BACTERIOLOGY OF CANNED MILK PRODUCTS

A Thesis

submitted to the University of Glasgow
in accordance with the Regulations for
the Degree of Doctor of Philosophy in
the Faculty of Science

by

AGNES ALLISON NICHOLS, B.Sc.(Agric.), M.S., N.D.D.

The Hannah Dairy Research Institute,
Kirkhill, Ayr, Scotland.

22nd December, 1938.

ProQuest LLC.
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106 – 1346

BACTERIOLOGY OF CANNED MILK PRODUCTS.

Contents.

BACTERIOLOGY OF CANNED MILK PRODUCTS.

Part I. Introduction.

I. Concentrated Milk Products.

Methods of manufacture and salient points of control of micro-organisms causing deterioration on storage.

1. Sweetened condensed milk.
2. Evaporated milk.
3. Sterilised cream.

II. Dried Milk Products.

Methods of manufacture and salient points in the control of micro-organisms present in the powder.

1. Roller dried.
2. Spray dried.

THE BACTERIOLOGY OF CANNED MILK PRODUCTS.

PART I. INTRODUCTION.

As the world's dairy industry has developed some countries have produced milk in excess of their own fluid requirements or in certain districts there has been a surplus over that required as market milk. This surplus may have existed over the whole year sometimes, or at certain seasons only. The storage of surplus milk for future consumption and its transportation to consuming districts has been facilitated by removing water by either condensing or drying and packing the resulting products in cans, in which they are expected to keep in a palatable condition almost indefinitely.

Milk contains numerous food factors required for growth, and so is an excellent medium for the cultivation of many species of bacteria, yeasts and moulds. Many micro-organisms associated with the contamination derived from the cow, cow-shed and dairy during the production and handling of milk, provided that other conditions of development are favourable, cause some kind of fermentation and render milk unfit for use. Hence the methods used to preserve these concentrated forms of the rapidly deteriorative food, milk, depend chiefly upon the addition of sugar and/or heat treatment in the case of the concentrated milks or desiccation alone for the dried milk products.

I. CONCENTRATED MILK PRODUCTS.

Gail Borden in 1856 first successfully manufactured concentrated milk. Since then the industry has steadily increased, and products can now be divided into three main groups, namely:-

1. Sweetened condensed or condensed milk.
2. Unsweetened condensed, evaporated milk, or concentrated milk.
3. Sterilised or canned fresh cream.

Sweetened and unsweetened condensed milks are made from both full cream or skimmed milk, and minimum legal standards for fat and total-solids content are laid down(1) in this country. The quality of these products and especially their keeping properties largely depends upon the micro-organisms which have survived the manufacturing process. The following account of the methods employed in manufacture is given, since the processing conditions have a direct bearing on the bacteriological problems.

1. Sweetened condensed milk manufacturing process.

In this process 2 to 2¾ lbs. fresh milk yield 1 lb. of condensed. As a preservative agent sugar of a high grade, usually sucrose, is added in about 15 to 16 lb. amounts to 100 lbs. of fluid milk. The aim is to have sufficient concentration of sucrose in the final product to inhibit growth of organisms surviving in the milk when the manufacturing process is completed.

Before processing the milk is standardised and is fore-

warmed. The numbers of organisms present when bulk milk is delivered at the condensery may be considerable, especially in hot weather, and one of the primary objects of forewarming is to reduce the number of these bacteria. The temperature used varies from 130°F. to above the boiling point. The higher the temperature employed the more thorough will be the destruction of the bacterial flora, but the forewarming temperature also affects the physical stability of the final milk and consideration has to be given to this in fixing the temperature. A temperature of about 170°F. is preferred in this country. Sugar is added to the milk after forewarming and the sweet milk is drawn into a vacuum pan, operating from about 130 to 145°F; the lower temperatures usually give the best results, but this temperature is suitable for the growth of certain thermophilic organisms which may lead to difficulties. Water is evaporated until the desired concentration is reached, after which the milk is cooled and filled into barrels or cans.

This product is not therefore free from micro-organisms, although many of the original bacteria of the milk have been destroyed by the heat treatment. A 40 per cent. concentration of sugar and a sugar-in-water ratio of 62.5 per cent. have been shown commercially to inhibit the bacteria usually present. This does not prevent the growth of all yeasts and moulds, and care must be taken to prevent re-contamination after the milk leaves the pan. Cooling tanks, filling machines and cans must be sterilised, so that they do not add deteriorative micro-

organisms to the milk and shorten the keeping quality.

The salient points in the control of micro-organisms causing deterioration on storage are therefore (a) the forewarming treatment, (b) the added sugar, and (c) re-contamination after the milk leaves the vacuum pan. Of these the last two points are of major importance.

2. Evaporated milk manufacturing process.

This process aims at the destruction of the milk flora by the heat of steam under pressure, although the methods adopted in removing water are similar to those employed in sweetened condensed milk. The bulked standardised milk is forewarmed, usually to a temperature of between 190° and 200°F., since the heat stability mainly depends on the temperature of forewarming. The milk is then condensed and withdrawn from the pan at the required concentration. It is homogenised to prevent fat separation, cooled and filled into tin containers which are sealed. The sterilisation process follows. When a continuous steriliser is used sterilisation starts immediately after filling and there is no time for bacteria to increase. If a batch steriliser is in use, which is similar in operation to an autoclave, it is loaded, heated up to a definite temperature and held for a fixed time, cooled and unloaded. There may be delays between filling the cans and loading the retort and, if so, it will be necessary to cold-store the cans. Uniformity of heating of all cans in the batch steriliser is important, and

rotation of the cans in the retort partly filled with water facilitates this. The manufacturer adjusts the combination of time and temperature of exposure to heat so that any bacteria capable of producing spoilage of the milk are likely to be destroyed; thus the product is virtually sterilised and should keep indefinitely. In practice, however, batches are encountered in which organisms appear to have survived the processing. To overcome this the heat treatment could be still further increased, but if the temperature is raised too high the consistency, flavour and colour may be seriously impaired as a result of chemical changes in the milk constituents. The temperature and time balance is therefore important. With a rotary steam steriliser a temperature of between 115° and 118°C. is maintained for 15 to 30 minutes and about the same time is necessary to heat up the steriliser and cool it down again. While the temperature recorded in the retort may appear to be sufficient to ensure destruction of any bacteria there is found in practice to be a marked lag in the rate of heating within the cans. Loveless[2], using a thermocouple inserted in a can of cream inside an autoclave similar in construction to a steriliser, showed that for cream the temperature indicated by the steriliser thermometer was never reached and that there was a difference of 2° to 3°C. on a 20 minutes holding time. This lag in heating is more marked with cream than milk and is greater in large than in small cans. The cooling process proceeds as rapidly as possible, cold water being used for the

purpose. If the water supply in the steriliser is unevenly distributed or insufficient, the cooling of some cans may be retarded and a considerable difference in pressure between the interior and exterior may occur, and this may cause some cans to bulge and, especially if there is a defective seam, may lead to leaking cans. Bacteria may therefore gain entrance into the milk product through such defective seams, and as growth occurs the can contents are spoiled. In practice cans are incubated for at least 10 days after manufacture at 80° to 97°F., so that defective cans will usually be revealed before release on the market.

The salient points in the control of micro-organisms causing deterioration on storage are (a) the bacteriological quality of the incoming milk, especially with regard to incidence of sporeformers, (b) the operation of the steriliser, i.e. temperature and time of exposure to heat, and (c) defective seams and/or faulty cooling leading to leaking cans. Of these the first two points are the most important.

3. Sterilised cream manufacturing process.

The object of the manufacture of sweet canned cream is also to destroy bacteria by the heat of steam under pressure and yet to preserve the stability of the cream so that the chemical and physico-chemical changes due to heat, such as heat coagulation or lumpiness, browning, fat separation and cooked flavour, are prevented.

Sweet cream is separated and standardised usually to

23 per cent. fat content. Only cream of low titratable acidity made from fresh sweet milk is used. Bicarbonate of soda, which acts partly as a neutralising agent and also improves the stability to heat, is added up to about 1 lb. per 100 gallons of cream. The cream is preheated to 110°F. and homogenised. After prompt cooling the cream is filled into cans, bottles or glass jars, which are sealed and sterilised. The sterilisation temperature found most satisfactory is 118°C. and is maintained for 25 minutes, 15 to 20 minutes being taken to heat the steriliser to 118°C. and again to cool it down. Above 118°C. it is difficult to maintain heat stability, but lower temperatures have been used successfully.

The control of bacteria causing deterioration on storage depends upon the same factors as have already been noted for evaporated milk.

II. DRIED MILK PRODUCTS.

Another method of preserving milk in a more or less non-perishable form so that it can be reconstituted later is by desiccating to dryness. Spoilage by micro-organisms is then reduced to negligible proportions, since the moisture content of the powders, which is seldom above 5 per cent., will not support growth. Whole milk, skim milk, butter milk and whey are all preserved by desiccation, but the commonest dried milk product is made from skim milk, for which there is a large and growing demand from the baking and ice cream industries.

There are two main types of dried milk products in commercial use, namely roller dried and spray dried.

1. Roller drying process for milk products.

The manufacture of roller dried milk entails the evaporation of water from a thin film of milk on hot revolving metal rollers and the scraping off of the dried milk as the roller rotates. The temperature of the rollers is, however, relatively high, and it has been calculated that the milk in drying attains a temperature of 270°F. Although the period of heating on the roller is relatively short, it is sufficient to kill the great majority of the micro-organisms which are found in milk, so that unless recontamination occurs after drying, the number of bacteria in the product is very low. For this reason roller dried powders were excluded from the present study.

2. Spray dried process for milk products.

The principle employed in the production of spray dried milk is simple and consists of reducing the milk to a fine mist in the presence of dry heated air. Evaporation of moisture from the milk mist is very rapid since the small particles present a large surface and the moisture is removed almost instantaneously. In practice the application of this principle has not been found to be so simple, and consequently there is a variety of spray driers. Generally the raw milk is heated to about 140° to 145°F. or sometimes to a lower temperature and the bulk of the water removed in a vacuum pan. The concentrated

milk is homogenised and passed under pressure to an atomiser from which the milk is sprayed into a drying chamber, where it meets hot dry air. The air has previously been filtered and heated to 220° to 300°F. The best operating temperature has been found to be 270°F. The dried milk drops to the bottom of the chamber from which it is usually removed mechanically. Some finer particles may be carried over in the air outlet stream, and are recovered in a dust collector. Spray milk powder plants usually operate continuously, although a few factories manufacture on the batch principle. The powder after sieving is packed in ½ cwt. or smaller airtight tins, but the commonest bulk package is a barrel.

If processing has been efficiently carried out the moisture content will be low, and provided that moisture is not allowed to penetrate during storage, no deterioration or spoilage due to the activity of micro-organisms should occur. If the powder is to be reconstituted before use, as for instance in the preparation of baby foods, ice cream, milk drinks, and starters, the type and kind of bacteria which are present is, however, important.

The control of bacteria present in the powders depends upon (a) the bacteriological quality of the incoming milk, with special reference to the bacteria which are resistant to heat, (b) the extent and management of heat treatment given in the plant, and (c) recontamination after drying. The first two of these points are of major importance.

PART II. CONCENTRATED MILK PRODUCTS.

I. Evaporated Milk and Canned Cream.

General investigation.

Experimental technique.

(i) Can opening.
(ii) Culturing.
(iii) Can contents.
(iv) Recovery of bacteria from unsterile cans and their identification.

Discussion and results.

1. Sterility of commercial samples.
   (a) Evaporated milk.
   (b) Canned cream.
2. Types of defective samples encountered and associated bacteriological flora.
   A. Spoilage due to non-sporeformers.
      (i) Gassy fermentation.
      (ii) Coagulation.
      (iii) Other defects.
   B. Spoilage due to sporeformers.
      Anaerobic sporeformers.
      Aerobic or facultative aerobic sporeformers.
      (a) Classification.
      (b) Induced spoilage.
      (c) Note on the relationship of aerobic sporeformers to curdling.
         (i) 'Sweet' curdling.
         (ii) Acid curdling.

II. Sweetened Condensed Milk.

Technique of examination.

Discussion.

1. Incidence of defective cans.
2. Types of defective samples.
   (i) Gassy fermentation.
   (ii) Progressive thickening.
   (iii) Buttons.

PART II. CONCENTRATED MILK PRODUCTS.

In the present investigation on concentrated milk products the greater part of the work was concerned with evaporated milk and canned cream. These two products have been considered together because, since their methods of manufacture are similar, it might be expected that bacteriological defects which have been reported in the past in evaporated milk might be encountered also in canned cream. In addition it was felt that a study of canned cream might be of special value because it is a relatively recent development of the canning industry and information regarding the bacteriological side is scanty.

I. Evaporated Milk and Canned Cream.

Previous investigations (see reviews 4 and 5), nearly all of which are confined to the products of one particular factory usually in connexion with a specific outbreak, indicate that although some workers invariably found evaporated milk to be sterile, others have occasionally found living organisms in the cans even when the contents have appeared to be sound. A few workers have also tested a general selection of samples with similar conflicting results. Thus while Weinzirl[4] investigated 23 market samples of evaporated milk, none of which revealed the presence of any living organisms, Park, Schroeder and Bartholow[4], examining a general collection of 37 tins from 19 brands found that they all contained living bacteria. Savage

and Hunwicke[4] tested 27 shop samples consisting of different brands together with 17 tins obtained from three different factories, 18 per cent. of which were unsterile.

General Investigation.

A general survey of a large number of samples of evaporated milk and canned cream produced by three condensed milk factories, designated X, Y and Z, operating in the West of Scotland, was undertaken to determine to what extent non-sterility occurred in commercial practice and what organisms were present after processing. Information was also desired as to the relative importance of different defects and their associated organisms. Samples were obtained at about weekly intervals for nearly two years, from 2 to 8 samples being examined from each individual batch.

A further number of tins were tested over a period of four years from other sources of manufacture. These samples were purchased from shops or obtained directly from other factories, making a total of 315 evaporated milk samples and 253 samples of canned cream. In addition to the above samples which were bought as sound, many samples have been examined which were either obviously defective from the apparent bulging of the cans themselves or from the "shake sound", or were sent in by firms as tins from a similar batch to that in which defective cans had occurred. About 150 of these special evaporated milk samples and 115 special samples of canned cream have been tested.

Experimental Technique.

(i) Can opening.

Samples from the factories were first incubated at 37°C. for varying periods of time; usually they had at least six days' but not more than 21 days' incubation. The remaining samples were also incubated before testing unless they were obviously faulty or old when they were opened at once. Several methods of sampling the contents were tried, since the opening of cans and the removal of part of the contents without aerial contamination presented obvious difficulties, especially if the can was extended by gas. Finally the following technique was adopted. The top of the can was washed thoroughly and in addition formalin was rubbed over it. Then about 5 ml. of absolute alcohol were ignited on the surface and a Bunsen burner held so that the flame played on the can top. A hole any size up to ½ inch in diameter was pierced by a cold chisel, which had previously been heat sterilised, and the tin was immediately covered by a sterile petri dish lid.

(ii) Culturing.

The culturing technique which was found to be most suitable was the heavy inoculation of the product into litmus milk, nutrient broth and dextrose broth. The inoculated media were then incubated at 37°C. for at least one week. The nutrient broth was prepared from .5% peptone, .3% marmite (or occasionally lemco), and tap water, but glucose broth had in addition 2%

glucose which was added before the final sterilisation. The final pH of the broths was about 6.8.

The inoculum was removed from the tin by a 1 ml. pipette, the tip of which had been widened to allow for the viscous nature of the product. At least 1 ml. was used, since it was found that if smaller inocula were employed the numbers of organisms present in the can were sometimes so small that they were not recovered. If the product was obviously spoiled but the presence of bacteria had not been demonstrated by the above technique, the inoculated media were further incubated at 55°C., in order to test for the presence of thermophilic organisms. Cooked meat media and dextrose agar shakes were inoculated from some of the samples to determine whether anaerobes were present, and occasionally inoculations were made into these media from the media already incubated at 37°C. if no other organisms had been recovered. At the beginning of the investigation agar plates were streaked with the product directly, and in addition plate counts were made to give an indication of the number of bacteria present. Plating was found, however, to be of little value and is not recommended. Esty and Stevenson[6], in similar examinations of canned foods, also abandoned direct plating as being of doubtful value. Films for microscopic examinations were made directly from the product and have given useful indications of the flora, but when the numbers are low, as in old samples, the absence of organisms on microscopic examination may be misleading.

(iii) Can contents.

The colour, odour, consistency and general condition of defective samples of evaporated milk and canned cream were noted at the time of testing so that it might be possible to determine whether specific defects were associated with certain types of bacteria.

(iv) Recovery of bacteria from unsterile cans and their identification.

When possible the organisms in the unsterile samples were purified by dilution and plate culture, and were further examined for morphological, cultural and biochemical characteristics. A list of tests used for the differentiation of the isolated bacteria is given below.

Type of cell and colony.
Motility.
Staining properties - Gram and with common dyes.
Spore formation.
Growth on agar slant,
in agar stab,
glucose agar stab,
gelatine stab at 22°C.
Growth in litmus milk,
broth,
glucose broth,
MacConkey's bile salt broth.
Final pH in glucose broth.
Carbohydrate fermentations - levulose, glucose, lactose, maltose, raffinose, salicin, mannite, starch.
Nitrate reduction 1% KNO_3.
Indol production - Ehrlich's method.
Voges-Proskauser test. Barritt's modification.
Reduction of methylene blue .1% in milk.
Splitting of sodium hippurate.
Splitting of aesculin.

As the media cultured did not differ appreciably from the recipes in general use no details have been stated. All the organisms were not examined by every test; a decision as to which tests were employed depended on whether the cultures were heat resistant. The incubation temperature was usually 37°C.

Results and Discussion.

1. Sterility of commercial samples.

(a) Evaporated milk.

100 per cent. of the tins which had been obtained from general sources were found to be sterile. It is probable that the lower value of the factory samples, namely 94 and 93 per cent. (Table 1), would be associated with some special circumstance of operation. With the samples from Factory X definite evidence of this was available as more than half of the unsterile samples from this factory were produced during a 10 day period, when it seemed likely that the processing was not being carried out as efficiently as usual.

Tests on the special samples which were examined because they were either obviously defective or associated with defective packs showed, as would be expected, a much higher percentage to be unsterile.

(b) Canned cream.

100 per cent. of the samples tested from general sources were again found to be sterile, but the percentage of sterile factory samples was markedly lower, being only 85 and 76 per cent. In seeking an

TABLE 1.

Sterility of commercial samples.

Source	Product tested	No. of samples examined	No. of batches from which samples obtained	No. of sterile samples	% of sterile samples	No. of samples not sterile	No. of batches from which unsterile samples obtained	% of unsterile samples
Factory X	Evaporated Milk	235	125	222	94	13	10	6
" Y		44	25	41	93	3	2	7
" Z		1	1	1	100	0		0
General sources shops, etc.		35	30	35	100	0		0
Total		315	182	299	95	16	12	5
Special samples faulty ?		152	52	93	61	59	22	39
Factory X	Canned Cream	52	22	44	85	8	4	15
" Y		6	3	6	100	0		0
" Z		178	82	136	76	42	24	24
General sources shops, etc.		17	17	17	100	0		0
Total		253	124	203	80	50		20
Special samples faulty ?		115	49	80	70	35	14	30

explanation of this it was found that the percentage of unsterile factory samples was considerably higher with 12 oz. cans than with smaller packs. The smaller containers examined consisted of 3 and 6 oz. can samples, together with a few small glass jars. It might be anticipated that it would be more difficult to sterilise the large cans, since there would be a lag in attaining the necessary temperature compared with the smaller containers. The difficulty in adjusting the process to compensate for this lag would be particularly marked where the factory had been unaccustomed to processing 12 oz. cans previously, and inquiry revealed that the majority of these samples were examined when the factories were processing the large cans for the first time. The figures given in Table 2 show clearly that the 12 oz. cans were chiefly responsible for the high non-sterility figures. Twenty per cent. of the total cans of cream examined were unsterile, but the corresponding percentage for 12 oz. cans was 77 per cent. while for the smaller packs it was only 8 per cent. It will be seen that unsterile large tins accounted for 65 per cent. of the total non-sterile canned cream figures.

2. Types of defective samples encountered and associated bacteriological flora.

Table 3 summarises the types of defective samples which were investigated and the associated bacteria isolated from them. It should be noted that in the classification into different defects the line of demarkation was not always so clear as would

TABLE 2.

Source	No. of samples examined		No. of sterile samples		% of sterile samples			No. of unsterile samples		% of unsterile samples			% of total non-sterile samples accounted for by 12 oz. cans
	Small containers	12 oz. cans	Small containers	12 oz. cans	Small containers	12 oz. cans	Total of all containers	Small containers	12 oz. cans	Small containers	12 oz. cans	Total of all containers	
Factory X	43	9	40	4	93	44	85	3	5	7	56	15	10
" Y	6	0	6	0	100	0	100	0	0	0	0	0	0
" Z	143	35	130	6	91	17	76	13	29	9	83	24	16
General sources shops etc.	17	0	17	0	100	0	100	0	0	0	0	0	0
Total	209	44	193	10	92	23	80	16	34	8	77	20	13

TABLE 3.

Defective samples and associated micro-organisms.

Source	No. of unsterile samples	Type of Defect						Product free from defect			No details of defect or organisms retained from these unsterile samples
		Bloats usually with coagulation	Coagulation	Coagulation lumpy	Soft coagulation some also putrid flavour	Putrefactive odour	Putrid flavour bitter lumpy, or thin thick	Bloat	Coagulation			
		Mixed flora	Non-spore-formers	Mixed flora spore-formers and non-spore-formers	Spore-formers	Spore-former	Spore-formers	Defect appearing to be bacteriological but no organisms isolated	Spore-formers present	Anaerobic spore-formers present	Mixed flora non-spore-forming present	
EVAPORATED MILK.												
Total samples from factories and general sources	16	3			7			2	1		1	2
Special	59	30	12	1	3	1	1	7			4	
CANNED CREAM.												
Total samples from factories and general sources	50	2	1		6		1		35	2		3
Special	35	4	1		3		2	22	1	1	1	

appear from the table, although it is probably true that the type of defect might have developed further on prolonged storage.

By using the resistance to heat of the organisms which were recovered as a broad basis of classification the defects have been divided into two groups, under which they will be considered. The two groups are: (A) a widely mixed general flora containing varieties of organisms which do not withstand high temperature, and (B) a restricted flora which are heat resistant and of the sporeforming type.

The reasons for differentiating the organisms into these two groups may be briefly stated as follows: Non-heat resistant bacteria will almost invariably gain access to the can during the cooling process when, through bad seaming or faulty sealing, leakages of air and water into the can may occur. Such contamination may therefore be looked upon as accidental. Heat resistant bacteria will, however, usually be present as a result of inadequate sterilisation. There will of course be exceptions to these two generalisations, e.g. gross understerilisation may result in the survival of non-heat resistant types, while sporeformers might be among the organisms which enter accidentally through mechanical defects in the cans.

A. Spoilage due to non-sporeformers.

In order to divide the organisms isolated from defective samples into one of the two main groups a study was made of their heat resistance. Small tubes of sterile skim milk were inoculated with the cultures and then heated for 20 minutes at

different temperatures in a thermostatically controlled glycerine bath. It was found that nearly all the non-sporeformers were killed at from 60 to 65°C., except for a few cocci which required a temperature of 69 to 70°C. Thus it is clear that, if they did not gain entrance to the cans after processing, the latter must have been grossly understerilised.

It is doubtful whether any importance can be attached to the actual type of non-heat-resistant organisms present in defective cans, since, if contamination is due to mechanical defects, the flora entering the can is likely to be mixed. A diversity of type of defect and of causative bacteria is therefore inevitable. Moreover, even if defects associated with non-heat-resistant organisms were due to serious understerilisation, a fairly large variety of types, probably chiefly heat-duric streptococci, would survive. It is probable that in an excellent medium like milk most of the contaminating organisms would find conditions suitable. It may be noted that when faulty containers are the cause of contamination spoilage usually occurs in casual cans.

Such spoilage may be classed under two general heads as follows:-

(1) Gassy fermentation. When this occurs the "blown" cans (or "bloats" or "swell heads" as they are usually termed) become bulged at the ends due to the evolution of gas which is usually the result of bacterial metabolism. In extreme cases, such fermentation may be accompanied by bursting of the seams.

Savage and Hunwicke[4] found that B. coli., B. cloacae

strains, a yeast, a streptococcus, a diplococcus, and some pleomorphic coccoidal organisms were responsible for "bloats" in evaporated milk, and that in most samples the milk was also clotted. Hammer[7] reported a case of fermentation due to a gas-producing streptococcus, in which the milk failed to clot, although the flavour was abnormal.

Nearly half the defective evaporated milk samples examined in the present survey (Table 3) were "bloats" and gassy fermentation was the commonest single cause of defective unsweetened canned milk. As regards the condition of the contents of the affected milk, the extent to which frothing occurred after opening varied, and the consistency of the contents also varied, although nearly all the samples were clotted. The samples often had, in addition, an unpleasant sour "cheesy" smell and a bad flavour. When the product was clotted it could be detected by the 'thumping sound and feel' when the tins were shaken. Canned cream "bloats" were similar in character to those described for evaporated milk, but were less numerous.

A variety of different bacteria was isolated from the "bloats", which often had a mixed flora. Cocci were the commonest types of organism isolated and many strains were recovered. Although almost all the cocci quickly produced acidity in sugar media and curdled milk they were not able to produce gas and therefore were not responsible for the "swells", although they obviously contributed to the curdling.

Of the organisms isolated those capable of pro-

ducing gas when reinoculated into canned products were entirely of the short rod, gram negative, Escherichia genus and related bacteria. This group of organisms ferments many carbohydrates with the production of acid and gas. It rapidly curdles milk and forms a gassy curd with unpleasant odour. Sixteen cultures were examined in greater detail and were finally classified by their reactions as:-

Strains of Escherichia coli communis	12
" " Aerobacter lactis aerogenes	1
" " Aerobacter cloacae	3

One species of yeast was also isolated, but since it did not ferment lactose it was obviously not responsible for the "swell".

(ii) Coagulation. Micro-organisms isolated from cans of which the contents were coagulated and sour, but in which gas production was absent, were almost entirely of the cocci type and were similar to those mentioned as isolated from "bloats".

It may be noted that several cultures of cocci have been recovered from products which did not exhibit any defect. These bacteria did not alter litmus milk or evaporated milk on prolonged incubation although they grew on agar media. They probably gained entrance by the same channels as the other non-heat-resistant non-sporeformers.

(iii) Other defects. Two further types of defect, namely bitterness[8] and fishiness[9], have been attributed in the past

to the non-sporeformers. Neither bitterness due to non-sporeformers nor fishiness was encountered in the present investigation.

In eleven defective samples, six of which were at least 3 months old, it was not found possible to recover viable organisms although the contents were either "bloated" or coagulated. In this connexion it may be noted that it was very difficult to keep these organisms alive for any length of time in the laboratory unless special precautions were taken. It therefore seemed probable that in these eleven defective samples the causative organisms had died out during storage.

B. Spoilage due to sporeformers.

Before dealing in detail with spoilage caused by sporeformers it should be observed that although such spoilage is usually more widespread than occurs with the non-heat-resistant types, the contents of many cans from an affected batch may be found to be sterile. Thus conclusions as to the sterility of any batch which are based on the examination of a single can may be misleading. Esty and Stevenson[6] have stressed this point and have suggested that a minimum of 24 cans should be used as a basis for diagnosis. As already noted, in this investigation up to 8 cans from each batch were examined.

Anaerobic sporeformers.

It has been reported by Savage and Hunwicke[4] that sporing anaerobes were responsible for unsoundness in 3 out of 47 samples, and that anaerobic sporeformers could be present in

sound samples also. Hunziker[10] reported epidemics of gassy fermentation which involved a number of cans which he found to be due to Plectridium foetidum. This obligate anaerobe had a thermal death-point between 245 and 250°F., and nearly always produced a foul odour and intense putrefaction.

In the present study over eighty sporeformers have been isolated but only two were anaerobic, and these were obtained from sound cans. The number of anaerobic sporeformers which normally survive processing and which cause defects in the products cannot therefore be of great importance.

Aerobic or facultative aerobic sporeformers.

In nearly all instances where aerobic or facultative aerobic sporeformers have been isolated only one species has been obtained from each batch of product where spoilage has been evident. The condition of the milk or cream in such cans has varied from a smooth, soft clot with or without lumpiness to a very thin consistency, the smell and flavour being putrid, bitter, or slightly indicative of protein breakdown. All combinations of consistency and off-flavour have been found, in spite of the fact that in the samples examined the bacteria isolated were all closely related.

(a) Classification of aerobic sporeforming organisms isolated from canned cream and evaporated milk.

Fifty-two cultures of aerobic or facultative aerobic sporeformers have been examined in detail. Included among these are some cultures which were isolated from products which were not noticeably defective when examined, although the organisms resembled very closely those isolated from faulty cans. For

instance, 35 cream samples appeared to be sound yet this type of sporeformer was isolated from them. These cans had, however, only been subjected to about a week's incubation at 37°C. It will be shown later that this incubation period may be insufficient to allow the full development of spoilage.

In the past there has been some confusion in the classification of this group. B. subtilis has been regarded as the type species, but different workers have employed various strains of this organism as a basis of classification. In 1936, however, the Second International Microbiological Conference decided that Gottheil's strain of B. subtilis (or the so-called Marburg strain) should be recognised as the type culture for the aerobic sporeformers. Since the type culture has been fixed the confusion has been reduced and the classification adopted here has been that suggested by Gibson[11,12,13]. The different strains isolated were of the same general type, i.e. they grew well on common media, spored readily and digested gelatine and litmus milk, all, except B. circulans, producing an alkaline reaction in the later stages. The numbers of each strain were found to be as follows:-

	No. of strains	% of total
B. subtilis*	36	69
B. licheniformis*	8	15
B. pumilus*	3	6
B. brevis	2	4
B. circulans	2	4
B. cereus	1	2
Total	52	100

* These cultures have been referred to earlier[14] as B.vulgatus B. subtilis and B. mesentericus. The names given here are now in agreement with the established type species.

The largest number of one species was 69 per cent. of B. subtilis, which together with B. licheniformis and B. pumilus are probably the most heat resistant, and occur commonly in milk, especially if the conditions of the byre are dirty. It may be noted that these organisms were found as spreaders on the $\frac{1}{10}$ and $\frac{1}{100}$ plates in the milk samples produced under dirty conditions which were examined and have been reported in the appendix. Thus bulk milk, unless it has a high standard of cleanliness during production, commonly contains numbers of heat resistant sporeformers, which are a potential source of spoilage in canned dairy products.

(b) Spoilage induced by aerobic sporeformers.

In order to confirm the relationship between aerobic sporeformers and the defects already mentioned, it was obviously desirable to investigate to what extent these bacteria (isolated chiefly from defective products) could reproduce spoilage on inoculation into sound cans. With this object 31 cultures were each inoculated into from 3 to 6 tins of evaporated milk or canned cream and incubated at 37°C. The cultures inoculated consisted of 22 strains of B. subtilis, 4 of B. licheniformis, 2 of B. brevis and of B. circulans and 1 strain of B. cereus. In addition some more strains of B. subtilis have been inoculated into canned cream only.

The following method was adopted for inoculating the cans. A small hole about $\frac{1}{8}$ inch in diameter was made in the top by the same method as was used for sampling the products. A

loopful of culture was introduced into the cream or evaporated milk and the can was re-sealed by soldering a sterilised circular piece of tin plate about 1 inch in diameter over the hole. The cans were opened after periods of incubation of from 1 week to 18 months, and were examined in a similar manner to those opened during the general survey. Control cans were also incubated and examined during the time the work was in progress.

From such artificial re-inoculation it might have been expected that a definite connexion between defective canned dairy products and the aerobic sporeformers, whether or not they were isolated from faulty or sound cans, would have been established. However a clear connexion between the two was not found to exist, as will be seen from the following summary of results.

(1) Only one outstanding defect was induced by the inoculation of the aerobic sporeformers, that of thinning and the associated bitterness of flavour. This defect was found to be of great commercial importance, 63 per cent. of the spoilage in the canned cream examined being of this type. It was therefore made the subject of a special study[15] which is detailed in Part III.

(2) There was, however, a slow development of a slightly proteolytic, putrid, and/or astringent flavour in many of the cans of evaporated milk and cream inoculated with strains of B. subtilis. Some of the other species also gave this slightly putrid and astringent flavour after months of incubation, but

the flavour development was not consistent.

(3) In addition some strains of B. subtilis after many months storage seemed to have thickened evaporated milk and to have produced a soft clot when compared with the condition of the control cans. It appeared that B. circulans had also thickened or formed small clots in evaporated milk. In interpreting this change in consistency allowance must, however, be made for the effect of the high temperature of incubation in causing normal thickening. In addition the heating of the outside of the can both at the time of inoculation and of examination may have been responsible for the observed differences in the consistency of the milk.

(4) All the cans inoculated with the four strains of B. licheniformis were very slightly "blown" after a period of from 2 weeks to 3 months. The consistency was, however, normal, although one or two cans had a slightly proteolytic flavour. It was thought at first that contamination had occurred but further investigation removed all possibility of this. B. licheniformis, although it does not usually produce gas from carbohydrate fermentation, can produce small gas bubbles between the glass and agar in dextrose agar stab cultures, and it is also the most tolerant to lack of oxygen of all the cultures examined. It seems probable therefore that B. licheniformis may produce a slight "swell" in these canned dairy products, especially as the plate counts which are given below were very high after 7 weeks, which indicated that growth had probably taken place in the tins.

Culture *B. licheniformis* Strain	Inoculated into	Plate count per ml. after 7 weeks incubation at 37°C.
No. 91	Canned cream	21,000
	Evaporated milk	43,000
92	Canned cream	approx. 60,000
	Evaporated milk	32,000
94	Evaporated milk	400

It will be seen that these samples with one exception had plate counts of over 20,000 per ml. In comparison plate counts of the bacteria causing bitterness and thinning which will be referred to in Part III were very low. Usually after a period of from 5 to 6 weeks storage at 37°C. the plate counts from such inoculated cans were under 1,000. Plate counts made from any other cans of cream or evaporated milk which had been inoculated with other cultures in order to induce spoilage were also low. (5) The aerobic sporeformers inoculated into cans of cream and evaporated milk survived for long periods even at the high storage temperature of 37°C. During the first 6 months of incubation the bacteria previously inoculated into the cans were recovered from practically every tin examined. After 18 months. however, the recovery of bacteria, even from the large inocula used, was exceptional. It seems probable that if a larger number of transfers from the can contents into media had been made a higher number would have been shown to contain viable bacteria. This point is apparent from the results of an examination made of a can of roasted veal over 100 years old by Wilson and Shipp (16).

They found that out of 64 tubes of media inoculated from the can contents all were sterile except 5 which contained strains of aerobic sporeformers.

x x x x x x

The above results show that the aerobic sporeformers when inoculated into canned milk or cream may be responsible for a variety of defects, varying from very definite faults such as bitterness and thinning to less evident defects such as minor changes in flavour and consistency. The results also show that, while in some cans an organism may not cause spoilage although it apparently survives for long periods after inoculation, when inoculated into other cans the organism may affect the product. It is clear, therefore, that under commercial conditions the manufacture should aim at a sterile pack, and it seems obvious from the survey undertaken that this should be ordinarily quite attainable.

The variable production of spoilage in canned cream and evaporated milk by the inoculation of these aerobic sporeformers and the variation in the extent of defects when they occur (as reported above) probably accounts for the considerable difference of opinion in the past regarding the connexion between aerobic sporeformers and deterioration in canned foods. Cameron and Esty[17] have reviewed this fully in their paper on the spoilage of non-acid canned foods. They examined the effect of

inoculating canned milk with six different strains of aerobic sporeformers and reported that no perceptible difference was noted up to 200 days' storage at room temperature. On the other hand, Savage and Hunwicke[4] claimed that when these bacteria were added to experimental tins in large numbers they might be able to produce enzymes and promote clotting with considerable acid production. However they stated that when present in small numbers and probably only as spores (as in some shop samples or as in lightly inoculated experimental tins) they did not multiply but remained comparatively harmless. These authors concluded that aerobic sporeformers never caused gas production and very rarely unsoundness, but that it was possible in some circumstances (which they did not elucidate) "that the bacteria might be able to multiply and produce sufficient acid to cause a solid clot". Savage and Hunwicke, as well as Cameron and Esty, apparently expected acid development to be brought about by the growth of the aerobic sporeformers which they inoculated into canned milk. However, the aerobic sporeformers examined in the present study (with the single exception of B. circulans) failed to change the reaction of litmus milk during early incubation or occasionally turned it slightly more alkaline, while on aging all the milk cultures showed a definite increase in alkalinity. A clot was either produced before digestion commenced or during digestion, but it was never hard like an acid clot and could be easily broken up on shaking. It appears therefore that faults such as are induced by inoculating strep-

tococci, coliform organisms or anaerobic sporeformers into sound cans are not to be expected from the action of the commonest species of aerobic sporeformers obtained from canned cream and evaporated milk. This conclusion is borne out by the results obtained with inoculated cans as has already been noted.

(c) Note on the relationship of the aerobic sporeformers to curdling in evaporated milk.

Before leaving the general subject of the aerobic sporeformers and spoilage it seems desirable to consider in some detail certain reports regarding the occurrence of curdling due to these organisms. Two different types of curdling will be mentioned.

(i) 'Sweet' curdling or curdiness without increase of acidity. Reports of this defect are of interest because many of the organisms already described were, so far as classification was possible, of similar species to those reported as causing sweet curdling, and therefore they were probably potential 'sweet' curdlers.

Thayer[18] investigated non-acid coagulation of evaporated milk. He reports that B. subtilis, the hay bacillus, figured actively in this spoilage, and that the thermal death point of the spores in milk was between 117 and 122°C. Hammer and Hussong[19] also identified B. cereus as responsible for curdy evaporated milk. They found that cultured evaporated milk in cans curdled slowly at 37°C. without change in odour and flavour unless more air was admitted, when curdling occurred rapidly with the production of a bad odour and flavour. A typical

B. cereus was again isolated by Kelly[20] from evaporated milk. The defective milk had a custard-like clot on top but no other change. In addition, two strains of an organism resembling B. vulgatus which were obtained from two widely separate districts were reported by Morrison and Rettger[21] to cause spoilage in evaporated milk.

(ii) Acid curdling or curdiness with increase of acidity. The curdling of evaporated milk as a result of the high acidity produced by aerobic sporeformers in the cans has also been reported. Hammer[22] isolated a rod shaped bacillus, B. coagulans, from this spoilage, and found that although it grew slowly at 37°C. it developed rapidly at 55°C. and that at 50°C.[23,24] it could clot milk in 3 to 4 days. Hussong and Hammer[25], investigating sour curdled evaporated milk in two separate condenseries, found the causative organism to be B. calidolactis which they had previously isolated from a plant drying skim milk. This sporeformer was of thermophilic character and failed to grow in milk at 37°C., but rapidly coagulated milk at 55°C. in under 20 hours.

In the present study, with the possible exception of two strains of B. circulans which produced an acid reaction with or without curdling in litmus milk, no sporeformers were recovered from the coagulated defective samples which produced any appreciable acidity on reinoculation into milk media. Nevertheless organisms producing acid curdling have been mentioned because sporeformers of this type were obtained in fairly large numbers

from spray dried milk powder plates incubated at 55°C. (see Part IV), and thus it would appear that in the bulk incoming milk at the factory they may be fairly common. The fact that none were, however, isolated even after incubating inoculated media at 55°C., a temperature at which both B. calidolactis and B. coagulans develop rapidly, would indicate that their survival in evaporated milk or canned cream under ordinary conditions in this country is rare and that as a cause of spoilage in these two products they are unimportant.

II. SWEETENED CONDENSED MILK.

The manufacture of sweetened condensed milk does not involve complete sterilisation, and this product invariably contains viable organisms. Under normal conditions the high sugar content, by exerting a high osmotic pressure, tends to plasmolyse the bacterial cells, which are effectively prevented from multiplication. Defects only occur when micro-organisms particularly resistant to the high sugar content survive the processing or gain access to the milk after it leaves the condenser.

A total of about 80 samples of condensed milk have been examined. Some samples were from general sources or from the special factories, but a few were received in connexion with complaints regarding the quality of the product.

Technique of examination.

The opening of cans, culturing, and examination of the can contents and identification of bacteria were carried out as outlined for evaporated milk. In addition the plate count and the presumptive coliform tests[26] were made on several of the samples so that the numbers of organisms present could be ascertained. It was difficult to pipette the milk, but this difficulty was reduced by warming the can in water prior to opening. A warm first dilution blank was also used to aid dilution.

Discussion.

1. Incidence of defective cans.

Of the 36 general samples of full-cream and skim sweetened condensed milk examined only 3 were defective. The number of samples tested was not sufficient to predict what percentage of defective samples might be expected in commercial practice. Two cans were "bloated" and one had a putrid flavour suggestive of bacterial proteolysis and was considerably thinned. Several other samples did not have the freshness of flavour which is usually characteristic of the product and could hardly be considered as first-class, though they could not be definitely classed as 'defective'. Plate counts, Table 4, made on some of these samples varied, the highest recorded (that of the putrid sample) being 267,000 per ml. of condensed milk. In addition this sample gave a positive reaction to the presumptive

TABLE 4.

Sweetened condensed milk samples.

	Plate count per ml.	Presumptive B. coliform test	Remarks regarding flavour and consistency of sample
	3,360	+ve $\frac{1}{10}$ ml.	Lacked freshness of flavour.
	8,400	-ve 1 ml.	do.
	25,600	-ve 1 ml.	Good.
	267,000	+ve $\frac{1}{10}$ ml.	Putrid and thin.
	17,800	-ve 1 ml.	Good.
	50,600	-ve 1 ml.	Good
12 samples about 18 months old when tested	Less than 500	-ve 1 ml.	Several of these cans had "buttons" and were thickened.

coliform test in $\frac{1}{10}$ ml. amounts. As noted by other workers, many of the plate counts were very low, especially after the samples were several months old.

About 40 tins were examined from one factory which had encountered "buttons". The examinations were made after several months storage, i.e. when sufficient time had lapsed for the defect to develop. The extent of this outbreak was considerable, since 77 per cent. of the cans sampled at random from 15 different days' manufacture spread over several months showed "buttons".

2. Types of defective samples encountered.

(i) Gassy fermentation. In both the "bloats" examined sucrose-

fermenting yeasts were responsible for the spoilage. Large numbers of bacteria of the staphylococci type were also isolated but did not produce gassy fermentation on re-inoculation into the sound product. The presence of these bacteria was therefore without significance so far as gas formation was concerned. Further samples from the same sources were not available so that it was not possible to investigate the extent of spoilage in greater detail. It has been reported (see reviews in (4) and (5)) that when "bloats" occur in condensed milk the spoilage is normally caused by sucrose-fermenting yeasts.

(ii) Progressive thickening. Thickening was only found in cans which were opened in connexion with the outbreak of "buttons". It was not found possible to isolate any bacteria which were capable of reproducing thickening. It seems probable, as none of the samples were unduly acid, that the fault was either associated with the formation of "buttons" or more probably that it was of physico-chemical origin, since the latter type of defect is more apparent on aging and the samples were from 9 to 18 months old when examined.

The thickening was obviously not of the same type as has been described by Rice and Downs[27] and by Downs[28]. They showed that certain cocci, similar to Staphylococcus albus, could promote progressive thickening and that such bacterial thickening was associated with increased acidity. A sucrose in water ratio of 64.5 was necessary to prevent these cocci from growing. Downs considered that acidity alone was not responsible for

thickening, but that the action of a rennin-like enzyme produced by the cocci was also instrumental.

(iii) "Buttons". This fault has been shown by Rogers et al.[29] to be due to the growth of a mould Aspergillus repens and by Knudsen[30] to be due to Aspergillus glacus and other moulds. The so-called "button" consists of a reddish-brown cheesy curd mass ¼" to ⅜" in diameter. The defect develops on storage, and after 8 to 10 weeks "buttons" have assumed a definite form. The growth of the mould itself probably ceases after 2 to 3 weeks, since at later stages mould cannot be isolated from the buttons easily, though the "button" continues to develop from the activity of a clotting enzyme.

From the outbreak already mentioned the "button" formation was similar to that described above, except that occasionally the hard curd tended to spread in an indefinite shape round the edges of the can. Three moulds (M 1-3) which appeared to be species which have not previously been reported as causing "buttons" were isolated. In view of the difficulties of identifying many species of mould, and particularly members of the Penicillium genus without specialised knowledge of the whole group, the author has considered it unwise to attempt to name the species. It is well known that many mould forms are considerably modified by the substrate in which growth occurs and that other conditions also are able to change the type of growth, and thus a single description or examination is often insufficient to enable identification, especially if the variations in form

which may occur within the species are not appreciated.

Mr. George Smith,[31] M.Sc., has kindly investigated the characteristics of these cultures and he has reported as follows:-

M 1 is a species of Actinomyces which is very similar in structure to some of the common soil forms. The hyphae and spores are however about 2½ times the usual diameter. There seems to be no previous reference in the literature to such a strain of Actinomyces, and Smith has never before encountered a culture like it.

M 2 is a strain of Penicillium terrestre Jensen, and it is classed in Thorn's group Asymmetrica-Funiculosa.

M 3 is also a species of the Penicillium genus.

Each culture was inoculated separately into sweetened condensed milk and incubated at 22°C. to confirm the fact that it was the cause of "button" formation. The condensed milk was first filled into wide small-neck glass bottles which were stoppered with cotton wool and heated in a bath to 140°F. for 30 minutes in order to destroy any mould spores which might have gained entrance during the transfer. After this heating the bottles were cooled and when the moulds had been inoculated the bottles were stoppered with sterile rubber stoppers and sealed with paraffin wax to ensure that they were airtight. The volume of air space inside was similar to that which would occur in a commercial can. The development of mould was observed through the glass and was first evident with M1 after two weeks'

incubation. This mould formed yellow brown colonies in the initial stages of growth, whereas the other two cultures were green. The growth of M 3 was not visible until after 3 weeks' incubation at 22°C., while M 2 developed even more slowly.

As the moulds grew on the condensed milk and even after development appeared to have been arrested, a reddish-brown to orange discolouration increased under the original growth and spread around it. The same kind of discolouration occurred with all three cultures whether the mould had been green or brown originally. The bottles of cultured condensed milk showed considerable discolouration over the major part of the surface of the milk after 9 months' storage. Hard cheesy masses of curd were evident, especially round the edges of the jars, and some samples were somewhat thick although the control samples remained unchanged.

This epidemic of defective samples was therefore very similar to those already reported in the literature. It appears, however, that the causative organisms were different, since one culture was a species of Actinomyces while the other 2 cultures were classified as species of the Penicillium genus.

PART III. BITTERNESS AND THINNING IN CANNED CREAM.

I. Bacteriological Studies.

A. Morphological and cultural characteristics.

B. Induced spoilage.

(i) Inoculations into normal canned cream and incubation.

(ii) Examination of inoculated cans.

(iii) Production of bitterness and thinning.

(iv) Plate counts and recovery of bacteria from inoculated cans.

II. Chemical Studies.

III. Application of the Results in Commercial Practice.

PART III. BITTERNESS AND THINNING IN CANNED CREAM.

It has already been noted that the development of bitterness and thinning was one of the most prevalent forms of spoilage in canned cream.

A development of a defective bitter flavour during the storage of canned dairy products has been previously reported. Thus Hammer[32] isolated sporeformers, which he named B. amarus, which caused coagulation with a characteristic bitter taste and generally with an increase in acidity. In litmus milk, however, it did not produce a clot and only in evaporated milk on prolonged incubation at 37°C. (89 days).

Spitzer and Epple[33] investigated an extensive outbreak of bitterness in evaporated milk, though this was not accompanied by any change in the physical appearance of the product. They found that the milk was infected with a spore-bearing organism, similar to B. panis (Migula, 1900), which survived heating at 250°F. for 8 minutes. When the organism was inoculated into sterile milk it caused considerable degradation of the proteins producing large amounts of peptone substances and some ammonia; there was also an increase in acidity. Grindrod[34] also reports two heat-resistant organisms which were responsible for bitterness in evaporated milk. Recently similar defects have been observed in canned cream. McMaster[35] reported both a marked bitter flavour and a water-like consistency in some defective batches of canned cream and was able

to demonstrate that the trouble was caused by a heat-resistant bacillus, similar to B. cohaerens, which withstood 10 minutes heating at 115.5°C.

When this defect was first observed in this study the contents of the cans were exceedingly thin and had a bitter taste resembling quinine. Only strains of B. subtilis were isolated from the defective cans and the presence of an abnormally high proportion of acid-soluble nitrogen showed that a considerable breakdown of protein had occurred. Preliminary experiments showed that bitterness and thinning could be reproduced in normal canned cream by inoculation with the cultures isolated from the affected cream. A detailed study of the bacteriological and chemical factors involved in the production of these two associated defects has been made. A full description of the chemical aspects has been reported in a joint publication by the author[15]. The bacteriological aspects are dealt with below.

I. BACTERIOLOGICAL STUDIES.

Three organisms, namely Nos. 6, 27 and 62, secured from different defective batches of canned cream have been used. The characteristics of these organisms have been considered together unless otherwise stated, and all cultures were incubated at 37°C.

A. Morphological and Cultural Characteristics.

Morphology. From broth, small rod-shaped organisms with rounded

ends occur singly, in pairs and in short chains. In 12 hour old cells there are often granules situated towards the extremities which disappear later. The cells from 24 hour old agar slant cultures measure between 1.5 and 3.0 , but are mostly 1.5 long and about 0.5 to 0.7 wide.

Motility. Young broth cultures are actively motile.

Staining properties. Cultures are Gram-positive and are readily stained by the common dyes.

Spore formation. Spores are formed readily from about 12 hours onwards on agar slants. Similar cells from 18 to 24 hours old are nearly all sporing, but with age the spores become free and usually do not retain the old cell wall attached to them. Spores are also formed on other solid media and rapidly in broth scums. They are generally in a central position or towards one end of the cell. The cell wall which is very slightly enlarged by the spore does not usually exceed 0.8 in width when grown on standard agar. The average width for young spores is 0.7 and the length from 1.0 to 1.5 .

Agar slants. There is abundant growth after 16 hours incubation which with all cultures is easily brushed off with in inoculating needle. The growth of No. 6 is raised and dull cream white to grey in colour with plumose undulating edge holding to the line of the inoculation neither wrinkled nor striated. It becomes drier and rather darker in colour with age. Cultures after several weeks at room temperature develop clear patches as though the organisms had disintegrated. The growth of No. 27 is not

as dense as that of No. 6 and shows a greater tendency to spread. No. 62 grows less profusely, and with age becomes almost colourless transmitting light easily.

Milk-agar stab. Stab growth is only villous and scanty, but development is heavy on the surface after 1 day.

Milk-agar plates. After 24 hours incubation surface colonies are 2 to 6 mm. in diameter, 3 to 4 mm. being the most usual size unless overcrowding occurs. Colonies tend to be circular but are spread if the agar is wet. The centres are dense and irregular in shape, do not transmit light, and are surrounded by a less dense granulated zone which is filamentous at the edges. Old surface colonies grow to 1 cm. in diameter and retain the dense dull centres surrounded by raised cream-coloured circular growth. As aging occurs the colonies are surrounded by clear zones in the agar where the small amount of casein (1 per cent. separated milk) is digested. Subsurface colonies are irregular and dense and about 1 mm. in diameter at 24 hours. Later they may break through to the surface and develop into the type of colony already described. In addition No. 62 produces mucoid or smooth colonies.

Glucose-agar stab. One-day-old cultures show abundant surface growth which is finely wrinkled, dull and light brown in colour. On further incubation these wrinkles usually increase and become dark brown in colour, while a pink red colour develops under the surface of the agar. The degree of wrinkling and darkening varies considerably. Growth occurs down the slant

to a considerable depth but is scanty. No. 62 has usually a smooth light-coloured surface growth which is viscous.

Gelatine stab. Liquefaction takes place readily at 22°C. After 3 to 5 days from one-third to one-half of the stab is liquid and a rough or smooth scum is formed.

Broth. A heavy scum covers the surface after 18 to 24 hours' incubation. At first there is practically no turbidity or deposit until parts of the pellicle are dislodged. The scum may become wrinkled and have a "honeycomb" appearance although No. 27 usually remains smooth.

Potato. After 1 day a profuse folded or wrinkled growth covers the whole potato surface. Since the organisms grow closely together the folds become almost obliterated with age and at the same time the potato blackens and the culture darkens considerably

Litmus milk. A soft coagulum is formed after 1 to 2 days and the milk clears rapidly from the surface downwards. All the curd is usually digested in from 3 to 6 days and sometimes digestion precedes clotting. As digestion advances the clear liquid at the surface becomes bright amber coloured, but after standing for some days the medium reverts to a port wine colour.

Other cultural characteristics. Further reactions brought about by the organisms during growth are shown in Table 5.

Heat resistance. Under laboratory conditions spore suspensions in sterile milk and cream have withstood temperatures up to 120°C. for as long as 40 minutes. The resistance, however, shows marked variations and will be discussed in the heat resistance section.

TABLE 5.

Cultural reactions of isolated strains.

Culture No.	Nitrate reduced to nitrite	Indol Ehrlich's method	Voges-Proskauer Barritt's modification	Carbohydrate fermentation							Starch hydrolysis
				Levulose	Glucose	Saccharose	Lactose	Raffinose	Salicin	Mannite	
6	+	-	+	A	A	A	-	-	A	A	+
27	-	-	+	A	A	A	-	-	-	-	+
62	-	-	+	A	A	A	-	-	A	A	+

A = production of acid.

B. Induced Spoilage.

(i) Inoculations into normal canned cream and incubation. In order to determine the conditions under which bitterness and thinning could be produced in canned cream, normal cans were inoculated with cultures of the above organisms. Cans of normal cream about 4 weeks old which had undergone age thickening were selected from the same batch manufactured at a local creamery. Nos. 6, 27 and 62 were each inoculated from a 2-day-old culture into forty cans. The method used for inoculating the cans has already been described in Part II. About twenty control cans were inoculated with a loopful of sterile water*. After inoculation the cans were well shaken and were reshaken occasionally during storage at different temperatures. Two cans of each batch were examined at definite intervals.

(ii) Examination of inoculated cans. The examination was similar to that used in the general survey of cans. Separate tubes of 1 per cent. glucose broth and litmus milk were each inoculated with 1 ml. and one loopful of the cream. These were incubated at 37°C. and were examined after 3 and 7 days for the presence of the organisms. Plate counts on the cream were carried out and cream was also streaked directly on milk-agar plates and slides prepared for direct microscopic examination.

(iii) Production of bitterness and thinning. Table 6 shows the extent to which bitterness and thinning were produced on storage at various temperatures. Only the results for cultures Nos. 6

*The results of the control inoculations were uniformly negative, and are therefore not detailed in the text.

TABLE 6.

Development of bitterness and thinning at different temperatures.

No. of days after incubation	Room temperature		25°C.		32°C.		37°C.	
	Flavour	Consistency	Flavour	Consistency	Flavour	Consistency	Flavour	Consistency
				Culture No. 6.				
5	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
9	Normal	Normal	Normal	Normal	Normal	Slight thinning	(1) Very bitter (2) bitter	Thin
15.	Normal	Normal	Normal	Slight thinning	(1) Slight putrefaction (2) bitter	Thin	(1) Slight putrefaction (2) bitter	Thin
22	Bitter	Thin	Bitter	Thin	Very bitter	Very thin	(1) Bitter (2) very bitter	Very thin
54	-	Thin	-	Thin	-	-	-	-
				Culture No. 62.				
4	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
8	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
14	Normal	Normal	Normal	Normal	(1) Slight putrefaction (2) bitter	Slight thinning	Slight putrefaction	Slight thinning
21	Normal	Normal	Normal	Normal	Slight bitter	Thin	(1) Normal (2) slight bitter	Thin
53	Normal	Normal	-	Thin	(1) Very bitter (2) bitter	Thin	-	-

Note: In each instance two duplicate cans were examined. If the results differed the cans have been recorded separately.

and 62 are recorded, since these exhibited the widest differences in their action on the cream. Bitterness and thinning developed most rapidly with culture No. 6. All the cans were incubated at 37°C. and most of those incubated at 32°C. were thin at the end of 2 weeks, while those incubated at 25°C. were just starting to lose their thick consistency. The cans kept at room temperature were practically unaltered. After 3 to 4 weeks cans stored at 32 and 37°C. were very thin, while those kept at 25°C. and at room temperature were thin. The development of bitterness usually, but not invariably, ran parallel with the thinning. The results for cans inoculated with culture No. 62 were similar throughout the periods of incubation, but the development of both bitterness and thinning was less rapid. Thus at the end of 3 weeks no abnormality was observed in cans kept at 25°C., while at room temperature the cans were still unaffected after $5\frac{1}{2}$ weeks' storage.

(iv) Plate counts and recovery of bacteria from inoculated cans. The organisms were at all times recoverable from the inoculated cans in pure culture. After about 6 weeks their presence could not be detected in films made directly from the cream, nor was it possible except in the early stages to demonstrate the persistence of the bacilli by streaking on agar plates. Small inoculations of the cream (one loopful) into litmus milk and glucose broth only produced growth in the early stages of the experiment but with larger inoculations (1 ml.) growth could be produced at all stages. The necessity for using such

large inoculations for the recovery of these organisms from the cans is further illustrated by the results of plate counts. Table 7 shows the counts of inoculated cans after incubation at 37°C. It is remarkable to find that bitterness and thinning can be produced in cream having such low numbers of viable organisms. The counts of such organisms after different periods of incubation show, moreover, unexplained variations which could not be correlated with the intensity of bitterness or the extent of development of thinning. This suggests that such defects may be partly caused by the action of extracellular bacterial enzymes. It may be mentioned that in only three instances were viable organisms recovered from the cans other than those originally used for the inoculations. Casual contamination of the cans with extraneous organisms was therefore very infrequent.

II. CHEMICAL STUDIES.

Although full details of the chemical aspects of this spoilage have (as already noted) been described in a recent publication[15] it will be of interest to mention briefly the main findings. These may be summarised as follows:-

(1) Both bitterness and thinning are associated with the breakdown of protein, as indicated by determinations of the non-protein nitrogen and of the peptone and subpeptone fractions.

(2) There is a relationship between the development of thinning and bitterness as revealed by a study of the correlation

TABLE 7.

Plate counts of inoculated cans after incubation at 37°C.

Culture No. 6		Culture No. 27		Culture No. 62	
No. of days of incubation before testing	Mean of plate counts of 2 cans opened on same date	No. of days of incubation before testing	Mean of plate counts of 2 cans opened on same date	No. of days of incubation before testing	Mean of plate counts of 2 cans opened on same date
7	46,000	10	1,000	5	140,000
14	2,400	17	180	12	500
21	99,000	24	180	19	70
28	38,000	31	780	26	110
35	6,900	38	70	33	130
42	650	45	30	40	160
49	70	52	90	47	240
56	720	59	20	54	120
77	3,550	82	60	66	140
143	2,900	150	- *	124	20
154	2,190	171	10	158	280
167	-	175	40	-	-

* None found in $\frac{1}{10}^{th}$ dilution.

coefficients between viscosity and non-protein nitrogen.

III. APPLICATION OF THE RESULTS IN COMMERCIAL PRACTICE.

Owing to the high thermal death-point of the aerobic spore-forming organisms which cause bitterness and thinning in canned cream it may be extremely difficult to control this defect under commercial conditions. It has already been stated that spore suspensions have withstood temperatures up to 120°C. for as long as 40 minutes. Thermal death-points, however, depend on a large variety of different factors and less drastic heat treatment may therefore be sufficient to kill off the organisms in commercial practice. It was noted earlier that there is a limit to the heat treatment to which tinned cream can be subjected so that even if the thermal-death-point of the organisms was known and the cream could safely be given the necessary heat treatment, because of the marked lag in attainment of the required temperature inside the can itself, it would in practice be difficult to ensure that the desired heat treatment had been given to the can contents. It appears, therefore, that in practice the prevention of bitterness and thinning must depend more on improving the quality of the incoming milk supply than on altering the conditions of sterilisation of the canned product. Hygienic production, especially with regard to the cleanliness of the cows' udders and the byres, provides the first line of defence against infection.

A second point of importance in the control of bitterness

is the identification of defective cans of cream before despatch from the creamery. It is apparent from the results detailed in Table 6 that the commercial practice of incubating canned cream for not more than 2 weeks at temperatures of about 75 to 85°C. would frequently be insufficient to reveal the defect. It appears desirable, particularly where there is any history of this defect at a creamery, to store the canned cream for a further period of, say, 4 weeks or more at room temperature in order that the defect may be sufficiently developed in any affected cans to enable them to be picked out. In this connexion it may be noted that thinning almost invariably accompanies bitterness, and this therefore provides a simple method of identifying the defect without opening the can.

It is, of course, desirable that there should be a careful bacteriological control of all batches of cream where laboratory facilities are available for this purpose. It has been pointed out, however, that if small inoculations of cream are used (for example, a single loopful) there is a serious risk that the presence of viable organisms will be missed. On the other hand, the isolation of organisms of the B. subtilis type from canned cream should not be taken as indicating that the cream will inevitably become bitter. In the examination of canned dairy products some thirty-six strains of B. subtilis have been isolated, and they all showed the same morphological and cultural characteristics as strains which produce bitterness and thinning. Yet only a very few of these thirty-six strains were capable of

producing the defect when inoculated into normal cream. It appears that the only certain method of identifying the typical organism is by its ability to produce bitterness and thinning in the normal product.

PART IV. DRIED MILK PRODUCTS.

General study undertaken.

Sources of powders.

A. Experimental Methods.

1. Methods of reconstitution of powder.
2. General methods of examination of powder.
3. Method of isolation of organisms.

B. Results and Discussion.

I. Bacteriological quality of powder.

(i) & (ii) Plate count and methylene blue reduction test.
(iii) Fermentation test.
(iv) Presumptive coliform test.
(v) Yeast and mould count.

II. Keeping quality of reconstituted milk and effect of storage at 60°F.

III. Bacterial flora of dried milk powder.

1. Litmus milk reactions of micro-organisms predominating at 37°C.
2. Classification of milk coagulating cultures obtained from plates incubated at 37°C.
3. Classification of milk coagulating cultures obtained from plates incubated at 55°C.
4. Other organisms examined.

IV. Factors affecting variability of bacteriological quality.

1. Variation in the powder of different factories.
2. Variation in the powder of the same factory.
3. Variation in the powder during the same day's operation.
4. Variation according to season of year.
5. Variation between full-cream and skim milk powders.
6. Variation during storage.
   (a) Variations at room temperature.
   (b) Variations at different temperatures.
   (c) Variations with storage in air versus nitrogen.

V. The use of the methylene blue reduction test as a method of assessing the bacteriological quality of milk powders.

VI. A note on the desirability of grading spray dried milk powders.

PART IV. DRIED MILK PRODUCTS.

The great heat to which milk is exposed in roller drying reduces the bacteriological problems to the surviving spore-formers or to recontamination with micro-organisms after the powder leaves the rollers. The numbers of bacteria are low and it will be seen from the review by Allen[5] that many of the bacteriological aspects have already been investigated. Roller dried milk cannot be reconstituted so completely as spray dried milk. The latter approaches a solubility of 100 per cent. and for this reason is increasing in popularity. The extent of heat treatment to which milk is subjected during spray drying is, however, much less than during roller drying, and it might therefore be expected that the bacterial flora of the former would be more numerous and varied. The investigations reported here have therefore been confined to the spray dried product since this presented the most interesting and least explored field, few investigations having been conducted with spray dried milk powder outside the United States.

At the outset it was felt that an insight into the bacteriological quality of spray dried powder could best be obtained by a general study of samples from the various factories operating in England and Scotland. Seven firms have installed plant for the production of spray dried milk powder, but during the time that samples were received one plant was not operating and another was drying the constituents of milk which had previously been altered to conform to a "baby food" formula.

Finally samples were obtained in 6 oz. sterile bottles from eight factories operated by five firms. Two of these factories were situated in the south-west of Scotland, one in the north of England, and five in the south-west of England, i.e. Somerset, Dorset and Devon. In all over 400 samples have been examined, and among this number were included samples from plants of the Milkal[3], Kestner[36] and Gray Jensen types. The plants are designated alphabetically throughout. About 10 per cent. of the samples were of whole milk powder and the remainder were made from separated milk. The examination of samples from two plants extended from June 1937 until September 1938, and from the other factories samples were received over the first eight months of 1938. A drying plant for economic operation is in use from 18 to 22 hours out of the 24 and samples were received in batches from a day's run of the plant. The first sample was usually obtained in the first hour of drying, another was usually taken at the end of the day's processing, and a third sample was obtained somewhere in the middle of processing. In some instances four other samples were taken at roughly regular time intervals between the first and last sample. It will be seen, therefore, that any conclusions regarding the bacteriological quality of the powders are based on fully representative samples since they are derived from different types of plant, situated in different localities and sampled throughout cold and warm seasons and at different stages in the day's run.

A. EXPERIMENTAL METHODS.

1. Method of reconstitution of spray dried powders.

The reconstitution of samples for analysis presented two difficulties, viz.

(1) the avoidance of contamination, and

(2) the preparation of a representative sample.

Regarding (1) there are two points of contamination,

(a) at weighing, and

(b) on reconstituting.

Quick weighing by using a large sample on a rough balance minimised contamination at weighing. The prevention of contamination on reconstitution was more difficult. Spray dried powder, although almost completely soluble when mixed with water, behaves in a manner similar to that of cornflour and unless it is stirred thoroughly into a paste first, lumps of undissolved powder occur which render it impossible to get a uniform solution. A method was tried in which a sterile glass rod was used to rub the powder into a paste in a wide test tube, but it was neither successful nor could the technique be considered satisfactory. The same criticism also held when a pestle and mortar was used to make the paste. Hence a closed bottle containing water and some beads was eventually adopted.

As regards (2), the necessity for having a representative sample was attained by

(a) using a large sample, and

(b) thoroughly reconstituting.

It will be seen that the technique devised to avoid contamination incidentally also met these two points. The American Dry Milk Institute[37] recommend weighing the powder directly into a sterile bottle containing the water blank and furnished with a sterile stopper. Other workers have weighed the powder directly into a sterile bottle and Ringer's solution has been added afterwards. These methods of weighing have obvious disadvantages and in addition it requires considerable shaking to get a completely dissolved powder, although the addition of lead shot has recently been suggested[37] to facilitate solution. It should be noted also that dilution blanks containing N/60 sodium or lithium hydroxide have been suggested[38] for the analysis of milk powder when difficulties are experienced in counting plates after incubation owing to undissolved flakes or particles of powder. The presence of undissolved flakes is common in plates made from roller dried powder but no difficulties were encountered during the examination of the more soluble spray dried skimmed milk powders, and hence weak alkaline dilution blanks were not used in this study. However, with spray dried whole milk powder, fat particles rendered plates of the $\frac{1}{10}$ and $\frac{1}{100}$ dilutions difficult to estimate with accuracy.

The method of reconstitution finally adopted was to weigh out 20 g. of powder into a pair of sterile petri dishes, and transfer the powder through a sterile paper cone into a glass bottle containing 180 ml. of sterilised water. The capacity of

the bottle, which was secured by a screw cap, was about 300 ml., and it contained about a dozen glass beads ($\frac{1}{5}$" diameter). By vigorous shaking all the powder was dissolved in from 1 to 3 minutes. Twenty g. of powder added to 180 ml. of water increase the total volume of reconstituted milk to between 190 and 195 ml. Hence it will be seen that the reconstituted milk was roughly a 10 per cent. solution and for all practical purposes 1 ml. could be taken as equal to 0.1 g. and could be considered as the $\frac{1}{10}$ dilution. Incidentally this reconstituted milk contains about 9.5 per cent. solids-not-fat which corresponds to the composition of separated milk itself.

2. General methods of examination of samples.

The following tests were carried out on all the powders:-

(i) Plate count was determined by the method described in Bulletin 46 of the Ministry of Agriculture[26] on milk agar at 37°C. and 55°C. Nearly all the dilutions were prepared in duplicate and were not made any lower than 1/1,000,000. It was necessary to incubate all plates at both temperatures for at least 3 days before the count was taken; even then many of the colonies were very small. In an attempt to improve the technique plates were prepared from 24 samples in this way, and were compared with a duplicate series to which $\frac{1}{2}$ ml. of defibrinated horse blood had been added as the agar was poured. It was found that the colonies on the plates to which blood had been added were usually larger and easier to count and that the number was somewhat higher. The disadvantages of this

method for routine purposes seemed to outweigh the advantages, however, especially as, when combined with the milk agar, the blood was haemolysed owing to the low pH, and any haemolysing organisms present could not be estimated.

(ii) The methylene blue reduction test was applied at 37°C. and 55°C. as described by Wilson[39] for whole milk.

(iii) The tubes of milk used for the reduction test were examined after 24 hours' incubation, and the type of fermentation noted.

(iv) 1 ml. of the reconstituted milk, and in some instances 10 and 20 ml., were tested by the presumptive coliform test[26] at 37°C.

(v) 1 ml. of the reconstituted milk was also added to litmus milk and incubated at 37°C. for three days, as it was considered that changes in the litmus milk would provide information as to the type of flora present.

(vi) Keeping quality tests[26] were carried out at 60°F. The reconstituted milks could not be graded from the first perceptible taint up to clotting, as any slightly developed taint was masked by the somewhat cooked and often acid flavour of the reconstituted milk itself. The cooked and/or acid flavours were more marked in the milk reconstituted from skimmed milk powder than in that made from whole milk powder. The keeping quality was therefore judged from the time of reconstitution until clotting took place on boiling or until a clot was formed in the bottle.

The following tests were carried out on some samples only:-

(vii) The testing was repeated on certain samples after the reconstituted milk had been stored at 60°F. for 24 hours.
(viii) About 40 powder samples were retained at laboratory temperature and about 100 re-tests were made after they were $1\frac{1}{2}$, 3, 6, 8 and 12 months old.

(ix) A further series were made up in cans, each of which held about 200 g. of separated milk powder, from a large bulk sample and were stored at 40°C., 22°C., cold room temperature (about 10°C.) and in a cold store (about 4°C.). Half the sample cans were evacuated of air and re-filled with an inert gas, nitrogen. In order to do this special equipment was supplied by the Metal Box Company, Worcester. The cans were examined at stated intervals by the methods already outlined.

(x) 1 ml. quantities of a few of the reconstituted samples were plated on beer wort agar, which had been buffered to a pH of 3.5 with citric acid and potassium citrate. This medium was selective for yeasts and moulds. The plates were incubated at 22°C.

3. Method of isolation of organisms.

About 1,700 colonies have been picked off the agar plates used for the plate count test. These colonies were inoculated into litmus milk since growth in this medium would be indicative of changes which might be expected in milk products made from the powder. The tubes were incubated at 37°C. or 55°C. according to the temperature at which the plate had previously been incubated. The changes in the litmus milk cultures were

recorded for about a week and most of the cultures, where no change was noted, were examined microscopically to ascertain whether growth had occurred. All the colonies from a plate or a portion of a plate were picked off, so that the cultures obtained were representative, and about 180 of them were purified and examined in detail.

B. RESULTS AND DISCUSSION.

I. Bacteriological quality of spray dried milk powder.

(i) & (ii) Plate count and methylene blue reduction test.

It will be seen from Table 8 that the arithmetic "weighted" mean of the plate count at 37°C. was 4,363,000 per g. of powder, and if this is considered as reconstituted milk it will approximate to a plate count of 400,000 per ml., which does not compare very favourably with good quality raw or pasteurised milk. Wide variation in the plate count was shown, and individual results varied from 1,400 to 149,000,000 per g. By using the logarithm of the plate count at 37°C. the extreme values are levelled out to a certain extent and the mean value was then found to be equivalent to a count of 800,000 per g. The "weighted" mean methylene blue reduction time at 37°C. was 8.1 hours, and varied from 3 to 14 hours or more.

The plate counts at 55°C. also varied widely, and ranged from less than 100 to 1,300,000 per g. The methylene blue reduction times were much higher than at 37°C. The lowest

TABLE 8.

Factory	Plate Count at 37°C. per g.			Log. of plate count at 37°C.				Methylene Blue Reduction Test at 37°C. in hours				
	No. of samples	Mean	Range Minimum Maximum	No. of samples	Mean	Standard Deviation	Coeff. of Variation	Range Minimum Maximum	No. of samples	Mean	Standard Deviation	Coeff. of Variation	Range Minimum Maximum
A	24	4,265,000	46,000- 20,200,000	24	6.05	0.7085	11.70%	4.66 - 7.31	23	8.18	2.033	24.9%	4.5 - 12.5
B	63	1,535,000	6,000- 24,800,000	63	5.74	0.7260	12.69%	3.78 - 7.39	63	7.65	1.217	15.9%	5.0 - 11.0
C	66	19,800	1,500- 129,000	66	4.07	0.3911	9.60%	3.18 - 5.11	61	11.03	1.482	13.45%	7.5 - 14.0
D	90	12,250,000	28,000-149,000,000	90	6.19	1.321	21.34%	4.45 - 8.17	94	6.84	1.426	20.8%	3.0 - 10.0
E	138	3,388,000	1,400- 45,500,000	138	5.93	0.9132	15.40%	3.15 - 7.66	139	7.81	1.767	22.8%	3.5 - 12.5
F	12	305,500	22,000- 1,170,000	12	4.89	1.946	39.70%	4.34 - 6.07	11	9.45	1.094	11.6%	7.5 - 11.0
G	12	106,300	15,600- 450,000	12	4.77	0.6124	12.84%	4.19 - 5.65	12	8.54	1.244	14.6%	6.0 - 11.0
Total	405	4,363,000*	1,400-149,000,000	405	5.65*	0.8161	14.45%	3.15 - 8.17	403	8.13*	1.805	22.3%	3.0 - 14.0

* Weighted mean.

time was 4½ hours but over 30 per cent. of the samples required 12 or more hours before decolorisation occurred.

The factors responsible for these variations will be dealt with later.

(iii) Fermentation test.

No definite or uniform relationship was established between the fermentation reactions of the methylene blue tubes examined after 24 hours' incubation and the plate count and reduction tests. Useful data were, however, occasionally obtained regarding the number and activity of thermophiles present when these tests were conducted at 55°C. Typical examples given in Table 9 illustrate this point. G 1 to 3 and C 19 and 20 show how deterioration in the results of the fermentation test may forecast an increase in the number of thermophiles or a change in the activity of the organisms (E 89 to 91). It was apparent from the results that some factories had more thermophiles present than others and that the activity of the organisms also varied. Factory C, for instance, usually had from 1,000 to 10,000 per g., although the consistency of the milk in the fermentation test was nearly always unchanged. It is probable therefore that the fermentation test is a better indication than the plate count at 55°C. of any difficulties which might be expected in the plant operation owing to increased acidity which had been produced by thermophiles.

(iv) Presumptive coliform test.

When 1 ml. quantities of the reconstituted milk were tested

TABLE 9.

Sample	No.	Plate Count at 55°C.	M.B.R.T. at 55°C.	Fermentation Reactions after 24 hours at 55°C. Unchanged	Coagulated	Whey	Digestion	Gas
G	1	100	>14½	+				
	2	2,400	13½		+			
	3	63,600	8½		+			
C	19	26,000	>18½	+				
	20	55,000	11½		+			
E	89	1,030	9½		+	+		
	90	1,430	4½		+	+	+	+
	91	560	6		+	+		

by the presumptive coliform test about 10 per cent. gave positive results. A few samples which gave negative results with 1 ml. showed positive reactions when tested in addition in 10 and 20 ml. quantities. It would seem, however, that the latter is too stringent a test, since 1 ml. quantities are considered sufficient to judge the quality of ordinary raw and pasteurised milk. It should be noted that positive tests were often due to the growth of anaerobic sporeformers and not to organisms of the coliform group. Crossley[40], working with the powder from two similar plants, reported that 65 per cent. of the positive presumptive coliform tests are due to anaerobes, and that the positive presumptive coliform tests actually caused by coliform organisms were more frequent in freshly made than in stored samples. From additional tests it did not, however, appear that the percentage of positive results due to anaerobes would normally be so high as Crossley reports, provided that the survey is (as in the present investigation) spread over samples from a large variety of plants.

(v) Yeast and mould count.

Since the heat of processing is sufficiently severe to kill yeasts and mould spores it is generally held that the sieving and packing of the powder constitute the only serious source of contamination with these organisms. However, the number of yeasts and moulds in the samples which were examined by plating on beer wort agar was found to be practically negligible.

One special sample was encountered which had a mouldy odour and taste, and a mould of the Penicillium genus was found to have grown near the surface, where the moisture content of the powder was very high. It is probable that this was due to damp gaining entrance to the barrel.

II. Keeping quality of reconstituted milk and effect of storage at 60° F.

It has sometimes been held that reconstituted milks only keep in a palatable condition for a very short time as compared with fresh milk. It was felt therefore desirable to investigate this point. It has already been stated that the keeping quality could not be determined by taste, and the figures in Table 10

TABLE 10.

Showing percentage of samples which clotted on boiling or were coagulated in the bottle after different periods of storage at 60° F.

Factory	Days storage							
	1	1½	2	2½	3	3½	4	4½
A				10	43	33	14	
B					37	38	25	
C					18	32	44	6
D	1	1	1	8	53	24	12	
E			1	5	54	22	17	1
F					25	50	25	
G				25	50	16	9	
Total	.25	.25	1	4.5	44	28	21	1

refer to the time between reconstitution and the formation of a clot on boiling or in the bottle. In order to convert these values to the actual keeping quality, i.e. to the time of development of an undesirable flavour, half a day at least should be deducted. If this deduction is made over 90 per cent. of the samples remained sweet for between $2\frac{1}{2}$ to $3\frac{1}{2}$ days, which allows ample time for the use of reconstituted milk for liquid consumption. Thus it appears that the belief that reconstituted milks have a short life is unwarranted.

Examination of the results obtained with the samples which were re-tested after 24 hours' storage at 60°F. showed that the plate count at 37°C. had generally increased and the reduction time had decreased, but that the changes were very much less than would have been expected. The reduction test did, however, appear to be the more sensitive indication of the additional aging of the milk, as will be seen from Table 11. The samples tabulated were chosen at random. It will be seen that the reduction time was approximately halved after 24 hours, whereas the increase in the plate counts was less regular. It is probable that other factors, such as the operation of a long initial lag phase before increase of numbers occurs, as well as the tendency for the organisms to clump when multiplication commences, may have affected the counts but not the activity of the organisms as shown by the reduction test. At 55°C. there was less uniformity in the results. In the main the tests showed no change, a result which would be expected at a storage

TABLE 11.

Showing tests on milk powder when first reconstituted and after 24 hours' storage at 60°F.

Sample No.	Time after reconstitution when tested (hours)	Plate count at 37°C.	Reduction Test at 37°C.	Plate Count at 55°C.	Reduction Test at 55°C.	Presumptive Coliform Test
131 A	0	1,070,000	7	20,800	>8	Acid
	24	730,000	5	8,600	7	Acid + Gas
109 D	0	20,200,000	4		9½	Acid
	24	25,000,000	1½		7½	Acid
114 D	0	160,000	6½	340	9½	Acid
	24	690,000	3½	220	9½	Acid
73 D	0	1,690,000	6½	40,000	5½	Acid
	24	9,200,000	4½	60,000	6	Acid + Gas
48 D	0	1,310,000	6½		6	Acid
	23	550,000	7		5	Acid
34 D	0	5,800,000	8½	30,000	8	Acid
	28	8,700,000	7½	18,800	8	Acid
142 E	0	94,000	12		14	Acid
	24	270,000	7		10½	Acid
140 E	0	5,200,000	6½	70,000	6	Acid
	28	12,600,000	3	45,000	7	Acid
125 E	0	4,600,000	7	34,000	10	Acid
	24	3,280,000	4	20,500	>8½	Acid + Gas
89 E	0	100,000	9½	1,030	8½	Acid
	24	224,000	7	90	>7½ <10½	Acid
79 E	0	246,000	9	90	10	Acid
	24	288,000	5	210	9½	Acid

temperature too low for thermophilic growth to occur.

It is apparent that the flora of reconstituted milk does not increase as rapidly as that of raw milk at 60°F., and this must be given consideration if the quality of the reconstituted milk which has been made some hours is to be assessed by the plate count alone. The work of Ayers, Cook and Clemmer[41] showed that averaging the results of 16 clean milk samples there was a 38-fold increase in count after 24 hours, i.e. from 3,243 to 123,562 per ml., and the average result of 13 samples of dirty milk increased 63-fold, i.e. from 707,761 to 48,550,923 per ml. In comparison, a 5-fold increase in the plate count of the reconstituted milk was exceptional, while in some samples there was actually a reduction in the count 20 hours after reconstitution.

III. Bacterial flora of dried milk powder.

1. Litmus milk reactions of micro-organisms at 37°C.

Examination of the litmus milk media which had been inoculated with 1 ml. of reconstituted milk after 3 days' incubation at 37°C. showed that in all cases the predominating flora at this temperature rendered the milk acid and coagulated it. The acidity produced was sufficiently high to cause the milk to "whey off" in 60 per cent. of the samples. Roughly 40 per cent. of these cultures also showed gas formation and about 20 per cent. appeared to be partially digested. It was, however, often difficult to differentiate whey production and digestion, particularly if the curd had shrunk and was broken up by gas.

2. Classification of cultures obtained from plates incubated at 37°C.

Tables 12 and 13 show that the majority of organisms which are present in spray dried milk powder produce souring readily. 80.5 per cent. of the 1,304 cultures picked off the plates incubated at 37°C. and 74 per cent. from the plates incubated at 55°C. produced acidity and coagulation in litmus milk media.

Macey[42] also showed a preponderance of acid-forming bacteria from milk powder, but his figures are lower unless the percentage of acid peptonising cultures are included with those producing acid coagulation which is hardly justifiable.

viz.	Percentage	of	cultures	producing	weak acid 26.0%
	"	"	"	"	acid coagulation 26.8%
	"	"	"	"	acid peptonisation 12.8%

Since the production of acid and coagulation in milk media was the most characteristic property of the organisms isolated, most of the cultures examined in detail were from the litmus milk inoculations which showed these changes.

The 124 cultures obtained in this way, originating from colonies which grew on agar plates incubated at 37°C. and produced acid coagulation in milk, were, with three exceptions, all streptococci. These have been classified according to the suggestions given by Sherman[43] in his review on the streptococci and, with one exception in the 'lactic' division, all fall into the 'viridans' and 'enterococcus' divisions.

Primary grouping. In this grouping two of the more important

TABLE 12.

Litmus milk reactions of cultures obtained from spray-dried milk powder plates incubated at 37°C.

Factory	Total No. of colonies inoculated into litmus milk	Litmus milk									
		Unchanged			Acid	Acid coagulation			Alkali	Peptonisation		
		No further examination made	Microscopic examination showed			Total	Litmus Reduction also	Whey production also		Alkali	Unchanged in reaction	Slightly acid reaction
			No bacteria present	Bacteria present								
A	208		16	6	15	170	43	84				2
B	181	4	3	3	7	159	108	47			4	
C	184	33	18	5	7	113	64	21		4	3	1
D	303	12	13	3	7	269	100	75		1		
E	323	4	30	2	7	274	72	81		6		
G	105	12	9	15	4	64	16	39				1
Total	1,304	65	89	33	47	1,049	403	347		11	7	4
%		5.0	6.9	2.3	3.6	80.5	31.0	27.0		0.9	0.5	0.3

TABLE 13.

Litmus milk reactions of cultures obtained from spray-dried milk powder plates incubated at 55°C.

Factory	Total No. of colonies inoculated into litmus milk	Litmus milk							
		Unchanged			Acid	Acid coagulation			Peptonisation Slightly acid reaction
		No further examination made	Microscopic examination showed No bacteria present	Microscopic examination showed Bacteria present		Total	Litmus Reduction also	Whey production also	
A	29	17	8			3			1
B	134	16	8	3	10	97	46	14	
C	147	10			22	115	84	2	
D	42		3			39			
E	7					7	7		
G	19					19			
Total	378	43	19	3	32	280	137	16	1
%		11.4	5.0	0.8	8.5	74.0	36.2	4.2	0.3

"tolerance tests", namely the growth in media of pH 9.6 and growth in the presence of 6.5 per cent. sodium chloride, have not given as sharp a line of distinction as the data given in Sherman's review suggests.

In glucose agar stabs of pH 6.8 the growth of the streptococci examined was invariably heavy and throughout the line of inoculation after a period of from 1 to 3 days' incubation at 37°C., but when the pH of the same media was raised to 9.6 growth was in several instances less definite. Occasionally slight growth occurred at the top end of the stab after 2 to 3 days but for some cultures this was variable. When the streptococci were inoculated into 2 per cent. glucose broth containing 6.5 per cent. salt a few cultures placed later in the 'enterococcus' division only grew very lightly. It was possible to pick out the cultures which developed as heavily as they would have done in the same media without salt, and also to select the large number of cultures which failed to develop at all. In between these extremes of development, however, the growth of about 15 per cent. of the 'enterococcus' strains was less marked. Tolerance of 6.5 per cent. salt was determined by changes in the pH of the glucose broth media since all the strains examined fermented glucose. In addition growth was indicated by an increase in the turbidity of the broth.

Detailed analyses of the strains of streptococci isolated are given in Tables 14 and 15. It will be seen from Table 14 that 66 per cent. of the cultures were classified into the 'viridans' division and 33 per cent. into the 'enterococcus'

TABLE 14.

Classification of cultures causing acid coagulation of litmus milk obtained from agar plates of milk powder incubated at 37°C.

Division	Group or Species	No. of cultures isolated		Percentage	
Pyogenic	S. pyogenes S. mastitidis S. equi "Animal pyogenes" The "Human C" "Minute hemolytic" Group G streptococci Group E streptococci Group H streptococci	None			
Viridans	S. salivarius S. equinus S. bovis Varieties of S. bovis S. thermophilus	80	1 none 6 3* 4 3* 69	0.8% 0 5.0% 3.3% 57.0%	66.1
Lactic	S. lactis S. cremoris	1		0.8%	0.8
Enterococcus	S. fecalis S. liquefaciens S. zymogenes S. durans	40	8 4* none none 32 2*	6.6% 0 0 26.5%	33.1
Total number of cultures examined		121		100	

* Cultures which showed sufficient variation from fixed type characteristics to make their inclusion in the strain subject to question; such cultures have, however, been included in the percentage figures.

division. All these organisms were thermoduric streptococci and grew at 45°C., many cultures producing sufficient acidity to coagulate milk in 12 hours at this temperature, and they all survived 60°C. for 30 minutes. Crossley[36], working with the powder from one type of plant only, has also reported that acid-forming bacteria nearly always predominate in the spray dried milk powder and that thermoduric cocci form the bulk of the flora.

The differential tests on which the grouping was based is given in Table 15 under each species. A number of strains, referred to later in detail, gave occasional reactions to some of the tests which were dissimilar to those which would be expected for a particular group as a whole, although it appeared from the other tests that they should be placed in that group. The exceptional reactions of these cultures have been given in the reaction column as an extension.

Although 7 out of 11 possible strains of the non-pyogenic streptococci were recovered from spray dried milk powder, 83.5 per cent. of the cultures were restricted to strains of S. thermophilus or S. durans, over twice as many strains of the former being isolated as of the latter.

'Viridans' Division. Regarding the differentiation of the streptococci placed in this division the greatest number, i.e. 86 per cent., of strains were of S. thermophilus and no difficulty was encountered in classifying them by the method advocated by Sherman[45], except in regarding their tolerance to 2 per cent.

TABLE 15.

		Viridans Division								Enterococcus Division							
		S. salivarius	S. equinus	S. bovis	*	Varieties of S. bovis	*	S. thermophilus	*	S. faecalis	*	S. liquefaciens	S. zymogenes		*	S. durans	*	*
No. of strains		1	0	3	3	1	4	45	24	4	4	0	0		24	6	1	1
Primary characteristics	Haemolysis	-	-	-		-		-		-		-	+	+	24-	6-	1-	1-
	Growth at 10°C.	-	-	-		-		-		+		+	+	+				
	45°C.	±	+	+		+		+		+		+	+	+				
	Growth in the presence of: 6.5% NaCl	-	-	-		-		-		+		+	+	+				
	pH 9.6	-	-	-		-		-		+		+	+	+				
	.1% methylene blue	-	-	-		-		-		+		+	+	+				
	Survival at 60°C. for 30 mins.	-	±	+		+		+		+		+	+	+				
	Strong reduction	-	-	-		-		-		+		+	+	-				
Additional characteristics	Growth in 2% NaCl	+	+	+		+		-	24 ? +									
	Starch hydrolysed	-	±	+		-		-		-		-	-	-				
	Aesculin split	±	+	+	3-	+	2+ 2-	-		+		+	+	+				
	Gelatine liquified	-	-	-		-		-		-		+	±	-				
	Milk curdled	±	-	±		±		+		+		+	+	+				
	Final pH in glucose broth	5.4 4.0	4.5 4.0	4.5 4.0		4.5 4.0		4.5 4.0		4.5 4.0		4.5 4.0	4.5 4.0	4.5 4.0				
	Acid from: Maltose	+	+	+		+		-		+		+	+	+				
	Sucrose	+	±	+		+	2+ 2-	+		±	3+ 1-	+	+	-	21- 3+	1- 5+	1+	1-
	Lactose	+	-	+		+		+		+		+	+	+				
	Raffinose	±	±	+		+	3+ 1-	±		±	2+ 2-	±	±	-				
	Glycerol	-	-	-		-		-		±	3+ 1-	+	+	-			1+	1+
	Sorbitol	-	-	±		-	1+ 3-	-		+	3+ 1-	+	+	-		6+		1+

* Strains which showed variations from type species of a particular group but which have been included in that group for classification purposes.

sodium chloride. 2 per cent. glucose broth, in which heavy growth occurred with these streptococci, was again used as the basic media for the 2 per cent. salt tolerance tests, and it was noted that about 35 per cent. of the strains of S. thermophilus grew slightly and irregularly in this salt broth. Sherman(44) reports that as the total number of cultures examined by the two percentage salt tolerance tests has been small he would be surprised to find on the examination of greater numbers that all strains of S. thermophilus were inhibited by this amount of salt. He suggests that as the salt tolerance of S. thermophilus is a unique property a slight increase in the percentage of salt might be better as an empirical test for this group.

'Enterococcus' Division. None of the cultures classified as S. durans were able to haemolyse defibrinated horse blood on poured (not streaked) agar plate culture, although blood haemolysis is considered by Sherman(43) to be one of the primary characteristics of S. durans. Sherman(44) reports more recently that a few strains of S. durans have been encountered in his laboratory which are non-haemolytic and he considers that it is logical to anticipate the existence of such strains, since the same type of relationship exists between the other so-called species in the 'enterococcus' division. It should be noted that 9 of the strains classified as S. durans produced acid from sucrose. Sherman in his review characterises S. durans cultures as failing to ferment sucrose, but more recently Smith and Sherman(45) report that about 25 per cent. of the strains of

S. durans isolated from human faeces produce acid. Six strains also classified as S. durans fermented sorbitol, which is usually considered to be unattacked.

3. Classification of milk curdling cultures obtained from plates incubated at 55°C.

Thirty cultures producing an acid coagulum in litmus milk have been obtained from colonies picked off agar plates previously incubated at 55°C. and examined in detail. All of these cultures were found to be obligate thermophilic sporeformers which grew strongly at a temperature of from 55°C. to 60°C. but not at 37°C. Acid production was very fast and it was not uncommon for litmus milk to be coagulated in 18 hours at 55°C. This type of thermophilic sporeformer was first encountered in February 1936 when Factory D, which works on a continuous system, encountered difficulties from (1) milk clotting in the nozzles of the spray and in the high pressure pipe line supplying it at temperatures of 130 to 160°F., (2) coagulation of batches of milk in the holding tanks at 160°F., and (3) high acidity in some of the dried powder. From tests of the incoming milk and of the milk throughout processing it was seen that, although the thermophilic organisms were present in small numbers in the incoming milk as might be expected in any similar bulk of mixed separated milk, they increased throughout the operation of the plant, reaching very high numbers in the final concentrated milk which was pumped to the spray. It was apparent that thermophiles were the cause of the trouble, for all the cultures isolated grew at 70°C. and produced sufficient acidity to coagulate milk in under

15 hours. The plant was running for about 20 hours out of 24, and some of the incoming milk was held at 160°F. for the whole of that time. Hence the conditions in the plant were ideal for the proliferation of the organisms and consequently it is not surprising that difficulties from the acid produced by them arose. The plant was not sterilised as thoroughly as the conditions of processing necessitated and this, together with the fact that some of the concentrated milk remaining from the previous day was dried first on the following day's operation, obviously continued to seed the plant from day to day.

A study of the characteristics of the 30 cultures mentioned above showed that they were all strains of B. calidolactis[25]. This organism, as already noted, was observed by Hammer and Hussong[25] in the first instance as the cause of the coagulation of separated milk which was held at 71 to 77°C. before drying, and it has also been reported by them to coagulate evaporated milk on storage at high temperatures.

Prickett[46] found from an extensive examination of different samples which included raw and pasteurised milk, milk powder, milk-stone and some cattle foods, that B. calidolactis was one of only two thermophilic sporeforming bacteria which he isolated that produced an acid curd in milk. B. thermoliquefaciens, the other sporing thermophile reported by him to curdle milk, was very similar to B. calidolactis, except that it liquified gelatine and always reduced nitrate, whereas reduction of nitrate by B. calidolactis is variable.

The cultures examined agreed with the description of B. calidolactis already recorded[25] except that some strains grew more readily on agar slants. This has also been noted by Prickett[46]. In addition it was found that after several cultivations in the laboratory on agar slants the extent of growth of some strains which had grown scantily at first increased and that the growth on milk agar slants was usually good.

4. Other organisms examined.

The numbers of bacteria causing peptonisation at 37°C. were very low and almost negligible at 55°C. The 10 cultures causing peptonisation at 37°C., which were examined in detail, were all aerobic sporeformers of the B. subtilis type, which are, as has been previously stated, common in most dairy products. The strains isolated were as follows:-

B. subtilis	4
B. licheniformis	4
B. pumilus	2

In addition one aerobic sporeforming thermophile was isolated, which produced large terminal spores readily on agar but only digested milk very slightly after several days. The identity of this organism was not definitely established and since it did not appear to be very common it is probably not of commercial importance.

Examination of the plates throughout the analysis showed that aerobic sporeformers were not present in large numbers and seldom occurred in plates made from less than .001 g. of powder

at 37°C. or .1 g. at 55°C. Thus they were found to be more prevalent in the lowest count samples and were particularly evident in the samples from Factories C and B.

About 14 per cent. of the inoculations from agar plates held at 37°C. which were made into litmus milk did not change the media visibly, and 9 per cent. were further examined microscopically to see if bacteria were present. About 7 per cent. of these inoculations showed no growth, so that either the inoculations had been faulty or the organisms did not grow in milk. The organisms which grew in the milk at 37 and 55°C., but caused no change under the conditions of test, were a mixed variety of cocci and bacteria, and since they have probably little significance as far as the reconstituted milk is concerned they were not examined further.

IV. Factors affecting variability in bacteriological quality of spray dried milk powders.

As already indicated, the bacteriological quality of the spray dried powders was found to vary widely, whether assessed by the plate count or by the methylene blue reduction test. It was felt desirable to study the figures in more detail in order, if possible, to determine the chief factors responsible for the observed variations.

1. Variations in the powder of different factories.

Table 8 (p. 57) shows that there were wide variations in the mean plate counts from one factory to another, extending

from roughly 20,000 per g. with Factory C to over 12,000,000 per g. with Factory D. The mean values of the reduction times showed corresponding variations, the figures varying from 11.03 hours with Factory C to barely half this period (6.84 hours) with Factory D. It is obvious therefore that differences in the quality of the incoming milk supply and/or the technique of manufacture at the various factories caused considerable variations in the quality of the final product.

2. Variations in the powder from the same factory.

Apart from variations in the mean counts from one factory to another, marked differences were found in the ranges of the counts obtained at each individual factory. These are to some extent apparent in the standard deviations and coefficients of variation of the logarithms of the plate counts (Table 8). But they are perhaps most clearly shown from a study of the maximum and minimum figures, which give the extreme ranges of the counts. Thus, whereas the counts for Factory C varied only from 1,500 to 129,000 per g., and for Factory G only from 15,600 to 450,000 per g. Factory A showed a range of from 46,000 to 20,200,000 per g. and Factory D the extreme values of 28,000 to 149,000,000. Corresponding variations were found in the reduction times. Apart therefore from any general differences in the technique of manufacture between the different factories, it may be said that in some factories there must have been a complete lack of consistency in the day-to-day management of the plant.

3. Variation in the powder from the same factory during the same day's operation.

It might be expected that, as many of the factories were operated continuously during the day's run, the bacteriological quality would deteriorate through the multiplication of thermophilic or thermoduric bacteria during processing. Such deterioration has, of course, been frequently observed in large scale pasteurising plants when tanks have been used for long periods without intermittent sterilisation. Thus powder produced during the first few hours of plant operation might be expected to be of a higher quality than that produced at the end of the day's run. The mean values of the plate counts and reduction times at 37°C. and 55°C. (Table 16) of powders sampled at the beginning of processing and towards the end of the same day's manufacture demonstrate that there is, in fact, some indication of a falling off in bacteriological quality, though the results are by no means consistent. It will be seen, however, that the results of the plate counts made at 55°C. are definitely more consistent than those made at 37°C., which appears to indicate (as would be expected) that any increase in count is attributable to thermophilic and thermoduric types.

It is doubtful, however, whether the figures in Table 16 can be adequately interpreted without reference to the exact method of processing involved at each individual factory. This point may be illustrated by reference to Factory E. At this factory the day's operations usually began by drying

TABLE 16.

Comparison of results of tests on powder sampled at the beginning and at the end of the same day's operation.

Factory	Samples	Log. Plate Count at 37°C. No. of samples	Log. Plate Count at 37°C. Mean	M.B.R.T.* at 37°C. No. of samples	M.B.R.T.* at 37°C. Mean	Log. Plate Count at 55°C. No. of samples	Log. Plate Count at 55°C. Mean	M.B.R.T. at 55°C. No. of samples	M.B.R.T. at 55°C. Mean
A	First	7	5.80	6	9.17	7	4.13	5	6.90
	Last	7	6.02	6	7.83	7	4.29	5	8.70
B	First	19	5.81	19	7.55	18	3.55	19	10.71
	Last	19	5.74	19	7.68	18	3.66	19	9.47
D	First	17	6.21	18	6.92	16	3.85	13	9.15
	Last	17	6.14	18	6.85	16	4.40	13	7.27
E	First	28	5.53	29	7.80	17	3.58	22	9.36
	Last	28	6.12	29	7.53	17	3.75	22	8.68
F	First	4	5.32	3	9.50	2	2.67	1	12.5
	Last	4	5.27	3	8.50	2	2.50	1	12.5
G	First	4	4.54	4	9.38	3	2.07	-	
	Last	4	5.13	4	7.63	3	3.63	-	
Total	First	79	5.71	79	7.78	63	3.66	60	9.46
	Last	79	5.93	79	7.53	63	3.90	60	7.58

* Methylene blue reduction test.

some of the precondensed milk which had remained from the previous day's manufacture. This practice increased the economy of working, but it is clear that the old precondensed milk (especially when inadequately cooled between the two different days' operations) would be heavily contaminated with both ordinary milk flora and thermoduric bacteria. Hence the first powder to be dried would probably have an abnormally high count; this count would then be diluted as fresh milk entered the precondenser, but the count of the latter (being well seeded with thermoduric bacteria from the old precondensed milk) would again gradually increase as the day's run progressed. That such a sequence actually occurred in Factory E was shown in tests carried out on samples of powder taken at intervals throughout a single day's run, the results of the tests being as follows:-

Factory E Sample No. July 1938	Hours after start of manufacture when sampled	Plate Count at 37°C.	Reduction Test at 37°C.	Plate Count at 55°C.	Reduction Test at 55°C.
103	0	36,000,000	4¼	1,300	7½
104	2	8,900,000	7½	370	10½
105	4	5,800,000	7½	550	10½
106	6	1,310,000	7	1,700	8½
107	9	2,150,000	6¼	2,600	7
108	11	5,300,000	5½	3,200	7½

Such a regular sequence is probably exceptional, since many adventitious factors may affect the bacteriological quality of the product at any given time. The above results serve, however, to show that while the figures given in Table 16 cannot be taken at their face value, they probably tend to under-

estimate rather than to over-estimate the detrimental effect of continuous operation on the quality of the powder.

4. Variation according to season of year.

An examination of the results obtained on samples of powder obtained from individual factories shows that seasonal variations in quality only occur where the general bacterial standard is normally poor. This fact is illustrated, in the forms of histograms, in figures 1, 2 and 3, where the average figures for a series of daily runs are shown separately for the summer and winter periods. Referring to figure 1 (plate counts at 37°C.), it will be seen that at Factory C (which has been shown in Table 8 to produce powder of uniformly good quality) there is no difference in the range or distribution of the counts between summer and winter. On the other hand at Factories D and E (which have been shown to produce powder of relatively poor quality) the distribution of the counts varies between summer and winter, the summer period having a markedly higher proportion of high counts. Similar results are shown in figure 2 (plate counts at 55°C), while from figure 3 it will be seen that the distribution of methylene blue reduction times shows corresponding variations, there being no difference between the summer and winter tests for Factory C, but marked differences for Factories D and E. These results show once again that with consistent management of the plant, a factory can produce milk powder of high quality throughout the year, while with poor management special difficulties will inevitably be experienced

FIGURE 1 LOCALITIES OF THE PLATE COUNT AT 37°C.

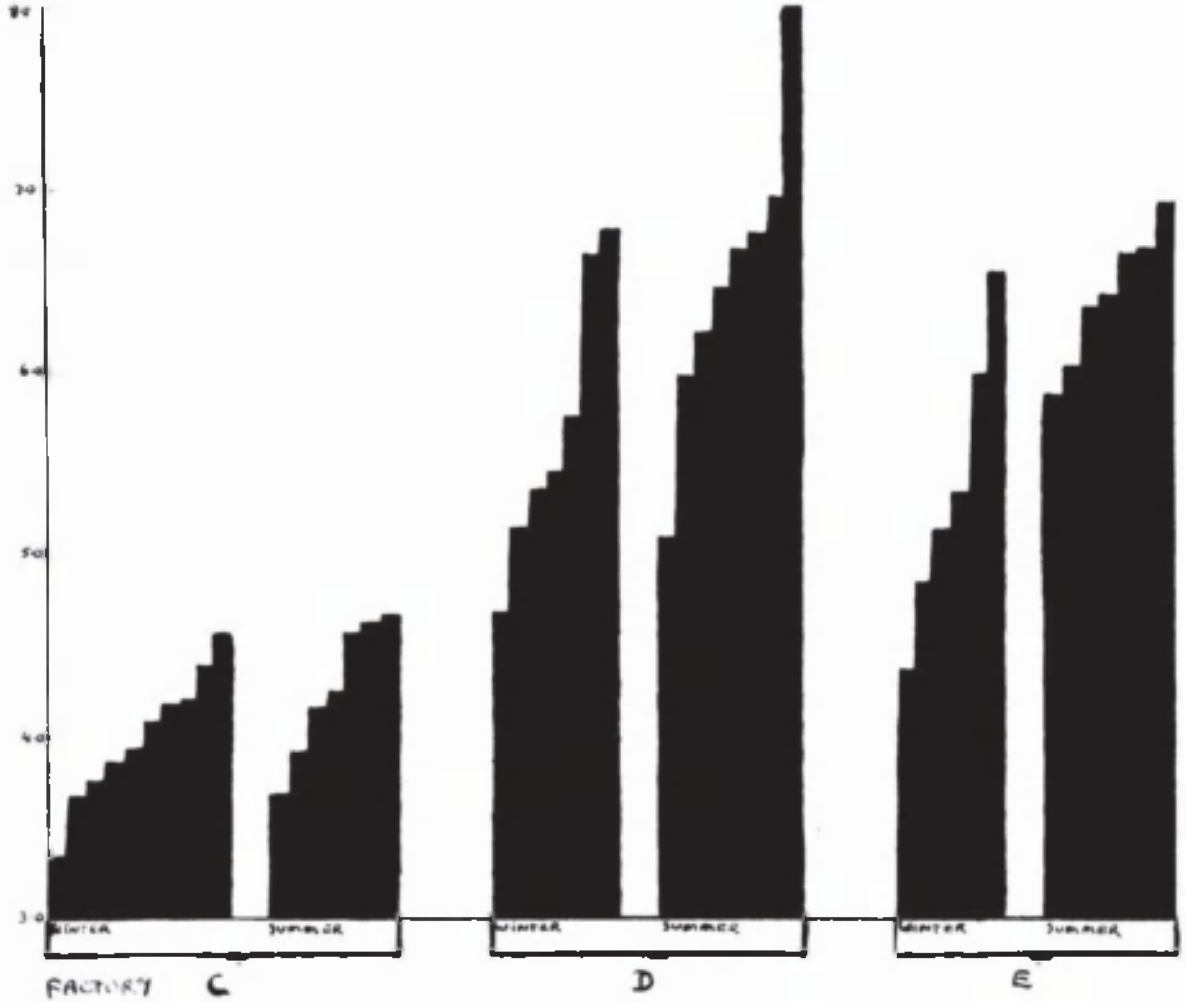
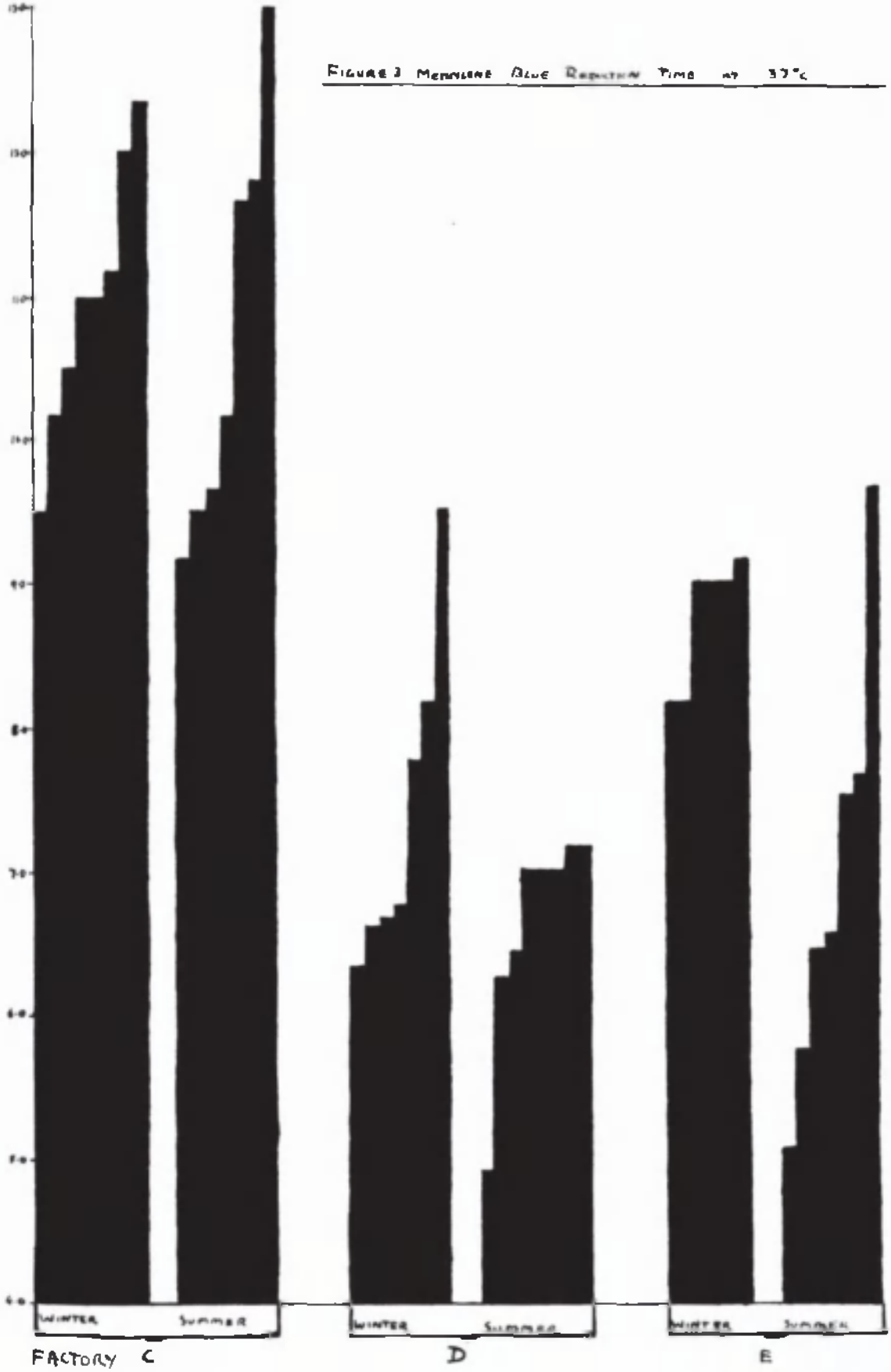


FIGURE 3 METHYLENE BLUE REDUCTION TIME AT 37°C



under summer conditions when not only the total bacterial content of the incoming raw milk but its content of heat resistant streptococci[47] will be exceptionally high.

Note on Figures 1, 2 and 3.

In the above figures the winter period was taken as extending from the beginning of October until the end of March, and the summer period consisted of the other six months of the year.

Each of the values plotted in the figures represents the mean value of all the samples tested from one particular day's operation at the three different factories, and they were usually the mean values calculated from 3 to 6 samples.

The plate counts at 37 and at 55°C. are given as logarithmic values and the reduction time at 37°C. in hours. The total number of samples on which the mean values for the histograms were based is given below:-

Factory	Winter Total No. of samples examined by:-			Summer Total No. of samples examined by:-		
	Plate Count at 37°C.	Plate Count at 55°C.	Reduction Test at 37°C.	Plate Count at 37°C.	Plate Count at 55°C.	Reduction Test at 37°C.
C	30	30	29	21	21	21
D	22	22	22	39	38	39
E	18	16	18	35	17	35

5. Variation between full cream and skimmed milk powders.

Since only a limited number of full cream milk powder samples was examined, and since the variations in the quality of samples obtained from individual factories have been shown to be large, a direct comparison between the bacterial content of full cream and separated milk powders has been limited to samples obtained from a single factory, i.e. Factory E. The relevant figures are shown in Table 17. It will be seen that the mean values of the plate counts differed appreciably, that of the skimmed milk powder being about eight times as high as that of the full cream product. Corresponding results were obtained for the methylene blue reduction tests. A careful study of the standard errors of the logarithms of the plate counts indicates moreover that, although the ranges of the counts did not differ materially, the means are probably significantly different. The same conclusion applies to the reduction tests, and it will be seen that in this case the skimmed milk powder actually had a wider range of reduction times, the minimum being $3\frac{1}{2}$ hours as compared with 6 hours for the full cream powder. It is possible that these differences are associated with variations in the quality of the incoming milk, since it might be expected that fresh whole milk would have a smaller bacterial flora than separated milk.

6. Variations during storage.

(a) Variations at room temperature. Previous workers[40,42] have shown that during prolonged storage the viable bacteria

TABLE 17.

Factory E.

		Powder made from: Whole milk	Skimmed milk
No. of samples examined		42*	107
Plate Count at 37°C.			
Mean		511,000	4,180,000
Range	Minimum	1,400	5,000
	Maximum	45,500,000	36,000,000
Log. of Plate Count at 37°C.			
Mean		5.31	6.00
Standard Deviation		1.278	0.844
Coefficient of Variation		24.07%	14.07%
Range	Minimum	3.15	3.70
	Maximum	7.66	7.56
Methylene Blue Reduction Time at 37°C.			
Mean		9.20	7.45
Standard Deviation		1.712	1.597
Coefficient of Variation		18.6%	21.5%
Range	Minimum	6.0	3.5
	Maximum	12.5	12.5

* 11 of these samples were not included in the total for Factory E given in Table 8 since they were examined after the statistical analysis had been made.

initially present in milk powders tend to die out. This observation was confirmed in the present study. The following table gives a summary of the results obtained in the periodic examination of a series of samples of typical spray-dried powders:-

Time stored at laboratory temperature	0	1½	Months 3	6	8	12
Mean % reduction in count		50%	67%	73%	76%	95%
Range of % reduction in count		1-96%	25-91%	5-99%	29-98%	91-98%
No. of samples examined	43	12	18	28	16	7

While the extent of the reduction in bacterial flora varied widely from sample to sample (as shown from the figures of the range of percentage reductions), the mean figures showed that there was a progressive fall during storage, plate counts made on powders kept in storage for 12 months giving counts which were less than 10 per cent. of their initial values. Thus (to take two typical samples) with sample D 72 an initial count of 330,000 per g. was reduced to 29,000 per g., while with sample E 140 an initial count of 5,200,000 per g. was reduced to 500,000 per g.

Other tests for bacteriological quality also indicated that the number of bacteria decreased on storage. Thus with

the keeping quality tests, the number of days before the samples clotted on boiling tended to increase by ½ to 1 day on 12 months' storage, particularly if the initial keeping quality was 3 or less than 3 days. When, however, the original keeping quality was 3½ to 4 days as with many of the samples which had a low plate count, the initial keeping quality did not increase appreciably on storage.

The reduction tests at 37°C. also generally reflected a reduction in bacteriological activity as the samples aged and reduction times increased, especially when the original times were short. For instance, the methylene blue reduction time of sample E 26 was 3½ hours initially; after 6 weeks' storage it had risen by 1 hour, and after 6 months it required twice as long for the methylene blue to be reduced. However, with sample F 5, the increase was less marked, the reduction time increasing to 10½ hours from 9½ hours after 6 months' storage.

The plate counts and reduction times at 55°C. indicated on the whole a reduction in numbers and bacteriological activity but the results were less marked and less consistent than for the same tests carried out at 37°C.

It may be noted that this apparent improvement in the bacteriological quality of milk powders during storage is of considerable importance in relation to grading standards, since it is quite probable that a powder which failed to attain a given standard when examined soon after production would qualify by the same standard when examined after several months' storage.

It is clear, therefore, that in formulating quality standards the approximate age of the powder at the time of testing would need to be stipulated.

(b) Variations at different temperatures. Table 18 shows that the bacterial flora tend to die out more rapidly as the temperature of storage is raised. The initial plate counts of the powders show considerable variations (probably ranging from 100 to 250 millions per g. of powder), but if the lower value be taken as a standard it will be seen that after six months' storage in a refrigerator the count has been reduced by 13 per cent. (i.e. to 87,000,000 per g.), whereas at room temperature it has been reduced by 82 per cent., at 22°C. by 98 per cent., and at 40°C. by 99.9 per cent. (i.e. to only 94,000 per g.). Results obtained with the methylene blue reduction tests confirmed in general these findings, the reduction times for samples stored in a refrigerator and at room temperature remaining more or less constant, those for samples stored at 22°C. rising from 6½ hours to 8½ hours, and those for samples stored at 40°C. rising from 6½ hours to 10½ hours.

(c) Variations with storage in air versus nitrogen. Opportunity was taken to determine the relative effects of storage in air with storage in an inert gas, namely nitrogen. In both cases the milk powder was packed in sealed containers, but with one set of samples the container had been exhausted of air and re-

TABLE 18.

No. of days storage	Storage Temperature: Refrigerator (approx. 4°C.) Plate Count at 37°C. Tins Air filled	Refrigerator (approx. 4°C.) Plate Count at 37°C. Tins Nitrogen filled	Room Temperature (approx. 10°C.) Plate Count at 37°C. Tins Air filled	Room Temperature (approx. 10°C.) Plate Count at 37°C. Tins Nitrogen filled	22°C. Plate Count at 37°C. Tins Air filled	22°C. Plate Count at 37°C. Tins Nitrogen filled	40°C. Plate Count at 37°C. Tins Air filled	40°C. Plate Count at 37°C. Tins Nitrogen filled
9	111,000,000	70,000,000	141,000,000	81,000,000	59,000,000	154,000,000	33,000,000	60,000,000
18	147,700,000	102,400,000	54,400,000	89,400,000	81,500,000	126,200,000	2,730,000	11,200,000
28	93,000,000	265,000,000	88,000,000	102,000,000	51,000,000	98,000,000	1,570,000	4,600,000
84	88,000,000	181,000,000	89,000,000	97,000,000	16,200,000	28,300,000	330,000	490,000
127	148,000,000	160,000,000	20,600,000	86,000,000	7,600,000	13,100,000	173,000	231,000
140	103,000,000	175,000,000	204,000,000	302,000,000	7,900,000	12,400,000	260,000	289,000
164	88,000,000	77,000,000	39,000,000	65,000,000	4,400,000	18,500,000	112,000	160,000
185	87,000,000	57,000,000	18,500,000	62,000,000	2,520,000	7,900,000	94,000	79,000

filled with commercial nitrogen*. An examination of Table 18 indicates that in general the rate of reduction in bacterial flora was considerably retarded by the substitution of nitrogen for air. Thus after six months' storage at room temperature the plate count of powder in the air-filled containers showed an 82 per cent. reduction while that of powder in the nitrogen-filled containers showed only a 38 per cent. reduction. The equivalent figures after six months' storage at 22°C. were 98 per cent. and 92 per cent. At 40°C. there were no significant differences after prolonged storage (i.e. after 6 months) since the counts of both sets of samples had been reduced by over 99.9 per cent., but the differences were clearly apparent at the earlier stages of storage (up to 28 days).

It may be noted that the above differences in the rate of reduction of bacterial flora were seldom accompanied by any perceptible differences in the methylene blue reduction times.

V. The use of the methylene blue reduction test as a method of assessing the bacteriological quality of milk powders.

In the Appendix evidence is presented regarding the relationship between the plate count, the methylene blue reduction test and the keeping quality of samples of raw milk produced under a variety of conditions, and the conclusion was drawn that

* The chief object of using nitrogen was to attempt to prevent or retard fat deterioration (tallowiness), which is recognised to be due to oxidation. The bacteriological studies were therefore incidental to the main object of the investigation.

for the general purpose of classifying milk according to bacteriological quality the methylene blue reduction test appears to have outstanding advantages, among which may be mentioned simplicity, rapidity and cheapness. It seemed desirable to make a similar comparison of the relative values of the plate count and the reduction test in assessing the quality of milk powders.

Owing to the relatively small range of variation in the keeping qualities of the majority of samples examined* it was not practicable to attempt to correlate either of the above tests with keeping quality. The comparison has, therefore, been limited to a direct correlation between plate count and reduction test. Full data for the tests carried out at 37°C. are given in the form of a frequency table in Table 19. It will be seen that the majority of the figures fall along a broad band, the discrepancies being somewhat more numerous than with samples of raw milk (see Appendix). This difference is reflected in the correlation coefficients for the two series, the value of 'r' for the dried milk samples being -0.723 as against -0.818 for the raw milk samples.

It may be noted, too, that the regression equations for the two series differ, as shown in the following table:-

* It will be seen on reference to Table 10 (p. 60) that 93 per cent. of the samples had keeping qualities of between 3 and 4 days, which would allow only three values for comparison, i.e. 3, 3½ and 4 days. It was felt that this would be quite inadequate to enable a valid correlation coefficient to be calculated.

TABLE 19.

Frequency Table.

Log. of the plate count at 37°C.	Methylene blue reduction time in half-hours at 37°C.																						Total
	3	3½	4	4½	5	5½	6	6½	7	7½	8	8½	9	9½	10	10½	11	11½	12	12½	13	13½	14	
3.01-3.25																			1	1				2
3.26-3.50														1	1					2		2		6
3.51-3.75							1						1				4	3	1	1	2			13
3.76-4.00														2	3	2	3	1	2	1	3		1	18
4.01-4.25											1	1	1	1	2		3	2	1	1		1		14
4.26-4.50							1			2			3	4	4	1	3		1				1	20
4.51-4.75										1	3	2	5	2	3	1	2							19
4.76-5.00						2		1	1	1	1	4	2	5		1			1					19
5.01-5.25								1	2	5	4	3	5	6	1	1								28
5.26-5.50					1	1			1	4	2	3	4	1		1				1				19
5.51-5.75						2			5	5	2	10		1	1	2			1					29
5.76-6.00					1	1	1	5	11	7	3	6	3	1	1	2								42
6.01-6.25						3	2	9	6	7	3	3	3	1	1		1							39
6.26-6.50					3	3	9	8	6	7	2	3	6	2	1									50
6.51-6.75				1	2	2	2	5	2	3	1		1											19
6.76-7.00						5	1	1	4	5		3	1	1			1							22
7.01-7.25				2				2			1													5
7.26-7.50		1		3		1	1	2		2														10
7.51-7.75				1		1		1	1															4
7.76-8.00	1							3																4
8.01-8.25	1					1	1	2	1															6
	2	1		7	7	22	19	40	40	49	23	38	35	28	18	11	17	6	8	7	5	3	2	388

TABLE 20.

	Regression equations	Value of y when plate count is 200,000 per ml., i.e. when x = log. 5.30 hrs. mins.		Value of x when y = 5.0 hrs.	Plate count per ml. equivalent to value in preceding column.
Raw milk samples	x = -0.269y + 6.514 y = -2.486x +18.17	4	59	5.17	148,000
Dried milk samples	x = -0.3877y+ 8.778 y = -1.349x +15.769	7	16	5.84*	690,000*

where x = logarithm of the plate count
y = time in hours required to reduce methylene blue in the reduction test.

From this table it will be seen that for a given plate count the bacterial flora of reconstituted milk powder show a less active reducing power than the flora of raw milk; e.g. when the count is 200,000 per ml., the equivalent reduction time for reconstituted milk powder is $7\frac{1}{4}$ hours compared with 5 hours for the raw milk. It seems clear that this must be due to the very specialised type of flora which (as has already been shown) normally occurs in milk powders.

Since part of this flora consists of thermophilic and thermoduric bacteria it was felt that a better correlation might

* These figures refer to the reconstituted milk powder, which will contain per ml. only one-tenth of the bacterial flora which is present per g. of powder.

TABLE 21.

Frequency Table.

Log. of the plate count at 55°C.	Methylene blue reduction test at 55°C. (in half-hours)																							Total
	3½	4	4½	5	5½	6	6½	7	7½	8	8½	9	9½	10	10½	11	11½	12	12½	13	13½	14	14½	
1.76-2.00							1	1							1			1						4
2.01-2.25						1			2	1	1			1							1			7
2.26-2.50						1		1		1	1		2	1										7
2.51-2.75							1	1			1	5	1	2	1		1		1					14
2.76-3.00						1			3				2		2	2	1	1	2	2	2		1	19
3.01-3.25								1	4	2	2	1	2	1	2		1							16
3.26-3.50				1				2	1	1	3	2	2	5	2	1	1		1	1		2		25
3.51-3.75									1	1	1	2	1	1	4			1	1	3	2	1		19
3.76-4.00						3		1	1		2	3	3	2		3	2	2	1	1	1	1		26
4.01-4.25					1				1	4	1	1	1			1				2				12
4.26-4.50	2				1		2	1	1	5	2	1		1	3		1		1			1		22
4.51-4.75						1	1	5	2	4	2	2	1	3	2	1	1	1	1					27
4.76-5.00				1	1			3	2	2		1	1	1	1	2						1		16
5.01-5.25			1			2	4	1	2	1	1	1		1								1		15
5.26-5.50						1		1	1	4		1		1	2									11
5.51-5.75						1	1		1	1	1	3	1	2										11
5.76-6.00											1													1
6.01-6.25							1				1													2
	2		1	2	3	11	11	18	22	27	20	23	17	22	20	10	8	6	8	9	6	7	1	254

exist between plate counts and reduction times when these were carried out at 55°C. Table 21 shows, however, that the frequencies are widely scattered, and calculation of the correlation coefficient gives the low value for 'r' of -0.221.

It appears from the above facts that when the reduction test is carried out at 37°C. it shows reasonable agreement with the plate count provided that the correct regression equation is used. On the other hand there is a lack of agreement between the reduction test at 55°C. and the numbers of thermophilic and thermoduric bacteria in the milk powder. This fact does not, however, invalidate the conclusion already stated on page 58 that the reduction test at 55°C. can provide useful information regarding the activity of such organisms.

VI. A note on the desirability of grading spray dried milk powder.

Data have been presented which show that wide variations exist in the bacteriological quality of spray dried milk powder but that these variations are least in the powder from the factories which generally produce the best quality powder. It has been indicated that powders of high bacteriological content are probably due to poor technique of operation at the factory. Hucker and Hucker[48] have also demonstrated this fact and have shown that by the introduction of special sanitary methods at drying plants the counts of commercially prepared infant foods were greatly reduced. Hence it seems reasonable to suppose

that milk powder of high bacteriological quality can be consistently produced.

The use of milk powder in the preparation of other food products is becoming of increasing importance, but it is essential to the manufacturer of a proprietary food product that he should maintain a uniform quality in the product from day to day. In order to do this he obviously requires ingredients of unvarying quality. It is clear therefore that the grading of milk powders would be of value to the purchaser. Moreover, the provision of definite grade standards of quality would also act as an inducement to raise the standards of operation at many existing milk drying plants. The value of such grading has already been appreciated in the United States where the American Dry Milk Institute[49,37] has successfully operated grades for skimmed milk powder since 1930.

It is only intended here to mention briefly some of the factors which might affect the bacteriological standards for milk powders. Bacteriological grades should probably be based entirely on the examination of the final product, but as a general safeguard the incoming milk, which should be fresh, clean and free from abnormal taste and smell, must be pasteurised, preferably by the holder system, at a minimum temperature of 145°F. for at least half an hour. It might in some operations be simpler to "flash" pasteurise the milk but the process must be controlled so that the incoming raw milk or partially heated milk is not mixed with that which has been

rendered free from disease-producing organisms by pasteurisation. Moreover from a chemical point of view the heat treatment should be sufficient to destroy any enzymes (e.g. lipase) which might adversely affect the keeping quality of the powder.

It would be important, if grades were adopted for spray dried milk powder, to specify that all the testing should be carried out within a definite time after manufacture, since aging seriously changes the quality. Three grades, namely First, Standard and Third Grade, are in operation in the United States for dried milk solids, and the plate count at 37°C. forms the basis of the bacteriological limits of the grades. Similar standards might well be adopted in this country. In order to determine what percentage of the samples would fall within the limits of the plate count specified by the American grades and within the limits of the same count expressed in terms of the reduction test, the results of the tests carried out during the present survey have been collected in Table 22.

It will be seen from this table that 36 per cent. of the samples of spray dried milk powder would fall within the American First Grade, and 48 per cent. within the Standard Grade, while over 40 per cent. would be classed as Third Grade. If the reduction times corresponding to the plate counts which have been obtained from the regression equation of these samples were to be used instead of the plate counts, 36 per cent. of the samples would be classed as First Grade, 54 per cent. as Standard and 48 per cent. as Third Grade. It would appear, therefore,

TABLE 22.

American Dry Milk Institute Grade Designation	Bacterial count per cc. of reconstituted milk for Grade	Log. of count of preceding column calculated per g. of powder	Reduction Times equivalent to log. of plate count of preceding column	Reduction Time taken as	Percentage of samples within the plate count standard of Grade	Percentage of samples within the reduction test equivalent to plate count of Grade
First	15,000	5.18	8.78 hours	9 hours	36	36
Standard	50,000	5.70	8.09 "	8 "	48	54
					Percentage of samples over the plate count which is lower limit for Grade	Percentage of samples over the reduction test equivalent to plate count of preceding column
Third	over 100,000	6.00	7.68 "	7½ "	41	48

that the reduction test, if used alone for grading, would impose a slightly more severe standard and that a reduction time of 8 hours or more might be taken to indicate a powder of relatively good bacteriological quality.

PART V. HEAT RESISTANCE OF MILK MICRO-ORGANISMS.

A. Heat Resistance of General Flora of Milk (i.e. Non-sporeformers).

1. The effect of the reaction (pH) of the milk on the destruction of milk bacteria by the heat of pasteurisation.

   (i) General considerations.
   (ii) Experimental.
   (iii) Discussion of results.

2. The effect of increased butterfat percentage on the destruction of milk bacteria by heat.

   (i) General considerations.
   (ii) Experimental.
   (iii) Discussion.

B. Heat Resistance of Aerobic Sporeformers.

1. Review of the literature.

2. Substrate container for heat resistance tests.

   (a) Glass capillary tubes.
   (b) Glass ampoules or bulbs.
   (c) Glass pasteur pipettes.
   (d) Test tubes.

3. General heat resistance trials on aerobic sporeformers.

4. Special studies on heat resistance of aerobic sporeformers.

   Preparation of spore suspensions.

   (i) The effect of the reaction (pH) of the substrate on the destruction of B. subtilis by heat.
   (ii) The effect of increased butterfat percentage on the destruction of B. subtilis by heat.

      (a) By survival percentage method.
      (b) By plate count method.

PART V. HEAT RESISTANCE OF MILK MICRO-ORGANISMS.

The literature dealing with the destruction of living micro-organisms by heat is extensive (see reviews (50) and (51)). It is clear that the heat resistance of sporeformers is a more complex process than for non-sporeformers, and that the resistance of bacteria to heat is affected by a great many subsidiary factors, as for example the composition of the substrate in which heating occurs, the strain and age of the culture, and the method of heating. Much of the work reported has been carried out under widely differing conditions of time, of temperature and of age of the cells, and thus it is often difficult to compare one set of results with another.

Since the types of bacteria which survive in the manufacture of canned dairy products and later cause spoilage either in the can or on reconstitution depend to a great extent on their heat resistance, a study has been made of certain aspects which might be expected to provide information on the subject. The work has been divided into investigations on the heat resistance of (A) the general flora of milk, i.e. of the non-sporeformers chiefly and of (B) a more specialised flora, the sporeformers.

A. Heat resistance of the general flora of milk (chiefly non-sporeformers).

Fore-warming, as outlined in Part I, in some form or other precedes the manufacture of all canned milks and milk powders.

This fore-warming may be a special process, or it may be equivalent to pasteurisation, or it may take place at the same time as condensing, but even if the method of fore-warming varies, in the preparation of all these products it has the same important function, that of reducing the initial count of the incoming milk. In the manufacture of sweetened condensed milk and spray dried milk powders, it is the most significant factor in the whole process in the reduction of bacteria.

1. The effect of the reaction (pH) of the milk on the destruction of milk bacteria by the heat of pasteurisation.

(i) General considerations.

The importance of the pH of the substrate on the resistance to heat of bacteria has long been recognised, and its effect on the destruction of the general flora of milk at low temperatures has been studied. Chick[52], for example, found that the rate of heat destruction of Bacterium typhosum suspensions in distilled water was increased by the addition of small quantities of acid or alkali. Bigelow and Esty[53] also demonstrated the importance of hydrogen ion concentration on the resistance to heat of bacteria.

It is generally supposed that when milk is pasteurised the relative destruction of micro-organisms is greater in milk of very high bacterial content than in milk containing comparatively few bacteria. Among the factors which might be responsible for this difference in percentage destruction it appeared possible that increasing acidity (associated with bacterial multiplication)

might be important. Moreover it seemed likely that this factor might be specially marked when the milk concerned was of the relatively low quality occasionally used for manufacture, such milk being either surplus to liquid requirements or being separated and therefore probably of high count. An investigation was conducted to determine the effect of alteration in pH on the destruction of milk bacteria by heat.

(ii) Experimental.

The following experiments[54] were carried out on samples of mixed milk from the districts which are shown in Table 23 in order to determine whether alteration in the hydrogen ion concentration would result in differences in the number of bacteria which survived the process of heating for 30 minutes at 145°F.

The samples of normal bulk raw milk were thoroughly mixed and divided into five portions of 100 ml. The experiments were carried out on this milk with its natural flora, so that tests are not subject to the objections which may be raised against the use of cultures added artificially to milk and then heated.

One portion of each sample, indicated by an asterisk in Table 23, was not treated in any way, but to the other four subsamples small volumes of N/10 NaOH or N/10 HCl were added slowly and with constant shaking. A titration curve of a representative sample of mixed milk, showing the shift in pH when any given quantity of acid or alkali was added, was used as a reference to determine roughly the quantities required for the subsamples in order to obtain a representative range of acidities.

TABLE 23.

No. of Experiment and source of milk	Sub-sample No.	pH of milk just before heating	Mean value of the plate count of the milk Before heating	After heating
I	1	6.92	183,000	4,100
Somersetshire	2*	6.71	179,000	3,500
	3	6.64	210,000	3,600
	4	6.50	190,000	5,300
	5	6.36	156,000	2,300
II	1	6.81	1,240,000	2,250
Berkshire	2	6.74	700,000	2,110
	3*	6.65	1,460,000	1,750
	4	6.48	820,000	2,000
	5	6.34	780,000	1,200
III	1	6.85	880,000	67,100
Somersetshire	2*	6.74	1,280,000	30,900
	3	6.52	780,000	9,300
	4	6.38	1,510,000	9,900
	5	6.24	1,120,000	8,800
IV	1	6.92	3,120,000	14,400
Somersetshire	2*	6.71	3,020,000	9,800
	3	6.60	4,340,000	9,400
	4	6.44	3,650,000	6,900
	5	6.30	3,610,000	9,100
V	1	6.87	113,000	11,660
Gloucestershire	2	6.76	86,000	5,020
	3*	6.64	82,000	2,180
	4	6.47	77,000	220
	5	6.27	90,000	200
VI	1	6.97	2,520,000	33,000
Somersetshire	2	6.78	2,800,000	20,800
	3*	6.60	2,900,000	14,300
	4	6.47	2,230,000	2,600
	5	6.25	2,600,000	2,100
VII	1	6.95	6,560,000	127,200
Somersetshire	2*	6.76	6,210,000	27,400
	3	6.62	5,620,000	6,400
	4	6.51	5,050,000	1,520
	5	6.31	5,420,000	1,250
VIII	1	7.00	30,500	1,770
Gloucestershire	2	6.85	28,500	1,030
	3*	6.66	22,900	520
	4	6.55	25,900	310
	5	6.41	27,700	510
IX	1	6.72	9,600,000	6,910
Somersetshire	2*	6.65	9,000,000	2,240
	3	6.59	8,700,000	1,330
	4	6.45	9,500,000	1,140
	5	6.37	10,800,000	730

* Indicates untouched raw milk.

The titration curve did not, of course, give exact information for all samples because of variations in buffer values, but it was a useful guide. After addition of acid or alkali the samples were left for at least 2 hours at 18°C. to come to equilibrium, after which the pH was determined by means of the quinhydrone electrode.

Immediately after the pH values had been ascertained the subsamples were heated in stoppered glass test-tubes at 145°F. ± 0.5° for 30 minutes by immersion in a thermostat. The temperature in the tubes was controlled by observing a standard thermometer fixed in one of them. After this pasteurisation the subsamples were quickly cooled and the dilutions plated in duplicate on milk agar. Each subsample of raw milk was also plated in duplicate immediately prior to heating. The plate counts of the samples before and after heat treatment were made after they had been incubated for 3 days at 37°C., and the results given in Table 23 are the mean values which were obtained from the counts of the duplicate plates.

(iii) Discussion of results.

The results show that in general the heat treatment has the greatest killing effect when the pH is lowest, i.e. as the acidity increases the number of bacteria which survive decreases. This appears to hold after the proper allowance has been made for sampling and other errors of the plate count, the counts of the raw milk subsamples secured from any one sample indicating the extent of the sampling error.

The data are, however, not quite homogeneous. In Experiments I, II and IV the differences are very small but the plate counts show the same general tendency to decrease with reduction of pH. In the remaining experiments when the pH exceeds 6.5 to 6.6 the number of colonies on the plates is notably greater than at the lower pH values. The indications are, however, that, although the tendency is for the surviving bacteria to decrease in numbers as the pH is lowered, additional factors also influence the percentage of the general flora which remain viable after heating, since at any given pH value the percentage destruction varies from sample to sample.

These results do suggest, however, that in pre-heating or fore-warming processes in the early stages of the manufacture of canned dairy products a greater reduction of initial count may be expected with the more acid milks.

2. The effect of increased butterfat percentage on the destruction of milk bacteria by heat.

(i) General considerations.

In addition to the pH of the substrate the composition of the medium itself has been shown(50) to be an important factor in the heat resistance of micro-organisms. Certain media have been reported to exert a protective action so that the killing effect of heat is reduced. Brown and Peiser(55) demonstrated this protective action for certain lactic acid bacteria by comparing the thermal death point, using 10 minute exposures to different temperatures, in cream of 25 per cent. butterfat

content, whole milk, separated milk, whey and bouillon. They showed that the temperature necessary to destroy all the bacteria in 10 minutes was lowest in broth, from 2° to 4°C. higher in whey, from 4° to 6°C. in separated milk, 8°C. in milk and from 12° to 14°C. higher in cream. The milk media were all made from the same milk sample and sterilised intermittently in flowing steam. Brown and Peiser attribute this marked increase in the heat resistance with the different milk media to the protective action of the butterfat alone.

At many stages in the various heat treatments of canned dairy products, there is a high butterfat content in the product. The fat content of milk is as high as 9 per cent. in the vacuum pan during the manufacture of condensed or evaporated milk. There is, moreover, from 23 to 25 per cent. of butterfat in cream during forewarming for the manufacture of canned cream. It was decided therefore to attempt to confirm the findings of Brown and Peiser by investigating the effect of increased fat percentage on the destruction by heat of non-sporeforming bacteria in milk.

(ii) Experimental.

The substrates were prepared by separating fresh mixed milk. The butterfat contents varied slightly with the different batches of media prepared and were as follows:-

Substrates	Butterfat content
Separated milk	< 0.1%
Whole milk	3.8-4%
Cream	10-12%
Cream	22-25%

The pH of these different media was determined by the quinhydrone electrode before and after sterilising. From the examples given below it will be seen that the differences before

	Before sterilisation	After sterilisation	Before sterilisation	After sterilisation
Separated milk	6.58	6.34	6.35	6.00
Whole milk	6.61	6.39	6.32	6.07
Cream (24% fat)	6.68	6.51	6.61	6.11

sterilisation were not great enough to allow further adjustment and that after sterilisation the differences in pH did not exceed a pH of 0.2. Hence in the range of media used the pH only varied very slightly, and since the pH of the media rose as the fat percentage increased any change in the pH of the cream would operate in favour of slightly greater heat resistance. All the media were homogenised, since this was desirable for the cream samples and was equivalent to the conditions which would prevail in manufacturing, and they were sterilised in bulk by

steaming on three successive days at 100°C. for 30 minutes.

Four non-sporeforming cultures, Nos. 15, 17, 31 and 33, which were species of the genus 'Escherichia' similar to some of the organisms used by Brown and Peiser and which produced an acid and gassy clot in litmus milk, were grown separately on several standard agar slants. In addition 2 cultures, No. 63 of S. thermophilus and No. 106 of S. durans, both of which produced acid coagulation in milk, were used. A suspension was prepared of each of the 6 cultures examined by washing the growth, after 2 days' incubation at 37°C., off the slants with sterile distilled water. The suspension was shaken thoroughly in a screw capped bottle in which glass beads were used to assist in breaking up the clumps, and was filtered through sterile cotton wool and a filter paper to remove any remaining clumps. An equal volume of the suspension was added to 200 ml. of skimmed milk, milk and cream of 10 per cent. butterfat. Cream substrate containing 23 per cent. butterfat was difficult to pipette if it was not used almost immediately, since it underwent age thickening, and it was only used in this experiment for Cultures Nos. 63 and 106. After thorough shaking the samples were plated on milk agar and 1 ml. amounts of the inoculated substrates were filled into small resistant glass test tubes and heated in triplicate or quadruplicate in a thermostatically controlled glycerine bath (see Part V (B), page 99, for discussion on container and method adopted for heat resistance studies). Three cultures, Nos. 33, 63 and 106 were

exposed for 10 minutes at different temperatures while the other three cultures were held at 60°C. for different lengths of time. In each experiment 2 minutes were allowed to lapse before recording the commencement of heating. After heating the tubes were immediately cooled in cold water, and they were then incubated at 37°C. Inspection of the media after incubation showed whether bacteria had survived heating.

(iii) Discussion.

The results are given in Table 24 (A) and (B). The plate counts of the original inoculated substrates before heating were very similar so that it is unlikely that any differences in heat resistance between them could be attributed to differences in the number of organisms present. The results of both increasing the temperature of holding for a fixed time (A) or increasing the time of holding at a fixed temperature (B) were not, from the somewhat inconsistent results, absolutely uniform. Even so, it is clear that the increase of butterfat had not increased the resistance of the micro-organisms to heat to any great extent; in fact the tendency in a few instances was for the heat resistance to be slightly less in cream.

B. Heat resistance of aerobic sporeformers.

1. Review of the literature.

The difficulties of investigations into the resistance of bacteria to heat are intensified when the organisms under consideration are in the form of spores. In work of this type a great variety of factors are involved and when any one of these

TABLE 24.

Bacterial resistance to heat in milk substrates of increasing butterfat percentage.

(A) Fixed time, variable temperature

	Substrate	Culture No. 33	Substrate	Culture No. 63
Plate count of inoculated substrate before heating	Skimmed milk	2,260,000	Skimmed milk	12,000
	Milk 3.8%	2,280,000	Milk 4.1%	14,100
	Cream 10%	2,620,000	Cream 12%	13,200
			Cream 22%	14,300

Temperatures of 10 minute exposures			Temperatures of 10 minutes exposures		
55°C.	Skimmed milk	+++	65°C.	Skimmed milk	++
	Milk	+++		Milk	++
	Cream	+++		Cream 12%	++
				Cream 22%	++
58°C.	Skimmed milk	+++	67°C.	Skimmed milk	++
	Milk	+++		Milk	++
	Cream	++		Cream 12%	++
				Cream 22%	++
60°C.	Skimmed milk	+++	69°C.	Skimmed milk	++
	Milk	+++		Milk	++
	Cream	+++		Cream 12%	++
				Cream 22%	++
62°C.	Skimmed milk	+++	71°C.	Skimmed milk	--
	Milk	+++		Milk	--
	Cream	++-		Cream 12%	--
				Cream 22%	++
64°C.	Skimmed milk	+--	73°C.	Skimmed milk	-
	Milk	+--		Milk	--
	Cream	++-		Cream 12%	--
				Cream 22%	--
66°C.	Skimmed milk	+--	75°C.	Skimmed milk	--
	Milk	++-		Milk	--
	Cream	---		Cream 12%	--
				Cream 22%	--
67½°C.	Skimmed milk	---	77°C.	Skimmed milk	--
	Milk	---		Milk	--
	Cream	---		Cream 12%	--
				Cream 22%	--

(B) Fixed temperature, variable time

	Substrate	Culture No. 106
Plate count of inoculated substrate before heating	Skimmed milk	1,450,000
	Milk 4.1%	1,580,000
	Cream 12%	1,390,000
	Cream 22%	1,830,000

Temperatures of 10 minutes exposures			Time of holding at 60°C.		Culture No. 15	Culture No. 17	Culture No. 31
				Skimmed milk	49,000	51,000	3,560,000
				Milk 3.8%	45,000	62,000	3,400,000
				Cream 10%	48,000	42,000	4,300,000
66°C.	Skimmed milk	++	5 mins.	Skimmed milk	++--	++++	++++
	Milk	++		Milk	++++	+++-	++++
	Cream 12%	++		Cream	++++	+++	++++
	Cream 22%	++					
69°C.	Skimmed milk	++	10 mins.	Skimmed milk	----	++-	++++
	Milk	++		Milk		++--	+++-
	Cream 12%	++		Cream	----	+---	++--
	Cream 22%	++					
71°C.	Skimmed milk	+-	15 mins.	Skimmed milk	----	+---	++++
	Milk	--		Milk	+---	----	++-
	Cream 12%	+-		Cream	----	----	+---
	Cream 22%	+-					
73°C.	Skimmed milk	--	20 mins.	Skimmed milk	----	----	++--
	Milk	--		Milk	----	----	+++-
	Cream 12%	--		Cream	----	----	----
	Cream 22%	--					
75°C.	Skimmed milk	--	25 mins.	Skimmed milk			++--
	Milk	--		Milk			++++
	Cream 12%	--		Cream			----
	Cream 22%	--					
77°C.	Skimmed milk	--	30 mins.	Skimmed milk			----
	Milk	--		Milk			----
	Cream 12%	--		Cream			----
	Cream 22%	--					
79°C.	Skimmed milk	--					
	Milk	--					
	Cream 12%	--					
	Cream 22%	--					
81°C.	Skimmed milk	--					
	Milk	--					
	Cream 12%	--					
	Cream 22%	--					

is being studied the others must be rigidly controlled and standardised. The whole problem, too, is often confused further by the dormancy or delay in germination of the spores themselves after they have been heated under the experimental conditions, so that even the numbers of surviving spores are often masked. These various difficulties will be better illustrated by referring briefly to some of the more important publications which have already appeared on this subject.

Bigelow and Esty[53] were probably among the earliest to examine the heat resistance of spores extensively. In this connexion they showed with studies on a thermophilic aerobic sporeformer the importance of hydrogen ion concentration and of the concentration of spores. Much work on C. botulinus and allied anaerobes followed and it was demonstrated[56] that the media in which the spores developed, the age of the spores, their concentration and the pH and salt concentration of the substrate all affected the thermal death rate.

Burke[57] showed that individual spores of C. botulinus might vary in the time required for germination and that they might germinate quickly or lie dormant for long periods after heating. It was also shown that cultures heated for periods up to a given temperature all grew uniformly when incubated under favourable conditions, but if heated for longer periods, when single tubes were used, the results would be irregular and inconsistent. Thus spore suspensions in a single tube series that had been treated for a time far in excess of those in which

uniform growth had been observed might contain viable organisms, even though several tubes in between were found to be sterile. These so-called "skips" were at first thought to be due to faulty technique but were later accounted for on the grounds of variation in the resistance of individual spores. Esty and Williams[58] therefore suggested a multiple tube method for recording the heat resistance and stated that by using 30 tubes of the same suspension heated for at least 4 different times at a fixed temperature a logarithm chart could be prepared from which it was possible to predict the resistance of the spores when one factor was varied. They demonstrated the usefulness of such a method in the control of C. botulinus. The work on the dormancy of this organism was extended to show that "skips" did not depend only on heat treatment but that delayed germination could mask the survival figures and that sudden changes in temperature or agitation accelerated germination after heat treatment. Dickson et al.[59] in pointing this out also stated that the heat resistance was greatest in broth which was covered with a thin layer of oil.

The heat resistance of B. subtilis and other common aerobic sporeformers including B. cereus, B. mycoides and B. mesentericus was investigated by Williams[60]. He found that the resistance of spores varied according to the kind of media on which they were produced and with the temperature of cultivation. but that dormancy was practically non-existant, since only 4 out of a total of 3,442 tubes which he tested failed to develop until

after the eighth day of incubation. Sommer[61] aimed at establishing a standard procedure for the mass production of heat resistant C. botulinus spores, and having developed resistant spores he sought to determine a technique for keeping them uniformly resistant. The results of several other workers were reviewed by him and of these the studies of Magoon[62,63] should be particularly noted. Magoon investigated several different types of sporeformers, amongst which were included the aerobic sporeforming bacteria. He concluded that a score of factors, many of them unknown, affected the development of heat resistant spores, and that there was no definite regularity of behaviour for different lots of spores under the methods of storage which he investigated. Williams[64] has attempted more recently to increase the heat resistance of spores by selecting single cells of 3 anaerobic and 3 aerobic sporeformers. He was, however, only able to show a significant increase with B. mycoides. He also noticed the "skip stop" phenomena with one of the anaerobic sporeformers which he examined.

Morrison and Rettger[21] found that the heat resistance and dormancy of 2 strains of bacteria, which have already been referred to as the cause of spoilage in evaporated milk, were variable when the spores were heated in water and subcultured in plain nutrient broth. However, when the spores were heated in evaporated milk with or without subculturing the results were regular and no dormancy was recorded, which suggested that unfavourable environment was the controlling factor. They

examined[65] the relationship of spore germination to environment further, and found that some organisms, e.g. B. subtilis and probably B. cereus and B. vulgatus. displayed no delayed germination, but B. megatherium behaved with inexplicable idiosyncrasies in certain media, and the strains which they had isolated from milk spoilage showed marked peculiarities in requirements for germination in different media.

Curran[66] investigated the influence of environmental factors on the thermal resistance of spores of B. cereus isolated from defective evaporated milk and concluded that the resistance of spores is not a fixed property and that the direction and extent of this change is largely determined by a number of physical and chemical forces operating outside the cell as, for example, temperature of storage and dryness.

It was thought therefore that, before attempting any special studies of the factors affecting the heat resistance of the sporeformers isolated from canned milk and cream, it would be advisable to obtain some general and approximate information on their heat resistance, when certain peculiarities of behaviour, as for instance dormancy, might become apparent which would modify further experiments. At this stage the choice of container for the heat resistance tests, to which reference has already been made, will be considered.

2. Substrate container for heat resistance tests.

The container in which inoculated substrate may be heated presents certain problems of technique. The difficulties

connected with the choice of a container are particularly evident at the high temperatures which are employed for the aerobic sporeformers and when milk or cream are used as the substrate. The points of importance in connexion with the container are:

(i) That it should be constructed so that the time necessary for the contents to reach the same temperature as the liquid in the bath is reduced to a minimum.

(ii) That few breakages should occur during operation.

(iii) That it should be easy to fill with substrate, or if the substrate has been sterilised inside the container it should be simple to inoculate it quickly.

(iv) That the examination for spore germination after heating should be simple and the possibilities of contamination of the substrate when transferred to other media be negligible.

A variety of containers has been suggested and the following have been tried:

(a) Glass capillary tubes. In these the temperature lag in heating the substrate was obviously very small, but in practice this advantage was outweighed by certain other factors. It was found, for example, that large numbers of capillary tubes broke or leaked when introduced into the hot bath and that even if the capillary tubes survived heating the transfer of the heated material into media for incubation without introducing outside contamination was difficult. This problem was particularly evident when the milk media clotted at some of the high

temperatures. In addition thick cream could not readily be drawn up into the capillaries.

(b) Glass ampoules or bulbs. Ampoules of glass sufficiently resistant to withstand autoclaving and heating without breakdown were not only difficult to seal quickly but were also difficult to break open for transference of the contents into media. Small ampoules were desirable so that the heating lag would be short, and consequently the ampoules were narrow necked and only pasteur pipettes could be used for filling them, and again it was not easy to do this when the substrate was cream.

(c) Glass pasteur pipettes were used as follows. The inoculated substrate was drawn up into the pipette which was then sealed at both ends. If pipettes were used which had a wide tip, cream could be drawn up readily by this method. These containers have thicker glass walls than the two already mentioned, and hence there would be a greater temperature lag in the heating of the substrate. It was found however that, although the filling was simplified, the breakages, which amounted to about 30 per cent., were so high that this container could not be employed satisfactorily.

If only a few heat resistant tests had been required probably any of these containers could have been used successfully, but as large numbers were usually necessary a method which involved frequent breakages did not seem practical. Hence a further method was explored in which use was made of special test tubes.

(d) Test tubes. Narrow tubes which had been sealed after the introduction of the inoculated substrate have been used successfully by several workers and the container finally adopted in this study was a test tube. The technique of filling the tubes was in general similar to that advocated by Bigelow and Esty(53).

Usually a large bulk of substrate was inoculated with the test culture, except in the trial tests on the sporeformers when the substrate contained in the tubes was inoculated directly from the culture. 1 ml. amounts of the inoculated substrate were transferred after thorough shaking by pipetting into small rimless test tubes, which were made of hard resistant glass. The tubes were 3" long and had an external diameter of $\frac{7}{16}$", and an internal diameter of $\frac{3}{8}$". The tubes were previously sterilised and plugged with cotton wool, but after filling were tightly corked by rubber bungs, which had been wrapped separately in grease-proof paper and sterilised in the autoclave in Kilner jars before use. The rubber bung usually extended down into the tube for at least $\frac{1}{2}$". The tubes containing substrate were placed in a small metal clamp which was screwed down so that the bung would continue to fit tightly even when exposed to high temperatures.

These small tubes fulfilled the requirements for a suitable container, since breakages were not very great. Such breakages as did occur were mainly with new batches of glassware or were caused before heating when the tubes were being bunged. Large

numbers of tubes could be filled quickly with approximately equal volumes of substrate, so that if the substrate was inoculated in bulk the number of organisms in each tube would be uniform. After heating the tubes could be incubated directly so that observations on the survival of bacteria might be made, or the whole of the contents could be transferred very quickly into tubes of other media either by pouring directly or by pipetting. It should be noted that in these studies litmus milk was the only medium used for reinoculation purposes, and that in the many tubes transferred in this way practically no contamination occurred. Regarding the heat transfer, this was obviously less efficient than with capillary tubes, but uniform tubes were used throughout so that the lag in attaining the maximum temperature was a constant factor. In the studies with non-sporeformers, 2 minutes were allowed to lapse before the recording of the time of heating began. With the sporeformers, however, because of the cooling effect on the glycerine when the cold tubes were introduced into the bath, the tubes were held for 2 minutes in boiling water first before being transferred to the bath, and the time was recorded from the moment of their immersion in the glycerine.

3. General heat resistance trials on aerobic sporeformers.

In order to obtain general information on the heat resistance of aerobic sporeformers cultures of these organisms were grown on milk agar slants for 2 days at 37°C. and held at room temperature for 3 days before being inoculated directly into

skimmed milk previously sterilised in the tube containers just described. Tubes were subjected to a much higher temperature than previously and the resistance to a time of 20 minutes instead of 10 minutes was investigated, since this time is more in keeping with that which is used commercially in canning. After heating, the tubes were cooled as before and incubated at 37°C. In most instances the survival of the organisms in the tubes was quickly apparent on incubation as the characteristic digestion of milk occurred, and microscopic examination confirmed this. Where, however, there was any doubt as to whether organisms had survived or not, or it appeared that none had withstood heating, the contents of the tube were transferred into litmus milk media and incubated for a minimum of 2 weeks but more often for 1 month. Usually the heat exposure tests were carried out in duplicate and frequently in triplicate. The results are shown in Table 25.

A similar general trial of heat resistance was carried out with some of the above strains and also with certain additional cultures which together totalled nearly 40 strains examined. In this trial, however, one inoculated tube of skimmed milk, another of milk and a third of cream (containing 23 per cent. butter fat) were heated at each temperature. As before, the tube contents were transferred into litmus milk and the survival in cream was read in nearly all instances by this method, since the consistency of this substrate made direct reading of growth difficult in the early stages. The results are shown in Table

TABLE 25

Resistance of sporeformers to heat in skimmed milk (20 minute exposures).

Temperature of exposure °C.	Species: B. licheniformis						B. cereus	B. brevis	B. circulans	
	Culture No. 29	91	92	93	94	95	40	20	49	67
70									++	
80									+-	++
90		+++	+++	+++	+++	++-	++			++ ++
100	+-	+++	+-	---	++-	--	++	++	+-	++ ++
105		++	--	--	--	+-	++	++		++
110	+-	+++	---	--	---	++-	+-	+-		-- +-
115	++	--	++	+-	--	---	--			
120	--						-			

Temperature of exposure °C.	Species - B. subtilis																
	Culture No. 1	2	4	7	13	24	26	27	62	79	80	87	88	89	90	77	78
70					++	++											
80					++	++											
90					++	++				++	++	+++	+++	+++	+++	++	++
100	+	+	+	++	++	++				++	++	+++	+++	+++	+++	++	++
105	++	++	++	++	++							++	++	++	++	++	++
110	++	++	+-	-	++	++		++	++	++	++	+++	+++	+++	+++	++	++
115	++	++	+-	--		++	++	++	++	++	--	+-	--	--	--	++	++
120	++	+-	--			++	+-	++	--	+-	--					--	--
125							--	--	--	--	--						

TABLE 26.

Resistance of sporeformers to heat in skimmed milk, milk and cream (23 per cent. butterfat) (20 minute exposures).

Temperature of exposure °C.	Substrate	B. brevis		B. circulans	B. licheniformis		B. subtilis																		
		20	22	49	29	54	1	2	3	4	5	6	7	26	27	27	54	55	57	58	59	60	62
80	Skimmed milk			+												+							
	Milk			+	+											+							
	Cream			+	+											+							
90	Skimmed milk	+	+	+												+							
	Milk	+	+	+	+											+							
	Cream	+	+	+	+											+							
100	Skimmed milk	+	+	+		+	+	+	+	+	+	+	+	+		+	+	+	+	+	+	+	+
	Milk	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	Cream	+	+	+	+	+	+	+	−	+	+	+	−	+	+	+	+	+	+	+	+	−	+
110	Skimmed milk	−	+	−		+	+	+	−	+	+	−	−			+	+	+	+	+	−	+	
	Milk	−	+	−	+	−	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	−	+
	Cream	−	+	−	+	+	+	+	−	+	+	−	−	+	+	+	+	+	+	+	−	+	+
115	Skimmed milk	−	+			−	−	−	−	+	−	−	−	+		+	−	+	−	−	−	−	
	Milk	−	−			−	+	+	−	+	−	−	−	+	−	+	−	−	−	−	−	−	+
	Cream	−	−			−	+	+	−	+	+	−	−	+	+	−	−	−	−	−	−	−	−
120	Skimmed milk	−	−			−	−	−	−	+	+	−	−		+	−	−	−	−	+	−		−
	Milk	−	−			−	−	−	−	+	−	−	−	−	+	+	−	−	−	−	−		+
	Cream	−	−			−	+	+	−	+	−	−	−		−	+	−	−	−	+	−		−
125	Skimmed milk										−						−	−	−	+	−		
	Milk										−						−	−	−	−	−		
	Cream										−						−	−	−	−	−		

From a study of Tables 25 and 26 it will be seen that the cultures of the greatest heat resistance were strains of B. subtilis and that the variation within strains of the same organism was considerable. Thus while the majority withstood the heat of 120°C. for 20 minutes, other strains, as for example No. 7, also isolated from processed milk, did not appear to have a great tolerance to heat. When the same culture was examined more than once absolute checks were not obtainable, but even allowing for the large temperature interval between the tests this lack of agreement was less in some cases than might have been expected from the review of previous work, since many of the precautions which have been shown necessary for uniformity were not taken in this general trial.

It will be noted that "skips" occurred in some tests, especially among the B. licheniformis species (Table 25) and among some of the other strains (Table 26). When cream, milk and separated milk were used as substrates there was no indication that the bacteria in one medium had a greater resistance to heat than in either of the others. Dormancy was not observed, although in some tubes the spores took one or two days longer than the majority to germinate. The survival of the spores was always evident within one week's incubation and more often after 3 to 4 days.

4. Special studies on the heat resistance of aerobic sporeformers.

It was decided to investigate in greater detail the heat resistance of some of the aerobic sporeformers, and for this purpose strains of B. subtilis were selected because it appeared both from the numbers isolated from cans and from the general trial of heat tolerance that organisms of this species are the most likely to survive commercial canning. Nos. 6, 27 and 62 were selected because they were known to be active in sterilised cream spoilage. They were representative also of the variation in heat tolerance of the strains of B. subtilis which were isolated from canned products, since No. 6 had a less pronounced heat resistance than that of the other two cultures.

Preparation of spore suspensions. Spore suspensions were prepared as follows:- The cultures were inoculated on to milk agar slants prepared in large flat sided medicine bottles. Each slant was about 20 square inches in extent, and they were incubated for 48 hours at 37°C. after inoculation. The cultures were then washed off with sterile distilled water and incubated for a further 24 hours at 37°C. to increase sporulation. The water suspension was well shaken up with glass beads to break up the clumps and filtered through a filter paper containing a heavy layer of sterilised absorbent cotton wool, all of which had been previously sterilised. The filtering process removed any remaining clumps, and absence of clumps of spores in the final suspension was confirmed by microscopic examination. The

suspension was heated to 190°F. for 10 minutes in water to destroy any vegetative forms and then it was cooled quickly and placed in a small screw capped bottle in a refrigerator at 40°F. until required. Since very good checks were obtained when several plates of the same dilution were counted and dormancy did not appear to occur, it seemed clear that counting the number of spores by specialised methods was not necessary, and the number of spores present in the suspension was therefore determined by the plate count on milk agar. It should be noted that when plate counts were made of the sporeformers sterile blotting paper and glycerine were placed inside the plates after they had been poured and inverted to absorb the water of condensation and prevent the colonies from spreading.

The effect of the two factors, namely reaction and butterfat content of the substrate, already studied in connexion with the heat resistance of non-sporeforming bacteria have also been investigated for sporeformers and will be dealt with separately.

(i) The effect of the reaction (pH) of the substrate on the destruction of B. subtilis by heat. Cream of 23.5 per cent. butterfat content prepared as previously described was the substrate used. By the additions of small quantities of $\frac{N}{10}$ NaOH or $\frac{N}{10}$ HCl, a titration curve was obtained for the particular cream, and by using the quantities indicated by the curve it was possible by adding sterile alkali and acid to adjust the sterilised substrate to different pH values. After this adjustment in pH had been made the subsamples of cream were

thoroughly shaken and were not used for heat resistance tests until at least 24 hours had elapsed when the pH had come to equilibrium. The pH was then determined by the quinhydrone electrode method and equal volumes of the same spore suspension which was prepared as described above were added to each of the cream subsamples.

The effect of changes in the reaction of the substrate on the destruction of B. subtilis strains Nos. 6 and 62 was investigated. Both cultures were tested in 4 subsamples of cream substrate. The pH and the plate counts of the subsamples, which were tested immediately before the heat resistance tests commenced, are given below.

Culture No.	Subsample No.	pH of subsample	Plate count per ml. of subsample	Temperature at which tubes were heated for resistance tests
6	1	7.0	1,700,000	116°C.
	2	6.7	1,610,000	
	3	6.3	1,540,000	
	4	6.1	1,520,000	
	5	5.95	1,440,000	
62	1	7.25	40,000	120°C.
	2	7.0	45,000	
	3	6.6	51,000	
	4	6.1	38,000	

It will be seen that the plate counts of the subsamples in both experiments were very similar. Since in commercial practice the range in pH of cream for canning does not vary very widely it will be noted that the range of pH values chosen for

these experiments was also small. After the substrate had been inoculated 1 ml. amounts were transferred to the tube containers already described. If there was an interval between the filling of the tubes and the heat treatment they were cooled down in iced water and retained at 40°F. The temperature of heating was kept constant in both experiments and the time of exposure to it was varied from 16 to 60 minutes. From 3 to 5 different lengths of heat exposure were given to each subsample and 25 tubes were used for each different time of exposure. As before the tubes were first held in boiling water for 2 minutes before they were placed in the bath, and they were cooled in cold water after heating and then incubated at 37°C. for one month. The numbers of tubes in which growth occurred after heating was recorded and has been plotted in figures 1 and 2 as the percentage of tubes in which spores survived, i.e. the survival percentage, against the length of time for which heating was carried out.

It will be seen that there is a lack of consistency in the results and that over the restricted range of pH examined it is impossible to state definitely the effect of change in the pH on the resistance of spores heated in cream. It appears from an examination of the results in general that increases or decreases in the values on either side of a pH of about 6.6 to 6.8 may exert a tendency to decrease the resistance of the spores to heat. It is clearly demonstrated, however, by this method that as the time of heating increases the survival percentage decreases.

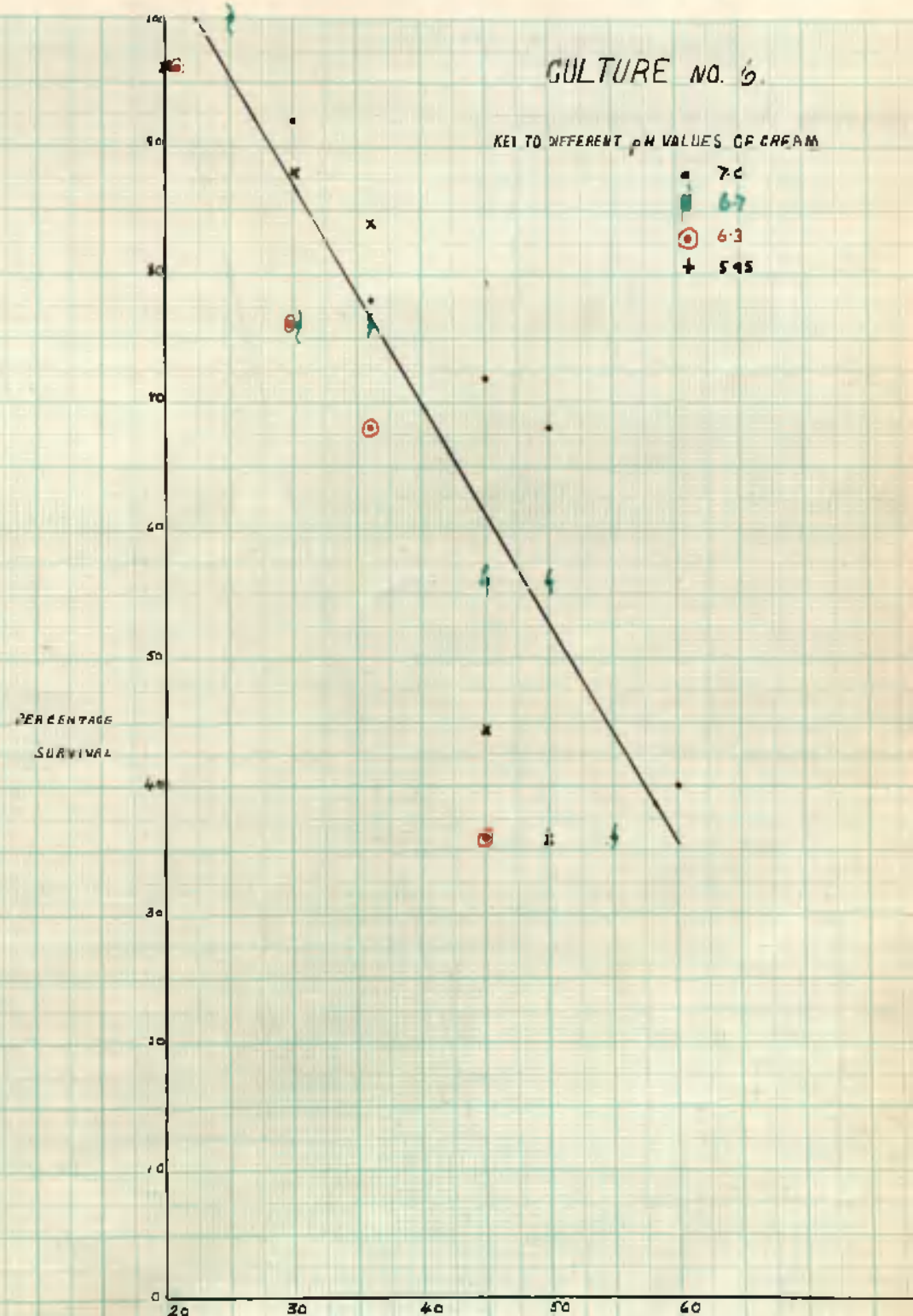

CULTURE NO. 6
KEY TO DIFFERENT pH VALUES OF CREAM
7·0
6·7
6·3
5·95
PERCENTAGE SURVIVAL
100
90
80
70
60
50
40
30
20
10
0
20
30
40
50
60
TIME IN MINUTES OF HEAT EXPOSURE

FIG. 1.

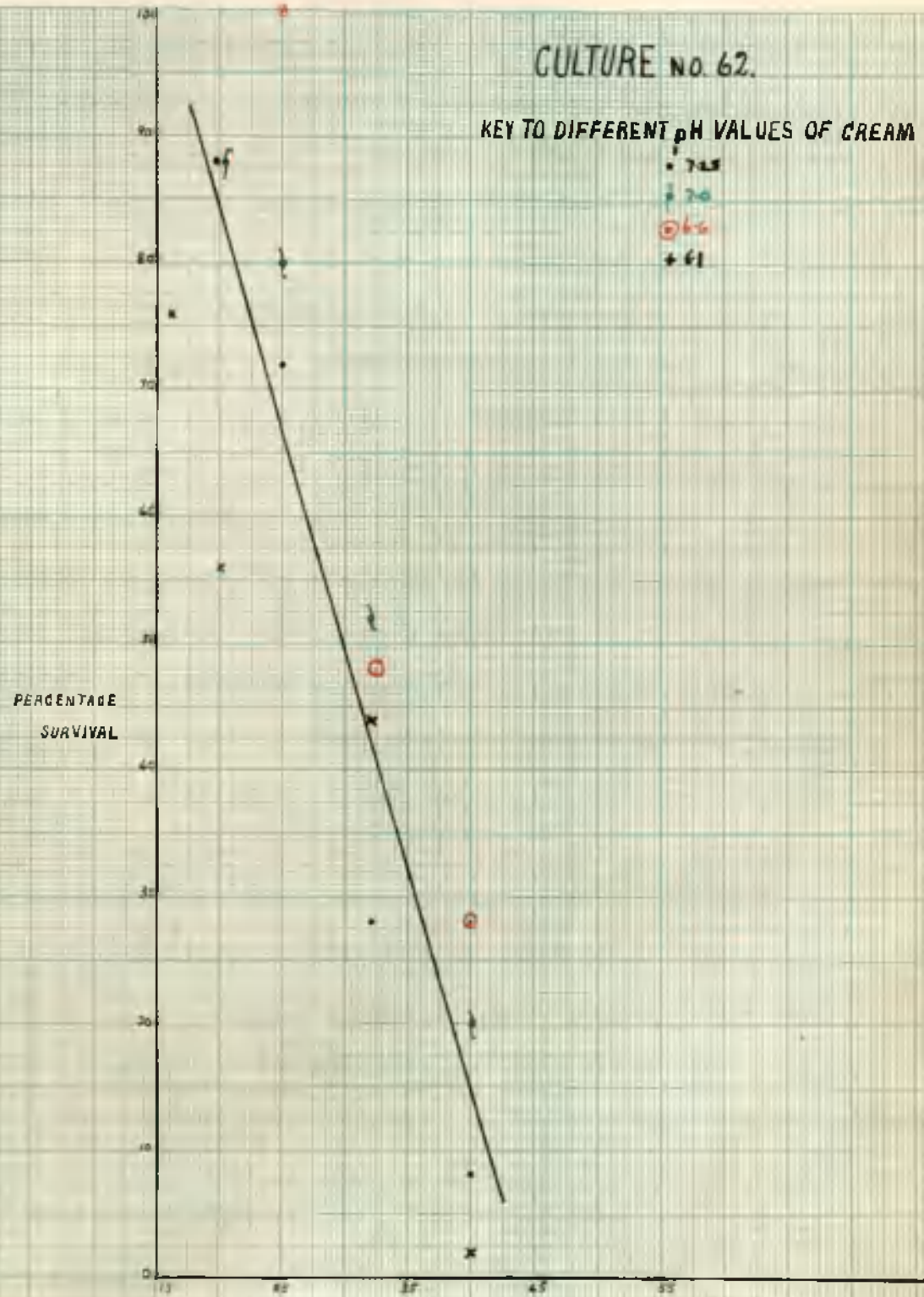


FIG. 2. TIME IN MINUTES OF HEAT EXPOSURE

(ii) The effect of increased butterfat percentage on the destruction of B. subtilis by heat.

(a) By survival percentage method. The effect of increasing the butterfat percentage on the heat resistance of B. subtilis in milk was investigated by two methods. The first method was similar to that which has just been described in the study of the effect of increasing acidity. The percentage of tubes in which spores survived in the different subsamples, which has been based on the results from about 25 tubes, is given in Table 27. The plate counts of the subsamples, together with the cultures examined and the different temperatures at which heat resistance experiments were carried out, is also stated.

It will be seen that there is little uniformity in the results of the tests, which again shows the difficulties of dealing with sporeformers in heat resistance experiments. However the results, such as they are, do show that raising the percentage of butterfat does not increase the resistance to heat of the organisms under test. In fact a general examination of the results indicates that the reverse is more probable, as has already been demonstrated with non-sporeformers. It might be suggested that the irregular results were due to casual contamination. It is, however, most unlikely that such a large number of tubes would have been contaminated after heating with the actual organisms under test. The possibility of contamination is also reduced by the fact that many of the tubes were incubated directly and were never uncorked.

TABLE 27.

Survival percentage.

Time retained at temperature mins.	Skimmed Milk	Milk 3.5% butterfat	Cream 10% butterfat	Cream 23% butterfat
A. Culture No. 6 heated at 110°C.				
20	97	100	100	93
22	82	100	100	100
25	64	100	96	93
27	61	90	83	70
30	36	79	73	50
35	3	35	30	40
38		17	20	
40		40	15	7
50	5			
B. Culture No. 62 at 115°C.				
7½	100	100		100
10	100	100		
12½	100		100	
15	96	100	100	100
20	88	100	68	100
22½	88	92	96	64
25	88	100	89	7
27½		76	84	10
30	96	88	88	0
32½			88	0
35		88	80	
37½		80		
40		80		
C. Culture No. 27 at 120°C.				
12	100		100	100
14	96	100		
16	100	100	88	88
18	92	92	68	
20	40	56	88	92
22	72	68	84	
25	48	40	52	80
30	48	76	68	40
35			76	36
40			88	12
50			88	
D. Culture No. 6 at 116°C.				
18	92			
21	100	88	76	88
24	80	64	88	84
27	92	64	72	80
30		88	76	68
33	96	80	80	80
36		72	64	
39	88	60	61	72
41	48			
42		48	60	48
45	32	72		
48		63	56	36

E. Culture No. 6 at 110°C.

Time retained at temperature mins	Skimmed milk	Time retained at temperature mins.	Milk 3.5% butterfat	Cream 11% butterfat
20	96	25	100	92
23	72	26½	100	
26	76	28	92	84
27½	92	29½	100	78
29	84	31	92	72
30½	76	34	72	92
32	52	35½	96	
35	72	37	100	76
38	56	40	84	80
41	44	43	96	60
44	72	46	88	
47	78	49	52	

Plate count per ml. of substrate.

	Skimmed milk	Milk 3.5% butterfat	Cream 10% butterfat	Cream 23% butterfat
A	950,000	1,225,000	1,190,000	1,270,000
B	180,000		160,000	167,000
C	285,000	806,000	297,000	293,000
D	2,110,000	2,880,000	1,950,000	1,570,000
E	2,160,000			3,200,000

(b) By a plate count method. A further attempt was made in order to show whether the increase of butterfat changes the resistance of sporeformers to heat. Substrates were prepared, as before, of different butterfat content from Grade A (T.T.) milk, but they were not sterilised. Spore suspensions of Culture 62 were added in the same way as in the previous experiments so that the count would be as nearly equal in each subsample as possible. Plate counts were made on the substrates before and after adding the spores, and approximately 4 ml. amounts of the subsamples both before and after the spores had been added were set up in the special tube containers for heat resistance tests. The tubes containing the inoculated substrate were held at different temperatures in the glycerine bath as previously for varying periods of time, and immediately after heating plate count tests were made. The results are given in Table 28.

It will be seen that the plate counts of the uninoculated subsamples before heating were low, and that after heating no bacteria could be detected on the plates of 1 ml. All samples were retained in iced water after inoculation if there was an interval before heating so that no multiplication of bacteria occurred, and it is clear therefore that the lack of sterility of the subsamples did not appreciably alter the results.

The plate counts indicated more definitely than the survival percentages that the increase of butterfat in the subsamples does not increase the resistance of the spores to heat.

It seems that the spores of these strains of B. subtilis

TABLE 28.

	No. 62	Heat treatment Temperature °C.	Time mins.	Plate count at 37°C. Skimmed milk	Milk 2.95% butterfat	Cream 12.5% butterfat	Cream 24.5% butterfat
Uninoculated substrate	Unheated	Control		6,200	6,000	3,700	6,000
	Heated	100	20	0	0	0	0
I. Inoculated substrate	Unheated	Control		4,300,000	5,500,000	5,500,000	7,500,000
	Heated	100	20	3,200,000	1,970,000	4,000,000	3,680,000
		110	20	1,200,000	1,152,000	1,440,000	1,616,000
		115	20	0	30	30	30
II. Uninoculated substrate	Unheated	as above					
	Heated						
Inoculated substrate	Unheated	Control		1,080,000	1,270,000	840,000	810,000
	Heated	110	20	1,180,000	1,220,000	1,320,000	1,430,000
		110	30	148,000	142,000	314,000	580,000
		115	10	138,000	117,000		222,000
		115	20	42,000	63,000	76,000	82,000
		115	15	2,800	2,780	2,090	2,060

	No. 62	Heat treatment Temperature °C.	Time mins.	Plate count at 37°C. Skimmed milk	Milk 3% butterfat	Cream 12% butterfat	Cream 22% butterfat	Cream 35% butterfat
Uninoculated substrate	Unheated	Control		1,500	2,000	1,400	600	1,200
	Heated	115	10	0	0	0	0	0
I. Inoculated substrate	Unheated	Control		3,000,000	2,000,000	2,300,000	4,200,000	4,000,000
	Heated	115	10	77,000	51,000	73,000	35,000	79,000
		115	15	1,140	1,290	4,000	1,480	4,000
		115	20	30	480	30	130	60
		110	40	0	0	0	0	50
II. Uninoculated substrate	Unheated	as above						
	Heated							
Inoculated substrate	Unheated	Control		2,320,000	2,020,000	1,690,000	2,100,000	2,020,000
	Heated	115	6	1,732,000	1,602,000	1,732,000	1,678,000	1,856,000
		115	10	77,000	64,000	63,000	53,000	54,000
		113	12	640,000	570,000	750,000	660,000	870,000
		110	30	480,000	890,000	880,000	460,000	210,000

are so resistant to heat that the minor changes which have been made in pH and in the composition of the substrates in these studies were not sufficient to change seriously the heat resistance.

APPENDIX.

BACTERIOLOGICAL QUALITY OF RAW MILK.

I. Routine methods for grading milk.

(A) General considerations.

(B) Comparative values of the plate count and the modified methylene blue reduction test. Criteria as to the suitability of the methylene blue reduction test.

(1) Comparison of reduction test with plate count.
(2) Comparison of plate count and reduction test with other measures of cleanliness.

(a) Correlations with other tests.
(b) Correlations with conditions of production.

(3) The effect of mastitis milk on the reduction test.
(4) The relative accuracy of the plate count and the reduction test.

Test recommended for routine bacteriological grading at factories.

II. Comparison between sensitivity of the plate count and reduction tests as conditions of milk production and of storage of the samples are changed.

(A) General plan of experiment.

(1) Conditions of milk production.
(2) Conditions of storage and age of milk samples on testing.
(3) Laboratory tests used.

(B) Results and discussion.

Comparison between sensitivity of the plate count and of the reduction test.

(i) On a percentage basis.
(ii) Using index numbers.
(iii) Using correlation coefficients.

APPENDIX.

BACTERIOLOGICAL QUALITY OF RAW MILK.

A supplementary investigation was undertaken into the bacteriological quality of the incoming raw milk in view of the far-reaching effects it may have on the manufacture and subsequent quality of canned dairy products. The work was divided into two parts. The first section deals[67] with data on the relative merits of the various routine tests for grading milk coming into factories, and the second deals[68] with a comparison between some of the tests as conditions of milk production are changed and as conditions of storage of the milk samples before testing are altered.

I. ROUTINE METHODS FOR GRADING MILK.

(A) GENERAL CONSIDERATIONS.

It was felt that it would be of particular value to processing factories if a quick test could be used on the platform as the milk was delivered and the results be available before the milk was tipped. Thus any poor quality samples could be kept out of the bulk which was destined for manufacture. With this object in view several tests which were capable of giving results in a very short time, among which were the brom-cresol-purple test, the acidity test and the alcohol test, were applied

to incoming samples of milk at factories in the south-west of Scotland. It was found that, with the exception of a few very poor samples obtained during a short period of hot weather, these tests were not sufficiently selective to assist in the grading of mixed milk. It was therefore obvious that more sensitive tests would have to be employed in grading, even though the results of these could not be obtained so rapidly. At the time that this investigation was commenced considerable controversy had arisen regarding two of the most common tests employed for routine milk grading, namely the plate count and the methylene blue reduction test. Since the latter test is far more rapid than the former, and has the additional merits of simplicity and cheapness, it was felt desirable to make a careful comparison of their relative accuracies as measures of bacteriological quality.

(B) COMPARATIVE VALUES OF THE PLATE COUNT AND THE MODIFIED METHYLENE BLUE REDUCTION TEST.

During the past twenty years the plate count has been employed as a means of assessing the hygienic quality of milk, for which it has proved to be of great value, and the marked improvement which has taken place in the quality of the milk of many producers is largely attributable to its use.

Increasing evidence has, however, been forthcoming that the plate count is subject to serious errors. In 1932 Malcolm (69), who studied the errors involved in plate counts, stated that

"it appears clear that in a very high proportion of samples ... the plating method of estimating the bacterial content of milk may yield highly misleading results if only one, or even two, plates are poured for each dilution". In 1935 Mattick et al.[70], investigating the plate counts obtained on the same milk at ten different centres, found gross variations, the range of 95 per cent. of the counts on identical samples extending from one-ninth of the mean to nine times the mean. These authors go so far as to state that "owing to the wide differences in the results secured by the examination of portions of the same milk by different observers in different laboratories, it is clear that the practice of comparing results from one laboratory with those of another must be abandoned". In his recent Report, Wilson[39] confirms in general the above conclusions and states that, even when a carefully standardised technique is employed, an allowance of ± 90 per cent. must be made in reporting the plate counts from single plates and of ± 64 per cent. in reporting counts from duplicate plates.

These facts have led Wilson to suggest the adoption of a modified methylene blue reduction test in place of the plate count as the standard method for the bacteriological grading of milk. A similar test has been used in other countries with apparent success. Thornton and Hastings[71], for example, found that the methylene blue reduction test was as accurate a measure of the keeping quality of milk as any method available, while Ellenberger et al.[72] state that the test is a more reliable

index of milk quality than the agar plate count. Since the adoption of the reduction test involved a fundamental change in the method of bacteriological grading commonly employed it appeared desirable to obtain further evidence regarding its suitability for this purpose.

Criteria as to the suitability of the methylene blue reduction test.

Any test which is to replace the plate count as a routine method of grading milk should fulfil the following requirements:

(1) The results of the test should show reasonable agreement with results obtained by the plate count.

(2) The test should show as good an agreement with other measures of cleanliness (e.g. farm inspection, keeping quality, etc.) as does the plate count.

(3) The test should not be invalidated by abnormalities in the milk.

(4) The accuracy of duplicate determinations should be closer than that obtained with the plate count.

The present investigation was planned to determine whether the methylene blue reduction test fulfils these requirements. For this purpose two series of samples have been employed. In the first series the samples, extending from June 1935 to December 1935, were secured from 200 different farms, and each consisted of a representative sample of the full morning or evening milking. The samples were stored at atmospheric temperature and evening samples were tested when 16 to 18 hours old,

but morning samples were tested when roughly 9 hours old.

In the second series about 800 samples, extending from December 1935 to July 1936, were taken from individual churns as tipped into the receiving tank at the creamery. All the samples were taken in the morning, but no information was available to indicate whether they were samples of evening or morning milk. The samples were taken to the laboratory and tested on arrival between 11 a.m. and 1 p.m.

The value of the tests employed throughout has been determined by calculating the correlation coefficient, which provides a convenient measure of the general relationship of any two variables over the full range of observations. Regression equations were determined where it was desired to express one variable in terms of another. In all calculations plate counts were expressed as logarithmic values.

(1) Comparison of reduction test with plate count.

The plate count and the reduction test measure two different properties of bacteria; the plate count is a measure of their number, the reduction test a measure both of their number and of their activity. Absolute agreement between them cannot therefore be expected, but since the plate count has been used successfully in grading up milk supplies it is obvious that, if the reduction test is to be equally successful, its results must run roughly parallel to those of the plate count.

In the present investigation the plate count was determined

by the method described in Bulletin 46 of the Ministry of Agriculture[26]. The methylene blue reduction tests were carried out as described by Wilson[39].

It will be seen from the results shown in Table 29 that the correlation between the two tests is remarkably high. In Table 30 the figures relating to the second series of tests have been recorded in the form of a frequency table, which shows that the values mostly fall within a definite band, and that the number of serious discrepancies between the plate count and the reduction test is not large.

In order to illustrate the extent of such discrepancies a further table has been constructed in which the reduction times corresponding to certain ranges of plate count are recorded (Table 31). The ranges chosen are (a) under 30,000 per ml., (b) 30,000-200,000 per ml., (c) 200,000-1,000,000 per ml., and (d) over 1,000,000 per ml. In Table 32 these figures are summarised as percentages. Percentages are also given for the reduction times of all samples under and over 200,000 per ml., since this figure has been commonly regarded in the past as the upper limit for good quality milks. Figures are included showing the percentage of samples which do not reduce methylene blue within 5 and 6 hours respectively. The latter figure has been given for comparison with Wilson's results; the 5 hour figure was selected because it represented the mean value adopted in the Milk (Special Designations) Order, 1936.

In general the figures show reasonably close agreement with

TABLE 29.

Correlation coefficient (r) for methylene blue reduction test and logarithm of plate count.

		r	Standard error
Series 1:	Morning samples	-0.791	0.041
	Evening samples	-0.768	0.040
Series 2:	All samples	-0.818	0.013

Regression equations.

			Value of y when plate count is 200,000 per ml., i.e. x=log.5.30 hr. min.		Value of x when y = 5.0 hr.	Plate count per ml. equivalent to value in preceding column
Series 1:	Morning samples	x = -0.310y + 6.34			4.79	61,700
		y = -2.02 x + 15.192	4	29		
	Evening samples	x = -0.284y + 6.312			4.89	77,600
		y = -2.070x + 15.667	4	42		
Series 2:	All samples	x = -0.269y + 6.514			5.17	148,000
		y = -2.486x + 18.17	4	59		

Where x = logarithm of the plate count, and y = time in hours required to reduce methylene blue in the reduction test.

TABLE 30.

Frequency table.

Log. of plate count	Methylene blue reduction time in half-hours																							
	0	½	1	1½	2	2½	3	3½	4	4½	5	5½	6	6½	7	7½	8	8½	9	9½	10	10½	11	Total
2.51-2.75																			1			1		2
2.76-3.00																		1		2		3		6
3.01-3.25									1					1				4	1	1	3	2		13
3.26-3.50														1			2	5	1	4	2			15
3.51-3.75									1				1		3	4	3	4	2	4	5	5	1	33
3.76-4.00										1		2	5	3	4	7	4	8	5	1	2	5	2	49
4.01-4.25										1	1	1	4	6	3	12	12	16	9	1	2	10	2	80
4.26-4.50			1					1	2		3	6	4	7	9	11	9	13	5	4	6	3	2	86
4.51-4.75							2		2	2		7	3	7	8	8	14	10	3	1	3	6		76
4.76-5.00					1	1			3	3	3	3	5	9	4	6	10	7	1		1	1	1	59
5.01-5.25			1	1		1	3	5	3	6	2	7	3	2	7	6	3	1			1	1		53
5.26-5.50				2		1		5	2	2	2	3	2	3	4		1	1	1		1	1		31
5.51-5.75	1	1	4		2	4	2	3	3	3	4	2	1	3			1							34
5.76-6.00	1	2	2	1	4		2		2	1							2							18
6.01-6.25	1	5	2	4	2	3	2	1	1	1							1							23
6.26-6.50		3	1	2	1		1	1		1							1							11
6.51-6.75	2	1		3	6				1		1													14
6.76-7.00	28	26	11	3	5		1					1							1					76
	33	38	22	16	21	10	13	16	21	21	16	32	28	42	42	54	63	70	30	18	26	39	8	679

TABLE 31.

Number of samples showing reduction times over certain ranges of plate count.

Range of plate count (per ml.)	Series	Methylene blue reduction time in half hours																						Total number of samples
		0	½	1	1½	2	2½	3	3½	4	4½	5	5½	6	6½	7	7½	8	8½	9	9½	10	10½	11	
Up to 30,000	First Series										1	4	2	13	11	17	22	12	17	8	7	1		3	118
	Second Series			1						4	1	4	9	13	18	21	35	30	51	23	17	20	28	7	282
	Total			1						4	2	8	11	26	29	38	57	42	68	31	24	21	28	10	400
30,000-200,000	First Series					1		1		2	7	3	3	4	7		2		1	4	1				36
	Second Series			1	1	1	2	5	7	8	12	6	18	11	19	21	20	28	18	5	2	7	9	1	202
	Total			1	1	2	2	6	7	10	19	9	21	15	26	21	22	28	19	9	3	7	9	1	238
200,000-1,000,000	First Series	1		1	2		2	5	4	2	3	2			1										23
	Second Series	2	3	6	3	6	5	4	7	7	6	5	4	4	4	3		2	1				2		74
	Total	3	3	7	5	6	7	9	11	9	9	7	4	4	5	3		2	1				2		97
Over 1,000,000	First Series		2	4		4			1		2	1													14
	Second Series	31	35	14	12	14	3	4	2	2	2	1	1					2		1					124
	Total	31	37	18	12	18	3	4	3	2	4	2	1					2		1					138

TABLE 32.

Percentage of samples showing reduction times over certain ranges of plate count.

Range of plate count (per ml.)	Percentage of samples showing reduction times of											Percentage not reducing in	
	0	½-1	1½-2	2½-3	3½-4	4½-5	5½-6	6½-7	7½-8	8½-9	9½-10	10½-11	6 hours	5 hours
Under 30,000	0	0.2	0	0	1.0	2.5	9.2	16.7	24.8	24.8	11.3	9.5	87.1	96.3
30,000-200,000	0	0.4	1.2	3.4	7.1	11.7	15.2	19.8	21.0	11.8	4.2	4.2	61.0	76.2
200,000-1,000,000	3.1.	10.3	11.3	16.5	20.7	16.5	8.2	8.2	2.1	1.0	0	2.1	13.4	21.6
Over 1,000,000	22.5	39.8	21.8	5.1	3.6	4.4	0.7	0	1.4	0.7	0	0	2.1	2.8
Under 200,000	0	0.3	0.5	1.2	3.3	6.0	11.4	17.9	23.4	19.9	8.6	7.5	77.3	88.7
Over 200,000	14.5	27.6	17.5	9.8	10.6	9.3	3.8	3.4	1.7	0.9	0	0.9	6.9	10.7

those recorded by Wilson. As regards the 5 hour figure it will be seen that in the two extreme ranges there is a clear differentiation by the reduction test. Of the samples giving plate counts below 30,000 per ml., 96 per cent. did not reduce methylene blue within 5 hours, while of those with plate counts above 1,000,000 per ml. 97 per cent. reduced methylene blue within this period. The two middle ranges show a similar inverse relationship; 76 per cent. of the samples between 30,000 and 200,000 per ml. did not reduce methylene blue within 5 hours, while 78 per cent. of the samples between 200,000 and 1,000,000 per ml. reduced methylene blue within this period. There is therefore an overlap of about 25 per cent. among these samples. This overlap is, however, reduced to about 10 per cent. when the samples are classified in two groups, i.e. above and below 200,000 per ml. It may also be noted that of the samples giving counts of over 1,000,000 per ml., about 85 per cent. reduced methylene blue within 2 hours.

The equivalent values of the plate count and reduction test have been determined from regression equations and are shown in the lower part of Table 29. In the first series the reduction time equivalent to a plate count of 200,000 per ml. falls between $4\frac{1}{2}$ and $4\frac{3}{4}$ hours, and for the second series the value is 5 hours. The figure interpolated from Wilson's data is roughly $5\frac{1}{2}$ hours. These discrepancies appear to be due to the different distribution of high and low plate counts in the various series. In the first series the counts of 81 per cent. of the samples fell below

200,000 per ml., and only 19 per cent. showed counts above this value. The equivalent figures for the second series were 71 and 29 per cent., and for Wilson's series 64 and 36 per cent. respectively. Similar differences are found in the regression equations relating reduction time to plate count. For example, a reduction time of 5 hours corresponds in the first series to a count of about 70,000 per ml., in the second series to a count of 148,000 per ml., and in Wilson's series to a count of over double this figure. It appears therefore that, if regression equations are used to relate plate counts to reduction times, it is essential that the samples examined should constitute a representative cross-section of the milk supply.

(2) Comparison of plate count and reduction test with other measures of cleanliness.

In order to determine whether the reduction test gives as accurate an index of the true cleanliness of milk as the plate count, it is necessary to ascertain how far each of these tests can be correlated with other measures of cleanliness. For this purpose two general methods were employed: (a) the tests have been correlated with three other laboratory tests, i.e. keeping quality, presumptive coliform test and laboratory post-pasteurisation count; and (b) the tests have been correlated with the conditions of milk production as judged by farm inspection.

(a) Correlation with other tests. Keeping quality and presumptive coliform test were determined by the method described in Bulletin No. 46[26]. The keeping quality samples were stored

at 60°F. and tasted at 9 a.m. and 6 p.m. The final results were expressed in ¼ days. Laboratory post-pasteurisation counts were also made(47).

The correlation coefficients are given in Table 33 A. Both the plate count and the reduction test show a fairly high correlation with the keeping quality and an equally high correlation with the presumptive coliform test. The correlations with the laboratory post-pasteurisation counts are only moderate. It seems possible that the exceptionally low correlation with the reduction test is associated with the effect of storage temperature on the reduction time. Any deterioration in the milk which is due to the multiplication of bacteria at high storage temperatures is not believed to affect the post-pasteurisation count seriously(73), where (as will be shown later) the reduction test is a very sensitive indication of high storage temperatures.

Taken as a whole, however, the results show that the reduction test gives as good correlations with other tests of cleanliness as does the plate count.

(b) Correlation with conditions of production. Detailed inspections were made of the conditions of milk production on the 200 farms of the first series. The scoring placed major emphasis on the methods of production (150 marks) rather than on the equipment (50 marks). Particular attention was paid to the treatment of utensils, one-third of the total marks for methods of production being allotted to this item.

Table 33 B shows that the correlations between farm

TABLE 33.

Correlation coefficients.

Variables	Morning samples r	Morning samples Standard error	Morning + evening samples r	Morning + evening samples Standard error	Evening samples r	Evening samples Standard error
A. Series 1:						
Methylene blue reduction test at 37°C. Keeping quality at 60°F.	+0.707	0.054	-	-	+0.639	0.056
Log. plate count at 37°C. Keeping quality at 60°F.	-0.535	0.077	-	-	-0.679	0.051
Methylene blue reduction test at 37°C. Log. post-pasteurisation plate count at 37°C.	-0.444	0.086	-	-	-0.194	0.091
Log. plate count at 37°C. Log. post-pasteurisation plate count at 37°C.	+0.539	0.076	-	-	+0.330	0.085
Series 2:						
Methylene blue reduction test at 37°C. Presumptive coliform test at 37°C.	-	-	-0.672	0.021	-	-
Log. plate count at 37°C. Presumptive coliform test at 37°C.	-	-	+0.566	0.024	-	-
B. Series 1:						
Methylene blue reduction test at 37°C. Total score farm inspection, i.e. equipment and methods of production	-	-	+0.093	0.073	-	-
Log. plate count at 37°C. Total score farm inspection, i.e. equipment and methods of production	-	-	-0.179	0.072	-	-
Methylene blue reduction test at 37°C. Total score for methods of production	+0.227	0.104	-	-	+0.141	0.104
Log. plate count at 37°C. Total score for methods of production	-0.347	0.073	-	-	-0.206	0.101
Methylene blue reduction test at 37°C. Score for general cleanliness and cleaning of cows before milking	+0.093	0.108	-	-	-	-
Log. plate count at 37°C. Score for general cleanliness and cleaning of cows before milking	-0.248	0.102	-	-	-	-
Methylene blue reduction test at 37°C. Score for cleanliness and sterilisation of utensils	+0.078	0.108	-	-	-	-
Log. plate count at 37°C. Score for cleanliness and sterilisation of utensils	+0.120	0.107	-	-	-	-
C. Methylene blue reduction test at 37°C. Temperature at testing	-0.430	0.088	-	-	-0.724	0.045
Log. plate count at 37°C. Temperature at testing	+0.158	0.105	-	-	+0.467	0.074

inspection scores and plate count or reduction test are, with the exception of that for the total score for methods of production, so low as to be of little significance. Wilson[39] found similar difficulty in obtaining satisfactory correlations between conditions of production and the two tests. He pointed out, however, the very considerable effect produced by the storage temperature of the milk subsequent to production. In this inquiry the temperatures of storage were not recorded, but the temperatures at the time of testing, which reflect roughly the temperature of storage, were noted. The correlation coefficients between the temperatures at time of testing and the two tests were therefore calculated. The results, shown in Table 33 C, illustrate the very marked effect of storage temperature on both plate counts and reduction times. The effect is also illustrated in Figure 3. From this figure it will be seen that for every increase of 4°F. in the temperature at testing there was on the average a decrease in reduction time equivalent to 70 minutes, and an increase in the plate count of roughly 150 per cent. The effect is, as might be expected, more marked in the evening samples than in the morning samples, the respective correlation coefficients being -0.724 and -0.430 for the reduction test and + 0.467 and + 0.158 for the plate count. It is obvious that the temperature of storage will seriously affect the farm inspection correlations, and this may largely account for the low correlation coefficients obtained. Moreover, it would be expected that the effect would be more marked in the evening

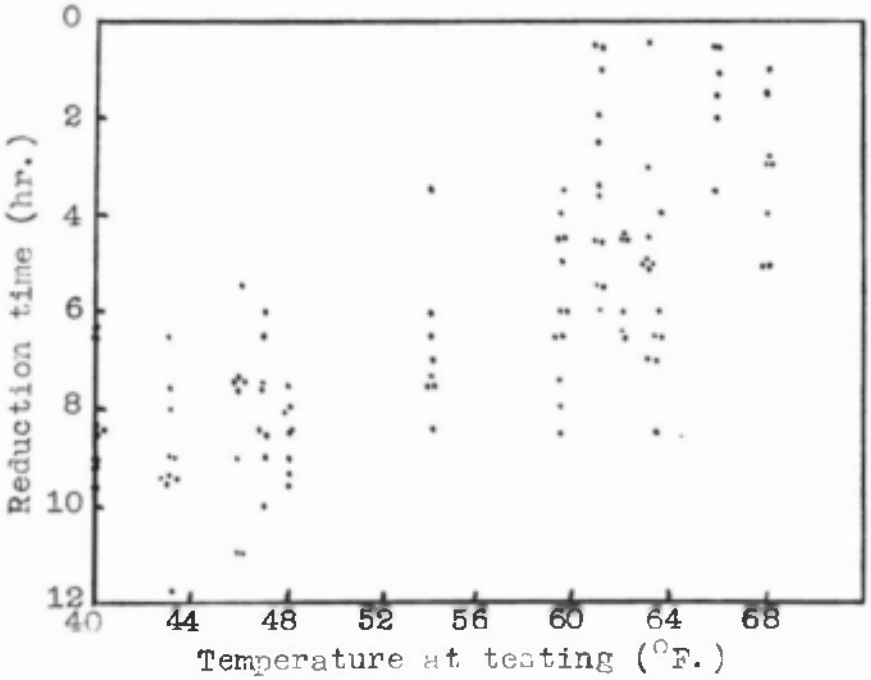


Fig. 3. Scatter diagram illustrating relation between reduction time and temperature at testing.

than in the morning samples. No direct comparisons are available, but it will be noted that the correlation coefficients for methods of production which were calculated for both morning and evening samples are lower than those calculated for morning milk alone. In addition it should be noted that the temperature of storage affects the reduction test to a greater degree than the plate count.

(3) The effect of mastitis milk on the reduction test.

It has been suggested that the results of the reduction test may be invalidated by the presence of abnormal milk, and particularly by milk from cows affected with bovine mastitis. If this were true it would certainly constitute a serious criticism of the test, since between 60 and 80 per cent. of mixed milk supplies from individual farms have been found to contain mastitis streptococci[74].

Abnormal milk might interfere with the results of the reduction test in one of two ways: the presence of cells in the abnormal milk might affect the reduction time, or the presence of mastitis streptococci might modify the rate of reduction of the methylene blue. As regards cell content, the weight of evidence reviewed by Wilson[39] shows that the presence of large numbers of leucocytes shortens the reduction time. Wilson himself, however, obtained somewhat indeterminate results, but although on occasion the presence of the added cells shortened the reduction time, in no instance did it increase it. Thornton et al.[75] found that newly calved cows' milk and milk at the end

of lactation both shortened the reduction time.

As regards mastitis streptococci, it is well recognised that the prevailing type (Str. agalactiae) does not reduce methylene blue at certain dilutions, and failure to reduce the dye at 1/20,000 dilution[76] is one of the properties employed for differentiating this organism. This does not imply that the 1/200,000 dilution of the dye (which is used in the reduction test) will inhibit the growth of Str. agalactiae. Frazier and Whittier[77] have shown that, in pure culture, the reduction of milk to a potential sufficient to decolourise methylene blue might take up to 24 hours. Ramsdell et al.[78] have shown that milk from infected quarters, tested almost immediately, failed to reduce methylene blue within 8 hours. Contrary evidence has been recorded showing[79] a definite decrease in the quality of milk from individual infected cows and infected quarters as determined by the reduction test.

Most of the evidence noted above was obtained either on specially inoculated samples of milk or on milk taken under sterile conditions from individual infected cows. Such results cannot be directly applied to mixed milks where a normal bacterial flora may be expected to predominate and, so that evidence might be obtained regarding this latter point, the samples used in the present investigation were examined to determine the presence or absence of mastitis streptococci.

It was decided that the criterion of infection using the selective medium devised by Edwards[80] should be the presence of

colonies of Str. agalactiae, either the β-haemolytic or the non-haemolytic varieties. With the β type diagnosis was based on colony appearance alone; if the non-haemolytic variety was suspected the nature of the colony was verified by fermentation tests.

In the first series it was possible to obtain a β-haemolytic plate count. In the second series the plates were inoculated directly with a saline suspension of centrifuged deposit. This enabled the samples to be differentiated into those showing and those not showing infection with mastitis, but provided no definite colony count of the milk.

The results may be considered from two aspects. Firstly, correlation coefficients were calculated to determine the relationship between the β-haemolytic count and the plate count and reduction tests. It will be seen from the figures in Table 34 that both tests show an unexpectedly high correlation with the β-haemolytic count, a high β-haemolytic count being associated with a high total plate count and a short reduction time. The presence of increasing numbers of β-haemolytic streptococci tends, therefore, to shorten rather than to lengthen the reduction time. Further, from the regression equation $x = 0.762y - 0.145$ relating β-haemolytic count to total plate count, it will be noted that for a total plate count of 200,000 per ml. the equivalent β-haemolytic count would constitute only a small fraction of the total plate count, namely about 3,800 per ml.

TABLE 34.

Correlation coefficients.

Variables		Morning and evening samples	Standard error
Methylene blue reduction test at 37°C. and log. β-haemolytic plate count at 37°C.	Series 1	-0.621	0.071
Log. plate count at 37°C. and log. β-haemolytic plate count at 37°C.	Series 1	+0.657	0.065
Samples showing the presence of mastitis organisms:			
Log. plate count at 37°C. and methylene blue reduction test at 37°C.	Series 1	-0.856	0.030
	Series 2	-0.818	0.020
Samples not showing the presence of mastitis organisms:			
Log. plate count at 37°C. and methylene blue reduction test at 37°C.	Series 1	-0.729	0.061
	Series 2	-0.792	0.019

Using the second method of comparison, correlation coefficients between total plate count and reduction test have been calculated for the infected and the non-infected groups for both the series of samples (Table 35). It will be seen that the correlation coefficient is higher in the infected than in the non-infected groups. The presence of infected milk has not therefore materially affected the correlation between plate count and reduction time.

A more striking illustration of this fact is available from a study of the regression equations. These are given in Table 35. They show that, taking a plate count of 200,000 per ml., the equivalent reduction times in the first series are for the infected group 3 hr. 56 min. and for the non-infected group 4 hr. 47 min., i.e. the infected group reduced the methylene blue more quickly than the non-infected. It is, however, probable that this difference is due to the small number of samples included in the first series: in the second series the equivalent times are 5 hr. 7 min. and 5 hr. 6 min. respectively.

It may be concluded therefore that, so far as mixed milks are concerned, the presence of milk from herds containing animals infected with streptococcal mastitis does not invalidate the methylene blue reduction test.

(4) The relative accuracy of the plate count and the reduction test.

It is difficult to make any direct comparison between the accuracy of the plate count and the reduction test owing to the

TABLE 35.

Regression equations.

		Value of y when x = log. 5.301	
		hr.	min.
Samples showing the presence of mastitis organisms:			
Series 1	$x = -0.276y + 6.20$ $y = -2.659x + 18.02$	3	56
Series 2	$x = -0.256y + 6.519$ $y = -2.620x + 19.012$	5	7
Samples not showing the presence of mastitis organisms:			
Series 1	$x = -0.271\ y + 6.00$ $y = -1.965x + 15.195$	4	47
Series 2	$x = -2.660y + 6.462$ $y = -2.363x + 17.614$	5	6

Where x = logarithm of the plate count, and y = time in hours required to reduce methylene blue in the reduction test.

relatively large intervals which are measured by the latter. It is possible from the regression equations given in Table 29 to express these intervals in terms of the plate count. Thus from the equation for the second series ($x = -0.269y + 6.514$) the equivalent plate counts for 5 and $5\frac{1}{2}$ hours' reduction time are 148,000 and 108,000 per ml. respectively. That is to say, the plate count equivalent to the 5-hour reduction is about 35 per cent. higher than that given by the $5\frac{1}{2}$-hour reduction. Since the plate counts are expressed logarithmically in the regression equation, this percentage difference per $\frac{1}{2}$-hour reduction time will hold throughout all ranges of plate counts and reduction tests. It may, however, be assumed that, where mistakes occur in determining the reduction time they will on the average be unlikely to exceed 15 minutes, i.e. the mean of two $\frac{1}{2}$-hour observations, which involves a possible error of only about 17 per cent.

It has, moreover, been found in practice that discrepancies between duplicate reduction tests are very infrequent. Of 367 duplicate tests made in the present investigation, 348 gave identical reduction times. Eighteen duplicates differed by $\frac{1}{2}$ hour and one by 1 hour. It may also be noted that only three out of these nineteen samples had reduction times of less than 6 hours, and only two of these fell within the critical range of $4\frac{1}{2}$ to $5\frac{1}{2}$ hours which form the present limits for officially graded milks. In the introduction of this appendix attention was drawn to the relatively large errors involved in determining

plate counts. It appears from the above facts that the reduction test, although it involves an inherent error of about 17 per cent. due to the ½-hour periods between readings, is likely to give more consistent and more easily duplicated results than the plate count.

Test recommended for routine bacteriological grading at factories.

For the general purpose of classifying milk according to bacteriological quality the modified methylene blue reduction test appeared to have outstanding advantages. This test seemed to fulfil the criteria suggested in that (a) the results show reasonable agreement with those obtained by the plate count; (b) the modified reduction test shows as good an agreement with other measures of cleanliness as does the plate count; (c) the modified reduction test, when applied to mixed milk, is not invalidated by the presence of mastitis milk; and (d) the variability of the modified reduction test is less than that of the plate count. It must not be inferred that the author considers the plate count to be redundant. It would, however, appear that its greatest value may be as a supplementary test; for example, to trace out individual sources of contamination. Other tests (for instance, the presumptive coliform test, the laboratory post-pasteurisation count, and the keeping quality test) also have their special uses.

II. COMPARISON BETWEEN SENSITIVITY OF THE PLATE COUNT AND REDUCTION TEST AS CONDITIONS OF MILK PRODUCTION AND OF STORAGE OF THE SAMPLES ARE CHANGED.

It has long been recognised that certain conditions of production at the farm adversely affect the bacteriological quality of milk and that these adverse effects can be shown by increases in the plate count and decrease in the keeping quality. Ayers, Cook and Clemmer[41] pointed out the usefulness of the plate count in indicating changes in conditions of production and concluded that the three essential factors in the production of milk of low bacterial count were: (i) sterilised utensils, (ii) clean cows, especially udders and teats, and (iii) small topped pails. They found that unsterile utensils introduced the greatest number of organisms into milk as shown by the counts and that the numbers from this source varied greatly.

In the same way in this country the plate count, the keeping quality and the presumptive coliform tests have been shown[81] to reflect sources of contamination to milk on the farm, as, for example, dirty churns and wet handed milking, and have been useful methods in the past for the determination of the source of contamination.

Results have been presented in the first section which showed that the reduction test is probably as good as any routine test for the grading of milk, and it was indicated that it may be a better test than the plate count. It therefore seemed desirable to investigate the sensitivity of the reduction test

to changing conditions of production on the farm. If the reduction test were not, at least, as sensitive as the plate count has been shown to be to varying conditions of production then the general usefulness of the reduction test compared with the plate count for advisory purposes might be reduced. Thornton et al.[82] appear to be the only workers who have attempted to interpret the different sources of milk contamination on the farm in terms of the methylene blue reduction test. They concluded that as clean milk improvement progresses bacteria from the udder and from unsterile utensils tend to become the predominating flora of the milk, and that plate counts are apt to be misleading due to the clumping tendency of the bacteria from these sources. The reduction test, on the other hand, being less influenced by clumping, measures these bacteria more accurately than the plate count does.

It has also been indicated previously that the storage of the milk before testing can have a masking effect on the value of farm inspection when correlated with plate count or reduction test. Since farm inspection scores should reflect conditions of production the temperatures of storage of samples before testing will obviously be of importance in a comparison of the plate count and reduction test as farm methods are altered. The importance of milk cooling and subsequent storage conditions has been emphasised by several workers[81,83], and it appeared therefore that it would be necessary to inquire into the extent to which differences in storage conditions would affect any

comparison between the test for grading milk as farm production methods are changed.

The object therefore of this investigation was to provide information which would help to answer the questions (1) how does the methylene blue reduction test compare with the plate count and other tests in revealing changes in the conditions of milk production, and (2) to what extent do differences in the conditions of storage of the milk samples before testing and the age of the samples on testing affect the comparison.

(A) GENERAL PLAN OF EXPERIMENT.

It was felt that a careful study of the milk from a limited number of cows would yield more reliable results than the study of bulk milk from a larger number of animals where the conditions of production could not be so well controlled. Whilst there are certain objections, the outstanding advantage obtained from using a small number of animals, i.e. the relative simplicity with which the same standard of milk production could be reproduced, seemed more than to counterbalance the disadvantages. The cows were milked twice daily and the milk from each animal and from each milking was examined as a separate unit. Each sample was therefore from a single cow's milk.

(1) Conditions of milk production.

Three factors of clean milk production which are generally accepted to be the main ones influencing milk production, viz. byre, cows and sterility of utensils were varied. Thus four

variables were introduced:- (i) the byre was clean or dirty; (ii) the cows were clean or dirty; (iii) the utensils were sterilised and stored in the cold, or were unsterilised and stored in the cold; (iv) or the utensils were unsterilised and stored in the warm. These factors were arranged in different combinations so as to provide nine experimental periods, each of which lasted for 7 to 8 days (Table 36).

TABLE 36.

Conditions of production.

	Period No.	Byre	Cows	Utensils	Storage of Utensils
Clean	1a 1b	Clean	Clean	Sterile	Cold
Moderately clean	2	Clean	Dirty	Sterile	Cold
	3	Dirty	Dirty	Sterile	Cold
Moderately dirty	4	Clean	Clean	Unsterile	Cold
	5	Dirty	Dirty	Unsterile	Cold
Very dirty	6	Clean	Clean	Unsterile	Warm
	7	Dirty	Clean	Unsterile	Warm
	8	Clean	Dirty	Unsterile	Warm
	9	Dirty	Dirty	Unsterile	Warm

The first period (representing ideal conditions) was duplicated at the end of the investigation and acted as a control. The experiment extended over twelve weeks from October 1937 to January 1938 and in April 1938. Altogether about 1,200 samples

were examined.

The small cow byre used fulfilled the requirements of a good modern milking byre. When clean byre conditions were operating the standard of cleanliness was similar to that which might be expected on the best graded milk producers' premises. When dirty conditions were required straw and manure were allowed to accumulate and throughout the period conditions became increasingly dirty. In no case, however, were the filthy winter byre conditions, where cows stand on old manure, reached, as it was felt that such a low standard of hygiene is becoming obsolete among the ordinary milk producer, and it is now a special rather than a general case as it was 25 years ago.

Two Ayrshire cows free from mastitis were used for each period of the experiment, and quarter samples of these animals throughout had a low count, 85 per cent. of the samples being below 500 per ml., while the higher counts all fell below 2,000 per ml. Altogether five different cows, all of which were in the last half of their lactation, were employed and were used for the different periods in the following order:-

Period	No. of 1st Cow	No. of 2nd Cow	Period	No. of 1st Cow	No. of 2nd Cow
1a	C	D	5	A	B
1b	A	B	6	C	D
2	A	B	7	C	D
3	C	D	8	A	B
4	C	E	9	A	B

During the operation of "clean" conditions the cows were washed and groomed but this was entirely omitted during the "dirty" period.

The same utensils were used throughout and consisted of two covered milking pails, one for each cow, a metal milk strainer and a tubular water cooler. It was thought that utensils stored in the cold might reduce the detrimental effect of unsterility in the resulting milk samples handled in them, since in some Scottish districts where cold night temperatures prevail for most of the year and where the day shade temperature is usually low except for a few weeks in the summer, it has been found difficult often to obtain a really poor sample of milk from some farms, although it is doubtful whether the utensils were even scalded. Attempts were made to estimate the contamination derived from the utensils under the different conditions of sterility. Rinsings were therefore taken with sterile saline and tested immediately. It will be seen from the data given below that when the utensils were steamed (Periods 1a and 3)

Period No.	Strainer and cooler*		Milking pails*	
	No. of organisms as shown by plate count to be present on utensils	Presumptive coliform test per ml. of rinsing water	No. of organisms as shown by plate count to be present on utensils	Presumptive coliform test per ml. of rinsing water
1a	-	Not found	-	Not found
3	-	" "	-	" "
4	120,000	" "	92,000	" "
5	18,700,000	+ 1/100	12,350,000	+ 1/100
6	400,000	Not found	5,800,000	Not found
7	18,000,000	" "	38,000,000	" "
9	240,000,000	+ 1/10,000	1,500,000,000	+ 1/100,000

* 2 litres of sterile saline were used for rinsing both the strainer and cooler and the milking pails.

they were practically sterile, but when unsterilised the number of bacteria which might be added to the milk from the utensils could vary considerably.

When sterilised utensils were required the usual methods of washing, rinsing and sterilising by steam were rigidly enforced after each milking. Where unsterile utensils were required they were only rinsed with cold water, and were stored at either a warm temperature in a boiler house, the range of which varied mainly between 68 and 78°F., or at a cold temperature seldom much above 50°F. About half-way through periods 6 to 9, where unsterilised utensils stored in the warm were used, it was found advisable, because of the accumulative effect, partially to sterilise the utensils. They were scrubbed in cold water and placed in the laboratory steamer for about 2 minutes, and then replaced in the boiler house in a wet condition as before.

For convenience of statistical treatment the nine experimental periods have been re-grouped into four main periods, which have for simplicity been designated: clean, moderately clean, moderately dirty and very dirty.

Clean periods 1a and 1b had exactly the same standard of cleanliness during milking, i.e. clean byre, cows and utensils.

Moderately clean periods 2 and 3 were very similar to the clean period except that the samples for period 2 were milked from dirty cows and for period 3 were milked when the byre was dirty.

Moderately dirty periods 4 and 5. The difference here was in the unsterility of the utensils which were stored in the cold and in addition for period 5 the byre and cows were dirty.

Very dirty periods 6 to 9. In all cases the utensils were not sterilised and were stored in the warm. The cleanliness of the byre and cows varied and were either both clean or both dirty, or if the cows were clean the byre was dirty and vice versa.

(2) Condition of storage and age of milk samples on testing.

Morning samples were stored under four different conditions:-

A was tested immediately after milking when it was at the most 2 hours old. The results should, therefore, indicate the initial contamination of the sample without the factor of bacterial growth having had the opportunity to operate, since the milk had had no appreciable storage period and the temperature at which it was held before testing was low.

B was held until the next morning, i.e. for 26 hours, at a cold room temperature of 53 to 58°F. The mean temperature of the milk on testing was 55°F. which further provides an indication of the fairly uniform temperature of storage.

C was also held for 26 hours before it was tested but at a higher temperature of 72°F. This temperature was selected to indicate the differences which might be expected if the milk was subjected to (1) high seasonal temperatures, (2) high temperature of storage by the consumer or (3) if there were no proper cooling facilities on the farm.

F was held under the same conditions as B, i.e. at cold room temperature until 6 p.m., after which it was stored at about 32°F. until 26 hours old, when it was tested.

Evening samples were stored under two different conditions:-

D was stored at cold room temperature, but was tested when 18 hours old. Hence the results of samples B and D so far as the conditions of storage influence them are comparable, except that D was stored for 8 hours less than B.

E was kept at 72°F. like samples C, with which the results may be comparable, except that E was stored for 18 hours only before testing.

It may be noted that samples F and D were stored and tested under conditions similar to those required by the Milk (Special Designations) Order 1936 for England and Wales.

Samples A and D were tested under conditions which would fall within the slightly different requirements of the same Order for Scotland. The Scottish Order requires that morning milk should be tested within 12 hours of milking and evening milk within 18 hours, but that if the tests are delayed after the samples are delivered to the laboratory they must be kept at from 0 to 5°C.

(3) Laboratory tests used.

The following tests were employed for all samples:-

(1) The plate count.
(2) The modified methylene blue reduction test at 37°C.
(3) The presumptive coliform test.
(4) The keeping quality test.
(5) The methylene blue reduction test at 60°F.

The technique throughout was similar to that given earlier in the Appendix, except that $\frac{1}{4}$-strength Ringer's solution[39] was used

instead of saline for dilution blanks and yeastrel[39] replaced lemco in the milk agar. The dilutions used varied according to the sample, and extended to $\frac{1}{10^7}$ for some periods of the experiment.

The methylene blue reduction test at 60°F. was set up exactly as for the test at 37°C. The tubes were brought to the desired temperature and placed in racks in an incubator, and observed at 9 a.m. and 6 p.m. This test had been noted in 1933[84] to give good agreement with the appearance of taint in the keeping quality test. Later it was shown[85] that only when the corked tubes are inverted at least twice daily was a close relationship between the reduction test at 15.5°C. and keeping quality, as determined by taste, obtained. Accordingly for 5 periods tubes were set up in duplicate and the tubes of one series were inverted twice at each reading, while the others were not.

The results of the plate count after the milk had been pasteurised in the laboratory were very low throughout and therefore the counts could not be used as a basis for comparison between the different periods and methods of storage. Only one count exceeded 1,000 per ml. while only about 4 to 10 colonies grew on most of the $\frac{1}{10}$ ml. dilution plates. Anderson and Meanwell[47] state that "heat resistant organisms frequently originate from the surface of unsterilised utensils" and they showed that when utensils are sterilised and a return was made to farm routine methods of cleaning which did not include

sterilisation, some days elapsed before heat resistant organisms reappeared in significant numbers in the milk. It is probable that the same factors operated in this experiment and that the periods were not of sufficient length for heat resistant bacteria to accumulate in large numbers on the unsterile utensils. It cannot be shown, therefore, what conditions of milk production are responsible for these bacteria gaining access to the utensils or what factors control their development on the utensils and in the milk samples before testing. Such information would have been of interest in view of the importance of heat resistant streptococci in spray dried milk powder.

"Spreaders" due to bacteria of the aerobic sporeforming group were very evident in at least 50 per cent. of the 1/10 dilution plates of all the periods except in the control periods. It appeared that although dirty byres, cows and utensils all seemed to contribute to the presence of aerobic sporeformers they were more evident when the cows and byres were dirty than when the utensils alone were dirty. This was only to be expected since hay and straw are known to be one of the main sources of the sporing aerobes, the 'hay bacillus' being one of the old names for B. subtilis, and contamination from both hay and straw might gain entrance if the byres and cows were dirty. The point of entry of these organisms is of interest because of their significance in the manufacture of evaporated milk and canned cream.

(B) RESULTS AND DISCUSSION.

Comparison between sensitivity of the plate count and of the reduction test.

(i) On a percentage basis. Firstly, the results of the methylene blue reduction test and the plate count for the different conditions of production and storage have been compared on a percentage basis as shown in Table 37. Arbitrary ranges of the plate count were chosen, and the methylene blue reduction times equivalent to these ranges have been calculated from the regression equation given in Table 29 (page 118) for bulk milk. The relative sensitivity of the two tests to alterations in production and storage conditions is shown from the distribution of the samples in the different ranges of the two tests. The changes in distribution of the samples will be seen from Table 37 to have occurred as production methods were progressively altered from "clean" to "very dirty".

If the results for samples retained at 72°F. for 26 hours prior to testing (Group C) are examined, it will be seen that the reduction test appears to be considerably more sensitive to increasingly dirty methods of production. This is especially marked in the very dirty periods (6 to 9), where the utensils are unsterilised and stored in the warm and the byre and/or cows are dirty. It will be seen that 42 per cent. of the plate counts are over 10,000,000 whereas the corresponding figure for the reduction test (i.e. of ½ hour or under) is almost

TABLE 37.

Samples and conditions of storage before testing	Plate Count at 37°C.	PERIODS 1a & 1b CLEAN %	2 & 3 MODERATELY CLEAN %	4 & 5 MODERATELY DIRTY %	6-9 VERY DIRTY %	Methylene Blue Reduction Time at 37°C.	PERIODS 1a & 1b CLEAN %	2 & 3 MODERATELY CLEAN %	4 & 5 MODERATELY DIRTY %	6-9 VERY DIRTY %
	Below 30,000	55	40	44	9	7½hrs. & over	21	18	14	2
C	30,000- 200,000	39	32	26	5	7 - 5 hrs.	42	28	25	
At 72°F.	200,001- 1,000,000	3	21	15	10	4½-3½ hrs.	28	40	14	5
for 26	1,000,001-10,000,000	3	7	15	34	3 - 1 hrs.	9	14	36	17
hours	Over 10,000,000				42	½ hr.or under			11	76
	No. of samples	29	28	27	58		33	28	28	58
	Below 30,000	100	93	82	34	7½ hrs.& over	71	79	68	11
E	30,000- 200,000		7	11	13	7 - 5 hrs.	26	21	18	9
At 72°F.	200,001- 1,000,000			7	18	4½-3½ hrs.			14	4
for 18	1,000,001-10,000,000				19	3 - 1 hrs.	3			27
hours	Over 10,000,000				16	½ hr.or under				49
	No. of samples	31	28	28	56		35	28	28	55
	Below 30,000	97	96	86	50	7½ hrs.& over	96	100	96	37
B	30,000- 200,000	3		14	11	7 - 5 hrs.	4		4	10
In cold	200,001- 1,000,000		4		17	4½-3½ hrs.				2
room for	1,000,001-10,000,000				15	3 - 1 hrs.				28
26 hours	Over 10,000,000				7	½ hr.or under				23
	No. of samples	29	28	28	54		28	25	27	57
	Below 30,000	100	100	90	66	7½ hrs.& over	100	96	100	60
D	30,000- 200,000			10	14	7 - 5 hrs.		4		17
In cold	200,001- 1,000,000				11	4½-3½ hrs.				7
room for	1,000,001-10,000,000				7	3 - 1 hrs.				14
18 hours	Over 10,000,000				2	½ hr.or under				2
	No. of samples	31	26	28	55		33	27	26	54
	Below 30,000	100	93	89	51	7½ hrs.& over	100	100	96	63
F	30,000- 200,000		7	11	24	7 - 5 hrs.			4	10
In cold	200,001- 1,000,000				7	4½-3½ hrs.				10
room for	1,000,001-10,000,000				16	3 - 1 hrs.				15
10 hrs. at	Over 10,000,000				2	½ hr.or under				2
32°F. till 26 hrs.old	No. of samples	28	28	27	55		28	23	27	51
	Below 30,000	100	100	93	60	7½ hrs.& over	100	100	96	73
A	30,000- 200,000			7	16	7 - 5 hrs.			4	13
Tested im-	200,001- 1,000,000				14	4½-3½ hrs.				12
mediately	1,000,001-10,000,000				10	3 - 1 hrs.				2
after	Over 10,000,000					½ hr.or under				
milking	No. of samples	29	29	27	58		27	24	25	52

doubled and is 76 per cent. This is shown also in the moderately clean periods (2 and 3), where 40 per cent. of the plate counts are below 30,000 but only 18 per cent. of the reduction tests are in the corresponding range of $7\frac{1}{2}$ hours or over.

The results of the samples retained at 72°F. for 18 hours before testing (Group E) show this same general effect.

The samples which have been stored at room temperature may be considered together. Group B has been held for 26 hours before testing and Group D for 18 hours. It will be seen that the reduction test is again the more sensitive of the two, but that the differences in sensitivity are only obtained under the very dirty conditions of production with unsterilised utensils stored in the warm. If the samples are produced by clean conditions (1a and 1b) or by moderately clean or moderately dirty methods, there is very little difference between the response of the two tests.

There appears to be little difference between the tests in their response to conditions of production throughout all the samples which were held at room temperature for the first 10 hours and were then placed in the refrigerator (32°F.). With these samples (Group F) in the clean periods 100 per cent. of the samples of both tests fell into the best range, i.e. below 30,000 for the plate count and $7\frac{1}{2}$ hours or over for the reduction test. Under the very dirty period also, the tests are very similar in their sensitivity, for 25 per cent. in the case of the plate counts and 27 per cent. in the case of the reduction tests are placed in the last three divisions of the range.

From a study of the results obtained from samples tested immediately after milking (Group A) very dirty methods are indicated most readily by the plate count. This might be expected, since the plate count measures numbers and not activit of bacteria and most of the bacteria have gained entrance to the milk just prior to the test and hence their activity is probably low. However, under the other conditions of production where, for instance, the utensils are unsterilised and stored in the cold and the cows and byre are either clean or dirty (4 and 5), this apparently greater sensitivity of the plate count is not found.

It will be noted that with the exception of samples stored at 72°F. both tests are relatively insensitive to dirty conditions of production. In most groups at least 50 per cent. of the plate counts of the very dirty samples fall below 30,000 and have reduction times of over $7\frac{1}{2}$ hours.

In other words it is shown that milk produced in dirty byres from dirty cows with unsterile utensils, if it is kept cold, will appear both by the plate count and by the reduction test to be of the best grade. This fact reinforces the importance of using relatively severe storage conditions when any attempt is made to judge production methods by either test, and it further indicates that the grading of milk in the winter months or in relatively cold districts as, for instance, in the north of England or in Scotland is of doubtful value as an

index of cleanliness of production unless some incubation of the milk occurs prior to testing.

(ii) Using index numbers. The second method of studying the different effect of changes in production or in storage on the milk tests made in this study was by the use of index numbers. Marks were allocated to the mean value of the logarithm of the plate count and to the mean value of the reduction times at 37°C. and 60°F. and of the keeping quality at 60°F. for each period and each condition of storage separately. The lowest value of the count and the highest value of all the other tests were awarded 100, and marks were given to the remainder of the values proportionately.

The treatment of the data by this method did not appear satisfactory and it was not used for the detailed comparisons, as, for example, between the sensitivity of the plate count and the keeping quality when the byres and cows were dirty and the samples were tested immediately after milking. It was possible, however, to draw some general conclusions from a study of the index marks.

(1) It appeared that unsterile utensils had a greater effect on the quality of the milk as shown by the different tests than any other condition of production. The adverse effect of the unsterile utensils was most marked when the byres and cows were dirty, but it was also important when the byres and cows were clean. When the effect of storing unsterile utensils in the warm was compared with storing them in the cold the effect was

shown to be as detrimental as that produced by unsterile utensils compared with sterile ones.

(2) Dirty byres and cows were shown to have a relatively small effect on the results of the different tests when the utensils were clean and, in fact, unsterile utensils appeared to outweigh the effect of dirty cows and byres. Since the "dirtiness" of the utensils could not be kept as uniform as the "dirtiness" of the byres and cows, this fact obviously contributed to the lack of consistency which was noted in the results.

(3) When assessed by the index number method the reduction test at 37°C. was usually the most sensitive of the tests to changing conditions of production and the plate count was often the least sensitive. The sensitivity of the keeping quality and reduction tests at 60°F. were, as might be expected, about equal.

(4) The examination of the effect of the different conditions of storage on the laboratory tests showed that for the four main periods the results of the reduction test at 37°C. were the most consistent.

(iii) Using correlation coefficients. The third method used to compare the relationship of the methylene blue reduction test at 37°C. and the plate count to conditions of production and storage consisted of calculating the correlation coefficients using an arbitrary scale of marks to assess production methods quantitatively. In awarding these marks for methods of production the clean periods scored 100, and 80 per cent. of the marks were

allocated for sterility of utensils and temperature of storage of utensils. This high score for utensils may appear to be out of proportion, but it was given after a careful, and more or less quantitative, study of the effects of the different factors had been made by the independent method of index numbers outlined above.

The results of the correlations are shown in Table 38. It will be seen that in general the results confirm those of Table 37 but are less consistent, especially with samples B and D. These groups give opposite results which are difficult to explain. Under the severe conditions of storage (C and E) the reduction test shows the greater sensitivity to methods of production, whereas under the most favourable conditions of storage, where the samples are tested immediately after milking, the plate count gives the higher values of r. This would be expected for there has been no time for the bacterial activity, as indicated by the reduction test, to increase before testing, although large numbers of organisms may be present.

Finally it may be of interest to consider briefly the correlation coefficients between the various tests which are shown in Table 39. It will be seen that the values are similar to those obtained by other workers in this field who have based their conclusions on experiments with mixed milk, although the coefficients are in some instances perhaps somewhat lower than might be anticipated. It is reasonable to assume, therefore, that the use of single cow samples in the present work has not seriously invalidated the results. The effect of storage con-

TABLE 38.

Correlation Coefficient Variables	Conditions of storage before testing.					
	At 72°F. for 26 hours	At 72°F. for 18 hours	In cold room for 26 hours	In cold room for 18 hours	In cold room for 10 hours at 32°F. till 26 hours old	Tested immediately after milking
Sample	C	E	B	D	F	A
Score for Conditions of Production versus log. of the Plate Count at 37°C.	-.6287	-.5506	-.4517	-.5036	-.4317	-.5456
Score for Conditions of Production versus Methylene Blue Reduction Test at 37°C.	+.7042	+.6757	+.5224	+.4149	+.4504	+.4154

TABLE 39.

Correlation Coefficient Variables	Conditions of storage before testing. At 72°F. for 26 hours	At 72°F. for 18 hours	In cold room for 26 hours	In cold room for 18 hours	In cold room for 10 hours at 32°F. till 26 hours old	Tested immediately after milking
	Sample C	E	B	D	F	A
Log. of Plate Count at 37°C : Methylene Blue Reduction Test at 37°C.	-.7688	-.6521	-.5999	-.5577	-.6094	-.5020
Log. of Plate Count at 37°C ; Keeping Quality at 60°F.	-.6837	-.5489	-.6023	-.5837	-.5384	-.4638
Methylene Blue Reduction Test at 37°C ; Keeping Quality at 60°F.	.7288	.7046	.6811	.5656	.5613	.4285
Methylene Blue Reduction Test at 60°F ; Keeping Quality at 60°F.	.8806	.7062	.7754	.6778	.7273	.7859
Log. of Plate Count at 37°C : Presumptive coliform test	.6573	.6583	.6530	.6604	.6558	.6166
Methylene Blue Reduction Test at 37°C : Presumptive coliform test	-.6248	-.5199	-.5588	-.4360	-.4889	-.3816
Keeping Quality at 60°F : Presumptive coliform test	-.5781	-.5028	-.6095	-.4904	-.5446	-.5454

ditions, both as regards time and temperature, on the correlations is clearly shown, the more severe conditions usually giving the higher correlations.

S U M M A R Y.

PART I. INTRODUCTION.

1. The methods adopted to store and preserve surplus milk in a concentrated form are discussed.

2. A description is given of the manufacture and salient points in the control of micro-organisms causing deterioration on storage in concentrated milk products. In particular it is pointed out that sweetened condensed milk is not a sterile product and that the aim of the manufacturer is to have sufficient concentration of sucrose in the final product to inhibit the growth of the surviving organisms. On the other hand, in the production of evaporated milk, although the methods used for removing water are similar to those employed in the manufacture of sweetened condensed milk, this process aims at the total destruction of the milk flora by steam sterilisation. It is pointed out that bacterial spoilage may occur in evaporated milk as a result of the survival of heat resistant sporeformers or to other bacteria which may gain entrance to the tins after heating. It is also shown that in the production of canned cream the same principle is employed as for the manufacture of evaporated milk in order to obtain a product of indefinitely long keeping quality.

3. The method of processing and the salient points of control in the preservation of milk by drying are discussed. Particular reference is made to the production of spray dried milk powder.

PART II. CONCENTRATED MILK PRODUCTS.

1. A survey of evaporated milk and canned cream samples from three condensed milk factories and from general sources has been undertaken to determine to what extent non-sterility occurred in commercial practice. In addition to the examination of sound samples many others have been tested which were obviously defective or which were associated with defective packs. 100 per cent. of the tins examined from general sources were sterile. The factory samples, especially those of canned cream, had a lower sterility percentage, although it appeared that this was associated with some special circumstance of operation and that a sterile pack should ordinarily be quite attainable.

2. An account is given of the methods used for sampling the can contents and for the recovery of bacteria from unsterile cans and their identification. The necessity for a large inoculum in such sampling is emphasised since although the bacteria may survive for many months in the tins they may be present in small numbers.

3. Data is presented regarding the types of defective samples encountered and the associated bacteriological flora. "Bloats" in evaporated milk and thin putrid cream were the two main causes of spoilage encountered.

4. By using the heat resistance of the organisms which were recovered from the cans as a basis of classification the defects have been divided into two groups, namely (1) a mixed general

flora which do not withstand high temperatures and (2) a restricted heat resistant flora which are of the sporeforming type.

5. The spoilage due to non-sporeformers has been shown to consist chiefly of "bloats" and/or curdling. A wide variety of bacteria were isolated from these defects, since such organisms gain access to the can during the cooling process through leaks caused by bad seaming or faulty sealing. Strains of the Escherichia genus were, however, the commonest producers of gassy spoilage.

6. Although 80 aerobic or facultative sporeformers have been isolated from sound and defective cans, only 2 cultures of anaerobic sporeformers have been obtained, both of which were from sound cans. The anaerobic sporeformers which survived processing are not therefore of importance. The aerobic sporeformers were all of the same general type, 69 per cent. being strains of B. subtilis and 15 per cent. strains of B. licheniformis. The condition of the contents in the cans from which these aerobic sporeformers have been isolated was in some instances sound, but when it was defective it varied from a smooth soft clot to a thin consistency, the smell and flavour being putrid or bitter. The relationship between aerobic sporeformers and the defects was investigated by the inoculation of 31 strains of these organisms into sound evaporated milk and canned cream. Inoculated cans were incubated

for up to 18 months and a clear connexion between the two was not found to exist in all instances. One outstanding defect, however, that of bitterness of flavour associated with thinness, has been induced. With some strains of B. subtilis a slightly putrid flavour developed slowly, but this was variable, and with some of the other aerobic sporeformers a soft clot was found in evaporated milk. Slightly "blown" cans resulted in all cans inoculated with B. licheniformis.

8. The variable production of spoilage in evaporated milk and canned cream by the inoculation of these aerobic sporeformers and the variation in the extent of defects when they occur has been suggested as probably accounting for the considerable difference of opinion in the past regarding the connexion between aerobic sporeformers and deterioration in canned foods. This has been discussed together with the conflicting literature on the subject.

9. Some sweetened condensed milk samples which invariably contain viable organisms have also been examined for defects due to micro-organisms. Two "bloats" and one sample with a putrid flavour were encountered. In addition 3 strains of mould, which were isolated from an extensive outbreak of "buttons" and which have not been previously reported as the cause of this defect, have been investigated.

PART III. BITTERNESS AND THINNING IN CANNED CREAM.

1. The results of a detailed cultural and morphological study which has been made of 3 strains of B. subtilis, namely Nos. 6, 27 and 62 are given. These bacteria, which have been isolated from thin and bitter samples of commercially canned cream, are shown to produce the same type of spoilage when they are re-inoculated into sound samples.

2. The rate of development of bitterness and thinning in experimentally inoculated cans has been investigated and it appeared to vary with the culture and with the temperature of incubation.

3. A brief reference is made to some of the chemical aspects of this spoilage.

4. The application of the results to the control of the defect in commercial practice is discussed and attention is drawn to 3 points in this connexion.

(i) It is indicated that it may be extremely difficult to control this type of spoilage under factory conditions owing to the high thermal death point of the causative bacteria. The heat treatment which can be given to tinned cream on account of the chemical breakdown which accompanies excessive exposures to heat is limited. It appears therefore that in practice the prevention of bitterness and thinning must depend more on improving the quality of the incoming milk supply than on

altering the conditions of sterilisation of the canned product.

(ii) It is stated that the identification of defective cans of cream before despatch from the creamery is of importance in the control of bitterness. It is shown therefore that it is desirable to store the canned cream, in addition to the usual 2 weeks of incubation at from 75 to 85°F. which are given in commercial practice, for a further period of 4 weeks or more at room temperature. By this means, the defect may be sufficiently developed in the affected cans to enable them to be picked out.

(iii) The desirability of careful bacteriological control of all batches of cream is emphasised. It is pointed out however that if only small inoculations of cream are used there is a serious risk that the presence of viable organisms will be missed. It is also shown that the isolation of organisms of the B. subtilis type from canned cream cannot be taken as indicating that the cream will inevitably become bitter, since the only certain method of identifying the typical organism is by its ability to produce bitterness and thinning in the normal product.

PART IV. DRIED MILK PRODUCTS.

1. Over 400 samples of spray dried milk powder from 8 factories operating in England and Scotland have been examined. These

samples were taken at different stages of the day's run and were obtained from several types of plant situated in widely different districts. Samples were taken in both the summer and winter months. The samples were, therefore, sufficiently representative to enable general conclusions to be drawn regarding the factors affecting bacteriological quality.

2. In reconstituting milk powders for bacteriological examination it is necessary to ensure (i) that the sample is sufficiently large to be representative and (ii) that external contamination is avoided during weighing and handling. The use of a 20 g. sample is advocated. Complete reconstitution of the powder is best obtained by shaking the sample with glass beads in a 180 ml. water blank which is contained in a screw capped bottle. Details are also given of the methods used in bacteriological examination of the samples and for the isolation of pure cultures from them.

3. From an analysis of the results the arithmetic "weighted" means of the plate count and methylene blue reduction test at 37°C. were found to be 4,363,000 per g. and 8.1 hours respectively. Individual plate counts varied widely, viz. from 1,400 to 149,000,000 per g., while the reduction tests varied from 3 to 14 hours. The plate counts at 55°C. also showed variations, although the range was smaller than at 37°C. The reduction times were longer at 55°C. than at 37°C. and 30 per

cent. of the samples required more than 12 hours' incubation before decolourisation occurred.

4. From a detailed study of the results information has been obtained regarding some of the factors responsible for these variations in bacterial quality. Wide variations in the mean of the plate counts of powders have been shown to occur from one factory to another and this appeared to be mainly associated with the technique of manufacture. It was also demonstrated that, since there was a marked difference in the range of variations of samples obtained from individual factories, there must have been a complete lack of consistency in the day-to-day management of certain of the plants. Some evidence is presented which tends to show that the bacteriological quality of milk powders may usually deteriorate during a continuous day's run owing to the multiplication of thermophilic and thermoduric bacteria during processing.

An examination of the data further demonstrated that seasonal variations in quality only occurred in plants where the general bacterial standard of the powder was normally poor. A comparison between a limited number of full cream milk powder samples and skimmed milk powder samples made at one factory indicated that the full cream powders were usually of a higher bacteriological quality. It is possible that these differences may have been associated with variations in the quality of the

incoming milk, since fresh whole milk might be expected to have the smallest bacterial flora. During prolonged storage the viable bacteria which were present in the milk powders died out. The mean percentage reduction in count after 12 months' aging at room temperature was 95 per cent. The bacterial flora tended to die out more quickly as the temperature of storage increased and decreased faster when stored in an atmosphere of air than in one of nitrogen.

5. The fermentation test has been shown to be mainly useful as an indication of the activity of thermophilic bacteria at 55°C.

6. About 10 per cent. of the samples tested in 1 ml. quantities gave positive presumptive coliform tests, but some of the positive tests were due to anaerobic sporeformers.

7. The number of yeasts and moulds present in the milk powder samples was found to be practically negligible.

8. The keeping quality of the reconstituted milk when stored at 60°F. has been examined. Over 90 per cent. of the samples remained sweet for between $2\frac{1}{2}$ and $3\frac{1}{2}$ days. Tests made on these samples after 24 hours' storage indicated that the flora of reconstituted milk did not increase as rapidly as that of raw milk and that the reduction test was the most sensitive indication of the additional aging of the milk.

9. The predominating flora of the reconstituted milk at 37°C. rendered it acid in reaction and clotted it. Eighty per cent. of the 1,304 cultures picked off the plates incubated at 37°C. and 74 per cent. from the plates held at 55°C. produced acidity

and coagulation in litmus milk media.

10. More than 97 per cent. of the pure cultures obtained from colonies on the plates incubated at 37°C. which produced acid coagulation of milk were found to be heat resistant streptococci. Further classification showed that 66 per cent. were strains of the "Viridans" division, 57 per cent. being S. thermophilus and 33 per cent. of the "Enterococcus" division, 26.5 per cent. of which were S. durans.

11. The milk curdling organisms obtained from the plates incubated at 55°C. have been identified as strains of B. calidolactis, which is one of the few thermophilic sporeformers capable of producing acid coagulation of milk.

12. A few bacteria causing peptonisation of milk at 37°C. have also been classified, and these were all found to be aerobic sporeformers of the B. subtilis type.

13. The use of the methylene blue reduction test as a method of assessing the bacteriological quality of milk powders has been explored and the conclusion is reached that this test gives reasonable agreement with the plate count at 37°C. provided that the correct regression equation is used. The reduction test at 55°C. does not appear, however, to be as useful as that at 37°C.

14. The desirability of establishing grades for spray dried milk powder is discussed.

PART V. HEAT RESISTANCE OF MILK MICRO-ORGANISMS.

1. It has been emphasised by a review of the literature that the destruction of living micro-organisms by heat is a complex process, and that the problem is particularly difficult when it is applied to the heat resistance of spores. Since the types of organisms which survive in the manufacture of canned dairy products and later cause spoilage depend to a great extent on their heat resistance, a study has been made of certain aspects which might provide information on the subject.

2. As regards the resistance of the general flora in bulk milk, when exposed to the heat of pasteurisation, it has been shown that the greatest killing effect occurs when the pH is lowest. These results suggest, therefore, that in pre-heating or pre-warming processes in the early stages of the manufacture of canned dairy products a greater reduction of initial count may be expected with the more acid milks.

3. It has been demonstrated by other workers that the resistance to heat of certain lactic acid bacteria was greatly increased in milk as the butterfat content was raised. Since at many stages of the various heat treatments of canned dairy products there is a high percentage of butterfat in the product, the effect of increasing the butterfat content has been studied with different milk bacteria which produce lactic acid. It was found that by raising the temperature of holding for a fixed time or by lengthening the time of holding at a fixed temperature the

increase of butterfat did not raise the heat tolerance of the organisms to any great extent. In fact the tendency in a few instances was for the heat resistance to be slightly less in cream.

4. The problems involved in the method of heating, and in the choice of a container for the inoculated substrate for heat resistance tests, is discussed with special reference to the difficulties involved at high temperatures such as are used in studies with spores of aerobic bacteria. Four different kinds of containers, namely (a) glass capillary tubes, (b) glass ampoules or bulbs, (c) glass pasteur pipettes and (d) test tubes, have been tried and a special kind of test tube was adopted to fulfil the necessary requirements. The tubes recommended should be made of heat resistant glass, 3" in length, with an internal diameter of $\frac{3}{8}$". In practice they were usually filled with inoculated substrate after sterilising and tightly corked by sterile rubber bungs. Metal clamps were used to ensure that the bungs fitted tightly throughout heating.

5. A trial test of the heat resistance has been conducted on nearly 40 strains of aerobic sporeformers isolated from canned milk products in order to obtain general information regarding the difficulties which might be expected later in more detailed studies. Many of the strains were found to be exceedingly heat resistant. No dormancy was apparent when the spores were heated and later incubated in milk products. "Skips" were

only observed to any great extent among strains of B. licheniformis.

6. Special studies on the heat resistance of 3 strains of B. subtilis which produced bitterness and thinning in canned cream have been made. Details are given of the methods of preparing spore suspensions for these tests and of preparing the substrates for subsequent inoculation so that equal concentrations of spores might be present in the final subsamples.

7. The effect of the reaction of the substrate on the destruction of 2 of these strains of B. subtilis by heat has been examined, but the results which have been expressed as survival percentages, over the narrow range of pH tested, lack consistency. Thus no definite change in the resistance of the spores, as has been shown for the non-sporeforming bacteria, could be demonstrated.

8. The results of similar experiments on the effect of increasing the butterfat percentage on the heat tolerance of B. subtilis also failed to show uniformity. In order to test the effect of this factor a second method, involving the use of plate counts, was explored. The plate count tests indicated more definitely than did the first method that the increase in butterfat in the subsamples did not increase the resistance of the spores to heat.

9. The most outstanding fact which was apparent from these studies with the aerobic sporeformers is their extremely high heat resistance. It is perhaps not surprising therefore that the small changes in the substrate as were explored in the tests reported failed to show clear-cut differences in resistance.

APPENDIX.

BACTERIOLOGICAL QUALITY OF RAW MILK.

1. The great importance of the bacteriological quality of the raw milk on the manufacture and subsequent quality of canned dairy products has been investigated and discussed.

2. It has been shown that certain tests which have the merit of giving results quickly are not usually sufficiently selective to assist in the grading of mixed milk from factories in the south-west of Scotland, and therefore that it is necessary to employ more sensitive tests. For this reason a comparison between two of the commonest tests used for this purpose, namely the plate count and the methylene blue reduction test, has been made.

3. The plate count has proved valuable in assessing the hygienic quality of milk in the past and criteria have been suggested which the reduction test should fulfil before the plate count could be replaced by it or any other test for milk grading.

4. Details of the experiments and of the methods of testing are given.

5. From the statistical analysis of the results the conclusion has been reached that for the general purpose of classifying milk according to bacteriological quality the modified methylene blue reduction test appeared to have outstanding advantages. This test fulfilled the criteria suggested in that (a) the results showed reasonable agreement with those obtained by the

plate count; (b) the modified reduction test showed as good an agreement with other measures of cleanliness as did the plate count; (c) the modified reduction test, when applied to mixed milk, was not invalidated by the presence of mastitis milk; and (d) the variability of the modified reduction test was less than that of the plate count.

6. A comparison between the sensitivity of the plate count and the methylene blue reduction test as conditions of milk production and of storage of the samples were changed has been made.

7. Details are given of the investigation. The samples were of single cow's milk. Four main factors of clean milk production have been varied to provide nine experimental periods, each period being of about a week's duration. The samples were stored under six widely varying conditions of temperature and for varying lengths of time before testing.

8. The results were analysed by three different methods, all of which indicated the same general findings. It was seen that for samples retained at 72°F. for 18 or 26 hours before testing the reduction test appeared to be considerably more sensitive to increasingly dirty methods of production than the plate count, and that this was also true when the samples were stored at room temperature (55°F.). At the lower temperature the effect was, however, only shown under the dirty conditions of production. Practically no difference in sensitivity was evident between the two tests when the samples were stored at room temperature for the first 10 hours and then in the refrigerator.

The results of samples tested immediately after milking showed very dirty methods of production most readily by the plate count test.

9. It should be noted that with the exception of samples stored at the warm temperature of 72°F. both tests were relatively insensitive to dirty conditions of production. This fact therefore shows the importance of using relatively severe storage conditions when any attempt is made to judge production methods by either test, and it further indicates that the grading of milk in cold weather or in relatively cold districts is of doubtful value as an index of cleanliness of production, unless some incubation of the milk occurs prior to testing.

REFERENCES.

(1) Public Health (Condensed Milk) Regulations (1923). Ministry of Health.

(2) Loveless A.H. (1935) Food 5, 50, 52.

(3) Scott A.W. (1932) Hannah Dairy Research Institute, Bull. 4.

(4) Savage W.G. and Hunwicke R.F. (1923) Dept. Sci. & Indus. Res. Food Invest. Board, Spec. Rep. 13.

(5) Allen L.A. (1932) Hannah Dairy Research Institute, Bull. 3.

(6) Esty J.R. and Stevenson A.E. (1925) J. Infect. Dis. 36, 986.

(7) Hammer B.W. (1927) Iowa Sta. Coll. J. Sci. 2.

(8) Hunziker O.F. and Wright W.R. (1910) Purdue Agric. Exp. Sta.

(9) Hammer B.W. (1917) Agric. Exp. Sta., Iowa Sta. Coll. Agric. Res. Bull. 38.

(10) Hunziker O.F. (1935) Canned Milk and Milk Powder, Ed.V, 445.

(11) Gibson T. and Topping L.E. (1936) Soc. Agric. Bact. Conf., Reading.

(12) _____ (1937) Soc. Agric. Bact. Conf., Edinburgh.

(13) _____(1938) " " " " Bristol.

(14) Howat G.R., Jackson C.J., and Nichols A.A. (1937) Milchwirtschaftlichen Weltkongresses 2, 241.

(15) Nichols A.A., Howat G.R., and Jackson C.J. (1937) J. Dairy Res. 8, 331.

(16) Wilson G.S. and Shipp H.L. (1938) Chem. Ind. 57, 834.

(17) Cameron E.J. and Esty J.R. (1926) J. Infect. Dis. 39, 89.

(18) Thayer L.A. (1912) Ver. Agric. Exp. Sta. Bull. 170.

(19) Hammer B.W. and Hussong R.V. (1932) J. Dairy Sci. 15, 220.

(20) Kelly C.D. (1926) Trans. Roy. Soc. Can. Section V, 387.

(21) Morrison E.W. and Rettger L.F. (1930) J. Bact. 20, 299.

(22) Hammer B.W. (1915) Agric. Exp. Sta., Iowa Sta. Coll. Agric. Res. Bull. 19.

(23) Hunziker O.F. and Cordes W.A. (1923) quoted from Hunziker - Condensed Milk and Milk Powder, Ed.V, 442.

(24) Sarles W.B. and Hammer B.W. (1932) J. Bact. 23, 301.

(25) Hussong R.V. and _____ (1931) Iowa Sta. Coll. Agric. J. Sci. 6, 89.

(26) Guide to the Conduct of Clean Milk Competitions (1934) Bull. 46, Min.of Agric.and Fish., London.

(27) Rice F.E. and Downs P.A. (1923) J. Dairy Sci. 6, 532.

(28) Downs P.A. (1925) ibid. 8, 344.

(29) Rogers L.A., Dahlberg A.O. and Evans Alice C. (1920) ibid. 3, 122.

(30) Knudsen (1922) Den. Kgl. Veterinaerog. Landbokojokele Aarsskryt, 282.

(31) Smith G. (1938) London School of Hygiene and Tropical Medicine. Private communication.

(32) Hammer B.W. (1919) Iowa Agric. Exp. Sta., Res. Bull. 52.

(33) Spitzer G. and Epple W.F. (1920) J. Dairy Sci. 3, 486.

(34) Grindrod (1928) Canning Age 9, 692.

(35) McMaster N.B. (1932-3) Rep. Fruit Veg. Pres. Sta., Campden, 71.

(36) Crossley E.L. (1938) Soc. Agric. Bact. Conf., Bristol.

(37) American Dry Milk Institute Inc., 221 North La Salle Street, Chicago (1936) The Grading of Dry Milk Solids.

(38) Sorensen C.M. (1938) Food Res. 3, 421.

(39) Wilson G.S.(1935) Spec. Rep. Ser. Med. Res. Coun. London, No. 206. H.M. Stationery Office.

(40) Crossley E.L., Aplin and Barret, Ltd., Yeovil. Private communication.

(41) Ayers S.H., Cook L.B. and Clemmer P.W. (1918) U.S. Dept. Agric. Bull. 642.

(42) Macey (1928) Quoted by Hucker and Hucker (1929) N.Y. (Geneva) Agric. Exp. Sta. Tech.Bull. 154.

(43) Sherman J.M. (1937) Soc. Amer. Bact., Bact. Rev. 1, 1.

(44) _____ (1938) N.Y. Sta. Coll. Agric., Cornell Univ. U.S.A., Private communications.

(45) Smith F.R. and Sherman J.M. (1938) J. Infect. Dis. 62, 186.

(46) Prickett P.S. (1928) N.Y. Sta. Agric. Exp. Sta., (Geneva), Tech. Bull. 147.

(47) Anderson E.B. and Meanwell L.J. (1933) J. Dairy Res. 4, 213.

(48) Hucker G.J. and Hucker A.M. (1929) N.Y.(Geneva)Exp. Sta., Tech. Bull. 154.

(49) Thompson E.C., Johnson A.H. and Kloser M. (1934) J. Dairy Sci. 17, 419.

(50) Buchanan R.F. and Fulmer E.I. (1930) Physiology and Biochemistry of Bacteria 2, 104.

(51) Robertson A.H. (1927) Vermont Agr. Exp. Sta., Bull. 274.

(52) Chick H. (1910) J. Hyg. 10, 237.

(53) Bigelow W.B. and Esty J.R. (1920) J. Infect. Dis. 27, 602.

(54) Mattick A.T.R. and Nichols A.A. (1935) J. Dairy Res. 6, 125.

(55) Brown C.W. and Peiser K. (1916) Mich. Agric. Coll., Exp. Sta., Tech. Bull. 30.

(56) Esty J.R. and Meyer K.F. (1922) J. Infect. Dis. 31, 650.

(57) Burke G.S. (1923) ibid. 33, 274.

(58) Esty J.R. and Williams C.C. (1924) ibid. 34, 516.

(59) Dickson E.C., Burke G.S., Beck D., and Johnston J. (1925) ibid. 36, 472.

(60) Williams O.B. (1929) ibid. 44, 421.

(61) Sommer E.W. (1930) ibid. 46. 85.

(62) Magoon C.A. (1926) J. Bact. 11, 253.

(63) _____ (1926) J. Infect. Dis. 38, 429.

(64) Williams F.T. (1932) J. Bact., 32, 589.

(65) Morrison E. and Rettger L.F. (1930) ibid. 20, 313.

(66) Curran H.R. (1935) J. Infect. Dis. 56, 196.

(67) Nichols A.A. and Edwards S.J. (1936) J. Dairy Res. 7, 258.

(68) _____ and Jackson C.J. (1938) Soc. Agric. Bact. Conf., Bristol.

(69) Malcolm J.F. (1932) J. Dairy Res. 4, 91.

(70) Mattick A.T.R., McClemont J., and Irwin J.O. (1935) ibid. 6, 131.

(71) Thornton H.R. and Hastings E.G. (1930) J. Dairy Sci. 13,221.

(72) Ellenberger H.B., Bond M.C., Robertson A.H. and Moody R.I. (1927) Utah Agric. Exp. Sta. Bull. 264.

(73) Hussong R.V. and Hammer B.W. (1931) Iowa Agric. Exp. Sta. Bull. 286.

(74) Pullinger E.J. (1935) J. Dairy Res. 6, 369.

(75) Thornton H.R., Strynadka N.J., Wood F.W. and Ellinger C. (1934) Canad. Dairy & Ice Cream J. 13, 8.

(76) Plastridge W.N., Anderson E.O., Brigham G.D. and Spaulding E.H. (1934) Storrs Agric. Exp. Sta. Bull. 195.

(77) FrazierW.C. and Whittier E.O. (1931) J. Bact. 21, 239.

(78) Ramsdell , Johnson and Evans (1935) J. Dairy Sci. 18,705.

(79) Bryon C.S. and Trout G.M. (1935) ibid. 18, 777.

(80) Edwards S.J. (1933) J. Comp. Path. 46, 211.

(81) Mattick A.T.R. (1937) Min. Agric. and Fish. Bull. 31.

(82) Thornton H.R., Strynadka N.J. and Wood F.W. (1934) Can. Pub. Health J. 284.

(83) Hoy W.A. and Neave F.K. (1938) Soc. Agric. Bact. Conf., Bristol.

(84) Hiscox E.R., Hoy W.A., Lomax R.L. and Mattick A.T.R. (1933) J. Dairy Res. 4, 211.

(85) Provan A.L., Dudley F.J. and Thomas S.B. (1936) Welsh J. Agric. 12, 130.

ACKNOWLEDGEMENTS.

The author is very much indebted to Dr. N.C. Wright for his valuable direction and constant help throughout the course of the work and to the members of the Staff of the Hannah Dairy Research Institute who, on so many occasions, have willingly given their assistance. She desires to thank Dr. T. Gibson for his help with the classification of the aerobic sporeformers, and Major A. Douglas, County Veterinary Office, for supplying some of the raw milk samples and carrying out farm inspections. She is also indebted to the firms who co-operated by supplying samples of spray dried milk powder and of canned milk and cream and, in particular, to the Scottish Milk Marketing Board for a grant which contributed towards the cost of part of this work.