I C E - C R E A M

and its

Relation to the Public Health

A

T H E S I S

F O R T H E D E G R E E

of

D O C T O R O F M E D I C I N E

Presented

to

THE UNIVERSITY OF GLASGOW

by

G E O R G E F. B U C H A N

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I hereby declare that the work for this Thesis has been done and the Thesis itself composed by me.

My thanks are due to Dr. John Robertson who suggested this investigation to me and who placed the facilities of the Public Health Department of the City of Birmingham at my disposal while the enquiry was being carried out.

The Bacteriological work was conducted in the Pathological Department of the University of Birmingham by kind permission of Professor R. F. C. Leith.

I desire also to record my indebtedness to Drs. R.M.Buchanan, A.C.Houston, Professor R.F.C.Leith, Dr. A.T.MacConkey, Professor E.J.McWeeney, and Dr. W.G.Savage for their kindness in sending me the various strains of organisms made use of during this investigation and tabulated in Table 9.

George F. Buchan.

ICE-CREAM AND ITS RELATION TO THE PUBLIC HEALTH.

The importance of ice-cream as a means by which infection may be carried and disease produced has not entirely escaped the attention of epidemiologists. In some recorded cases a particular ingredient of the ice-cream has been at fault while in others it has been the ice-cream as a whole which has become contaminated.

Recorded outbreaks due to the Ingredients in Ice-cream.

Scarlet Fever. In 1875 Buchanan [1] in a report to the Privy Council and Local Government Board called attention to the fact that ice-pudding had caused cases of Scarlet Fever and Sore-throat at South Kensington. In this outbreak the infection was traced to the cream which had been used in the manufacture of the ice-pudding.

Irritant Poisoning. Other ingredients of ice-cream have likewise been accused of causing illness, especially the colouring matter. 130 persons were attacked with symptoms of irritant poisoning in New York in 1884, [2a] and this was put down to the colouring material used in the ice-cream of which they all partook.

Recorded Outbreaks due to Ice-Cream.

Typhoid Fever. In 1891 Turner [3] traced the origin of an outbreak of Typhoid Fever occurring at Deptford to specifically infected ice-cream made by Italians living in Mill Lane. His investigation during this outbreak into the

conditions under which ice-cream is manufactured led him to conclude that the sale of ice-cream "should be regulated in the same way as the sale of milk".

In his Report on the Health of Liverpool, for 1897, Hope [4] records 27 cases of Typhoid Fever, 25 of which had partaken of ice-cream at a village fair and 2 of chip potatoes bought from the same vendor at the same time. A case of Typhoid Fever was resident in this vendor's house at the time of the fair.

The spread of Enteric Fever by ice-cream was again well illustrated in 1904, by the outbreak which occurred at Govan and was reported upon by Barras,[5] Bacteriologist to the Public Health Department there. 19 cases occurred and these all obtained ice-cream from a vendor who was suffering from an attack of Typhoid which he considered to be Influenza. Reference is made in this paper to a similar outbreak on a much larger scale investigated by Munro[6] in Renfrewshire in 1893.

Typhoid Fever and Gastro-Enteritis. Anderson[7] contributed to the May Number of Public Health, 1896, the results of an enquiry into the manufacture and storage of ice-cream which was popularly looked upon as the cause of certain cases of Typhoid Fever and Gastro-Enteritis at Blackpool. The

investigation however was begun late in the season and no definite conclusion was arrived at.

Diarrhoea. An interesting outbreak of Diarrhoea occurred in the middle of August, 1900, in the Rochdale District and was investigated by Henry.[8] 146 persons between the ages of 1 and 5 years were affected, and 141 of these had eaten ice-cream supplied by one man. The severity of the symptoms ranged from slight sickness to severe vomiting and dysentery. Death occurred in one case. The baby in the house of the vendor was suffering from Diarrhoea and Dr. Henry reports that on visiting the premises he found the baby's napkins being washed within a few inches of the strainer used in the process of manufacture of the ice-cream.

"Gaertner" Infection. A very complete record of an outbreak of disease due to the consumption of ice-cream is to be found in the Annual Report of the Medical Officer of Health[9] for Birmingham, 1905. 52 cases were recorded, 4 being adults and the remaining 48 children under 14 years of age. All had partaken of ice-cream supplied by one vendor and a sample of this was submitted to Leith for bacteriological analysis. The examination revealed the presence of a bacillus belonging to the Gaertner group, and to this organism the outbreak was consequently attributed.

Small-Pox. The British Medical Journal, 1901, Vol.2, contains a report by the Medical Officer of Health[10] for Rowley Regis on the spread of Small-Pox in that District by ice-cream obtained from a vendor who had a boy suffering from Small-Pox at home. The history of the outbreak is not clear and the evidence against the ice-cream not conclusive.

Ice-Cream Poisoning. Vaughan[2b] in 1886 chemically examined a sample of ice-cream which had caused illness in

18 persons. He inclined to the view that the poisoning was due to a chemical poison allied to tyrotoxicon.

Three cases of illness, one of which was fatal, occurred in St.Pancras in 1888 and in all of them ice-cream had been taken and was considered the etiological factor. At the inquest on the fatal case the possibility of the "leaden or pewter pot" in which the article was frozen having determined the symptoms of poisoning was freely put forward.[20]

An outbreak of poisoning by ice-cream occurred at Antwerp in 1898.[11] Some twenty cases were removed to hospital and the most striking symptoms of the illness were low temperature and cyanosis. Curiously enough there were no symptoms of gastro-intestinal irritation. The Analyst reported that the implicated ice-cream was good.

Collingridge[12] attributed the illness of 18 boys in the telegraph department in the City of London, in July 1902, to the consumption of ice-cream. The main symptoms were epigastric pain, colic, headache, nausea and nervous depression, associated in some instances with vomiting and diarrhoea. Klein was appointed at that time to conduct a bacteriological examination of samples of ise-cream taken in the City. Twenty-four samples were submitted and Klein reported that the number of organisms per cubic centimetre varied greatly and that thirteen of the samples were proved poisonous by inoculation into guinea-pigs. Many organisms of the Coli group were isolated during the investigation and one of them was an extremely virulent bacillus. The majority of the organisms were non-sporing and Klein was therefore of opinion that contamination had occurred after boiling, i.e., during the cooling and freezing processes.

Individual cases of illness due to Ice-Cream.

In addition to these various outbreaks ample reference may be found to individual cases of illness caused by ice-cream consumption. Hamer's Report[11] on the Preparation and Sale of Food in London to the London County Council in 1899, gives a good account of them and to that number may be added the case of a boy of 14 years (Lancet,Vol.2, 1900, p.1391) who succumbed to ptomaine poisoning as a result of eating ice-cream.

Previous Bacteriological Studies of Ice-Cream.

The foregoing accounts show that ice-cream may be the carrier of very many diseases. The first systematic study of the frozen commodity from the bacteriological point of view was conducted in this country by MacFadyen and Colwell[13] in 1895. Their investigations included chemical, microscopic and bacteriological examinations. Bed bugs, bugs' legs, fleas, straw, human hair, cats' and dogs' hairs, coal dust, woollen and linen fibres, tobacco, epithelial scales and muscular tissue were all revealed as occasionally polluting this material. The maximum number of organisms per cubic centimetre in ice-cream which these observers found present in shop samples was just over 1,000,000 and in barrow samples over 7,000,000.

Nield-Cook [14] gave attention to the subject in the following year. He obtained 14,280,000 microbes per cubic centimetre in ice-cream and was able to isolate from several samples the Bacillus Coli Communis, Proteus Vulgaris, the Bacillus Fluorescens Liquefaciens and many Cocci.

Wilkinson[15] published his bacteriological results in the January Number of Public Health, 1899 and the examinations by Klein[12] in 1902 of twenty-four samples taken in the City of London have been already referred to.

In America the Annual Report[35] of the Boston Health Department for 1906 furnishes records of the bacteriological examinations of various samples of ice-cream and hokey-pokey taken during 1906 in which the highest bacterial count was 150,000,000 per cubic centimetre and the lowest 1,000,000. An account of similar work conducted in the bacteriological laboratory of the City of Philadelphia by Pennington and Walter[24] during 1905 and 1906 appeared in the November Number of the New York Medical Journal for 1907. 68 samples were examined and among these the lowest count recorded was 50,000 per cubic centimetre and the highest more than 151,200,000 and called innumerable. Their examination included an enumeration of leucocytes and a search for streptococci as well as a sanitary inspection of the premises on which the article was manufactured.

Scope of the present investigation.

The present research has been undertaken in Birmingham with a view to ascertaining how and to what extent ice-cream is contaminated. It has been limited to the ice-cream prepared by Italians and small retail English confectioners and has included

(1) An enquiry into the methods of preparation,

(2) A sanitary inspection of the premises on which ice-cream is made, and

(3) A bacteriological study of the mixture at various

stages in the process of manufacture.

Composition of Ice-cream in this Country.

The ingredients vary according to the quality of the article sold. The simplest form of ice-cream is composed of milk, sugar and cornflour. In others the cornflour is replaced by "ice-cream powder", and to either combination eggs may be added in the proportion of 4 to 12 to the gallon. Sometimes a colouring agent is added and the finished product varies in colour from white to orange.

Methods of preparation.

The methods of preparation by 50 different vendors (33 English and 17 Italian) were investigated.

Heating. The first stage of the process varies according to the practice of the manufacturer. In 4 instances (all English) the milk and sugar were boiled in an enamel pot and poured with stirring onto the remaining ingredient or ingredients in a galvanised iron bucket.

In 17 instances (4 Italian and 13 English) some form of "water bath" was used for heating all the ingredients together. In only one instance (No.2 in Table 2 Italian) was a boiler reserved for use as a water-bath; the other 16 manufacturers using the washhouse boiler or a large pot. In this method of heating, the ice-cream ingredients were put into the freezer which in its turn was placed in the water of the boiler and gradually heated.

In the remaining 29 instances (16 English and 13 Italian) the milk and sugar were boiled in enamel or iron pots directly over an open fireplace or stove and the cornflour or ice-cream powder and eggs, if any, were gradually added with stirring and maintenance of the temperature.

The duration of heating varied greatly. In the first method the milk and sugar were merely brought to the boil, while in the second and third methods the manufacturers stated that boiling was continued in some cases for a few minutes and in others for one hour and a half.

Cooling. After this treatment the ice-cream commodity is set out in galvanised iron buckets to cool, or it may remain in the freezer if the first stage was carried out in that vessel. In only one instance (No. 6 in Table 2 Italian) were these buckets reserved solely for use in the manufacture of the ice-cream. In the remaining 49 cases they were used for ordinary domestic purposes as well, and in one instance for tripe cleansing also. In 10 cases (8 Italian and 2 English) the ice-cream was covered while cooling and with regard to the 8 Italians it may be said that in no instance was the covering effident. It consistedvof curtain material with a wide mesh work which would prevent only large particles of dirt gaining access. The 2 English vendors used fine muslin.

1. Cooling in shed with Curtain Cover.
(No 2 in Table 2)

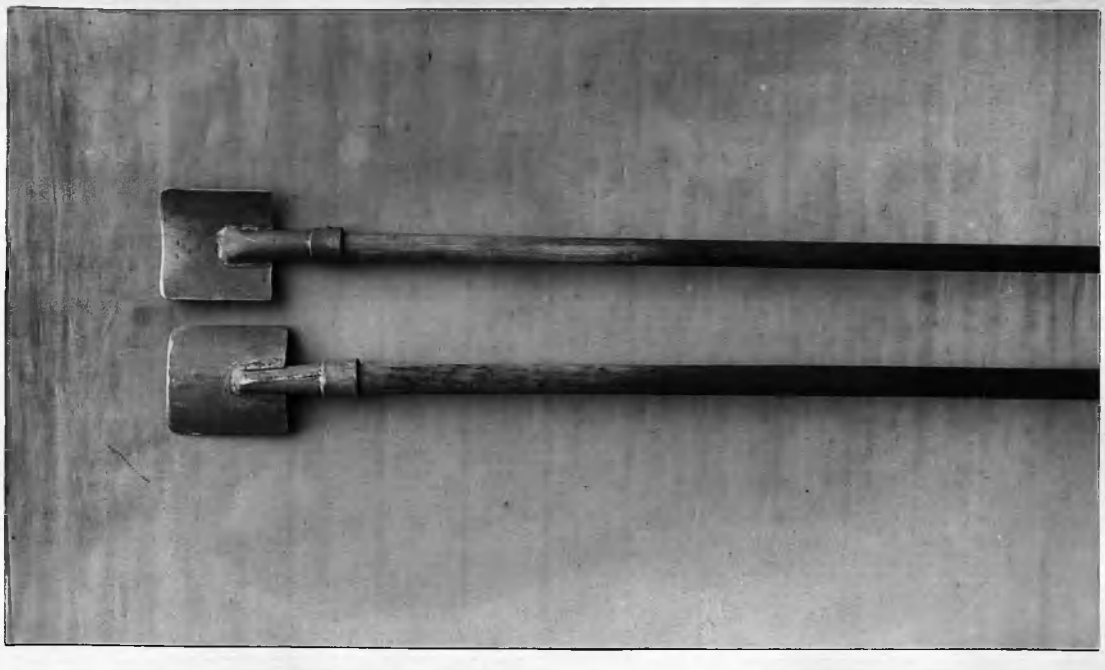
No special attention was given to the washing or cleansing of these coverings which were used over and over again until obviously unclean. In several cases the covering dipped into the ice-cream while cooling. The vessels in which the ice-cream was cooled were usually placed on a slope so as to expose a larger surface to the air for cooling and were allowed to stand in this position over night.

Freezing. On the following morning the mixture was strained into the freezer (in those cases in which it had not cooled in the freezer) through a metal sieve. The freezers used were either of the English or the American pattern and the freezing mixture was invariably salt and ice. The salt and ice were usually specially supplied by a trader for the purpose, but in a few instances salt was used which had been previously employed in the curing of bacon.

The English Freezer. During the process of freezing the inner vessel containing the mixture is in constant rotation. Where the English freezer is used the ice-cream has to be stirred up frequently to hasten and complete its solidification. For this purpose a special metal spade with a wooden handle is employed, and the lid of the vessel is removed and usually not replaced. The spade is worked by the hands which have not been washed before freezing is begun, and which, sliding up and down the handle of the spade and being used occasionally for tasting the ice-cream, greatly add to the contamination of the finished product.

2. English pattern. Method of"Spading" (No 2 in Table 2)

Note that the lid is placed on the ground during the process.



3. English pattern. Spades used in freezing (No.13 in Table 2). Note their rough construction and the lighter area on the shafts where the operator's hands have worked up and down.

The American Freezer. When the American Freezer is used the lid is kept on constantly during the whole freezing process and the necessary stirring of the ice-cream is done by a mechanical contrivance, inside the apparatus. This freezer obviously greatly diminishes the risks of pollution and it ought to be in general use.

4. American pattern. Freezing.

5. American pattern. Shewing mechanical contrivance for stirring, removed from interior of freezer.

The Premises and their Surroundings.

Twelve manufacturers (all Italians) had sheds erected for the preparation of the article. These sheds were situate in a common yard and were constructed of wood carelessly nailed together with a corrugated zinc roof on which were to be found old baskets, rabbit skins, and other rubbish. The floors were badly paved. Only one of these twelve manufacturers (No.6 in Table 2) had a place used solely by himself. The others used sheds in common with other vendors. In many cases tubs overflowing with vegetable refuse were close at hand. It is very difficult to get Italians to make a proper use of W.Cs. and in consequence these were commonly found in a filthy state in the same common yard as that in which the ice-cream was manufactured. Reference has already been made to the fact that one manufacturer (No.2 in Table 2) had a boiler which he reserved for the heating of the ice-cream. This, however, was placed in a small dark corner of the yard and was surrounded with decaying vegetable matter and human excreta. Heating and cooling of the ice-cream material are carried out in these badly constructed sheds and during the cooling stage the commodity is exposed through the open door, defective joints and roof, to contamination by the dust and dried human excrement from the surface of the ill-paved and dirty common yards in which the manufacturing process is conducted. Amongst the remaining thirty-eight vendors the process of boiling or heating is carried on in the living room, kitchen or scullery of the house* or in the

* In the case of Italians the house is frequently overcrowded. One such example will suffice. No.4 in Table 2 - 3 small bedrooms and 2 small living-rooms were occupied by 7 adults (over 15 years) and 6 children (under 14 years).

washhouse common to several families in the same yard. The ice-cream may then be put on the sink in the scullery or on the doorstep to cool. Freezing is done in the common yard or sometimes at the door in the street.

Photographs to Illustrate Insanitary Conditions.

6. Shed used for heating and boiling by Nos 3,5,8,13, 14,15,16 and 21 in Table 2.

Note the defective roof, ill fitting boards, unpaved floor and uncovered dustbins close by.

Photographs illustrating Insanitary Conditions (Contd).

7. Interior of shed shewn in Photograph 6.

8. Inside the corrugated zinc shed (belonging to No 2 Table 2) there is a boiler which is reserved for the manufacture of ice-cream.

Photographs illustrating Insanitary Conditions (Contd).

9. Interior of shed shewn in Photograph 8. Note accumulation of vegetable refuse and human excreta at side of boiler.

Photographs illustrating Insanitary Conditions (Contd)

10. A recess in a shop over cellar head where heating of ice-cream mixture is done. (No 22 in Table 2).

11. Cooling in a shed. (No 2 in Table 2).

Photographs illustrating Insanitary Conditions (Contd).

12. A very common type of premises (No 4 in Table 2).

13. The cleanest premises inspected (No 6 in Table 2).

The Storage of Ice-Cream.

Where no sheds have been built for the purpose of the trade in ice-cream as in the case of the 12 manufacturers already alluded to, the frozen product is stored, usually uncovered, in a dirty underground cellar to which the dust from the street or yard gains easy access through a perforated iron grating or grid. This cellar is also used for the storage of other materials e.g., wood, coal, etc.

The Cleansing of Vessels.

All the manufacturers stated that the vessels employed in the preparation and sale were washed out with hot soda water, scalded, and thoroughly rinsed after use. It is doubtful if this process is always carried out, as in the majority of cases boiling water can be obtained in sufficient quantity only by heating over the kitchen fire in the same pot as was used for the preparation of the ice-cream. (With regard to the glass vessels in which ice-cream is sold to the purchaser it is well to note that in the case of street trollys the vendor has only a very small and limited supply of water to cleanse them for the use of subsequent customers.)

The storage of vessels.

For the most part ice-cream is prepared on a Friday for sale during the week-end and for the rest of the week the utensils used are stored anywhere as convenient, no special place being provided for them.

Sanitary Classification of Premises.

All the foregoing points were taken into consideration in classifying the various premises inspected, into clean, fair, dirty and filthy (Vide Col.,11,Table 2).

The following classification gives the results of the premises inspected:-

5 or 10% were considered clean.

18 or 36% were considered fair.

23 or 46% were considered dirty.

4 or 8% were considered filthy.

Collection of the Samples.

The bacteriological examination was begun on the 7th of July, 1908. Three series of Samples were taken called respectively, "a" "b" and "c".

"a" Samples: taken from each of 50 manufacturers immediately after the ice-cream material had been boiled. These samples were taken directly out of the vessels in which the ingredients were heated.

"b" samples: taken after the commodity had been cooled for variable periods of time.

"c" samples: taken after the material had been frozen and put on the market.

All these samples - "a" "b" and "c" in Table 2 - were taken with sterile precautions in wide mouthed bottles of about 200 cubic centimetres capacity and conveyed to the laboratory in a specially constructed case without delay. The frozen ice-cream never reached the laboratory in a melted condition.

Dilution of the Samples for Bacteriological Examination.

It was always possible to deal with the "a" samples without

dilution, as the preliminary quantities were put into the various media while the ice-cream material was still hot and before it had set. It was also practicable to deal in a similar way with several of the other samples. But with many of the cooled and all the frozen samples, dilution was necessary. This was invariably carried out with sterile distilled water in the sample bottles which were graduated at two points A and B, the capacity of the bottle to B being twice that to A.

The Bacteriological Examination.

The routine examination of these samples was as follows:-

Test 1. An enumeration of the colonies capable of growing on nutrient gelatine (reaction + 1%) at 20° - 22° C in 72 hours.

Test 2. A similar estimation using nutrient agar (reaction + 1%) and incubating at 35° - 37° C for 48 hours.

Test 3. Various quantities were put into MacConkey's glucose broth, and after incubation at 35° - 37° C for 48 hours the reaction produced was noted. In those cases in which acid and gas were produced in this medium a looplet was plated after appropriate dilution on MacConkey's Neutral Red Bile Salt Agar, and an endeavour made to determine the identity of the colonies by 16 different tests.

Test 4. An estimation of the presence or absence of the Bacillus Enteritidis Sporogenes, and also of the numbers in which this organism existed, was made by incubating decimultiple quantities of the ice-cream, after heating to destroy non-sporing organisms, under anaerobic conditions at 35° to 37° C for seven days.

The method employed for this last test is of interest.

Ice-cream is a substance which itself contains milk in large proportion. Fifty cubic centimetres of Sterile water were put into sterile flasks, with long necks, of 150 cubic centimetres capacity, and 100 cubic centimetres of ice-cream were added, and vaseline, into which a little hard paraffin had been put, was poured over in the usual way. Similarly 10 cubic centimetres of ice-cream were added to about 10 cubic centimetres of sterile water in a tube and sealed as above. In the case of 1 cubic centimetre of ice-cream this was added to about 10 cubic centimetres of sterile milk and water (half and half), the mixture of vaseline and hard paraffin being poured over as before to exclude the air. After heating to kill non-sporing organisms incubation was allowed to proceed at 35° - 37°C. In the earlier samples examined milk was used as above instead of water, but owing to the consistency of the ice-cream the typical "enteritidis change" was not well defined. The substitution of water for milk greatly improved the results and increased the ease of the manipulation as well.

A typical reaction resulted in many cases in 24 hours, but the above period -seven days- was allowed to elapse before a negative result was entered. Positive results were recorded only in those cases in which a typical torn and irregular pinkish clot was formed with a moderately clear whey and evolution of gas. Microscopic examination of the whey revealed the presence of large bacilli and spores when the conditions had become, by the forcing out of the plug of vaseline, no longer anaerobic.

Test 5. Neutral red glucose broth was used as the medium for incubating with deci-multiple quantities of the ice-cream for determining the presence of streptococci. After

incubation at 35° - 37° C for 48 hours the sediment was microscopically examined, and the character of the chains when present noted. In those samples where the number of streptococci as seen by the microscope appeared abundant attempts were made to isolate the organism sometimes on Drigalski's medium, and sometimes on glucose Agar.

The following table shews the various quantities used for examination in the case of each sample:-

Table 1

Shewing quantities of "a" "b" and "c" Samples put on for the 5 primary tests.

Tests	"a" Samples	"b" Samples	"c" Samples
Test 1	B & D*	D & F	D & F
Test 2	"	"	"
Test 3	10 cc., 1 cc., A & B	10 cc., 1 cc., A, B, C & D.	10 cc., 1 cc., A, B, C & D.
Test 4	100 cc., 10 cc., & 1 c.c.	100 cc., 10 cc., & 1 c.c.	100 cc., 10 cc., & 1 c.c.
Test 5	10 cc., 1 cc., A & B	10 cc., 1 cc., A, B, C & D.	10 cc., 1 cc., A, B, C & D.

* A = ·1 cubic centimetre
B = ·01 " "
C = ·001 " "
D = ·0001 " "
E = ·00001 " "
F = ·000001 " "

incubation at 35° - 37° C for 48 hours the sediment was microscopically examined, and the character of the chains when present noted. In those samples where the number of streptococci as seen by the microscope appeared abundant attempts were made to isolate the organism sometimes on Drigalski's medium and sometimes on glucose Agar.

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Shewing quantities of "a" "b" and "c" Samples put on for the 5 primary tests.

Tests	"a" Samples	"b" Samples	"c" Samples
Test 1	B & D *	D & F	D & F
Test 2	"	"	"
Test 3	10 cc., 1 cc., A & B	10 cc., 1 cc., A, B, C & D.	10 cc., 1 cc., A, B, C & D.
Test 4	100 cc., 10 cc., & 1 c.c.	100 cc., 10 cc., & 1 c.c.	100 cc., 10 cc., & 1 c.c.
Test 5	10 cc., 1 cc., A & B	10 cc., 1 cc., A, B, C & D.	10 cc., 1 cc., A, B, C & D.

* A = ·1 cubic centimetre
B = ·01 " "
C = ·001 " "
D = ·0001 " "
E = ·00001 " "
F = ·000001 " "

For ease of reference it is well to set out the results of the primary tests now, and this is done in the following table:-

Table 2.

General Bacteriological

Results.

(Vide pp. 25 - 35)

T a b l e 2.

General Bacteriological Results.

1	2		3	4	5	6		7	8	9	10	11
	Stage in process											
No. of Sample.	No. of hours cooling.	No. of hours frozen.	When examined.	Number of colonies per cubic centimetre capable of growing on Nutrient Gelatine (reaction +1%) at 20° - 22° C. in 72 hours.	Number of colonies per cubic centimetre capable of growing on Nutrient Agar (reaction+1%) at 35° -37° C. in 48 hours	Smallest amount of Ice-cream producing a reaction in MacConkey's Glucose Broth at 35° - 37° C. in 48 hours		Smallest amount of Ice-cream giving typical reaction of Bacillus Enteritidis Sporogenes when cultured anaerobically in Milk at 35° - 37° C.	Smallest amount of Ice-cream shewing microscopically the presence of Streptococci in Neutral Red Glucose Broth in 48 hours at 35° - 37° C.	Nature of Streptococcal chains.	English or Italian manufacturer.	Sanitary condition of premises. (see note page 20)
						Acid	Acid and Gas					
1 a			3 pm 7 Jul	2,100	5,000	A		10 c.c.	B	Long	E*	Fair
b	21		12 noon 8 "	6,600,000	230,000	B		10 c.c.	B	Long & short		
c	40	4	11 am 9 "	21,000,000	5,100,000		B	10 c.c.	B	" " "		
2 a			4.30pm 7 Jul	less than 100	200	1 cc		10 c.c.	B	Long	I®	Filthy
b	18		10.30am 8 "	180,000	20,000		B	10 c.c.	B	Long & short		
c	41		9.30am 9 "	7,000,000	5,000,000		D	10 c.c.	B	" " "		

1		2		3	4	5	6		7	8	9	10	11
3	a			12 noon.16 Jul.	10,000,000	2,000,000		B	10 c.c.	B	Long	I	Dirty
	b	28		4pm. 17 "	12,000,000	5,600,000		C	10 c.c.	B	Short		
	c	44	2	10am. 18 "	2,000,000,000	500,000,000		D	10 c.c.	D	Short		
4	a			12 noon.16 Jul.	80,000	50,000	1 cc		100 c.c.	A	Long	I	Dirty
	b	28		4pm. 17 "	50,000,000	42,000,000		B	10 c:c.	B	Long&short		
	c	44	3	11am. 18 "	2,200,000,000	400,000,000		D	10 c.c.	C	Long		
5	a			12 noon.17 Jul.	20,000,000	8,000,000		1 c.c.	10 c.c.	1 c.c.	Long	I	Dirty
	b	22		10am. 18 "	28,000,000	12,000,000		B	10 c.c.	B	Long		
	c	41	4	9am. 19 "	3,800,000,000	250,000,000		D	1 c.c.	D	Long & short		
6	a			5.30pm. 21 Jul	less than100	2,000	10 cc		100. c.c.	absent	-	I	Clean
	b	22		3 30pm. 22 Jul	liquified 3dys	100,000	A		10 c.c.	C	Long		
	c	38	5	12.30pm 23 "	12,000,000	20,000,000		B	10 c.c.	D	Long		
7	a			2pm 22 "	liquified 2dys	300,000	A		100 c.c.	A	Long	I	Dirty
	b	22		12 noon.23 "	38,000,000	50,000,000	A		10 c.c.	B	Long		
	c	22	5½	5.30pm. 23 "	3,200,000,000	65,000,000	D		10 c.c.	D	Long		

1		2		3	4	5	6		7	8	9	10	11
8	a			2.30pm 23 Jul	20,000	44,000	A		10 c.c.	Absent	-	I	Dirty
	b	20		10.30am 24 "	100,000,000	4,000,000	B		1 c.c.	Absent	-		
	c	41	1	8.30am 25 "	2,400,000,000	124,000,000		D	1 c.c.	A	Short		
9	a			7.pm 27 Jul	10,000	2,000	A		1 c.c.	Absent	-	E	Clean
	b	14		9.am 28 "	270,000	200,000		1 c.c.	1 c.c.	Absent	-		
	c	14½	3	12.30pm 28 "	560,000	320,000		A	1 c.c.	Absent	-		
10	a			7 pm 27 Jul	2,400	1,900	10 c.c		100 c.c.	Absent	-	E	Fair
	b	14		9 am 28 "	80,000	60,000		A	10 c.c.	B	Long&Short		
	c	20½		3.30pm 28 "	230,000	100,000		B	1 c.c.	C	" "		
11	a			7.30pm 27 Jul	17,800	15,000	A		100 c.c.	Absent	-	E	Fair
	b	14		9.30am 28 "	50,000	20,000		1 c.c.	100 c.c.	Absent	-		
	c	15½	4½	3.30pm 28 "	510,000	350,000		B	1 c.c.	Absent	-		
12	a			12.30pm 28 Jul	62,500	58,000	1 c.c		10 c.c.	Absent	-	E	Clean
	b	21		9.30am 29 "	Liquified 2dys	290,000		A	10 c.c.	A	Short		
	b	42	3	9.30am 30 "	500,000	850,000		C	1 c.c.	C	Short		

1	2	3	4	5	6	7	8	9	10	11
13 a		9.30am 31 Jul	Liquified 3dys	23,000	1 c.c	Absent	Absent	-	I	Dirty
b	7	4.30pm 31 "	" "	1,550,000	B	Absent	Absent	-		
c	23	8.30am 1 Aug	" "	2,280,000	B	Absent	A	Long		
14 a		2.15pm 31 Jul	2,100	2,000	1 c.c	Absent	A	Long	I	Dirty
b	3	5.15pm 31 "	30,000	100,000	A	Absent	A	Long		
c	18¼	8.30am 1 Aug	2,500,000	160,000	1 c.c.	10 c.c.	C	Long		
15 a		2.15pm 31 Jul	Liquified 3dys	64,000	1 c.c	Absent	1 c.c.	Long	I	Dirty
b	3	5.15pm 31 "	" "	1,000,000	A	100 c.c.	A	Long		
c	18¼	8.30am 1 Aug	" "	11,500,000	A	10 c.c.	C	Long		
16 a		2.30pm 31 Jul	1,300	8,000	No change	Absent	Absent	-	I	Dirty
b	2½	5. p m 31 "	20,000	20,000	A	100 c.c.	Absent	-		
c	18	8.30am 1 Aug	Liquified 3dys	1,130,000	1 c.c.	100 c.c.	A	Long		
17 a		5. p m 31 Jul	Liquified 3dys	4,800	No change	1 c.c.	Absent	-	E	Fair
b	18	11 a m 1 Aug	" "	60,000	A	1 c.c.	Absent	-		
c	19½	12.30pm 1 "	" "	380,000	B	1 c.c.	Absent	-		

1		2	3	4	5	6		7	8	9	10	11
18	a		11 p m 31 Jul	Liquified 3dys	4,300		1 c.c.	100 c.c.	Absent	-	E	Filthy
	b	10	9 a m 1 Aug	" "	11,000,000		B	1 c.c.	Absent	-		
	c	13½	12.30pm 1 "	" "	11,700,000		B	1 c.c.	Absent	-		
19	a		4.30pm 31 Jul	Liquified 3dys	60,000	1 c.c.		10 c.c.	A	Long	E	Dirty
	b	16	8.30am 1 Aug	" "	560,000		A	10 c.c.	A	Long		
	c	20	12.30pm 1 "	" "	3,600,000		B	10 c.c.	C	Long		
20	a		4.30pm 31 Jul	Liquified 3dys	480,000	1 c.c		Absent	A	Long	E	Dirty
	b	16	8.30am 1 Aug	" "	9,400,000		B	Absent	B	Long		
	c	20	12.30pm 1 "	" "	16,000,000		B	Absent	D	Long		
21	a		10 p m 31 Jul	2,000	7,000		A	Absent	A	Long	I	Dirty
	b	10	8 a m 1 Aug	70,000	20,000		A	10 c.c.	A	Long		
	c	12	10 a m 1 "	Liquified 3dys	300,000		B	1 c.c.	C	Long		
22	a		7.30am 1 Aug	2,800	3,000	1 c.c.		Absent	Absent	-	I	Filthy
	b	1½	9 a m 1 "	Liquified 3dys	20,000		1 c.c.	100 c.c.	Absent	-		
	c	3½	11 a m 1 "	50,000	30,000		1 c.c.	10 c.c.	A	Long		

1		2		3		4	5	6		7	8	9	10	11
23	a			10 a m	3 Aug	846,000	460,800	1 c.c		10 c.c.	A	Long	E	Filthy
	b	6		4 p m	3 "	10,800,000	4,400,000		B	1 c.c.	A	Long		
	c	6	1	5 p m	3 "	25,200,000	9,920,000		B	1 c.c.	D	Long		
24	a			11 a m	3 Aug	Liquified 3dys	1,750,000	1 c.c		10 c.c.	A	Long	E	Fair
	b	5		4 p m	3 "	102,240,000	38,880,000		B	1 c.c.	C	Long		
	c	5	1	5 p m	3 "	119,080,000	42,960,000		C	1 c.c.	C	Long		
25	a			11 a m	3 Aug	Liquified 2dys	1,220,000		A	10 c.c.	A	Long	E	Fair
	b	5		4 p m	3 "	" "	15,840,000		C	1 c.c.	B	Long		
	c	5	½	4.30pm	3 "	" "	19,440,000		C	1 c.c.	D	Long		
26	a			11 a m	3 Aug	Liquified 2dys	184,000		A	1 c.c.	A	Long	E	Clean
	b	22		9 a m	4 "	" "	1,260,000		C	1 c.c.	C	Long		
	c	32	2	9 p m	4 "	" "	13,000,000		D	1 c.c.	C	Long		
27	a			11 a m	3 "	Liquified 1 dy	460,800		10 c.c	10 c.c.	Absent	-	E	Fair
	b	5		4 p m	3 "	" 2dys	16,000,000		B	10 c.c.	10 c.c.	Long		
	c	5	2	6 p m	3 "	" "	25,920,000		C	1 c.c.	C	Long		

Classification of the "b" samples according to the results of Test 1.

	No. of organisms per cubic centimetre.
10 samples contained	between 10,000 and 100,000
9 " "	" 100,000 and 1,000,000
5 " "	" 1,000,000 and 10,000,000
9 " "	" 10,000,000 and 100,000,000
2 " "	over 100,000,000
15 were liquefied in three days.	

"c" Samples.

These samples were all taken after the material had been frozen. In one instance freezing was done after the sample had cooled 3½ hours, but in two instances cooling had gone for 44 hours before freezing was begun. The average time which elapsed between heating and freezing was about 20¾ hours for all the 50 samples examined. This supports the statements of the manufacturers that their usual practice is to freeze within 24 hours of boiling. The "c" samples were taken on an average 2½ hours after freezing. 38 of these samples were purchased when the article was exposed for sale in the street in the case of the Italian hawkers, or in a shop in the case of the small confectioners. The remaining 12 samples were obtained immediately freezing was completed, and before the finished product was placed on the market. In every case there was an enormous increase of bacteria in these frozen samples over the corresponding cooled samples, the average for the former being

372,213, 421 as against 13,042,857 colonies per cubic centimetre for the latter. This average for frozen samples is higher than that obtained by previous investigators who, however, examined only a few samples. It is mainly accounted for by the samples yielding more than 1,000,000,000 organisms per cubic centimetre (5 in number), excluding which (gives) an average is obtained of 16,470,515 organisms per cubic centimetre - a number in accord with other workers-.

Classification of the "c" Samples according to the results of Test 1.

	No. of organisms per cubic centimetre.
2 samples contained	between 10,000 and 100,000
13 " "	" 100,000 and 1,000,000
5 " "	" 1,000,000 and 10,000,000
11 " "	" 10,000,000 and 100,000,000
7 " "	over 100,000,000
12 samples were liquefied in 3 days.	

Causes of the increase in the Number of Organisms in the "c" Samples.

1. Multiplication of organisms during further period of cooling. This increase is due in part to multiplication during the further time the material was cooling namely, 5 hours on the average.

2. Multiplication of Organisms during frozen period.

It is also due to multiplication of organisms during the frozen period, as shewn by the following experiment:-

Experiment. On the 9th of April, 1909, ice-cream was obtained from a manufacturer in a freezer, which was brought to the laboratory and kept surrounded by ice and salt.

On its arival at the laboratory

the "gelatine count" of the ice-cream was 327,000 colonies per cubic centimetre

After 3 hours

the "gelatine count" was 388,000 " "

After 6 hours

the "gelatine count" was 459,000 " "

After 9 hours

the "gelatine count" was 603,000 " "

After 12 hours

the "gelatine count" was 611,000 " "

The "agar count" of the ice-cream on its arival at the laboratory was

93,000 colonies per cubic centimetre

After 3 hours

the "agar count" was 112,000 " "

After 6 hours

the "agar count" was 126,000 " "

After 9 hours

the "agar count" was 130,000 " "

After 12 hours
the "agar count" was 139,000 colonies per cubic centimetre.

During the experiment the temperature of the ice-cream varied between 28°F and 28·8 F.

The multiplication of organisms in Ice-Cream alternately Thawed and Frozen.

Alternate thawing and freezing did not probably occur in the samples under consideration, but under these conditions Pennington[25] has shewn that the organisms in ice-cream increase very rapidly, and this observation was confirmed during the enquiry by the following experiment:

Experiment.

Ice-cream freshly prepared by a manufacturer was purchased in the freezer in which it was made and brought to the laboratory. The initial bacterial count was taken, and the mixture of the salt and ice surrounding the ice-cream was thereafter renewed daily and bacterial counts taken at regular intervals. The maximum and minimum temperatures of the ice-cream during the intervals of sampling were recorded. The results are given in Table 3.

T a b l e 3.

Shewing multiplication of organisms in ice-cream alternately thawed and frozen.

Date	Hour	Temperature in Degrees F.		No. of organisms per cubic centimetre capable of growing on nutrient gelatine (reaction + 1%) at 20° to 22° C in 3 days.	No. of organisms per cubic centimetre capable of growing on nutrient agar (reaction + 1%) at 35° to 37° C in 2 days.
		Max.	Min.		
1909 March					
4	6 pm	Initial count		28,000	688,000
7	9.30am	50°	29°	1,970,000	12,480,000
10	6 pm	50°	29°	21,760,000	26,880,000
13	11.30am	50°	30°	144,240,000	182,880,000
16	2 pm	46°	29°	1,232,000,000	1,456,000,000
19 *	12.30pm	46°	29°	1,872,000,000	1,236,000,000
22	12 noon	41°.7	33°.3	5,732,000,000	1,156,000,000
25	6 pm	39°.5	28°	18,120,000,000	1,220,000,000
28	11.15am	46°.8	25°	56,160,000,000	860,000,000
31	3.45pm	47°	27°	54,200,000,000	790,000,000

* By this date the ice-cream had become clotted and was obviously unsound.

The results recorded in the foregoing experiment shew the importance in the interests of a pure ice-cream supply of not allowing re-frozen ice-cream to be exposed for sale.

Diminution in Number of Organisms at Low Temperatures.

In order to prevent effectually the multiplication of organisms in ice-cream it is necessary to keep it frozen at low temperatures.

Ice-cream was placed in a cold storage room by permission of the Linde Refrigerator Company, Birmingham, and bacterial counts taken at stated intervals gave the following results:

T a b l e 4.

Shewing Diminution of the Number of Organisms in Ice-Cream during cold storage.

Date 1909	Fahrenheit Temperature		No. of Organisms per cubic centimetre capable of growing on nutrient gelatine (reaction +1%) at 20° to 22° C. in 5 days.	No. of Organisms per cubic centimetre capable of growing on nutrient agar (reaction +1%) at 35° - 37°C in 2 days.
	Max	Min		
1	2	3	4	5
Mar.4.	Initial count		28,000	688,000
" 5	-	13°.5	29,000	700,000
" 6	18°.5	10°.0	28,000	654,000
" 7	18°.5	11°.0	20,000	628,000
" 10	22°	13°	10,000	220,000

1	2	3	4	5
Mar.13	18°·5	13°	8,000	55,000
" 16	19°·5	12°	7,000	13,000
" 19	20°	13·5°	3,200	4,800
" 22	22°	15°	3,100	4,500
" 25	21°	14°	1,760	2,880
" 28	20°	12°	1,510	2,720
" 31	22°	18°	1,484	2,040
Apl. 3	20°·5	14°	1,024	1,350
" 6	22°	15°	1,088	990
" 21	20°	16°	760	960
" 24	21°	15°	720	672
" 27	20°	15°	690	640
" 30	19°	15°	654	572
May. 3	20°	16°	608	564
" 6	21°	16°	592	560
" 9	20°	15°	541	524
" 12	22°	17°	535	479

A scrutiny of Table 4 shews that there was a very slight increase on the first day after storage. This, however, is hardly beyond the limit of experimental error and, if it occurred, may have taken place before the ice-cream got down to the temperature of the cold room. Similar experiments have been conducted by Stiles[26] in Washington, at a temperature varying between 0° and 10° F and by Pennington[25] in Philadelphia at a temperature of −5.8° F. Stiles found a slight increase up to the 3rd day, and then a regular fall in the number of organisms till the 27th day, when there was a second short sharp increase with a steep fall. This second rise was not found in the present experiment. Pennington occasionally found an increase at the end of 24 hours, but thereafter a gradual diminution in the original number of bacteria per cubic centimetre. Stiles' experiments lasted over a period of 34 days, Pennington(s) 9 days and those recorded by the writer 60 days.

3. The Addition of Organisms during the Freezing Process.

The high average count amongst the "c" samples is also to be accounted for by the addition of microbes during the process of freezing by the dirtiness and carelessness of the manufacturer as already noted. This point is well illustrated by the following table giving the results of the examination of samples taken from vendors immediately before and after freezing:

Table 5.

Shewing Number of Bacteria per Cubic Centimetre in Ice-Cream immediately before and after freezing.

Manufacturer Vide Table 2	Date of examination 1909	No. of Organisms capable of growing on nutrient gelatine (reaction + 1%) at 20° to 22° C in 3 days.		No. of Organisms capable of growing on nutrient agar (reaction + 1%) at 35° to 37°C in 2 days.	
		Before freezing	After freezing	Before freezing	After freezing
No. 6.	Feb.28.	10,000	30,000	230,000	470,000
No. 7.	Apl.30.	8,000	14,000	5,000	12,000
No.26.	May. 1.	2,300	36,000	18,000	29,000

Experiment.

A laboratory experiment was carried out to prove that it was possible to freeze without the introduction of microbes in excessive numbers. The ice-cream ingredients were duly boiled for 10 minutes and a looplet of the mixture was put into broth. No growth resulted. This sterile product was then poured while still hot into an ordinary freezer previously sterilised in the autoclave. The lid which had also been sterilised was put on and kept and the ice-cream was frozen by rotation in the mixture of salt and ice. The process occupied 2 hours. The ice-cream was then stored in the laboratory store room the only precaution

that was taken being to keep the lid on, 5 minutes, 30 minutes, and 90 minutes after freezing a looplet was put into broth but no growth resulted. 24 hours afterwards, 1 cubic centimetre of the ice-cream, which had been kept frozen, was put into nutrient gelatine and found to contain 200 organisms.* (Vide also experiments recorded on pages 101 and 102.

Test 2. Number of Organisms capable of growing on Nutrient Agar (reaction 1%) at 35 to 37 C in 2 days.

The agar plates were all counted after 2 days' incubation. Speaking generally, the number of colonies growing at 35° to 37° C., was found to be less than the corresponding number of colonies growing at 20° to 22° C. The same gradual increase in numbers is to be registered with this count as the manufacture of ice-cream is proceeded with. The following classification and averages indicate this :-

Classification of the "a" "b" and "c" samples according to results of Test 2.

No. of Organisms per cubic centimetre.	Samples		
	"a"	"b"	"c"
Containing less than 100	1	0	0
" between 100 and 1000	4	0	0
" " 1,000 and 10,000	18	1	0
" " 10,000 and 100,000	15	15	6
" " 100,000 and 1,000,000	7	12	14
" " 1,000,000 and 10,000,000	5	11	10
" " 10,000,000 & 100,000,000	0	11	16
" over 100,000,000	0	0	4

Average No. of organisms per cubic centimetre in
"a" Samples = 379,010
"b" " = 6,861,600
"c" " = 34,467,000

* Incidentally the appearance of 200 microbes per cubic centimetre supports the experiments and conclusions regarding the multiplication of organisms in frozen ice-cream.

Test 3. The Glucose Bile Salt Broth Test.

All samples were submitted to this test, and the reaction produced within 48 hours noted. The quantities (used put on) have already been given (Vide Table 1) the total for each of the "a" samples being 11.11 cubic centimetres and for each of the "b" and "c" samples, 11.1111 cubic centimetres. Only two samples gave "no change" in the total quantity put on immediately after boiling. In none of the other samples of the three series was "no change" recorded. This is not as it should be. Ice-Cream made in the laboratory -freezing being carried out immediately after boiling- produced "no change" in this medium after it had stood frozen 24 hours and 72 hours respectively, when as much as 20 cubic centimetres were examined. This fact shews how serious is the contamination of ice-cream with The "Glucose fermenters" or organisms of the intestinal type.

The following table sets out the results of this test with the samples of three series :-

Table 6. Classifying the Results given by the "a", "b" and "c" Samples with Test 3.

	"a" Samples	"b" Samples	"c" Samples
Producing no change in 11.11 c.c	2		
" acid in 10 c.c. but not in 1 c.c.	3		
" " in 1 c.c. " " in A	16		
" " in A " " in B	20	10	1
" " in B " " in C		8	1
" " in D		1	1

	"a"	"b"	"c"
Producing Acid and Gas in 10 c.c. but not in 1 c.c.	1	2	
" " 1 c.c. " " A	2	3	5
" " A " " B	5	7	5
" " B " " C	1	10	19
" " C " " D		7	7
" " D		2	11
Total	50	50	50

Nine of the "a" samples therefore produced the complete change -acid and gas- in varying quantities in this medium, one of these producing it in the smallest quantity put on, viz. B or ·01 cubic centimetre. Thirty-one of the "b" series produced this reaction, 2 giving it in D or ·0001 cubic centimetre. In the third series of samples, i.e., those taken after freezing, 47 out of the 50 samples examined showed fermentation, no less than 11 producing acid and gas in D or ·0001 cubic centimetre. The gradual increase in the number of samples producing this change, as well as in the number producing it in very small quantities of the ice-cream, is clear evidence of the contamination to which this article of food is subjected in the process of manufacture, and bears out the previous results of the gelatine and agar counts.

The Glucose Fermenters.

The next part of Test 3, was the isolation and identification so far as possible of the organisms which produced acid and gas in glucose taurocholate broth. From the smallest quantity in each series shewing this change a plate was made on MacConkey's Neutral Red Lactose Agar and the nature of the colonies developing in 24 hours at 35° to 37° C noted. The colonies varied from the red colonies with production of haze in the surrounding medium supposed to be typical of the Coli Group, to red without haze, pink and lastly white. 95 such plates were made, and the following classification gives the results as regards the nature of the colonies produced.

21	plates	shewed only red colonies with haze.
22	"	" red with haze and white colonies.
11	"	" only red colonies.
2	"	" red and pink colonies.
1	plate	" red, pink and white colonies.
19	plates	" red and white colonies.
6	"	" only pink colonies.
11	"	" " white colonies.
2	"	" no growth.

In 6 instances viz., 3b, 5c, 12¼ 18c, 43c and 48b, plates were made from different dilutions of the same sample, and these results are included in the foregoing classification. In these six, with one exception, 48b, the red colonies with haze or red colonies, when

present, were found in the lesser dilutions and the white colonies in the higher. So far as practicable, 3 colonies were selected from each plate - two red and one white as a rule- and these were each subjected to 16 fermentation and other tests for the purposes of differentiation and classification.

In this way 258 colonies were fished from the 95 plates, 108 being red colonies with haze, 69 red, 22 pink, and 59 white.

The Differential Reagents.

The reagents employed were :-

Glucose for fermentation.
Lactose " "
Neutral Red for fluorescence.
Peptone water for indol.
Glucose broth for Voges and Proskauer's reaction.
Mannite for fermentation.
Maltose " "
Galactose " "
Lævulose " "
Adonite " "
Saccharose " "
Raffinose " "
Inulin " "
Salicin " "
Dulcite " "
Gelatine " liquefaction.

Glucose, mannite, maltose, galactose, and laevulose invariably yielded positive results, and are therefore

excluded from the following table which gives the complete reactions of all the colonies examined, as well as the samples from which they were taken.

T a b l e 7.

Shewing reactions of "Glucose Fermenters" isolated from the "a" "b" and "c" Samples of Ice-Cream.

(Vide pp. 55 - 64)

TABLE 7.

Shewing reactions of "Glucose Fermenters" isolated from the "a" "b" and "c" Samples of Ice-Cream.

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
No. of Sample	No. of Organism	Dilution plated on "Rebipelagar"*	Nature of colonies developing on "Rebipelagar"* in 24 hours at 35° - 37° C.	Nature of colonies selected.	Lactose	Fluorescence	Indol	Voges and Proskauer's Reaction	Adonit	Saccharose	Raffinose	Inulin	Salicin	Dulcite	Liquefaction of gelatine
1c	1	B	Red and White	Red	+	+	−	+	−	+	+	−	+	−	−
	2			Red	+	+	+	−	−	+	+	−	+	+	−
2b	3	B	Red and White	Red	+	+	−	−	+	+	+	+	+	+	−
	4			Red	+	+	−	+	+	+	+	+	+	+	−
	5			Red	+	+	+	−	+	+	+	+	+	+	−
2c	6	D	Red and White	Red	+	+	+	−	−	+	−	−	−	−	−
	7			Red	+	+	+	−	−	+	−	−	−	−	−
	8			Red	+	+	+	−	−	−	−	−	−	−	−
3a	9	B	Red with haze	Red with haze	+	+	+	−	−	+	−	−	−	+	−
	10			Red with haze	+	+	+	−	−	+	−	−	−	+	−
	11			Red with haze	+	+	+	−	−	+	−	−	−	+	−
3b	12	B	Red with haze and White	Red with haze	+	+	+	−	−	+	+	−	−	−	−
	13			Red with haze	+	+	+	−	−	+	+	+	+	+	−
	14			Red with haze	+	+	+	−	−	+	+	+	+	+	−
	15	C	White	White	+	+	−	+	+	+	+	+	+	+	−
	16			White	+	+	−	+	+	+	+	+	+	+	−
	17			White	+	+	−	+	+	+	+	+	+	+	−
3c	18	D	Red with haze	Red with haze	+	+	+	−	+	+	+	−	−	+	−

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
4 b	19	B	Red with haze and white.	Red with haze	+	−	+	−	−	+	+	−	−	−	−
	20			Red with haze	+	+	+	−	−	+	+	−	−	−	−
	21			Red with haze	+	−	+	−	−	+	+	−	−	−	−
4 c	22	D	White	White	+	+	−	−	−	+	+	+	+	+	−
	23			White	+	+	−	−	+	+	+	+	+	+	−
	24			White	+	+	−	−	+	+	+	+	+	+	−
	25	B	Red with haze and white	Red with haze	+	+	+	−	+	+	+	+	+	+	−
	26			Red with haze	+	+	+	−	+	+	+	+	+	+	−
5 a	27	1 cc	Red with haze and white	Red with haze	+	+	+	−	+	+	+	+	+	−	−
	28			Red with haze	+	+	+	−	+	+	−	+	−	−	−
	29			Red with haze	+	+	+	−	+	+	−	+	−	−	−
5 b	30	B	Red with haze	Red with haze	+	+	+	−	−	+	+	−	−	+	−
	31			Red with haze	+	+	+	−	−	+	+	−	−	+	−
	32			Red with haze	+	+	+	−	−	+	+	−	−	+	−
5 c	33	C	Red with haze and white	Red with haze	+	+	−	−	−	+	+	+	+	+	−
	34			Red with haze	+	+	−	−	−	+	+	+	+	+	−
	35			Red with haze	+	+	−	+	−	+	+	+	+	+	−
	36	D	White	White	+	+	−	−	−	+	+	+	+	+	−
	37			White	+	+	−	−	−	+	+	+	+	+	−
	38			White	+	+	−	+	−	+	+	+	+	+	−
6 c	39	B	Red	Red	+	+	+	−	−	+	+	−	−	+	−
	40			Red	+	+	+	−	−	+	+	−	−	+	−
	41			Red	+	+	+	−	−	+	+	−	−	+	−
8 c	42	D	Red	Red	+	+	+	−	+	−	−	−	−	−	−
9 b	43	1 cc	Pink	Pink	+	−	−	−	+	+	+	−	+	−	−
	44			Pink	+	−	−	−	+	+	−	−	+	−	−
	45			Pink	+	−	+	−	+	+	+	−	+	−	−
9 c	46	A	Pink	Pink	+	−	+	−	−	−	+	−	−	+	−
	47			Pink	+	+	+	−	−	−	+	−	−	+	−
	48			Pink	+	+	+	−	−	−	+	−	−	+	−
10b	49	A	Pink	Pink	+	+	+	+	+	+	+	−	+	+	−
	50			Pink	+	+	+	+	+	+	+	−	+	+	−
	51			Pink	+	+	+	+	+	+	+	−	+	+	−

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
10c	52	B	Pink	Pink	+	+	−	−	+	+	+	−	+	−	−
	53			Pink	+	+	+	−	+	+	+	−	+	−	−
	54			Pink	+	+	+	−	+	+	+	−	+	−	−
11b	55	1cc	Pink	Pink	+	+	−	−	−	−	−	−	−	−	−
	56			Pink	+	+	−	−	−	−	−	−	−	−	−
	57			Pink	+	+	−	+	−	−	−	−	−	−	−
11c	58	B	Pink	Pink	+	−	+	−	−	+	+	−	−	+	−
	59			Pink	+	−	+	−	−	+	+	−	−	+	−
	60			Pink	+	−	+	−	−	+	+	−	−	+	−
12b	61	1 cc	Red	Red	+	+	−	−	−	−	−	−	−	−	−
	62			Red	+	+	−	−	−	−	−	−	−	−	−
	63			Red	+	+	−	−	−	−	−	−	−	−	−
	64	A	Red	Red	+	+	+	−	−	−	−	−	−	−	−
	65			Red	+	+	+	−	−	−	−	−	−	−	−
	66			Red	+	+	+	−	−	−	−	−	−	−	−
12c	67	C	Red	Red	+	+	+	−	−	−	−	−	−	+	−
	68			Red	+	+	+	−	−	−	−	−	−	+	−
	69			Red	+	+	+	−	−	−	−	−	−	−	−
14c	70	1 cc	Red with haze	Red with haze	+	+	+	−	−	−	−	−	−	−	+
	71			Red with haze	+	+	+	−	−	−	−	−	−	+	−
	72			Red with haze	+	+	−	−	+	+	−	+	+	−	−
16c	73	1 cc	White	White	+	+	−	−	+	+	+	−	+	−	+
	74			White	+	+	−	−	+	+	+	−	+	−	+
	75			White	+	+	−	−	+	+	+	−	+	−	+
17b	76	A	Red	Red	+	+	−	−	−	−	+	−	−	+	−
	77			Red	+	+	−	−	−	−	+	−	−	+	−
	78			Red	+	+	−	−	−	−	+	−	−	+	−
17c	79	B	Red	Red	+	+	−	−	−	+	+	−	+	+	−
	80			Red	+	+	−	−	−	+	+	−	+	+	−
	81			Red	+	+	−	−	−	+	+	−	+	+	−

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
18a	82	1cc	White	White	+	+	−	−	−	−	−	−	−	−	−
	83			White	+	+	−	−	−	−	−	−	−	−	−
	84			White	+	−	−	−	−	−	−	−	−	−	+
18b	85	B	Red	Red	+	+	−	−	−	+	+	+	+	+	−
18c	86	A	White	White	−	+	−	−	−	−	+	−	+	+	−
	87	B	White	White	+	+	−	+	+	+	+	+	+	+	+
	88			White	+	+	−	+	+	+	+	+	+	+	+
	89			White	+	+	−	+	+	+	+	+	+	+	+
19b	90	A	Red with haze	Red with haze	+	+	−	−	−	+	+	−	−	−	−
	91			Red with haze	+	+	−	−	−	+	+	−	−	−	−
	92			Red with haze	+	+	−	−	−	+	+	−	−	−	−
19c	93	A	Red and Pink	Red	+	+	−	−	−	−	+	−	−	−	−
	94			Pink	+	+	−	−	−	−	+	−	−	+	−
	95	B	Red	Red	+	+	−	−	−	−	−	−	−	−	−
20b	96	B	Red and Pink	Red	+	+	−	+	−	−	+	−	−	−	−
	97			Pink	−	+	−	+	−	−	+	−	−	−	−
	98			Pink	−	+	−	−	−	+	+	+	+	−	−
20c	99	B	Red with haze	Red with haze	+	−	−	−	+	+	+	+	+	+	−
	100			Red with haze	+	−	−	−	+	+	+	+	+	+	−
	101			Red with haze	+	−	−	−	+	+	+	+	+	+	−
21a	102	A	Red and White	Red	+	+	+	+	+	+	+	+	+	+	−
	103			Red	+	+	+	+	+	+	+	+	+	+	−
	104			Red	+	−	−	−	+	−	−	−	−	−	−
21b	105	A	Red and White	Red	+	+	−	−	−	+	+	+	+	−	−
	106			White	−	+	−	−	−	+	+	+	+	−	−
	107			White	−	+	−	+	−	+	+	−	+	−	−
21c	108	B	Red with haze and white	Red with haze	+	+	+	−	−	−	−	−	−	−	−
	109			White	−	−	−	+	−	+	+	+	+	+	−
22b	110	1c.c.	Red with haze and White	Red with haze	+	+	−	−	+	+	+	−	+	+	−
	111			Red with haze	+	+	−	−	+	+	+	−	+	−	−
	112			White	+	+	−	−	+	+	+	−	+	−	−

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
22c	113	1 cc	Red with haze and White	Red with haze	+	+	−	−	+	+	+	+	+	+	−
	114			White	−	−	−	−	−	+	+	−	+	−	+
23b	115	B		Red with haze	+	+	−	−	−	+	+	−	−	−	−
	116			Red with haze	+	+	−	−	−	+	+	−	−	−	−
23c	117	B	Red with haze and White	Red with haze	+	+	−	−	−	+	+	−	+	−	−
	118			Red with haze	+	+	−	−	−	+	+	−	+	−	−
	119			White	+	+	−	−	−	+	+	−	+	−	−
24b	120	B	Red with haze	Red with haze	+	+	+	−	−	−	−	−	−	+	−
	121			Red with haze	+	+	+	−	−	−	−	−	−	+	−
	122			Red with haze	+	+	+	−	−	−	−	−	−	+	−
24c	123	C	Red with haze and white	Red with haze	+	+	+	−	−	−	−	−	−	−	−
	124			Red with haze	+	+	+	−	−	−	−	−	−	−	−
	125			White	−	+	−	−	−	+	+	+	+	−	−
25a	126	A	Red with haze and White	Red with haze	+	+	−	−	+	+	+	+	+	−	−
	127			Red with haze	+	+	−	−	−	+	+	−	+	−	−
	128			White	+	+	−	−	−	+	+	−	+	−	−
25b	129	C	Red with haze and White	Red with haze	+	+	−	−	−	+	+	−	−	−	−
	130			Red with haze	+	+	−	−	−	+	+	−	+	−	−
	131			Red with haze	+	+	−	−	−	+	+	−	+	−	−
25c	132	C	Red with haze and White	Red with haze	+	+	+	−	−	+	−	−	−	−	−
	133			Red with haze	+	+	−	−	−	−	−	−	−	−	−
26a	134	A	Red with haze	Red with haze	+	+	−	+	+	+	−	+	−	−	−
	135			Red with haze	+	+	−	+	+	+	−	+	−	−	−
	136			Red with haze	+	+	−	+	+	+	−	+	−	−	−
26b	137	C	Red with haze	Red with haze	+	+	+	−	+	+	+	−	+	−	−
	138			Red with haze	+	+	−	+	+	+	+	−	+	−	−
	139			Red with haze	+	+	−	+	+	+	+	−	+	−	−
26c	140	D	White	White	−	+	−	−	−	−	+	−	+	−	−
	141			White	−	+	−	−	−	−	+	−	+	−	−
	142			White	−	+	−	−	−	−	+	−	+	−	−

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
27a	143	10c	Red with haze and White	Red with haze	+	+	−	−	+	+	+	−	+	−	−
	144			Red with haze	+	+	−	−	−	+	+	−	−	−	−
	145			Red with haze	+	+	−	−	−	+	+	−	−	−	−
27b	146	B	Red with haze and White	Red with haze	+	+	−	−	−	−	+	−	+	−	−
	147			Red with haze	+	+	−	−	−	−	+	−	+	−	−
	148			White	−	+	−	−	−	−	+	−	+	−	−
27c	149	C	Red and White	Red	+	+	−	+	−	−	+	−	+	−	−
	150			Red	+	+	+	−	−	+	+	−	+	−	−
	151			White	−	+	−	+	−	+	+	−	+	−	−
28b	152	B	White	White	+	+	−	−	−	+	+	−	+	−	−
	153			White	+	+	−	−	−	+	+	−	+	−	−
	154			White	+	+	−	−	−	+	+	−	+	−	−
28c	155	B	Red with haze and White	Red with haze	+	+	−	−	−	+	+	−	+	−	−
	156			Red with haze	+	+	−	−	−	+	+	−	+	−	−
	157			White	−	+	−	+	−	+	+	−	+	−	−
29a	158	A	Red with haze and White	Red with haze	+	+	−	−	−	+	+	−	+	−	−
	159			Red with haze	+	+	−	−	−	−	−	−	−	+	−
29b	160	D	Red with haze and White	Red with haze	+	+	−	−	−	−	−	+	+	−	−
	161			White	+	+	−	+	−	−	+	−	+	−	−
	162			White	+	+	−	−	−	−	+	−	+	−	−
29c	163	D	Red with haze and White.	Red with haze	+	+	+	−	−	+	+	−	−	+	−
	164			Red with haze	+	−	+	+	−	+	+	−	+	−	−
	165			White	−	+	−	+	−	+	+	−	+	−	+
30a	166	A	Red with haze and White	Red with haze	+	+	−	−	−	+	+	−	+	−	−
	167			Red with haze	+	+	−	−	−	+	+	−	+	−	−
	168			White	+	+	−	+	−	+	+	−	+	−	−
30b	169	C	Red with haze	Red with haze	+	+	−	−	−	+	−	−	−	+	−
	170			Red with haze	+	+	−	−	−	+	−	−	−	+	−
	171			Red with haze	+	+	−	−	−	+	−	−	−	+	−
30c	172	C	White	White	+	+	−	−	−	+	+	−	−	−	−

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
30c	173	C		White	+	+	−	−	−	+	+	−	−	−	+
	174			White	+	+	−	−	−	+	+	+	+	+	−
31c	175	B	Red and White	Red	+	+	+	−	−	−	−	−	−	−	−
	176			Red	+	+	+	−	−	−	−	−	−	−	−
	177			White	−	+	+	−	−	−	−	−	−	−	−
32c	178	B	Red and White	Red	+	+	−	+	−	−	+	−	−	−	−
	179			Red	+	+	−	+	+	+	−	−	−	−	−
	180			White	−	+	−	+	−	+	−	−	−	−	−
33b	181	10cc	Red with haze	Red with haze	+	+	−	+	−	+	−	−	−	−	−
	182			Red with haze	+	+	−	+	−	+	−	−	−	−	−
	183			Red with haze	+	+	−	+	−	+	−	−	−	−	−
33c	184	B	Red and White	Red	+	+	−	+	−	+	+	−	−	−	−
	185			White	−	+	−	+	+	+	+	+	+	−	+
34c	186	A	Red and White	Red	+	+	−	+	−	+	−	−	−	−	−
	187			Red	+	+	−	+	−	+	+	−	−	−	−
	188			White	−	+	−	−	−	−	+	−	−	+	+
35b	189	10cc	White	White	−	+	−	+	−	+	+	−	+	−	−
35c	190	B	Red and White	Red	+	+	+	+	+	−	+	−	+	−	−
	191			Red	+	+	−	−	+	−	+	−	+	−	−
	192			White	+	+	−	+	+	−	+	−	+	+	−
36b	193	A	Red and White	Red	+	−	−	+	+	+	+	−	+	+	−
	194			Red	+	−	−	+	+	+	+	−	+	+	−
	195			White	+	−	−	+	+	+	+	−	+	+	−
36c	196	B	Red and White	Red	+	+	−	−	+	+	+	+	+	−	−
	197			Red	+	+	−	−	+	+	+	+	+	−	−
	198			White	−	+	−	+	+	+	+	−	+	−	−
37c	199	B	Red and White	Red	+	+	+	−	+	+	+	+	+	+	−
	200			Red	+	+	+	−	+	+	−	−	+	+	−
	201			White	−	+	+	+	−	+	−	−	−	−	−
38b	202	B	Red with haze	Red with haze	+	+	+	−	−	−	−	−	−	−	−

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
38b	203	B		Red with haze	+	+	+	−	−	−	−	−	−	+	−
	204			Red with haze	+	+	+	−	−	−	−	−	−	+	−
38c	205	C	Red with haze	Red with haze	+	+	+	−	+	−	−	−	−	−	−
	206			Red with haze	+	+	+	−	+	−	−	−	−	−	−
	207			Red with haze	+	+	+	−	+	−	−	−	−	−	−
39b	208	C	Red with haze	Red with haze	+	+	+	−	−	−	−	−	−	+	−
	209			Red with haze	+	+	+	−	−	−	−	−	−	+	−
	210			Red with haze	+	+	+	−	−	−	−	−	−	+	−
39c	211	D	Red and White	Red	+	+	+	−	−	−	+	−	−	+	−
	212			Red	+	+	+	−	−	−	+	−	−	+	−
	213			White	−	+	−	+	−	+	−	−	−	−	−
40c	214	A	Red, Pink and White	Red	+	+	+	−	+	+	+	−	+	−	−
	215			Red	+	+	−	+	+	+	+	+	+	+	−
	216			Pink	+	+	+	−	−	−	−	−	−	−	−
41c	-	A	No growth												
42c	217	1cc	Red with haze	Red with haze	+	+	−	−	−	−	−	−	−	−	−
	218			Red with haze	+	+	−	−	−	−	−	−	−	−	−
	219			Red with haze	+	+	+	−	−	+	+	−	−	+	−
43b	220	A	Red and White	Red	+	+	−	−	−	−	+	−	−	+	−
	221			Red	+	+	−	−	−	−	+	−	−	+	−
	222			Red	+	+	−	−	−	+	+	−	−	+	−
43c	223	C	Red and White	Red	+	+	−	−	−	+	+	−	−	+	−
	224			Red	+	+	−	−	−	+	+	−	−	+	−
	225			White	−	+	+	−	−	−	−	−	−	−	−
	226			White	−	+	+	−	−	−	−	−	−	−	−
	227			White	−	+	+	+	−	−	−	−	−	−	−
	228	A	Red	Red	+	+	−	−	−	−	−	−	−	−	−
44b	229	C	Red and White	Red	+	+	−	−	−	−	+	−	−	−	−
	230			Red	+	+	+	−	−	−	−	−	−	−	−
	231			White	−	+	−	−	−	+	+	+	+	−	−
44c	232	D	Red with haze	Red with haze	+	+	−	−	−	−	−	−	−	−	−

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
44c	233	D		Red with haze	+	+	+	−	−	−	−	−	−	−	−
	234			Red with haze	+	+	+	−	−	−	−	−	−	−	−
45c	235	A	Red with haze	Red with haze	+	+	+	−	−	+	+	−	−	−	−
	236			Red with haze	+	+	+	−	−	+	+	−	−	−	−
	237			Red with haze	+	+	+	−	−	+	+	−	−	−	−
46c	238	1cc	Red	Red	+	+	+	−	+	+	+	+	+	−	−
	239			Red	+	+	−	−	+	+	+	+	+	−	−
	240			Red	+	+	−	−	+	+	+	+	+	+	−
47c	241	D	Red with haze and White	Red with haze	+	−	−	−	+	+	+	−	−	−	−
	242			Red with haze	+	−	+	−	+	+	+	−	−	−	−
	243			White	−	−	−	+	+	+	+	+	+	−	−
47c	244	D	Red with haze	Red with haze	+	+	+	−	−	−	−	−	−	−	−
	245			Red with haze	+	+	+	−	−	−	−	−	−	−	−
	246			Red with haze	+	+	+	−	−	−	−	−	−	−	−
48b	247	C	Red with haze	Red with haze	+	+	+	−	+	+	+	−	+	−	−
	248			Red with haze	+	+	+	−	+	+	+	−	+	−	−
	249			Red with haze	+	+	−	−	+	+	+	+	+	−	−
	250	B	Red and White	Red	+	−	−	−	+	+	+	+	+	+	−
	251			Red	+	+	+	−	+	+	+	+	+	+	−
	252			White	−	+	−	−	+	+	+	−	−	−	−
48c	253	D	Red with haze	Red with haze	+	+	+	−	−	+	+	−	−	−	−
	254			Red with haze	+	+	+	−	−	+	+	−	−	−	−
	255			Red with haze	+	+	+	−	−	+	+	−	+	−	−
49c	256	B	Red with haze	Red with haze	+	+	−	−	−	−	−	−	−	+	−
	257			Red with haze	+	+	−	−	−	−	−	−	−	+	−
	258			Red with haze	+	+	−	−	−	−	−	−	−	+	−
50c	-	B	No growth												

* "Rebipelagar" = MacConkey's Neutral Red Lactose Agar.

† + = Production of acid and gas,
presence of indol,
presence of Voges and Proskauer's reaction,
or liquefaction of gelatine according to
the column in which it appears.

‡ — = absence of above according to column,
or slight production of acid without gas
or slight production of acid with only a
bubble of gas.

The Composition of the Media Employed.

For Fermentation:

Where the fermenting power of an organism was to be tested gelatine media were employed having the following composition :

Sugar or alcohol	1%
Peptone	2%
Lemco	1%
Gelatine	10%
5% KHO Solution	1%
Distilled water tinted with litmus	85%

For Fluorescence:

A medium similar in composition to the foregoing was used, litmus being omitted and neutral red replacing the sugar or alcohol so as to give a brilliant color to the gelatine.

For Indol:

Peptone	1%
Salt	0·5%
Distilled water	98·5%

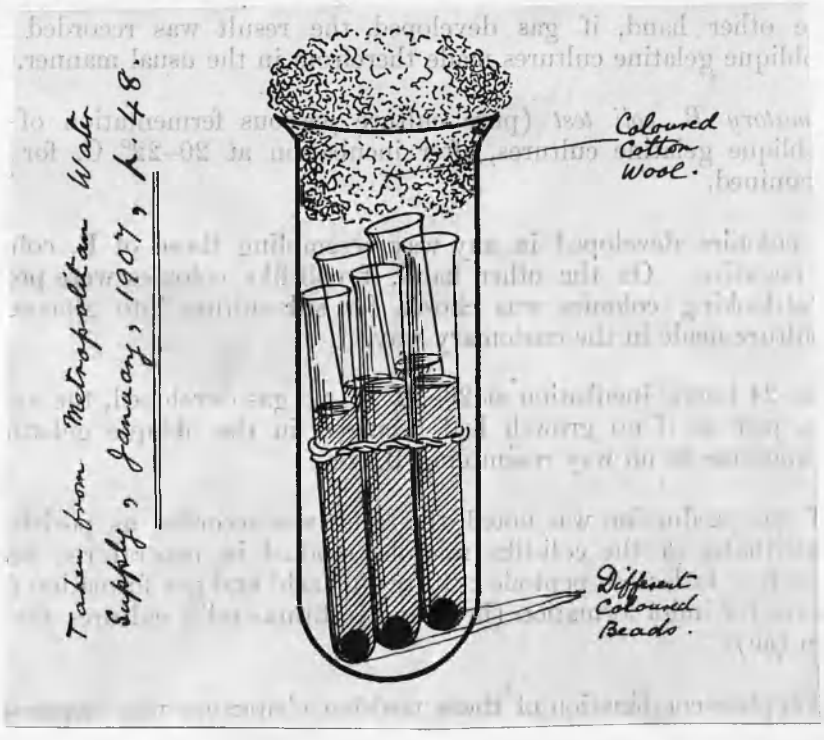
For Voges and Proskauer's Reaction:

Glucose	0·5%
Peptone	1%
Lemco	0·5%
Distilled water	98·0%

The Method of Conducting the Differential Tests.

The method employed in carrying out these differential tests was based on that devised by Houston[16e]

and described by him in the Reports on the Metropolitan Water Supply, January and June, 1907. The media were put up in small tubes (2" x ½") which in their turn were placed inside wide tubes (3" X 1"). The various media were distinguished where necessary by colored beads in the small tubes and colored wool in the wide. When sterilised they were ready for use. One such wide tube containing 5 of the small size with colored beads at the bottom of the latter is shewn in the accompanying sketch.



As 3 colonies were selected for each plate a complete set of tubes for one operation was as follows:

Three of the following:

A. Wide tube (white wool) containing 5 small tubes as follows:

1.	litmus glucose gelatine	-	white bead
2.	" lactose "	-	brown "
3.	" mannite "	-	blue "
4.	" maltose "	-	green "
5.	" galactose "	-	black "

Three of the following:

B. Wide tube (blue wool) containing 4 small tubes as follows:

1.	litmus laevulose gelatine	-	no bead
2.	" adonit "	-	red bead
3.	" saccharose "	-	yellow bead
4.	" raffinose "	-	indigo "

Three of following:

C. Wide tube (brown wool) containing 4 small tubes as follows:

1.	litmus salicin gelatine	-	blue bead
2.	" dulcite "	-	green "
3.	" inulin "	-	brown "
4.	neutral red "	-	no bead.

One of following:

D. Wide tube (white wool) containing 6 small tubes as follows:

3 with glucose broth (blue bead)

3 with peptone water (no bead)

When a colony was fished from a "Rebipelagar" plate it was emulsified in sterile water in one of these small tubes. It is easy to get these tubes full of sterile water by boiling them in water and to reduce the quantity of water in them to the desired amount by jerking the excess out.

The wires used for landing the colonies and for emulsification were those recommended by Houston and easily obtained from florists who stock them for floral decorative purposes. They are soft iron wires of nice length (about 7 inches) thickness and balance. They can be sterilised in bulk, cost almost nothing and can be used over and over again.

When a colony was completely emulsified in the sterile water in a small tube 14 additional wires were added, making 15 in all. As 3 such colonies were selected from each plate, before commencing the inoculation of the media there were obviously 3 small tubes each containing an emulsified colony and 15 infected wires (45 wires in all).

During inoculation, the small inner tubes containing the media were removed from the outer by means of sterile forceps and placed in a special rack.

13 of the 15 wires in an emulsified colony were used for infecting the 13 small tubes in one of the sets A, B and C. For the 3 colonies, therefore, 39 wires were used and all the sets A, B and C. Six infected wires were still left, 2 each in each emulsified colony, and these were used for inoculating the three sets of glucose broth and peptone water in D, the small tubes being marked with one, two or three

transverse bars to identify the colony.

When thus inoculated the small tubes were replaced in their original containing-tubes which were appropriately marked for the identification of the microbe with which the media had been infected. All were allowed to incubate at 35° to 37° C; but at the end of 3 hours the gelatine media (A, B and C) were removed and placed for half an hour in an ice-chest and afterwards allowed to incubate at 20° to 22° C. The primary incubation at the higher temperature melts the gelatine, allows of some multiplication to take place and enables the organisms to distribute themselves throughout the medium. The production of acid or gas is readily indicated by the change of color of the litmus or the presence of a bubble of gas in the medium.

It took on an average 15 minutes to inoculate a complete series of these tubes i.e. A, B, C and D corresponding to the 3 colonies fished from a "Rebipelagar" plate, and certainly no set was exposed to the air for a longer period than 20 minutes. It has been suggested that during this time the gelatine may become contaminated with air-borne organisms and the results therefore vitiated. But it must be remembered that the surface of the gelatine is small and at the foot of a sterile column of air, which acts as a protective layer to the nutrient media. An experiment was conducted in which the tubes were inoculated in the usual way but with sterile wires and

the period of inoculation and exposure was extended to one hour. Incubation was then allowed to proceed, but no growth resulted, nor was any change in any of the media apparent. Houston[16e] also makes reference to the fact in his first description of the method that there is no appreciable danger of air contamination during inoculation of the small saline tubes which he employs.

The tubes were looked at daily for fermentation up to 7 days. It was not found to be practicable on account of the numbers in use to keep them longer than that time. Those shewing definite production of acid and gas within that period are indicated by the sign + in the appropriate column. No record of the degree of acidity or amount of gas production is possible by this method. At the same time it was clear that the organisms isolated, although yielding an unmistakably positive result, differed greatly in their powers of splitting these reagents. Houston[16e] (Metropolitan Water Supply, September, 1907 Report, pp 23-25) has pointed out the extreme delicacy of gelatine media for these fermentation tests. This conclusion was amply supported during the present inquiry, and the sign — indicates (a) production of neither acid nor gas in 7 days, (b) slight production of acid without gas, and (c) slight production of acid with only a bubble of gas.

It is also worthy of note that liquefaction of the gelatine may set in before fermentation has begun, making gas production impossible of recognition.

This, however, did not happen in the case of any of the microbes investigated. But it raises the question if these gelatine sugar media are as useful for the differentiation of unknown microbes as they certainly are for the purpose to which Houston puts them, viz. the recognition of bacillus coli communis and bacillus typhosus, neither of which liquefy gelatine.

Paradimethylamidobenzaldehyde and persulphate of potassium were used for the indol test, the addition of 50 per cent of the volume of peptone water of each of these giving the best results. Within the 7 days allotted for the completion of these results it may be said that the intensity of the coloration depended on the length of growth. But from the experience of a few instances specially tested, it may be said that the indol reaction, if not detectable within 24 hours of incubation, is not given later.

Voges and Proskauer's reaction was carried out in the following manner. Incubation in glucose broth was allowed to proceed for three days when about 25% of the volume of the broth was added of a 2% solution of caustic soda. The tubes were then allowed to remain at room temperature for 4 days longer, daily observations being made during this time. The color, like that of a dilute alcoholic solution of eosin, appeared as a rule within 24 hours, and in some instances went through varying shades to a pale green with a brown sedimentous deposit within the 4 days. All tubes showing the brownish fluorescent coloration on standing are indicated by the + sign in

the tables.

The following table indicates the number of positive and negative results together with the percentages, recorded for each of the tests other than glucose, mannite, maltose, galactose, and laevulose, all of which were positive:-

Table 8.

(Vide p. 73)

Shewing the positive and negative results with corresponding percentages yielded by the various colonies tested with the different reagents.

Nature of Colony on MacConkey's Neutral Red Lactose Agar.	Red with haze				Red				Pink				White				Total positive and negative results and percentages.			
	+	−	% +	% −	+	−	% +	% −	+	−	% +	% −	+	−	% +	% −	+	−	% +	% −
Fermentation of Lactose	108	-	100	-	69	-	100	-	20	2	90·9	9·1	32	27	54·2	45·8	229	29	88·8	11·2
Fluorescence	100	8	92·6	7·4	65	4	94·2	5·8	15	7	68·2	31·8	53	6	89·8	11·2	233	25	90·3	9·7
Indol	54	54	50·0	50·0	28	41	40·6	59·4	13	9	59·1	40·9	5	54	8·5	91·5	100	158	38·8	61·2
Voges & Proskauer's reaction	10	98	9·3	90·7	15	54	21·7	78·3	5	17	22·7	77·3	24	35	40·7	59·3	54	204	20·9	79·1
Fermentation of Adonite	29	79	26.9	73.1	23	46	33·3	66.7	9	13	40.9	59.1	18	41	30.5	69.5	79	179	30.6	69.4
" " Raffinose	61	47	56·5	43·5	47	22	68·1	31·9	17	5	77·3	22·7	49	10	83·1	16·9	174	84	67·4	32·6
" " Inulin	21	87	19·4	80·6	16	53	23·2	76·8	1	21	95·5	4·5	19	40	32·2	67·8	57	201	22·1	77·9
" " Salicin	38	70	35·2	64·8	29	40	42·0	58·0	10	12	45·5	54·5	45	14	76·3	23·7	122	136	47·3	52·7
" " Dulcite	37	71	34·3	65·7	33	36	47·8	52·2	18	4	81·4	18·6	10	49	16·9	83·1	98	160	38·0	62·0
Liquefaction of Gelatine	1	107	0·9	99·1	-	69	-	100	-	22	-	100	12	47	20·3	79·7	13	245	5·0	95·0
Fermentation of Saccharose	72	36	66.3	33.1	38	31	55.1	44.9	13	9	59.1	40.9	43	16	72.9	27.1	166	92	64.5	35.5

The Value of the Various Reagents Employed for Differentiation.

So far as the results obtained by a minute examination of the 258 microbes isolated are concerned, it is possible to say that mannite, maltose, galactose and laevulose are useless for purposes of differentiation of glucose-fermenters as all the organisms isolated produced acid and gas in these media. MacConkey[27a] has already made the same statement.

The position of lactose is interesting, and may be summoned up thus :-

(1) All colonies definitely red in colour on MacConkey's neutral red bile salt agar are lactose fermenters.

(2) Pink or white colonies on this medium may or may not split lactose. There were tested 22 pink and 59 white, and the majority of these produced lactose fermentation. MacConkey[27b] has referred to this fermentation of lactose by colourless colonies in the Journal of Hygiene, Vol VIII, p 324.

Fluorescence was produced by 233 out of the 258 colonies examined, or by 90.3%. This is a higher percentage than obtained by the writer[19] when examining glucose fermenting organisms isolated from mussels. In this latter case the percentage was 50. A positive reaction as regards fluorescence is not always given by glucose-fermenters, but the statement of Houston[16a] that it is by the great majority is probably correct.

Indol was invariably produced by those red colonies

producing definite haze in the surrounding medium, and it becomes questionable, if it is valuable as a further test in these cases.

The position and exact interpretation of Voges and Proskauer's reaction has not yet been definitely decided. It is generally accepted as a reaction given only by bacilli of the B. lactis aerogenes and B.cloacae groups. Fifty-four out of the 258 colonies examined, or 20.9% gave the reaction. It is not, therefore, a reaction given by every organism as MacL.Harris[28] has stated and MacConkey[27c] has already contraverted. But of the 54 positive organisms only 2 could be placed in the Lactis aerogenes group by their other reactions, and only 2 more in the Cloacae group. It is, therefore, difficult to place organisms in any particular group by means of this test. It seems rather to be a reaction given by a large number of organisms of different classes, although in the present state of our knowledge it may be well to accept it as a necessary qualification of the organisms belonging to the groups mentioned.

No particular mention requires to be made of the other reagents employed, which, judging by the percentage of positive and negative results given by each, are all more or less of diagnostic value. Too much importance must not be attached to the last test for liquefaction of gelatine where the results are negative, as the time given (7 days) was short for this test. So far as it goes it is interesting to note that 12 out of the 13 liquefiers were colourless colonies on MacConkey's Neutral Red Bile Salt Agar.

The Nature of the Organisms Isolated.

Referring to Table 7 it will be seen that in only three of the ice-creams was it possible to isolate an organism giving the same reactions in two successive stages, viz:-

Organisms Nos. 64,65 and 66 in sample 12b (cooling 21 hours), and organism No. 69 in sample 12c (cooling 42 hours and freezing 3 hours);

organisms Nos. 127 and 128 in sample 25a (immediately after boiling) and organisms Nos. 130 and 131 in sample 25b (cooling 5 hours);

organism No. 222 in sample 43b (cooling 18 hours) and organisms Nos 223 and 224 in sample 43c (cooling 19 hours and freezing 3 hours).

In the case of the organisms isolated from samples Nos. 25a and 25b, No. 128 was white on MacConkey's Neutral Red Lactose Agar, while Nos 127, 130 and 131 all shewed red with haze. These facts indicate the multiplicity of glucose fermenters which abound in ice-cream and lend strong presumption to the view that fresh organisms are added by contamination at each stage of the manufacture.

For purposes of classification of these various isolated organisms, it was necessary to put known organisms through the same tests in the same way. By the kindness of Drs. R.M.Buchanan, A.C.Houston, Professor R.F.C.Leith, Dr.A.T.MacConkey, Professor E.J.McWeeney and Dr.W.G.Savage, the writer was able to obtain strains of various organisms and the following table gives the reactions obtained with these bacilli as well as the sources from which they were got.

T a b l e 9

Shewing Reactions of known Organisms with the differential Media employed in the present investigation.

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Name of Organism	Obtained from	Glucose	Lactose	Fluorescence	Indol	Voges & Proskauer's Reaction	Mannite	Maltose	Galactose	Laevulose	Adonite	Saccharose	Raffinose	Inulin	Salicin	Dulcite	Liquefaction of gelatine
B.Coli communis (Escherich)	Dr.A.T.MacConkey	+	+	+	+	−	+	+	+	+	−	−	−	−	−	+	−
B.Acidi lactici (Hüppe)	"	+	+	+	+	−	+	+	+	+	+	−	−	−	−	−	−
"	Dr.A.C.Houston	+	+	+	+	−	+	+	+	+	+	−	−	−	−	−	−
"	Král through Dr.R.M.Buchanan	+	+	+	+	−	+	+	+	+	+	−	−	−	−	−	−
B.lactis aerogenes	Dr.A.T.MacConkey	+	+	+	−	+	+	+	+	+	+	+	+	−	+	−	−
B.cloacae	"	+	−	+	−	+	+	+	+	+	−	+	+	−	−	−	+
"	Dr.A.C.Houston	+	−	+	−	+	+	+	+	+	+	+	+	−	−	−	+
"	Král through Dr.R.M.Buchanan	+	−	+	−	+	+	+	+	+	−	+	+	−	−	−	+
B.paracoli (Widal)	Král through Dr.R.M.Buchanan	+	−	+	−	−	+	+	+	+	−	−	−	−	−	+	−
B.enteritidis (Gaertner)	Dr.A.C.Houston	+	−	+	−	−	+	+	+	+	−	−	−	−	−	+	−
"	Král through Dr.R.M.Buchanan	+	−	+	−	−	+	+	+	+	−	−	−	−	−	+	−
"	Original strain through Dr.W.G. Savage.	+	−	+	−	−	+	+	+	+	−	−	−	−	−	+	−

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
B.paraty phosus B (Schottmüller)	Dr.A.C.Houston	+	−	+	−	−	+	+	+	+	−	−	−	−	−	+	−
" "	Král through Dr.R.M.Buchanan	+	−	+	−	−	+	+	+	+	−	−	−	−	−	+	−
" " (Achard)	Král through Dr.R.M.Buchanan	+	−	+	−	−	+	+	+	+	−	−	−	−	−	+	−
" " (Schottmüller)	Dr.W.G.Savage	+	−	+	−	−	+	+	+	+	−	−	−	−	−	+	−
" " (McWeeney)	Prof.E.T.McWeeney	+	−	+	−	−	+	+	+	+	−	−	−	−	−	+	−
" " A (Schottmüller)	Dr.A.T.MacConkey	+	−	+	−	−	+	+	+	+	−	−	−	−	−	+	−
" "	Dr.A.C.Houston	+	−	+	−	−	+	+	+	+	−	−	−	−	−	+	−
" "	Král through Dr.R.M.Buchanan	+	−	+	−	−	+	+	+	+	−	−	−	−	−	+	−
" "	Dr.W.G.Savage	+	−	+	−	−	+	+	+	+	−	−	−	−	−	+	−
" " (Brion & Kayser)	Král through Dr.R.M.Buchanan	+	−	+	−	−	+	+	+	+	−	−	−	−	−	+	−
B.aertryoke	Dr.A.T.MacConkey	+	−	+	−	−	+	+	+	+	−	−	−	−	−	+	−
B.Sinpestifer	Prof.Uhlenhuth through Dr.W.G. Savage	+	−	+	−	−	+	+	+	+	−	−	−	−	−	+	−
B.psittacosis	Dr.A.T.MacConkey	+	−	+	−	−	+	+	+	+	−	−	−	−	−	+	−
B.Friedlander (Nicolle)	"	+	+	+	−	−	+	+	+	+	+	+	+	−	+	+	−
B.Friedlander	Dr.A.C.Houston	+	+	+	−	−	+	+	+	+	+	+	+	−	+	+	−
B.levans	Dr.A.T.MacConkey	+	−	+	−	+	+	+	+	+	−	+	−	+	+	−	+
B.Grünthal	"	+	+	+	+	−	+	+	+	+	−	+	−	−	−	−	−
B.cavicida(Brieger)	"	+	+	+	+	−	+	+	+	+	−	−	−	−	A*	−	−
B.oxytocus perniciosus	"	+	+	+	−	−	+	+	+	+	+	+	+	+	+	+	−
B.coscoroba	"	+	+	+	+	−	+	+	+	+	−	+	A	−	−	−	−

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
B.typhosus	Prof.R.F.C.Leith	*A	–	–	–	–	A	A	A	A	–	–	A	–	–	–	–
B.rhinoscleromatis	Dr.A.T.MacConkey	+	+	+	–	–	+	+	+	+	+	+	+	+	+	+	–

The fermentation tests in this table were all carried out in gelatine media.

*A = Acid

\+ = Vide notes to Table 7.

– = " " "

Notes to Table 9.

MacConkey (Journal of Hygiene 1905 Vol.V page 350) describes

B.Coli Communis (Escherich)	as Raffinose +
B.Acidi Lactici	as Raffinose +
B.Cloacae	as Lactose +
B.Paracolon (Day)	as Raffinose + and Dulcite A.
B.Enteritidis (Gaertner)	as Raffinose +
B.of the Hog-cholera group	as Raffinose A and Dulcite A.
B.Psittacosis	as Raffinose +
B.Friedlander	as Lactose A and sometimes Indol +

MacConkey (Journal of Hygiene 1906 Vol VI page 397.) describes

B.Lactis Aerogenes	as Indol +
B.Levans	as Lactose + and Saccharose —
B.Grünthal	as Saccharose —
B.Cavicida (Brieger)	as Dulcite +
B.Oxytocus perniciosus	as Voges and Proskauer's +
B.Rhinoscleroma.	as Indol + and Inulin —

Morgan (British Medical Journal 1905 Vol I. page 1259) describes

B.Coli	as Saccharose + Dulcite —
	or Saccharose + Dulcite +
B.Cloacae	as Liquefaction of Gelatine —
B. "	as Lactose +
B.Paracoli	as Dulcite —
B.Paratyphoid B.	as Indol a trace
B. " A.	as " "
B. of the Hog-cholera group	as " "
B.Grünthal	as Saccharose —

Comparing the reactions of the organisms isolated from ice-cream (Table 7) with those given in Table 9, we find that 66 out of the 258 can be classified as follows:

(Vide Table 10. p 82.)

Table 10.

Shewing the nature of 66 of the organisms isolated from the "a" "b" and "c" Samples examined.

	Red with haze	Red	Pink	White
B.Oxytocus perniciosus (V & P +)		4,215,		15,16,17,
B.Oxytocus perniciosus (V & P −) B.Rhinosclerom (Indol − Inulin +)	115	2,240,		23,24,
B.Coli (Sacc + Raff + Dulc +)	30,31, 32,165, 219	39,40, 41,		
B.Coli (Sacc − Raff + Dulc +)		211,212	47,48,	
B.Cloacae (Lact + Liq. of gelatine −)		184,187.		
B.Coli (Sacc + Raff − Dulc −) B.Grünthal B.Coscoroba	132	6,7,		
B.Coli (Sacc − Raff − Dulc −) B.Cavicida (Dulc −)	108,123, 124,202, 233,234, 244,245, 246.	8,64, 65,66, 69,175, 176, 230.	216	
B.Coli (Sacc + Raff − Dulc +)	9,10,11			
B.Acidi lactici (Raff −)	205,206, 207,	42		
B.Coli communis (Sacc − Raff − Dulc +)	71,120, 121,122, 203,204, 208,209, 210.	67,68.		
B.Friedlander (Indol −)	110			
B.Lactis aerogenes (Indol −)	138,139			

N.B. The figures refer to the organisms numbered on Table

Coli Group.

In this table we find that the Coli group predominates, no less than 47 out of the 66 recognised organisms belonging to this group, 11 of these being the typical bacillus coli communis. In only 3 instances did these members of the coli group not yield red colonies on MacConkey's neutral red lactose agar and even these three were pink. It is interesting to note that 27 out of the total 47 coli-like microbes produced red surface colonies with haze in this medium. This indicates that while the colonies to be specially selected when using MacConkey's Neutral Red Lactose Agar should be those producing haze, other varieties must not be neglected in the search for Bacillus Coli.

Organisms of this group have long been associated with contamination by faeces, human or otherwise. Doubtless they gain entrance to ice-cream from the dried particles in the court yards as well as from the makers' hands which are not washed before commencing its manufacture.

B. Coli, B. Grünthal, B. Coscoroba, B. Cavicida.

So far as these tests go it is impossible to distinguish between certain varieties of the B.Coli and the B.Grunthal, B.Coscoroba and B.Cavicida as seen on Tables 9 and 10. The B.Grünthal has been placed in the Colon group by Morgan[29] (British Medical Journal, Vol. I. 1905, p 1258). B.Coscoroba is stated to have been

the cause of an epidemic occurring in swans. This was studied by Tritrop[30] and his results published attributing the outbreak to this bacillus in Ann. de l' Inst. Pasteur, XIV, 1900, p.224. The B.Cavicida has been isolated from faeces by Brieger,[31] who gives an account of the organism in Ueber Spaltungs-producte der Bakterien (Leitschr. fur phys. Chemie, VIII 1884, et Berlin klin. Wochenschr. 1884 No. 14). It is pathogenic for guinea-pigs when inoculated subcutaneously, but is without effect when taken with the food. According to Brieger it is allied to Bacillus Coli Communis and Bacillus Lactis Aerogenes.

B.Oxytocus perniciosus and B.Rhinoscleromatis.

Next to the Coli group of organisms the organism found in most abundance was the Bacillus Oxytocus perniciosus. This is an organism first described by Wysskowitsch[35] and isolated by him from old milk. It is pathogenic for mice and rabbits when large doses are used.

MacConkey[27c] identified this organism 16 times in 170 organisms isolated from milk.

A glance at Table 10 will shew that it has been necessary to associate a variety of this bacillus as regards its fermentation tests with bacillus rhinoscleromatis. Including both varieties here indicated, the bacillus oxytocus perniciosus has been found 10 times in the 258 microbes specially studied.

B.Cloacae.

The B.Cloacae has been isolated twice. A good account of this organism is to be found in MacConkey's[27a] article on Lactose Fermenting Bacteria in Faeces which appeared in the Journal of Hygiene, Vol. 5, 1905. With the strains there examined liquefaction of gelatine did not occur within six weeks, and this result supports the classification of organisms Nos. 184 and 187 as B.Cloacae, the time allowed for liquefaction in the present investigation being one week only.

B.Lactis Aerogenes and B.Pneumoniae (Friedlander).

The B.lactis aerogenes has been identified twice and the B.pneumoniae (Friedlander) once.

Authorities are not agreed as to the relationship between these organisms. Some consider them identical. Others consider the B.lactis aerogenes as identical with the B.coli communis. The presence of Voges and Proskauer's reaction is the most important test yet put forward for the identification of the B.lactis aerogenes. MacConkey[27c] isolated the latter twice from various samples of milk, but was unable to identify the B.pneumoniae (Friedlander) during this investigation. He considered that the B.cloacae and B.lactis aerogenes could be found in milk in greater abundance after it had been kept some time.

B.Acidi Lactici.

The B.acidi lactici has also been recognised. MacConkey[27a] has suggested that this organism while occurring in faeces disappears so quickly that it may provide an excellent test for the nearness or remoteness of pollution. In any case authorities are agreed that it is a close ally of B.coli communis and as such must be regarded as evidence of serious contamination. It was found in two of the samples investigated.

Classification of the remaining organisms.

With regard to the remaining 192 organisms these may be divided into 69 different groups as shewn in the following table :-

T a b l e 11.

(Vide pp. 87, 88, 89, 90.)

T a b l e 11.

Classification of the 192 unrecognised organisms isolated from the "a" "b" and "c" Samples examined.

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Red with haze	Red	Pink	White	Lactose	Fluorescence	Indol	Voges & Proskauer's Reaction	Adonite	Saccharose	Raffinose	Inulin	Salicin	Dulcite	Liquefaction of gelatine
	102,103			+	+	+	+	+	+	+	+	+	+	−
			88,89,87.	+	+	−	+	+	+	+	+	+	+	+
		49,50,51.		+	+	+	+	+	+	+	−	+	+	−
25,26	5,199,251			+	+	+	−	+	+	+	+	+	+	−
27				+	+	+	−	+	+	+	+	+	−	−
13,14.				+	+	+	−	−	+	+	+	+	+	−
35			38.	+	+	−	+	−	+	+	+	+	+	−
			185.	−	+	−	+	+	+	+	+	+	−	+
	190.			+	+	+	+	+	−	+	−	+	−	−
137,247, 248.	214	53,54.		+	+	+	−	+	+	+	−	+	−	−
18				+	+	+	−	+	+	+	−	−	+	−
	200			+	+	+	−	+	+	−	−	+	+	−
	2			+	+	+	−	−	+	+	−	+	+	−
			192	+	+	−	+	+	−	+	−	+	+	−
126,249.	196,197, 238,239.			+	+	−	−	+	+	+	+	+	−	−
			73,74,75.	+	+	−	−	+	+	+	−	+	−	+

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
33,34,	85,		22,36,37, 174.	+	+	−	−	−	+	+	+	+	+	−
	193,194.		195.	+	−	−	+	+	+	+	−	+	+	−
99,100, 101.	250			+	−	−	−	+	+	+	+	+	+	−
28,29				+	+	+	−	+	+	−	+	−	−	−
255,-	150			+	+	+	−	−	+	+	−	+	−	−
134,135, 136				+	+	−	+	+	+	−	+	−	−	−
	1.		168.	+	+	−	+	−	+	+	−	+	−	−
111,143,		52,	112,	+	+	−	−	+	+	+	−	+	−	−
72.				+	+	−	−	+	+	−	+	+	−	−
	105,			+	+	−	−	−	+	+	+	+	−	−
	79,80,81			+	+	−	−	−	+	+	−	+	+	−
164				+	−	+	+	−	+	+	−	+	−	−
		45		+	−	+	−	+	+	+	−	+	−	−
			198	−	+	−	+	+	+	+	−	+	−	−
			165	−	+	−	+	−	+	+	−	+	−	+
			243	−	−	−	+	+	+	+	+	+	−	−
			109	−	−	−	+	−	+	+	+	+	+	−
12,20,253 254.				+	+	+	−	−	+	+	−	−	−	−
	179			+	+	−	+	+	+	−	−	−	−	−
	149		161	+	+	−	+	−	−	+	−	+	−	−
	191			+	+	−	−	+	−	+	−	+	−	−
158,166, 167,117, 118,127, 130,131, 155,156.			119,128, 152,153, 154.	+	+	−	−	−	+	+	−	+	−	−
	222,223, 224.			+	+	−	−	−	+	+	−	−	+	−
			173	+	+	−	−	−	+	+	−	−	−	+
242				+	−	+	−	+	+	+	−	−	−	−
		58,59,60.		+	−	+	−	−	+	+	−	−	+	−

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
		43,		+	−	−	−	+	+	+	−	+	−	−
			104,151, 157,189.	−	+	−	+	−	+	+	−	+	−	−
		98	106,125, 231.	−	+	−	−	−	+	+	+	+	−	−
235,236, 237.				+	+	+	−	−	−	+	−	−	−	−
70.				+	+	+	−	−	−	−	−	−	−	+
181,182, 183.	186.			+	+	−	+	−	+	−	−	−	−	−
	96,178.			+	+	−	+	−	−	+	−	−	−	−
144,90,91 92,115, 116,129.			172	+	+	−	−	−	+	+	−	−	−	−
169,170, 171.				+	+	−	−	−	+	−	−	−	+	−
146,147.			162.	+	+	−	−	−	−	+	−	+	−	−
	76,77,78, 220,221	94		+	+	−	−	−	−	+	−	−	+	−
160				+	+	−	−	−	−	−	+	+	−	−
19,21				+	−	+	−	−	+	+	−	−	−	−
		46		+	−	+	−	−	−	+	−	−	+	−
241				+	−	−	−	+	+	+	−	−	−	−
		44		+	−	−	−	+	+	−	−	+	−	−
			201	−	+	+	+	−	+	−	−	−	−	−
			252	−	+	−	−	+	+	+	−	−	−	−
			188	−	+	−	−	−	−	+	−	−	+	+
			114	−	−	−	−	−	+	+	−	+	−	+
		57		+	+	−	+	−	−	−	−	−	−	−
	93,229			+	+	−	−	−	−	+	−	−	−	−
159,256, 257,258,				+	+	−	−	−	−	−	−	−	+	−
			227	−	+	+	+	−	−	−	−	−	−	−
			180,213	−	+	−	+	−	+	−	−	−	−	−
		97		−	+	−	+	−	−	+	−	−	−	−
			140,141, 142,148,	−	+	−	−	−	−	+	−	+	−	−

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
			86	−	−	−	−	−	−	+	−	+	+	−
183,217, 218,232,	61,62,63, 95,228	55,56,	82,83	+	+	−	−	−	−	−	−	−	−	−
	104			+	−	−	−	+	−	−	−	−	−	−
			84	+	−	−	−	−	−	−	−	−	−	+
			177,225, 226.	−	+	+	−	−	−	−	−	−	−	−

N. B. The figures refer to the Organisms numbered on Table

It will be noted that the method of classification here drawn up is purely arbitrary. The tests have been put down in the order in which they were conducted and the organisms giving the greatest number of positive reactions have been placed first on the table, the organisms with the greatest number of successive positive reactions taking precedence where the total number of positive reactions is the same.

In this way it has been possible to arrange the 192 unrecognised organisms in 69 different groups. Many of these groups approximate to the known organisms already described, but the differences are such as to prevent their inclusion in known groups. MacConkey lays considerable stress on the value of these fermentation tests, and the above results support his conclusions. The labour involved in working out these reactions for the various organisms is considerable, and doubtless prevents this method of differentiation from becoming popular. It is equally true that in the present state of our knowledge no organism can be absolutely recognised without them, and it is hoped that the present contribution will help to add something to our knowledge of the subject. It might serve a useful purpose if bacteriologists decided upon some definite method for the application of these tests and the classification of results. The method of classification I have employed is empirical but it is at least suggestive and might conceivably be used as a working basis for discussion.

Test 4.

The Enteritidis Test.

The next step in the enquiry was to ascertain in what numbers the spores of the Bacillus Enteritidis Sporogenes existed in the samples examined. The method of conducting this test has been indicated (vide p. 2/). In no case was a smaller quantity of ice-cream than 1 cubic centimetre used, and the following table gives the results :-

T a b l e 12.

Classifying the Results given by the "a" "b" and "c" Samples with Test 4.

	"a" Samples	"b" Samples	"c" Samples
Producing no change in 111 c.c.	9	4	2
" the "enteritidis change" in 100 c.c. but not in 10 c.c.	18	14	8
" " " in 10 c.c. but not in 1 c.c.	19	21	17
" " " in 1 c.c.	4	11	23
Total	50	50	50

In only nine of the samples taken immediately after boiling was no reaction produced in all the three quantities put on, viz. 100 cubic centimetres, 10 cubic centimetres and 1 cubic centimetre. All the other 41 samples shewed the "Enteritidis change" in one or other of these quantities. It has already been pointed out that the ice-cream product should be a sterile fluid immediately after heating, and a mixture of milk, sugar, and cornflour heated in the laboratory as already described (vide p. 50) did not give the "enteritidis change" when 100 cubic centimetres were tested. After freezing and standing 72 hours, 100 cubic centimetres of the ice-cream material were still free from the Bacillus Enteritidis Sporogenes.

Presence of the Bacillus Enteritidis Sporogenes in the Ingredients of Ice-cream.

So far as the ingredients of ice-cream are concerned, the presence of the Bacillus Enteritidis Sporogenes was found in samples of milk, and cornflour, but not in sugar put on for this test. Milk, however, contains the organism in by far the highest numbers, for while the "enteritidis change" was given with as little as .1 cubic centimetre of milk, this reaction was not produced with less than 10 grammes of cornflour in the present investigation. The initial boiling of these ingredients should destroy the organism as shewn by the experiment recorded in the preceding paragraph, and its

entrance later in the manufacture must come from other sources.

A Consideration of the Increase in Numbers of the Bacillus Enteritidis Sporogenes.

(a) By Multiplication.

As regards the increase in numbers of this organism in the "a" "b" and "c" samples, the same may be said as has already been stated for the "counts" and the "glucose-fermenters". There is doubtless multiplication due to the fact that air has been driven out of the milk, sugar, and cornflour compound while boiling, and the cream of the milk rising to the surface maintains the anaerobic conditions suitable for the growth and multiplication of this bacillus.

(b) By addition from contaminated sources.

Organisms of this type are also added from dust and the sweepings of dirty court yards in which ice-cream is manufactured. Houston[16a,b.] has shown that this organism is to be found in relatively great numbers in soil, and other observers have supported his conclusions, while Hewlett[17] and Klein[18a,b.] have shown that this organism is widespread in its distribution and especially prevalent in dust.

Test 5.

Streptococci.

Next to the glucose taurocholate broth test, the test for this group of organisms is considered by some bacteriologists to be the best as an index of pollution. In the present series of experiments, with a few exceptions, the test has been a microscopic one after incubation of the quantities already stated (vide Table I) in neutral red glucose broth for 48 hours. In a few instances where microscopically these organisms were apparently present in great numbers, plates were made on Drigalski's medium and sometimes on glucose agar. In one instance as many as 67 of the minute colonies developing on one or other of these media were examined without any one of them proving to be streptococcal; and in none of the samples examined by this process was a colony of streptococci landed. This is in accordance with my previous experiences recorded in Mussels[19] and Typhoid Fever; City of Birmingham Health Reports, 1908, although in this latter investigation after prolonged search of 25 samples of mussels 3 colonies were isolated and their reactions studied. The methods described by Andrewes[32] and Horder[20] Gordon,[21a,b,c.] Houston,[16c,d.] and Savage[22a,b.] all indicate the difficulty of successful isolation of these organisms, and the value of this test, therefore, for routine work must depend on the presence or absence of this group of organisms, and the kind of chains they form as seen by the microscope.

Doubtless as our knowledge of the subject increases it will be possible to apply other tests with rapidity and certainty, which will enable an accurate differentiation of these organisms to be quickly made.

The following table gives the results obtained for streptococci in the present enquiry :-

T a b l e 13.

Classifying the Results given by the "a" "b" and "c" Samples with Test 5.

	"a" Samples	"b" Samples	"c" Samples
Shewing no streptococci in the quantities examined	31	23	7
" stretococci in 10 c.c. but not in 1 c.c.	-	1	2
" " in 1 c.c. " " " A	3	-	1
" " in A " " " B	13	8	6
" " in B " " " C	3	15	7
" " in C " " " D	-	3	15
" " in D	-	-	12
Total	50	50	50

The Sources of Streptococci.

Streptococci have been found in enormous numbers in milk. In 31 of the samples of ice-cream, taken immediately after boiling, no streptococci were to be seen. It is therefore a fair conclusion that the original streptococci of the milk had been destroyed during the heating process. Only 7 of those 31 samples were still free from streptococci after freezing. In 24 samples, therefore, streptococci had entered. Streptococci are constantly found in faeces, manure, dust, and air and from one or other of these sources the 24 samples must have derived the organisms. It is here that more knowledge of the subject of streptococci is necessary. It is highly desirable that we should be able to associate a particular variety of streptococcus with a particular source, and for this purpose isolation and rapid and complete differentiation as above indicated are essential.

Suggested Bacteriological Standards.

In dealing with the subject of standards it is well to remember that a standard is not merely a matter of bacteriological average. It has to be considered also in the light of the attainable and as a result of the experience of observers as regards the potential disease-producing power of the material in question. The outbreaks recorded at the commencement of this paper clearly shew that ice-cream may be the carrier of very many diseases. Unfortunately only in the outbreak investigated by Robertson[9] was the implicated material

submitted to bacteriological analysis, which, however, was conducted with a view to determining the causal agent and not the amount of pollution. If information on this latter point had also been provided investigators would have been in possession of valuable data on which to base the consideration of standards. Under these circumstances the question must be decided by the other two factors here mentioned.

A Consideration of Standards based on Tests 1 and 2.

The average number of colonies capable of growing on nutrient gelatine or agar (vide pp 36-49) in all these samples was very high. This average cannot be taken as a fair test of what the standard of purity should be, for except in the cases of 7 "a" samples yielding less than 1,000 organsims per cubic centimetre on nutrient gelatine, the initial heating could not be considered as in any degree satisfactory. If on the other hand these 7 samples be carefully considered at stages "a" "b" and "c" it is possible to arrive at a fair conclusion as to what should be the limit of the bacterial content of frozen ice-cream. The following table shows the results of enumeration so far as these seven samples are concerned.

T a b l e 14.

Shewing Number of Organisms per Cubic Centimetre in the Seven "a" "b" and "c" Samples in which the "a" Samples yielded less than 1,000 Organisms per Cubic Centimetre capable of growing on Nutrient Gelatine (reaction + 1%) at 20° to 22°C in 5 days.

1	2			3		
Number of Sample	Number of Organisms per cubic centimetre capable of growing on Nutrient Gelatine (reaction + 1%) at 20° to 22° C in 5 days.			Number of Organisms per cubic centimetre capable of growing on Nutrient Agar (reaction + 1%) at 35° to 37° C in 2 days		
2a	Less than 100			200		
b		130,000			20,000	
c			7,000,000			5,000,000
6a	Less than 100			2,000		
b		Liquefied in 3 days			100,000	
c			12,000,000			20,000,000
41a	200			900		
b		70,000			70,000	
c			100,000			660,000
42a	600			200		
b		20,000			10,000	
c			50,000			30,000
46a	Less than 100			Less than 100		
b		50,000			Less than 10,000	

1	2			3		
46c			100,000			20,000
49a	600			5,600		
b		200,000			80,000	
c			2,720,000			140,000
50a	600			3,000		
b		1,280,000			880,000	
c			4,600,000			2,000,000

It will be noted that the best of these samples are Nos. 42 and 46, with No 41 not very far behind. The sanitary conditions of the premises on which these were manufactured were fair. The premises of No. 2 were filthy, No.50 dirty, No.6 clean and No.49 again fair. The high counts in sample No. 6 require explanation. This manufacturer showed special care in the making of his ice-cream. He reserved buckets and an outhouse for its preparation. But this latter was situated in a close, confined, crowded, ill-paved yard common to two houses. The water-closet of the other tenant was out of repair at the time this sample was taken, and the ashbin a few feet away was full. Undoubtedly these insanitary and dirty arrangements close by, combined with the long period of cooling and freezing -43 hours-, contributed largely to the high counts obtained in the finished article. Another consideration is this,

that the frozen sample was obtained from the manufacturer's employé off a trolley stationed in one of the busiest parts of Birmingham five hours after freezing had taken place. The dust of the traffic and the continual serving of customers in the open street by the not over clean seller must also have largely contributed to contamination and therefore high counts. Bearing all these facts in mind, and taking into consideration the figures in the seven samples set forth in Table 14, it seems reasonable to assume that ice-cream prepared under the various conditions laid down in the text should follow the course of the majority of these seven samples and not contain more than 1,000,000 organisms per cubic centimetre capable of growing at 20° to 22° C or 35° to 37° C.

The experiment already quoted (vide p. 48) shews that it is possible to manufacture ice-cream in the laboratory, which does not shew more than 200 organisms per cubic centimetre after standing frozen 24 hours. It is impossible for manufacturers to work under laboratory conditions, but they can readily prepare ice-cream which will pass the standard of 1,000,000 indicated. This is shewn by the results of the examination of ice-cream prepared as follows by manufacturers Nos. 6,7,26,1,11 and 12 in Table 2.

Method of preparation: The ice-cream mixture was boiled in a pot directly over the fire for 15 minutes

in the case of Nos. 6,7 and 26, and afterwards poured into the freezer through a metal sieve. In the case of the other manufacturers the so-called "water-bath" method was used in which the freezer containing the ice-cream mixture was put into cold water in a large pot or boiler. The water was brought to the boil and kept boiling for 30 minutes (Nos 1 and 11) and for 45 minutes (No 12) respectively.

In each case freezing was carried out immediately after heating.

All the utensils used including the spade* were thoroughly cleansed with soda and hot water and scalded with hot water immediately before use.

The manufacturer's hands and arms were carefully scrubbed and washed.

The following table gives the results :

Vide Table 15

* It is much to be desired from the hygienic point of view that this article be made in one piece (all metal) so that it can be thoroughly cleaned.

Table 15.

1	2	3	4
Manufacturer	Time of taking Sample	Number of colonies per cubic centimetre capable of growing on nutrient gelatine (reaction + 1%) at 20° to 22° C in 3 days.	Number of colonies per cubic centimetre capable of growing on nutrient agar (reaction + 1%) at 35° to 37° C in 2 days.
6	Immediately after boiling	1,200	10,000
	" " freezing	3,300	16,000
	22 hours " "	27,000	41,000
	92 " " "	119,000	127,000
7	Immediately after boiling	2,000	11,000
	" " freezing	2,000	16,000
	22 hours " "	14,000	48,000
	70 " " "	77,000	110,000
26	Immediately after boiling	Less than 1,000	Less than 1,000
	" " freezing	" " "	" " "
	20 hours " "	4,500	6,000
	44 " " "	41,000	35,000
1	Immediately after boiling	Less than 1,000	3,000
	" " freezing	1,100	4,000
	48 hours " "	63,000	71,000
	62 " " "	112,000	125,000

1	2	3	4
11	Immediately after boiling	Sterile	Sterile
	" " freezing	200	400
	26 hours " "	9,000	16,000
	46 " " "	30,000	26,000
12	Immediately after boiling	Sterile	Sterile
	" " freezing	200	400
	20 hours " "	7,000	10,000
	44 " " "	35,000	17,000

The results obtained(immediately after heating) by the water-bath method (Nos 1, 11 and 12) are better than those obtained when the mixture is boiled directly over the fire. This is due to the fact that during heating by the latter method constant stirring with the exposure of a large surface has to be practised to prevent the material being burnt and this does not conduce to obtaining a sterile article.

The Board of Public Health of the State of Victoria[23] require that ice-cream shall not contain more than 50,000 organisms per cubic centimetre. But this standard is probably too severe considering the fact that in an investigation conducted in Philadelphia by Pennington and Walter[24], a certain manufacturer "endeavoured to preserve the strictest cleanliness possible" and yet

produced ice-cream with organisms varying in number from 6,535,000 to 33,120,000 per cubic centimetre. The standard of 1,000,000 here laid down may be called lenient, yet it condemns 35 or 70% of the samples examined.

That this standard is reasonable and easily attained, if the ice-cream is properly manufactured and not stored for too long a period (which should not be longer than 48 hours after heating) is clearly shewn by Table 15.

A Consideration of a Standard based on Test 3.

None of the seven samples which were taken immediately after boiling and found to contain less than 1,000 organisms per cubic centimetre and which have been discussed in "A Consideration of Standards based on Tests 1 and 2", produced acid and gas in glucose bile salt broth in any of the quantities examined.

The ice-cream which was manufactured as above by Manufacturers Nos. 6,7,26,1,11 and 12 produced acid and gas as follows:

No.6 - after freezing 92 hours:

Present in 10 c.c. and 1 c.c.

No.7 - after freezing 70 hours:

Present in 10 c.c. and 1 c.c.

No.1 - after freezing 62 hours:

Present in 10 c.c. and 1 c.c.

The complete reaction was not given in any of the

samples of the other manufacturers or in any of the preceding samples of Manufacturers Nos. 6, 7 or 1 examined (vide Table 15).

Ice-cream prepared in the laboratory (vide p. 50) after 72 hours' freezing failed to shew the presence of "glucose fermenters" in as much as 20 cubic centimetres of the ice-cream.

Bearing these facts in mind, it is not too much to require that ice-cream prepared with due observance of the various conditions laid down should not contain "glucose fermenters" in less than 0.1 (or A in the nomenclature used) cubic centimetre of the finished product. In fact this is a very lenient standard. Yet on this basis, only 13 or 26% of the samples examined would be passed, a fact in strong support of the uncleanly conditions under which this article is manufactured.

A Consideration of a Standard based on Test 4.

In the case of the ice-cream prepared by the six manufacturers according to the method laid down on page 101 the Bacillus enteritidis sporogenes was found, after the mixture had stood frozen in all cases, in 100 cubic centimetres, and in 3 instances in 10 cubic centimetres as well. In no case was it found in 1 cubic centimetre.

The ice-cream which was prepared in the laboratory (see p. 93) and afterwards allowed to stand frozen and

covered for 72 hours did not shew the "enteritidis change" when 100 cubic centimetres were examined.

It is further interesting to observe that in two of the frozen samples (Nos. 15c and 20c) this change was not given in 111 cubic centimetres.

With these facts before us it is allowing a wide margin for unavoidable accident to state that a well made, well stored ice-cream should not shew the presence of this bacillus in less than 10 cubic centimetres. Houston[16d] in his report on "The Bacteriological Examination of Milk" to the London County Council, 1905, states that a sample of milk immediately cooled and maintained at a temperature of 10° C should be objected to if it gives the "enteritidis change" in less than 1 cubic centimetre. Orr[34] in his report on the Yorkshire milk supply, 1908, supports this standard. In only 2 out of 75 samples of milk was he able to find this bacillus in a less quantity than 1 cubic centimetre. If these observers reckon this a fair standard for milk merely cooled and kept cool, it is not unfair to ask for the higher standard of 10 cubic centimetres for milk, sugar, and cornflour, which is first boiled and immediately frozen and kept frozen. On this standard 27 or 54% of the Samples of ice-cream examined were satisfactory.

A Consideration of a Standard based on Test 5.

In the light of our present knowledge as regards

streptococci it is a difficult matter to set up a definite standard. The difficulty is increased by the consideration of an experiment in the manufacture and storage of ice-cream conducted in the laboratory under the same conditions as similar experiments already quoted. In the mixture thus prepared and stored streptococci, although absent immediately after boiling, made their appearance in 20 cubic centimetres within 24 hours. In the same time neither the presence of "glucose fermenters" or the bacillus enteritidis sporogenes could be demonstrated in the same amount.

In the ice-cream specially prepared by Manufacturers, Nos. 6,7,26,1,11 and 12 (vide p. 101) streptococci were present in 0.1 or A cubic centimetre in the frozen Samples of 4 of these makers and in 0.01 or B cubic centimetre as well in the case of one of these four. These organisms were absent from 10 c.c. in the samples of the two remaining manufacturers, and in no case were they present in .001 or C cubic centimetre.

Keeping these results in view, I am not disposed to urge in the present state of our knowledge regarding the significance of streptococci that they must be absent from large amounts of frozen ice-cream, and would suggest that ice-cream be accepted which does not shew their presence in less than .001 (or C) cubic centimetres. On this standard 38 or 76% of the samples would pass. Houston[16d] in his report on milk to the London County Council for like reasons

suggests a lenient standard as regards streptococci. He suggests that the presence of this class of organism in less quantity than .0001 cubic centimetre lays the milk open to objection from the bacteriological standpoint. In asking for a higher standard in the case of ice-cream, it is because it is comparatively easy to prepare ice-cream which is initially sterile, while it is a difficult matter to procure freshly drawn milk which does not shew the presence of these organisms .

SUMMARY .

(1) The premises of 50 manufacturers of ice-cream were inspected, their methods investigated, and bacteriological examinations made of samples taken

"a" immediately after heating.

"b" after cooling.

"c" after freezing.

(2) The trade is not carried on under the conditions or with the precautions necessary to secure a clean product.

THE SOURCES OF THE CONTAMINATION OF ICE-CREAM.

(3) Bacteriologically polluted ice-cream is due to :

(A) Insufficient initial heating and the multiplication of the organisms, not destroyed

by heat, during the periods of cooling and freezing.

(B) The use of unclean vessels.

(C) The addition of organisms during cooling and freezing :

(a) from the unclean linen or muslin covers (used by 10 manufacturers) dipping into the mixture while cooling.

(b) from the dried dust of the court yards in which the processes are carried on.

(D) The addition of organisms during storage :

(a) from lack of a proper covering for the vessel containing the ice-cream or ice-cream mixture.

(b) from the dust of the street or courtyards, which gains entrance to the cellars or rudely constructed sheds in which storage is effected.

(E) The addition of organisms while on sale in streets or shops :

(a) from the dust of streets

(b) from the unclean hands and sleeves of sellers.

THE SCIENTIFIC METHOD OF THE MANUFACTURE OF ICE-CREAM.

(4) To secure a pure Ice-cream :

(a) The premises on which ice-cream is manufactured should be approved and registered by the local authority and should be constantly supervised.

(b) All vessels should be thoroughly cleansed

immediately before use and reserved for the manufacture of ice-cream. They should be stored in a clean place.

(c) The manufacturer's hands and forearms should be thoroughly scrubbed and cleansed before each stage of the process. The clothing likely to come in contact with the ice-cream should also be clean.

(d) Fresh milk should be used in its manufacture.

(e) The ingredients should be boiled directly over a fire for ten minutes, or heated by means of a water-bath at boiling point for 30 minutes. The latter method is the better as the former is liable to burn the mixture.

(f) The mixture should be frozen, immediately after boiling, preferably in a freezer of the American pattern. Thereafter the ice-cream should be kept frozen while in the vendor's possession.

(g) No ice-cream should be exposed for sale 48 hours after boiling.

BACTERIOLOGICAL STANDARDS.

(5) Ice-cream made under the conditions laid down in (4) :

(a) Should not contain more than 1,000,000 organisms per cubic centimetre capable of

growing on nutrient gelatine (reaction + 1%) at 20° - 22° C. in 3 days. 35 or 70% of the samples fail to pass this standard.

(b) Should not contain more than 1,000,000 organisms per cubic centimetre capable of growing on nutrient agar (reaction + 1%) at 35° - 37° C. in 2 days. 30 or 60% of the samples fail to pass this standard.

(c) Should not produce Acid and Gas in MacConkey's Glucose Broth with a less quantity than 0.1 cubic centimetre. 37 or 74% of the samples fail to pass this standard.

(d) Should not contain the Bacillus Enteritidis Sporogenes in less than 10 cubic centimetres. 23 or 46% of the samples fail to pass this standard.

(e) Should not contain Streptococci in less than .001 cubic centimetre of ice-cream. 12 or 24% of the samples fail to pass this standard.

THE "GLUCOSE FERMENTERS".

A. Value of the Differential Tests employed.

(6) Fermentation Tests. Lactose, Adonite, Saccharose, Raffinose, Inulin, Salicin and Dulcite were all found very useful. On the other hand Mannite, Maltose, Galactose and laevulose all yielded positive reactions with this group of organisms and were useless for purposes of differentiation.

Lactose fermenting colonies on Neutral Red

Lactose Agar may vary in character from red with haze, to red, pink or lastly white.

(7) Fluorescence. This was produced by 255 or 90.5% of the glucose fermenters studied.

(8) Voges and Proskauer's Reaction. This was given by 54 or 20.9% of the organisms studied. Only four of these organisms, however, could be identified as belonging to the Bacillus lactis aerogenes or Bacillus Cloacae groups.

(9) The Test for Indol. A few experiments specially carried out shewed that the indol test using paradimethylamidobenzaldehyde and persulphate of potassium if not distinguishable after 24 hours is not given after longer incubation; but if given in 24 hours the intensity of the coloration increases up to seven days - the time over which the experiments lasted.

Indol was invariably produced by microbes shewing red colonies with definite haze on MacConkey's Neutral Red Lactose Agar.

B. The Isolated Organisms.

(10) Two hundred and fifty eight "glucose fermenters" were isolated and 66 of these were recognised.

One or other variety of Bacillus Coli was recognised 47 times.

The Bacillus Oxytocus Perniciosus, or the Bacillus Rhinoscleromatis, was recognised 10 times.

The Bacillus Acidi Lactici was identified 4

times.

The Bacillus Cloacae was isolated twice.

The Bacillus Lactis Aerogenes was identified twice.

The Bacillus Pneumoniae (Friedlander) was isolated once.

(11) By an empirical method put forward it was possible to arrange the remaining 192 unrecognised organisms in 69 classes.

GROWTH OF ORGANISMS AND TEMPERATURE.

(12) The Organisms in ice-cream mixture

(a) multiply rapidly under the influence of alternate thawing and freezing. The temperature varied in this experiment between 25° F and 50° F.

(b) multiply more slowly when the mixture is kept frozen. The temperature varied in this experiment between 28° F and 28.8° F.

(c) diminsh in number when the mixture is kept frozen in cold storage. The temperature varied in this experiment between 10° and 22° F.

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