheroids isolated from aseptically-drawn milk with C. bovis and Bacterium lipolyticum. It is of interest to note that this organism is not heat-resistant having, according to Evans, a thermal death point of 52°C. for 30 minutes or 63°C. for 30 seconds. 125 strains of C. bovis were isolated in this investigation mainly from the aseptically-drawn milk samples. Their appearance among the predominant flora of market raw/

raw milk was scarce and confined to two clean certified samples. The very much-encountered and the very much-neglected corynebacteria in milk are those whose most conspicuous property is their high heat resistance. The first mention of these organisms was by Orla-Jensen (49) who grouped them in a genus Microbacterium. Jenkins (40) mentioned that among the types of organisms which survived pasteurisation at temperatures from 59-68.8°C. for 30 minutes, a very short gram-negative bacillus, which corresponded to Orla-Jensen's "Microbacterium", was found in a few samples. Robertson (58), examining the heat resistance of bacteria producing pin-point colonies on pasteurised milk plates, found that Microbacterium lacticum Orla-Jensen was the most heat resistant organism among the thermophilic and thermoduric non-spore-forming organosms examined by him. Jensen (41) regarded Microbacterium lacticum Orla-Jensen and Microbacterium liquefaciens Orla-Jensen as typical Corynebacterium types. In this study, more than 1200 strains belonging to these two species have been isolated from raw and pasteurised milk, utensils etc. On examination no character could be found to differentiate these organisms from the genus Corynebacterium whose definition is usually regarded as "non-motile bacteria without endospores formation, gram-positive, not acid fast, generally rod shaped but with a marked tendency to show involution forms and multiplying by a characteristic 'snapping' division of the cells which causes the bacteria in microscopical preparations to appear in V or III-line arrangements or irregular groups sometimes compared to Chinese letters".

Nine hundred and sixty-eight strains belonging to this

group/

group of corynebacteria isolated from raw and pasteurised milk were tested for their action on gelatin, casein, butter fat or cotton seed oil and litmus milk; for ability to reduce nitrate, to produce acid from dextrose and lactose and for the production of diastase, pigments and catalase. The results obtained showed that liquefaction of gelatin was variable, (703 strains liquefied gelatin and 265 did not); casein was slightly digested by only 5 strains; butter fat or cotton seed oil was not hydrolysed; diastase was produced by all the strains; reduction of nitrate was variable; dextrose and lactose were always fermented with the production of acid, and action on litmus milk was variable a few strains producing no change while the rest formed acid with or without curdling of the milk. Chromogenesis and type of growth were variable among the cultures. Some were white, some produced lemon yellow colour the intensity of which varied greatly among the strains. The majority of the strains produced soft growth while a few produced wrinkled tough growth, difficult to remove from the surface of the agar and also difficult to emulsify. The morphology of the organisms was almost identical in all the strains with the exception of some variations in the length of the rods. No correlation could be found between any of the characters tested. Confronted with such findings it was decided to carry out a more detailed study of a collection of cultures isolated and purified during the examination of the milk samples. This collection, which was made up of 68 strains, contained types showing all the variations encountered. These cultures were tested, in addition to the previous properties, for the fermentation of mannite, maltose/

maltose, raffinose and starch. The last three carbohydrates were used by Orla-Jensen (49) for the distinction of the members of his genus Microbacterium. The strains were also tested for their ability to grow at 37°C. ability to grow on potato and the ability to survive heating at 75°C. for 15 and 30 minute periods. The result of this study revealed that, regarding the sugars tested, maltose was fermented by all the strains, starch by 54 strains, mannite by 34 strains and raffinose by 5 strains. None of the strains grew at 37°C.; all produced lemon yellow growth on potato with variation in the intensity of the colour and amount of growth; all strains survived heating at 75°C. for 15 minutes and, with the exception of the 5 strains which fermented raffinose, 75°C. for 30 minutes. The ability to ferment raffinose was the only property which showed correlation with the other tests carried out except mannite fermentation and nitrate reduction. Whether this correlation will still exist when a larger number of strains from different sources are examined, cannot be predicted and must await further work. Similar variations to what was obtained here have been found by Jensen (41) in his Corynebacterium liquefaciens group. In this connection, he states: "The strains, although similar to each other, are too different to be united into a single species and since no two strains agree well, I have refrained from naming any new species". Table 39 shows a summary of the results obtained from the 68 strains examined in some detail. The strains were divided according to their action on raffinose.

In this report, the designation C. lacticum was used for all the strains which failed to liquefy gelatin, and C. liquefaciens for those which liquefied gelatin. Such division was undertaken to suit the general trend of classifying this group although/

TABLE 39 - Cultural reactions of 68 strains of Corynebacterium lacticum group

	No. of strains	Survive 75°C. for 15 min.	Survive 75°C. for 30 min.	Pigment Present on Agar slopes	Pigment Present on Potato	Growth at 37°C.	Gelatin liquefied	Casein digested	Fat hydrolysed	Diastase produced	Nitrate reduced	Acid production from Dextrose	Acid production from Lactose	Acid production from Mannite	Acid production from Maltose	Acid production from Starch	Litmus milk: Acid	Litmus milk: Acid Coagulated	Litmus milk: No change	Growth on Potato: Heavy	Growth on Potato: Poor
Raffinose +	63	63	63	49	63	0	29	2	0	63	39	63	63	31	63	49	13	48	2	18	45
Raffinose -	5	5*	0	0	5	0	0	0	0	5	3	5	5	3	5	5	5	0	0	0	5
Total	68	68	63	49	68	0	29	2	0	68	42	68	68	34	68	54	18	48	2	18	50

* survive in reduced numbers

although it is felt that such division is artificial.

Some 68 strains belonging to the genus Corynebacterium were isolated from the raw milks. With the exception of one strain which was identified according to Bergey's Manual as C. erythrogenes, no effort was made to name the rest of the strains as their presence in the milk was considered to be infrequent. These were referred to in the previous sections as Corynebacterium sp.

ALCALIGENES GROUP OF BACTERIA IN MILK

Organisms belonging to the genus Alcaligenes were found to form quite a big proportion of the flora present in raw and pasteurised milks. They have not been isolated from aseptically-drawn milk, a finding which suggests that they gain access to the milk after it leaves the udder.

One of the more common ropy milk organisms is Alcaligenes viscosus which was probably first isolated by Adametz (13) from water. After noting the action of the organisms in milk and predicting that it might be the cause of ropiness in this product, Adametz obtained it from ropy milk. Since then it has been isolated and studied by many other workers (13) in connection with outbreaks of ropy milk. Long and Hammer (44) reported that organisms which are like Alcaligenes viscosus except for the failure to produce ropiness in milk and other liquid media, are commonly encountered among organisms isolated from dairy products. The designation Alcaligenes viscosus var. dissimilis was suggested for them. These investigators noted that one of the important characteristics of Alcaligenes viscosus is its ability to hydrolyse fat. There is a considerable amount of confusion

in/

in the literature as to whether this organism is gram-positive or gram-negative. Adametz and Ward (13) state that it is gram-positive. Löhnis (13) notes that it is frequently not decolorized when stained by gram but in his own experience he finds many organisms gram-negative in the course of their development. (cited by Buchanan and Hammer, 13.) Harrison, Buchanan and Hammer (13) and Long and Hammer (44) state that it is always gram-negative. Six hundred and seventy-one strains out of the 787 Alcaligenes strains isolated in this investigation from the raw and pasteurised milk appeared to belong to this type of organism. Some differences, however, were noted between the strains isolated mainly from the pasteurised milk and those which were isolated from the raw milk. Both types were similar in morphology but those isolated from the pasteurised milk regularly tended to resist the decolourizing action of alcohol during staining with gram. The raw milk types were always gram negative. Also, while the raw milk types produced good growth on ordinary agar slopes, those isolated from the pasteurised milk produced poor growth. This difference disappeared when both types were grown on tryptone infusion agar slopes. Spore-like unstainable granules were frequently present when films were prepared from the poor growth produced by the pasteurised milk types on ordinary agar slopes. These unstainable granules became unnoticeable when the organisms grew luxuriantly. The other alcaligenes type isolated from the raw milk did not reveal the presence of such unstainable granules. Harrison (1) seems to be the only worker who stated that spore-like granules were sometimes present but never true spores. Another difference between the pasteurised milk strains and those isolated from the raw milk was that the first type always failed to hydrolyse either butter fat/

fat or cotton seed oil, while the latter type always hydrolysed both fats. When the above differences were observed, representative cultures of both types isolated from most of the milk samples examined were purified by the pouring plate method and then subjected to a more detailed study. The results obtained pointed to two distinct species. Table 40 gives a summary of the results obtained from the study of 45 strains isolated from 5 different raw milk samples, referred to in the table as Alcaligenes viscosus a. and 68 strains isolated from freshly pasteurised, incubated pasteurised and incubated certified milk, referred to as Alcaligenes viscosus b. Both types would have been included in the species Alcaligenes viscosus according to most of the previous descriptions. However, the uniform difference between the two types in their heat resistance, ability to utilise ammonium phosphate as the only source of nitrogen and citrate salts as the only source of carbon, ability to hydrolyse fat, growth on potato, growth on ordinary agar slopes, resistance to the decolourizing action of alcohol and the presence of unstainable globules would justify their division into two separate species. Alcaligenes viscosus a. was represented by 224 out of the 247 strains isolated from the raw milks. Alcaligenes viscosus b. was represented by the 35 strains isolated from the freshly pasteurised milk, the 405 strains isolated from the incubated pasteurised milk and 7 strains isolated from the raw milk.

Alcaligenes viscosus a. agrees well with the description given by Long and Hammer (44) for the ropy strains, Alcaligenes viscosus and the non-ropy strains, Alcaligenes viscosus var. dissimilis. The characters shown by Alcaligenes viscosus b. might explain the controversy in the literature concerning the behaviour of the organisms/

TABLE 40. - Morphological and Cultural reactions of Alcaligenes viscosus a. and Alcaligenes viscosus b.

	Alcaligenes viscosus a.	Alcaligenes viscosus b.
Morphology	Rods 0.6-1μ by 0.8-2.5μ. Young cultures are almost coccus like. Occasionally filamentous involution forms are observed. Cells arranged in pairs, singly or short chains	Similar to Alc. viscosus a. In poor growing agar slope cultures cells tend to be larger in size and frequently contain unstainable granules.
Motility	Non motile	Non motile
Spores	Not present	Not present.
Staining reac-	Stains readily with ordinary stains. Gram-negative	Stains readily with ordinary stains. Cells tend to resist the decolourizing action of alcohol in the Gram staining.
Ordinary agar slope	Moderate to abundant, greyish-white, viscid or soft, shiny growth.	Poor greyish-white shiny growth
Tryptone infusion agar slope	Abundant greyish-white viscid or soft shiny growth	Similar to Alc. viscosus a.
Tryptone infusion agar colonies	Greyish-white, soft or viscid, shiny round with entire edge 3-5 mm. in diameter (5 days at 30°C.)	Similar to Alc. viscosus a.
Gelatin stab	No liquefaction.	No liquefaction.
Broth	A thin pellicle, turbidity, sediment.	Similar to Alc. viscosus a.
Litmus milk	Alkaline reaction, reduction at bottom of tube. Ropiness caused by some strains.	Similar to Alc. viscosus a.
Potato	Good growth, dirty grey becoming brown. Potato discoloured.	No growth
Nitrates	Not reduced.	Variable.
Ammonium salts	Utilised as the only source of nitrogen.	Not utilised as the only source of nitrogen.
Citrate	Utilised as the only source of carbon	Not utilised as the only source of carbon
Carbohydrates	Not utilised	Not utilised

Casein/

TABLE 40 (Cont.)

	Alcaligenes viscosus a.	Alcaligenes viscosus b.
Casein	Not digested.	Not digested.
Butter Fat) Cotton seed) oil)	Actively hydrolysed.	Not hydrolysed.
Oxygen	Strict aerobe.	Strict aerobe.
Heat resistance	Thermal death point below 60°C. for 30 min.	All strains survive 60°C. for 30 min. and, in reduced numbers, 63°C. for 30 min.
Temperature range of growth	Min. below 10°C., Opt. 30°C., Max. 37°C.	Min. below 10°C., Opt. 30°C., Max. above 37°C.

organisms to gram-stain and the unconfirmed observation of Harrison about the presence of unstainable granules.

From the examination of the 224 strains of Alcaligenes viscosus a. and the 447 strains of Alcaligenes viscosus b. it seems doubtful that the present classification of these organisms as primarily ropy types with the occurrence of non-ropy variants, expresses the correct picture of the group. Slime-producing variants are not uncommon among many species which are regarded as non-slime producing. In addition, slime production is not a stable character for employment as a basis for classification purposes. Indeed, Long and Hammer (44), after describing the common existence of non-ropy strains of Alcaligenes viscosus, stated that some of the cultures which did not produce ropiness when first isolated, gave ropiness after a number of transfers in milk. They also found that typical Alcaligenes viscosus cultures varied widely in the extent of the ropiness produced and in the time required to bring about this change. The idea of Alcaligenes viscosus being a primarily slime-producing organism arose from the fact that almost all the studies carried out on this group were in connection with outbreaks of ropy milk. However, the results obtained in this work revealed the common presence of these organisms in raw and pasteurised milk and that the ropy strains made up only a small proportion of the total number encountered. Thus it seems more likely that the ropy strains are variants of the non-ropy ones than the reverse. The name Alcaligenes viscosus var. dissimilis, suggested by Long and Hammer for the non-ropy strains becomes, therefore, questionable.

The remaining 16 organisms which belonged to the genus Alcaligenes belonged to three species. Alcaligenes bookerii (Ford)

(Ford) Bergey et al. (6) was represented by three strains isolated from a raw certified milk sample held at 22°C. for 24 hours. Alcaligenes albus Bergey et al. (6) was represented by seven strains isolated from another certified sample incubated at 22°C. for 24 hours. The remaining six strains did not agree with any of the Alcaligenes species listed in Bergey's Manual. These strains were isolated from a clean certified sample and possess the following characters:

Morphology: The organism is a short rod, measuring about 0.6-8 µ by 1.5-2.5 µ. Organisms appearing as pear-shaped egg-shaped, long rods, wide in the centre with tapering ends and filamentous forms are very common.

Motility: Motile: involution forms do not exhibit motility.

Spores: Not present.

Staining: Involution forms do not stain uniformly. The organism is gram-negative.

Agar slope: Leathery growth difficult to lift from the agar, white, with slight light yellow shade, growth not spreading, shiny.

Agar colonies: Light cream in colour, leathery, lifts as one part, about 3 mm. in diameter.

Gelatin stab: Liquefaction appears after 7 days' incubation at 22°C.

Broth: The organisms grow near the surface producing a slimy growth.

Litmus milk: Develops alkaline reaction. The organisms produce a very mucoid growth in the top part of the liquid.

Nitrate: Reduced.

Ammonium salts: Not utilised as the only source of nitrogen.

Carbohydrates: Not utilised.

Casein: Not digested

Butter fat: Not hydrolysed.

Temperature relationships: Optimum temperature about 30°C. Thermal death point below 60°C. for 30 minutes.

Oxygen/

Oxygen: The organism is a strict aerobe.

Classification of other types.

For the characterisation of the aerobic spore-formers the key proposed by Gibson and Topping has been used successfully.

Bergey's Manual of Determinative Bacteriology (6) was employed for the identification of the rest of the organisms isolated.

SUMMARY

1. Methods used for the classification of the organisms isolated in this study were discussed.

2. A test was devised for the separation of the hetero-fermentative from the homofermentative streptococci based on the production of gas by the former group.

3. A preliminary classification for the micrococci was advanced as a result of a study comprising 245 strains isolated from a variety of sources. This was based on ability to survive 60°C. for 30 minutes, to produce acid from dextrose, Barritt's modification of the Voges-Proskauer test, diastase production, liquefaction of gelatin, chromogenesis, ability to utilise sodium citrate as the only source of carbon and Gram reaction.

4. Classification of the corynebacteria was discussed. No satisfactory means of separating species within the heat-resistant group was arrived at.

5. It was found that Alcaligenes viscosus strains which were encountered in both raw and pasteurised milk could be separated into two distinct species.

SOURCES OF THE IMPORTANT GROUPS OF BACTERIA IN PASTEURISED MILK

There is uniform agreement among previous reports that the thermoduric organisms present in milk originate principally in dirty farm utensils and that cleaning up these utensils results in reduction of the post-pasteurisation counts. (39, 1, 15, 46, 50, 43, 69). These findings are not surprising since it has been shown that stable air (62) and insanitary stables in general (54) have little effect in increasing the original bacterial contamination of milk, while utensils (55), and especially milking machines (63), supply most of this original contamination. Most of the work that had been done with regard to the sources of the thermoduric bacteria in milk was only quantitative in nature. The method usually employed was to pasteurise in the laboratory samples of milk from individual farms and note the plate counts. The tests were repeated after the utensils and the dairy equipment employed on the farms had been properly sterilized. The two series of the post-pasteurisation counts were then compared and conclusions were drawn. Qualitatively, little information is available in the literature about the sources of the different types of organisms which usually constitute the predominant flora of pasteurised milk. A few studies Whiting (71), Robertson (56, 57) have been carried out on the flora of dairy utensils among which some of the known heat-resistant types (Micrococcus conglomeratus, Micrococcus luteus, Micrococcus varians) were noted. These

studies/

studies were carried out on the general flora and therefore would be expected to yield but little information with regard to the presence and the abundance of the thermoduric types which usually form a small proportion of the total flora. In some instances, some types of organisms which appeared on pasteurised milk plates were observed to be common in some dairy equipment. Thus, Eglinton and Yale (20) observed that yellow heat-resistant micrococci (not identified) which were often present in pasteurised milk, were common in milking machines and milk cans. Recently a study by Hileman et al. (12) was published in which an effort was made to trace the source of the thermoduric bacteria in milk pasteurised at 161°F. for 16 seconds. The method consisted of pasteurising at 161°F. for 16 seconds rinsings of pails and equipment on 19 farms. Five hundred and twenty colonies were picked and found to group as follows:- Streptococci 5%, Micrococci 70%, Sarcinae 15.6% and rods 9.4%. Only 17 cultures were identified and the results were:- Micrococcus varians, 29.4%, Micrococcus luteus, 28,4% Micrococcus candidus, 17.6%, Streptococcus thermophilus, 11.8% Micrococcus conglomeratus, 5.9% and Micrococcus freudenreichii, 5.9%. Hucker's key for the classification of the micrococci was employed. The presence of Corynebacterium lacticum types is likely to have been overlooked as a result of incubating the plates at 37°C. Contrary to the conclusion arrived at by these workers that the udder is the ultimate source of the micrococci in milk, pasteurisation tests in the present investigation revealed that the udder flora is not heat resistant.

This short review of the literature shows that the information concerning the sources of the pasteurised milk flora is/

is far from complete.

In order to trace the source of the bacteria which were uniformly found to constitute the predominant flora of pasteurised milk, the following method was planned. Visits were made to five dairies, two of which produced certified milk. The possible sources of contamination were examined before the start of milking. For the examination of the utensils, milking machines, teats and the outside of the udder, sterile cotton swabs which were wetted in sterile milk immediately before use, were employed. Samples of fresh faeces, dried faeces and dust were collected in sterile Petri dishes with the aid of sterile spatulas. Dandruff and dried hairs from the flank of two cows were scraped in a Petri-dish by means of sterile scalpels. Samples of the water supply and vacuum pipes' discharge were collected in sterile tubes. Several persons were asked to rub their hands with sterile milk which was present in sterile Petri dishes. On arrival at the laboratory, the swabs were shaken well in about 6 ml. of sterile milk and then squeezed as much as possible on the wall of the tube before being removed. The other samples - faeces dust etc. - were added to the same quantity of sterile milk and mixed well. Each sample was then transferred aseptically to two sterile tubes, great care being taken not to contaminate the walls above the surface of the milk. One set of the tubes was pasteurised at 63°C. for 30 minutes and then plated in quadruplicate. Half the plates were incubated at 40°C. for two days, the other half were incubated at 22°C. for 6-7 days. Incubation at 40°C. favours the growth of the streptococci encountered in pasteurised milk and, at the same time, eliminates the growth of the corynebacteria and micrococci which are liable to predominate on the plates incubated at 22°C. The second set of the tubes was heated at 75°C. for 15 minutes and then plated in duplicate. The plates were incubated at 30°C. for 5 days. This heat treatment will kill almost all the vegetative cells of the bacteria present with the exception of

Corynebacterium/

Corynebacterium lacticum types.

Meat extract agar containing 1% meat extract, 1% peptone, and 0.25% dextrose was used for plating. Immediately before pouring plates which were to receive less than 1 ml. of inoculum, 0.5 ml. of sterile milk was added to each tube of agar. After incubation a representative number of the various types of colonies were picked for characterisation.

Table 41 illustrates the results from the main possible sources of the four groups of bacteria which formed the main flora of pasteurised milk. Although the method employed is not a quantitative one, the number of the different types of organisms on the plates can be taken to indicate the abundance of these groups at the time of examination. Thus, the frequency with which the bacterial groups were encountered is shown in the table by +, ++ or +++. + indicates 1000 colonies or less, ++ 1000-100,000 and +++ more than 100,000. Dairies 'A' and 'B' produced certified milk while dairies 'C', 'D' and 'E' produced milk with no grade.

Dairy 'A' employed a conveyor milking machine. Immediately after milking is over cold water is run through the machine followed by hot soda water then hot water. All churns were heated in a steamer for 30 minutes. The receiver, strainer and surface cooler were cleaned by the same method used for the conveyor machine. The teat cups and rubber tubings were ~~taken down to pieces~~ dismantled twice weekly and cleaned with boiling water and a chlorine disinfectant.

Milking machines were used in dairy 'B'. All metal utensils were sterilized in a steamer immediately before use. The receiver, surface cooler and bottler were cleaned with boiling water/

TABLE 41. - Sources of the important groups of bacteria in pasteurised milk.

*Source	[1]Streptococci					[2]Micrococci					[3]Corynebacteria					Alcaligenes viscosus b.				
	Dairies					Dairies					Dairies					Dairies				
	A	B	C	D	E	A	B	C	D	E	A	B	C	D	E	A	B	C	D	E
Teat cups	-	-	0	0	+++	-	-	0	0	+++	+	++	0	0	-	-	-	0	0	++
Rubber tubing	-	0	0	0	+++	-	0	0	0	++	-	0	0	0	++	-	0	0	0	-
Pail of milking machine	0	-	0	0	+++	0	-	0	0	++	0	++	0	0	+++	0	-	0	0	+
Releaser tap	-	0	0	0	0	-	0	0	0	0	+	0	0	0	0	-	0	0	0	0
Milking pail	0	-	+	+++	0	0	-	++	+++	0	0	+	+	+++	0	0	-	+	+	0
Churn	-	-	-	+	++	-	-	+	+++	++	-	+	+	++	+++	-	-	+	++	++
Milk receiver	-	-	+++	+	0	+	+	+++	+	0	+++	++	+++	+++	0	+++	-	++	+	0
Milk strainer	-	-	0	0	0	-	-	0	0	0	+	+	0	0	0	+	-	0	0	0
Surface cooler	-	-	+++	++	0	+++	++	+++	++	0	++	+++	+++	+++	0	-	+	++	++	0
Rubber tube connection bet.bottler & cooler	0	-	0	0	0	0	++	0	0	0	0	+++	0	0	0	0	+	0	0	0
Bottler	0	-	0	0	0	0	++	0	0	0	0	+++	0	0	0	0	+	0	0	0
Bottling valves	0	-	0	0	0	0	+	0	0	0	0	+++	0	0	0	0	-	0	0	0
Vacuum water (beside teat cups)	-	0	0	0	0	-	0	0	0	0	+++	0	0	0	0	-	0	0	0	0
Vacuum discharge	+	0	0	0	0	+	0	0	0	0	+++	0	0	0	0	+	0	0	0	0

1 - Mainly S. thermophilus and S. bovis
2 - M. luteus group
3 - C. lacticum type

* 0 = not examined.

water. The teat cups and rubber tubings were cleaned daily with boiling water.

Hand milking was practised in dairy 'C' where the utensils were cleaned with cold followed by very hot water. The receiver and surface cooler, however, were placed on the top of the boiler! Hand milking was also practised in dairy 'D' where the utensils and other equipment were cleaned by cold followed by hot soda water then hot water. Machine milking was practised in dairy 'E'. Although cold and hot water was supposed to have been drawn through the rubber parts of the milking machine after each milking in addition to taking them to pieces for cleaning with hot water twice weekly, the condition in which these parts were found at the time of examination was anything but satisfactory. The rubber tubings contained very sour milk refuse of which a very small part curdled the sterile milk used for making the suspension during the heat treatment. The churns which were supposed to have been cleaned and steamed in the pasteurising plant were found in a wet condition with about an eighth of an inch of milky water at the bottom.

In the first two dairies, where an effort was made to produce low-count milk, the streptococci group was not encountered except for 3 strains of S. faecalis which were isolated from the vacuum discharge. On the other hand, Corynebacterium lacticum types were commonly present on most of the equipment. The milk receivers, surface cooler, bottler and bottling valves which were not steamed showed big numbers of both corynebacteria and micrococci. The absence of the streptococci and the common occurrence of the corynebacteria and, to a less extent, micrococci, is in agree-

ment/

agreement with the results reported earlier regarding the predominant flora of freshly pasteurised certified milk samples. As this part of the investigation was carried out during February, March and the first half of April, which belong to the cold part of the year it is realised that different results may occur if the work is to be carried out during the warm summer season.

In contrast to the results obtained from the grade milk dairies, the utensils and equipment examined in the other three dairies revealed gross infection with thermoduric streptococci, micrococci, corynebacteria and Alcaligenes types. The highest plate counts secured were obtained from the examination of the parts of the milking machines in dairy 'E'. These were in excess of 3,000,000. Streptococcus thermophilus and Streptococcus bovis were the only two types of streptococci encountered. All the micrococci strains which were characterised proved to belong to M. luteus group.

In addition to the sources listed in table 41, the outside of 5 udders, teats of 5 cows, dandruff and scrapings of the flanks of 3 cows, 2 samples of dust present in the two grade milk dairies, 2 samples of fresh faeces, 2 samples of dried faeces and the hands of 5 different persons were also examined in the above manner. All yielded great numbers of spore formers along with a few actinomyces. However, a small number of Corynebacterium lacticum types appeared on the plates of 2 udders and 2 teats and the hands of one person. The latter source showed also 2 strains of Streptococcus thermophilus and 2 strains of Streptococcus faecalis

SUMMARY

Sources of the principal bacterial groups encountered

in/

in pasteurised milk were traced in two dairies producing certified milk and three ordinary dairies.

In the first group of dairies, the main source of contamination was the equipment which was not subjected to steaming. This revealed large numbers of Corynebacterium lacticum types, Micrococcus luteus group and Alcaligenes viscosus b. variety.

Of this equipment, the Surface cooler, Milk receiver, Milk bottler and Bottling valves were the most infected.

In the remaining dairies, where conditions of milk production were not satisfactory, Streptococcus thermophilus, Streptococcus bovis, Micrococcus luteus group and Alcaligenes viscosus b. type were commonly present in large numbers on the utensils and equipment tested.

ACKNOWLEDGEMENTS

I wish to acknowledge my indebtedness to Dr. T. Gibson, under whose guidance the work was carried out; to Dr. A. Cunningham for so kindly providing the aspetically-drawn milk samples and the silage strains of heterofermentative lactobacilli and streptococci; to Mrs B. Sutton, B.Sc. for her kind help.

REFERENCES

1. ANDERSON, E.B. and MEANWELL, L.J. (1933) J.Dairy Res, 4, 213
2. AYERS, S.H. and JOHNSON, W.T. (1913) U.S.D.A. Bureau of Animal Industry, Bull, 161.
3. AYERS, S.H., JOHNSON, W.T. and MUDGE, C.S. (1924) J.Inf. Dis., 34, 29
4. AYERS, S.H., RUPP, PHILLIP and MUDGE, C.S. (1921) J.Inf. Dis., 29, 235
5. BERGEY, D.H. (1904) Penn.Dept.Agr.Bull. 125 Cited in ref.(6)
6. BERGEY (1939) Manual of Determinative Bacteriology. 5th Edition.
7. BERRY, J.A. (1933) J. Bact., 25, 433
8. BIGGAR, J.W. BOLAND, C.R. and O'MEARA, R.A. (1927) J.Path. and Bact., 30, 261.
9. BLACK, L.A. (1941) J.Bact., 41, 99.
10. BLACK, L.A., PROUTY, C.C. and GRAHAM, R.A. (1932) J.Dairy Sci., 15, 99
11. BREED, ALICE F. (1928) Tech. Bull.N.Y.(Geneva) St.Agric.Exp.Sta.No.132
12. BRITISH STANDARDS INSTITUTION (1940) Bull.895
13. BUCHANAN, R.E. and HAMMER, B.W. (1915) Res.Bull.Iowa St.Coll. Agric.Exp.Sta. No. 22
14. CUNNINGHAM, A. and SMITH, A.M. (1940) J.Dairy Res., 11, 243
15. DAVIES, B. (1933) Proc.Intern.Assoc.Milk Dealers, Laboratory Sect. 26, 59. Cited in Ref(30)
16. DAVIS, J.G. and THIEL, C.C. (1939) J.Dairy Res., 10, 455.
17. DORNER, W. (1930) Tech.Bull.N.Y. (Geneva) St.Agric.Exp.Sta.No. 165
18. DOTTERER, W.D. (1939) Dairy Sci.Abst.1, 217, also cited in ref.(30)
19. DUDGEON, L.S. (1908) J.Path. and Bact.12, 242
20. EGLINTON, R. and YALE, M.W. (1931) Ann.Rep.N.Y.State Assoc. Dairy and Milk Inspectors 3,63 - cited in ref (30)

21. EVANS/

21. EVANS, A.C. (1916) J.Inf. Dis., 18, 437

22. EVANS, A.C. (1917) J.Bact., 2, 185

23. EVANS, A.C. (1918) J. Inf. Dis., 22, 580

24. GIBSON, T. and ABDEL-MALEK, Y. (1940) Proc.Soc.Agric.Bact,, 19.

25. GIBSON, T. and TOPPING L. (1938) Proc. Soc.Agric. Bact., 33

26. HAMMER, B.W. (1938) Dairy Bacteriology. New York: John Wiley and Sons.

27. HAMMER, B.W. and BAKER, M.P. (1923) Res.Bull.Iowa St.Coll.Agric. Expt. Sta. No. 81.

28. HARNED, H.H. (1940) Iowa St.Coll. J.Sci., 14, 43.

29. HASSOUNA, M.M. and ALLEN, L.A. (1939) J.Dairy Res., 10, 7

30. HILEMAN, J.L. (1940) J.Dairy Sci., 23, 143

31. HILEMAN, J.L. LEBER, H. and SPECK, M.L. (1941) J.Dairy Sci., 24, 305

32. HOFFSTADT, R.E. and YOUMANS, G.P. (1932) J. Inf. Dis., 51, 216

33. HOFFSTADT, R.E. and YOUMANS, G.P. (1934) J. Bact., 27, 551

34. HUCKER, G.J. (1924) Tech. Bull. N.Y.(Geneva) St.Agric.Expt.Sta.No.100

35. HUCKER, G.J. (1928) Tech. Bull. N.Y.(Geneva) St.Agric.Expt.Sta.No.134

36. HUCKER, G.J. (1928) Tech.Bull.N.Y. (Geneva) St.Agric.Expt.Sta.No.135

37. HUCKER, G.J. and PEDERSON, C.S. (1931) Zbl. Bakt.II, 85, 65

38. HUCKER, G.J. and RETTGER, LEO.F. (1925) Zbl. Bakt.II, 65, 273

39. HUSSONG, R.V. and HAMMER, B.W. (1931) Res.Bull.Iowa St.Coll.Agric. Expt.Sta. No. 286.

40. JENKINS, H. (1926) J.Hyg., 25, 273

41. JENSEN, H.L. (1934) Proc.Linn.Soc.N.S.W., LIX, 19

42. JONES, F.S. (1921) J.Exp. Med., 31, 347

43. KRUEGER/

43. KRUEGER, P.F. (1939) — Dairy Sci. Abst., 1, 216

44. LONG H.F. and HAMMER B.W. (1936) — Iowa St.Coll.J.Sci.10, 261

45. MACY, H. (1939) — Dairy Sci. Abst, 1, 339

46. MEANWELL, L.J. (1939) — Dairy Ind.4, 382 - also Dairy Sci.Abst.1, 261

47. MELLON, R.R. and CALDWELL, D.W.(1932) — J. Bact., 12, 409

48. NEUMANN, R. (1897) — Arch.J.Hyg., 30, 1

49. ORLA-JENSEN, S. (1919) — The Lactic Acid Bacteria. København

50. PARFITT, E.H. (1939) — Dairy Sci. Abst.1,308

51. PINNER, M. and VOLDRICH, M. (1932) — J.Inf. Dis., 50, 185

52. FRAZIER, W.C. and RUPP, F . (1928) — J.Bact., 16, 57

53. PROUTY, C.C. (1934) — J. Dairy Sci., 17, 115

54. PRUCHA. M.J. and WEETER, H.M. (1917) — Ill.Agr.Expt.Sta.Bull. 199

55. PRUCHA, M.J.WEETER, H.M. and CHAMBERS, W.H. (1918) — Ill.Agr.Expt.Sta.Bull.204.

56. ROBERTSON, A.H. (1924) — Tech.Bull.N.Y. (Geneva)St. Agric. Exp.Sta.No. 105

57. ROBERTSON, A.H. (1925) — Tech.Bull.N.Y.(Geneva) St. Agric. Exp.Sta.No. 112

58. ROBERTSON, A.H. (1927) — Tech.Bull.N.Y.(Geneva) St. Agric.Exp.Sta.No. 131

59. ROBERTSON, A.H. (1927) — Vermont Agric.Exp.Sta.Bull. 275; also Biol Abst.,3,8539

60. ROGERS, ASSOCIATES of (1935) — Fundamentals of Dairy Science New York, Reinhold Publishing Corporation.

61. ROWLANDS, A. and PROVAN, A.L.(1941) — Proc.Spc.Agric.Bact. 29.

62. RUEKLE, G.L.A. and KULP, W.L. (1915) — N.Y.(Geneva) Agr.Exp.Sta. Bull. 409.

63. RUEKLE, G.L.A., BREED, R.S. and SMITH, G.A. (1918) — N.Y.(Geneva) Agr.Exp.Sta. Bull.450

64. SCOTT, A.W. and WRIGHT, N.C. (1935) — The Hannah Dairy Res.Inst. Bull. 6.

65. SHERMAN, J.M. (1937) — Bact. Rev.1, 1 - 97.

66. SMITH/

66. SMITH, F.R. and SHERMAN. J.M. (1939) J.Inf.Dis., 65, 301

67. STECK, W. (1921) Landw.Jahrb.Schweiz, 35 511 - cited in ref.(60)

68. STARK, C.N. and SCHEIB, B.J. (1936) J.Dairy Sci., 19, 191

69. TAYLOR, G.B. (1924) Intern.Assoc.Dairy and Milk Inspectors, Ann.Rep., 13, 287 - cited in ref.(30)

70. THURSTON, L.M. and OLSON, H.C. (1936) Biol. Abst., 10, 1416

71. WHITING, W.A. (1923) Tech.Bull.N.Y.(Geneva)St. Agric.Exp.Sta. No. 98

72. WILSON, G.S. (1935) Med.Res.Council, London, Spec.Rept. 206.

73. WORKMAN, W.T. (1939) Ann.Rep.N.Y.State Assoc. Dairy and Milk Inspectors, 13, 212 - cited in ref.(30)

74. CUNNINGHAM. A. (1933) J.Dairy Res., 4, 197

A P P E N D I X

TABLES I to XVIII

TABLE I - The predominant bacterial groups in aseptically-drawn milk according to the action on lactose and casein.

Sample	Percentage			
	Lactose + Casein -	Lactose + Casein +	Lactose - Casein +	Lactose - Casein -
1	97.2	2.8	-	-
2	100.0	-	-	-
3	1.7	-	-	98.3
4	95.0	-	5	-
* 5	-	100.0	-	-
ø 6	48.0	52.0	-	-
7	48.0	8.0	-	44.0
* 8	29.3	63.4	-	7.3
9	-	34.8	-	65.2

* From a cow infected with Streptococcus agalactiae.
ø From a cow that had been infected with Streptococcus agalactiae but was free from infection when sample was collected.

TABLE II - The effect of pasteurisation on the bacterial groups in clean certified milk according to the action on lactose and casein.

	Sample and Treatment	Percentage			
		Lactose + Casein -	Lactose + Casein +	Lactose - Casein +	Lactose - Casein -
1	Raw	50.0	14.3	3.6	32.1
	Pasteurised	-	-	(100)	-
2	Raw	50.0	50.0	-	-
	Pasteurised	-	-	-	-
3	Raw	54.5	33.3	-	12.1
	Pasteurised	-	-	-	-
4	Raw	18.2	-	42.4	39.4
	Pasteurised	-	-	(100)	-
5	Raw	23.3	2.3	4.7	69.7
	Pasteurised	-	-	-	-

TABLE III - The effect of pasteurisation on the bacterial groups in certified milk according to the action on lactose and casein

	Sample and Treatment	Percentage			
		Lactose + Casein -	Lactose + Casein +	Lactose - Casein +	Lactose - Casein -
1	Raw	64.0	25.7	-	10.3
	Pasteurised	95.0	-	2.5	2.5
2	Raw	59.6	12.6	25.5	2.2
	Pasteurised	100.0	-	-	-
3	Raw	63.6	-	22.8	13.6
	Pasteurised	89.4	-	2.1	8.5
4	Raw	47.5	10.0	7.5	35.0
	Pasteurised	97.8	2.2	-	-
5	Raw	82.1	5.1	5.1	7.7
	Pasteurised	100.0	-	-	-
6	Raw	57.5	2.5	15.0	25.0
	Pasteurised	100.0	-	-	-
7	Raw	-	-	-	100.0
	Pasteurised	100.0	-	-	-
8	Raw	20.7	3.4	44.8	31.0
	Pasteurised	100.0	-	-	-

TABLE IV - The effect of pasteurisation on the relative proportions of the predominant bacterial groups in tank milk according to the action on lactose and casein.

Sample and Treatment		Percentage			
		Lactose + Casein -	Lactose + Casein +	Lactose - Casein +	Lactose - Casein -
1	Raw	72.4	6.9	13.9	6.9
	Pasteurised	94.9	5.1	-	-
2	Raw	60.6	9.1	27.3	3.0
	Pasteurised	92.0	-	-	8.0
3	Raw	68.6	8.6	11.4	11.4
	Pasteurised	100.0	-	-	-
4	Raw	81.5	3.7	14.8	-
	Pasteurised	100.0	-	-	-
5	Raw	62.8	9.3	14.0	14.0
	Pasteurised	100.0	-	-	-
6	Raw	66.7	8.3	19.4	5.6
	Pasteurised	100.0	-	-	-
7	Raw	25.6	-	35.9	38.5
	Pasteurised	90.6	9.4	-	-
8	Raw	89.5	-	-	10.5
	Pasteurised	100.0	-	-	-
9	Raw	78.6	-	3.6	17.8
	Pasteurised	100.0	-	-	-
10	Raw	59.4	3.1	3.1	34.4
	Pasteurised	97.1	-	2.9	-

TABLE V - The average percentages of predominant bacterial groups in the different grades of raw milk according to the action on lactose and casein.

Raw Milk	Lactose + Casein -	Lactose + Casein +	Lactose - Casein +	Lactose - Casein -
Aseptically-drawn	46.6	29.0	0.6	23.8
Clean certified	38.2	20.0	10.1	30.7
Certified	49.4	7.4	15.1	28.1
Tank	66.6	4.9	14.3	14.2

TABLE VI - The average percentages of predominant bacterial groups after the pasteurisation of the different grades of raw milk according to the action on lactose and casein.

Pasteurised Milk	Lactose + Casein -	Lactose + Casein +	Lactose - Casein +	Lactose + Casein +
Aseptically-drawn	-	-	-	-
Clean certified	-	-	(100)	-
Certified	97.8	0.3	0.6	1.4
Tank	98.0	1.2	0.2	0.6

TABLE VII - The effect of holding clean certified milk prior to pasteurisation at 22°C. for 6 hours and at 9-11°C. for 24 hours on the predominant bacterial groups in raw and pasteurised milk according to the action on lactose and casein.

Sample	Treatment of Raw Milk	Percentage							
		Raw				Pasteurised			
		Lactose + Casein -	Lactose + Casein +	Lactose - Casein +	Lactose - Casein -	Lactose + Casein -	Lactose + Casein +	Lactose - Casein +	Lactose - Casein -
1	* 0	54.5	33.3	-	12.1	-	-	-	-
	6 hrs. at 22°C.	79.4	20.6	-	-	-	-	(100)	-
	24 hrs. at 9-11°C.	15.6	21.9	34.3	28.1	-	-	(100)	-
2	* 0	18.2	-	42.4	39.4	-	-	(100)	-
	6 hrs. at 22°C.	22.9	17.1	34.3	25.0	-	-	(100)	-
	24 hrs. at 9-11°C.	2.3	4.7	72.1	20.9	-	-	(100)	-

* 0 = examined at time of arrival.

TABLE VIII - The effect of holding certified milk prior to pasteurisation at 22°C. and at 8-14°C. for 24 hours on the predominant bacterial groups in the raw and pasteurised milk according to the action on lactose and casein.

Sample	Raw Milk Held for 24 hrs.at:	Percentage							
		Raw				Pasteurised			
		Lactose + Casein -	Lactose + Casein +	Lactose - Casein +	Lactose - Casein -	Lactose + Casein -	Lactose + Casein +	Lactose - Casein +	Lactose - Casein -
1	-	47.5	10.0	7.5	35.0	97.8	2.2	-	-
	22°C.	28.6	31.4	-	40.0	100.0	-	-	-
2	-	82.1	5.1	5.1	7.7	100.0	-	-	-
	22°C.	26.1	-	13.0	60.9	94.9	5.1	-	-
	10-13°C.	-	-	-	100.0	100.0	-	-	-
3	-	57.5	2.5	15.0	25.0	100.0	-	-	-
	22°C.	58.3	-	18.8	22.9	97.1	-	-	2.9
	12-14°C.	17.1	-	-	82.9	20.7	-	-	79.3
4	-	20.7	3.4	44.8	31.0	100.0	-	-	-
	22°C.	52.6	-	2.6	44.7	97.2	-	-	2.3
	8-10°C.	28.0	-	32.0	40.0	100.0	-	-	-

TABLE IX - The effect of holding tank milk prior to pasteurisation at 22°C. for 6 hours and at 10-14°C. for 24 hours on the predominant bacterial groups in the raw and pasteurised milk according to the action on lactose and casein.

Sample	Raw Milk Held at:	Percentage							
		Raw				Pasteurised			
		Lactose + Casein -	Lactose + Casein +	Lactose - Casein +	Lactose - Casein -	Lactose + Casein -	Lactose + Casein +	Lactose - Casein +	Lactose - Casein -
1	-	62.8	9.3	14.0	14.0	100.0	-	-	-
	11-13°C.for 24 hrs.	63.0	-	11.1	25.9	100.0	-	-	-
2	-	66.7	8.3	19.4	5.6	100.0	-	-	-
	22°C.for 6 hrs.	51.1	2.3	37.3	9.3	100.0	-	-	-
	10-11°C.for 24 hrs.	32.4	-	64.9	2.7	100.0	-	-	-
3	-	25.6	-	35.9	38.5	90.6	9.4	-	-
	22°C.for 6 hrs.	50.0	7.1	2.4	40.5	100.0	-	-	-
	12-13°C.for 24 hrs.	39.5	15.1	12.1	33.3	100.0	-	-	-
4	-	59.4	3.1	3.1	34.4	97.1	-	2.9	-
	22°C.for 6 hrs.	40.6	-	-	59.4	88.6	-	2.8	8.6
	12-14°C.for 24 hrs.	72.2	-	-	27.8	87.2	-	-	12.8

TABLE X - The effect of 24 hours' holding at 22°C. and at 8-14°C. on the predominant bacterial groups in pasteurised milk according to the action on lactose and casein.

Ø Sample	Held 24 hrs. at:	Percentage			
		Lactose + Casein -	Lactose + Casein +	Lactose - Casein +	Lactose - Casein -
Cl.C.1	-	-	-	-	-
	22°C.	-	-	(100)	-
	10-12°C.	-	-	(100)	-
Cl.C.2	-	-	-	100.0	-
	22°C.	26.7	-	73.3	-
	10-12°C.	-	-	85.7	14.3
C.1	-	100.0	-	-	-
	22°C.	17.4	-	-	82.6
	10.13°C.	94.4	2.8	-	2.8
C.2	-	100.0	-	-	-
	22°C.	50.0	-	-	50.0
	12-14°C.	97.1	-	-	2.9
C.3	-	100.0	-	-	-
	22°C.	3.2	-	-	96.8
	8-10°C.	91.9	-	-	8.1
T.1	-	100.0	-	-	-
	*22°C.	97.1	2.9	-	-
	10-11°C.	100.0	-	-	-
T.2	-	90.6	9.4	-	-
	22°C.	100.0	-	-	-
	12-13°C.	100.0	-	-	-
T.3	-	97.1	-	2.9	-
	22°C	6.7	-	-	93.3
	12-14°C.	47.8	-	-	52.5

Ø Cl.C. = Pasteurised Clean Certified milk; C. = Pasteurised Certified milk; T. = Pasteurised Tank milk.

*This sample was held 6 hours only.

TABLE XI - The changes in the predominant microflora and the bacterial groups according to the action on litmus milk in pasteurised milk sample I held at 22°C.

	Freshly Pasteurised Milk	Holding period in days		
		1	2	7
Plate count per ml.	34,000	3,200,000	25,000,000	420,000,000
Per cent Streptococci	-	-	-	29.4
" Micrococci	-	-	-	26.5
" Corynebacteria	100.0	3.2	4.2	26.5
" Alcaligenes	-	96.8	95.8	17.6
" Spore-formers	-	-	-	-
Per cent Acid-forming	100.0	3.2	4.2	55.9
" Acid-peptonizing	-	-	-	-
" Peptonizing	-	-	-	-
" Alkali-forming	-	96.8	95.8	17.6
" Inert	-	-	-	26.5

TABLE XII - The changes in the predominant microflora and the bacterial groups according to the action on litmus milk in pasteurised milk sample I held at 10-11°C.

	Freshly Pasteurised Milk	Holding period in days				
		1	2	7	15	21
Plate count per ml.	34,000	41,000	58,000	950,000	115,000,000	350,000,00
Per cent Streptococci	-	-	-	-	38.9	33.3
" Micrococci	-	-	3.4	4.2	2.8	-
" Corynebacteria	100.0	91.9	72.5	4.2	2.8	-
" Alcaligenes	-	8.1	24.1	91.6	55.5	44.5
" Spore-formers	-	-	-	-	-	22.2
Per cent Acid-forming	100.0	91.9	72.5	4.2	41.7	33.3
" Acid-peptonizing	-	-	-	-	-	-
" Peptonizing	-	-	-	-	-	22.2
" Alkali-forming	-	8.1	24.1	91.6	55.5	44.5
" Inert	-	-	3.4	4.2	2.8	-

TABLE XIII - The changes in the predominant microflora and the bacterial groups according to the action on litmus milk in pasteurised milk sample II held at 22°C.

	Freshly Pasteurised Milk	Holding period in days		
		1	2	3
Plate count per ml.	6900	360,000	102,000,000	440,000,000
Per cent Streptococci	2.9	-	2.8	30.6
" Micrococci	5.9	3.3	-	2.8
" Corynebacteria	91.2	3.3	-	5.6
" Alcaligenes	-	93.3	97.2	61.1
" Spore-formers	-	-	-	-
Per cent Acid-forming	97.1	6.7	2.8	36.2
" Acid peptonizing	-	-	-	-
" Peptonizing	2.9	-	-	-
" Alkali-forming	-	93.3	97.2	63.8
" Inert	-	-	-	-

TABLE XIV - The changes in the predominant microflora and the bacterial groups according to the action on litmus milk in pasteurised milk sample II held at 12-14°C.

	Freshly Pasteurised Milk	Holding period in days			
		1	2	3	15
Plate count per ml.	6900	11,700	665,000	5,750,000	95,500,000
Per cent Streptococci	2.9	4.3	-	-	48.7
" Micrococci	5.9	-	-	-	7.7
" Corynebacteria	91.2	43.5	-	10.0	17.9
" Alcaligenes	-	52.2	96.9	33.3	25.6
" Spore-formers	-	-	3.1	56.7	-
Per cent Acid-forming	97.1	47.8	-	10.0	74.4
" Acid-peptonizing	-	-	-	-	-
" Peptonizing	2.9	-	3.1	56.7	-
" Alkali-forming	-	52.2	96.9	33 3	25.6
" Inert	-	-	-	-	-

TABLE XV - The changes in the predominant microflora and the bacterial groups according to the action on litmus milk in pasteurised milk sample III held at 22°C.

	Freshly Pasteurised Milk	Holding period in days					
		1	2	3	4	7	9
Plate count per ml.	78,000	1,210,000	42,000,000	126,000,000	125,000,000	250,000,000	390,000,000
Percent Streptococci	80.5	90.4	82.9	100.0	79.6	31.0	63.1
" Micrococci	5.6	4.8	-	-	-	-	5.3
" Corynebacteria	13.9	-	-	-	-	1.0	-
" Alcaligenes	-	4.8	17.1	-	20.4	69.0	31.6
" Spore-formers	-	-	-	-	-	-	-
Percent Acid-forming	100.0	95.2	82.9	100.0	79.6	31.0	63.1
" Acid-peptonizing	-	-	-	-	-	-	-
" Peptonizing	-	-	-	-	-	-	-
" Alkali-forming	-	4.8	17.1	-	20.4	69.0	31.6
" Inert	-	-	-	-	-	-	5.3

TABLE XVI - The changes in the predominant microflora and the bacterial groups according to the action on litmus milk in pasteurised milk sample III held at 9-11°C.

	Freshly Pasteurised Milk	Holding period in days					
		2	4	7	9	15	20
Plate count per ml.	78,000	72,000	80,000	spreaders 10^5	spreaders 10^6	60,000,000	810,000,000
Per cent Streptococci	80.5	85.7	60.0	-	-	16.7	97.1
" Micrococci	5.6	-	-	-	-	-	-
" Corynebacteria	13.9	-	20.0	-	-	10.0	-
" Alcaligenes	-	14.3	20.0	-	-	73.3	2.9
" Spore-formers	-	-	-	100.0	100.0	-	-
Per cent Acid-forming	100.0	85.7	80.0	-	-	26.7	97.1
" Acid-peptonizing	-	-	-	-	-	-	-
" Peptonizing	-	-	-	100.0	100.0	-	-
" Alkali-forming	-	14.3	20.0	-	-	73.3	2.9
" Inert	-	-	-	-	-	-	-

TABLE XVII - The changes in the predominant microflora and the bacterial groups according to the action on litmus milk in pasteurised milk sample IV held at 22°C.

	Freshly Pasteurised Milk	Holding period in days					
		1	2	3	4	7	9
Plate count per ml.	67,000	1,070,000	10,000,000	69,000,000	131,000,000	30,000,000	59,000,000
Per cent Streptococci	69.4	90.0	97.1	65.6	88.6	80.0	50.0
" Micrococci	-	-	-	3.1	-	-	36.7
" Corynebacteria	30.6	10.0	2.9	21.9	5.7	20.0	13.3
" Alcaligenes	-	-	-	9.4	5.7	-	-
" Spore-formers	-	-	-	-	-	-	-
Per cent Acid-forming	100.0	100.0	100.0	90.6	94.3	100.0	100.0
" Alkali-forming	-	-	-	9.4	5.7	-	-

TABLE XVIII - The changes in the predominant microflora and the bacterial groups according to the action on litmus milk in pasteurised milk sample IV held at 9-11°C.

	Freshly Pasteurised Milk	Holding period in days					
		2	4	7	9	15	20
Plate count per ml.	67,000	68,000	70,000	920,000	25,300,000	174,000,000	139,000,000
Per cent Streptococci	69.4	40.0	87.5	86.7	100.0	83.8	100.0
" Micrococci	-	10.0	-	-	-	-	-
" Corynebacteria	30.6	50.0	8.3	13.3	-	9.7	-
" Alcaligenes	-	-	4.2	-	-	6.5	-
" Spore-formers	-	-	-	-	-	-	-
Per cent Acid-forming	100.0	100.0	95.8	100.0	100.0	93.8	100.0
" Alkali-forming	-	-	4.2	-	-	6.2	-