THE CONTAMINATION OF ICE-CREAM.

A SANITARY AND BACTERIOLOGICAL STUDY.

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Plate VII.

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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.

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Recorded outbreaks due to ice-cream.

Scarlet Fever. Buchanan (1875) called attention to the fact that ice-pudding had caused cases of this disease. He traced the infection to the cream which had been used in the manufacture of the implicated food-stuff.

Typhoid fever. Turner (1892) 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.

Munro (1894) also traced an epidemic of typhoid fever in Renfrewshire to infected ice-cream.

Hope (1897) records 25 cases of typhoid fever as resulting from the consumption of ice-cream bought at a village fair. A case of typhoid fever was resident in the house of the vendor at the time.

Nineteen cases of typhoid fever are recorded by Barras (1904) as having been due to ice-cream sold by a vendor who was suffering from typhoid fever, which he considered to be influenza.

Diarrhoea. Henry (1900) describes an epidemic of this disease involving 146 persons with 1 death which he attributed to ice-cream. The baby in the house of the vendor was suffering from diarrhoea, and Henry reports that its napkins were washed within a few inches of the strainer used in the manufacture of ice-cream.

"Gaertner" Infection. A record of an outbreak of disease due to the consumption of ice-cream is contributed by Robertson (1905). 52 cases were recorded, 4 being adults and 48 children under 14 years of age. All had partaken of ice-cream supplied by one vendor, and a sample of this was submitted 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.

Peacock (1909) records an outbreak of ice-cream poisoning in Attleborough, in which 67 persons were affected between the ages of 18 months and 50 years. The *Bacillus enteritidis* of Gaertner was found in a sample of faeces obtained from one of the sufferers, while the blood of of another agglutinated a laboratory culture of this bacillus.

Ice-cream poisoning. Collingridge (1902) attributed the illness of 18 boys in the telegraph department in the City of London to the

consumption of ice-cream. The main symptoms of the illness were epigastric pain, colic, headache, nausea and nervous depression associated in some instances with vomiting and diarrhoea.

Previous bacteriological studies of ice-cream.

The foregoing accounts show that ice-cream may be the carrier of 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 (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.

Neild-Cook (1896) 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, Bacillus fluorescens liquefaciens* and many cocci.

Wilkinson (1899) has also given attention to the bacteriology of ice-creams.

Klein (1902) gave the results of the bacteriological examination of twenty-four samples of ice-cream collected in the City of London. He 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 occurred after boiling, *i.e.* during the cooling and freezing processes.

Rickards (1906) furnishes records of the bacteriological examinations of various samples of ice-cream and hokey-pokey in which the highest bacterial count was 150,000,000 per cubic centimetre, and the lowest 1,000,000. Reference must also be made to an account of similar work conducted in the bacteriological laboratory of the City of Philadelphia by Pennington and Walter (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 of 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 four instances (all English) the milk and sugar were boiled in an enamel pot and poured with stirring on to 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 this method the ice-cream ingredients are put into a freezer, which is then placed in water and gradually heated. In only one of the seventeen instances was a boiler reserved for use as a water-bath, the other 16 manufacturers using washhouse boilers or large iron pots which were used also for other purposes.

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 added gradually 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 heating was continued in some cases for a few minutes and in others for an hour and a half.

Cooling. After heating the ice-cream commodity is set out in galvanised iron buckets to cool, or is allowed to remain in the freezer if the first stage was carried out in that vessel. In only one instance were these buckets reserved solely for use in the manufacture of ice-cream. In the remaining 49 cases they were used for ordinary domestic purposes as well, and in one instance for tripe cleansing also. The vessels in which the ice-cream was cooled were usually placed on a slope so as to expose a large surface to the air for cooling, and were allowed to stand in this position over night.

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 efficient. It consisted of curtain material with a wide mesh work which could prevent only large particles of dirt gaining access. The two English vendors used fine muslin. 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 covers dipped into the ice-cream while cooling.

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. Salt and ice formed the freezing mixture employed and 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. Lumps of ice are frequently put into the ice-cream mixture to hasten freezing. As no precautions are taken to secure that the ice is clean, its addition in this way greatly adds to the contamination of the frozen product.

The English freezer. Where an 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[1] 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.

The American freezer. When an American freezer is used the lid is kept on constantly during the freezing process, and the necessary stirring 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.

The premises and their surroundings.

Twelve manufacturers (all Italians) had sheds erected for the preparation of the article. Only one of these twelve had a place used solely by himself. The others used sheds in common with other vendors. These sheds were situate in a common yard and were usually constructed of wood carelessly nailed together, with a corrugated iron roof on which were to be found old baskets, rabbit skins, and other rubbish. The floors were badly paved. In several cases tubs overflowing with vegetable refuse were close at hand, and the w. c.'s in the common yard in which the manufacture of ice-cream was going on were frequently found in a filthy state, as a result of improper use by the Italian population.

Amongst the remaining thirty-eight vendors the process of heating was carried on in the living room, kitchen or scullery of the house[2], or in the washhouse common to several families, the ice-cream mixture afterwards being put on the scullery sink or the doorstep to cool. Freezing was done in the common yard or at the door in the street.

The storage of ice-cream.

Where no sheds had 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 was stored, usually uncovered, in a dirty underground

[1] This spade should be made in one piece (all metal) so that it can be thoroughly and easily cleansed.

[2] In the case of Italians the house is frequently overcrowded, *e.g.*, in one case three small bedrooms and two small living rooms were occupied by seven adults (over 15 years) and six children (under 14 years).

cellar, to which the dust from the street or yard had easy access through a perforated iron grating or grid. This cellar was 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 could 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 to be noted that in the case of street trolleys the vendor has only a very small and limited supply of water with which to cleanse them after use.

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.

The following classification gives the results:

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.

"*c*" *Samples*: taken after the material had been frozen.

All these samples 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 samples taken immediately after boiling 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 definite dilutions (usually half and half) with sterile distilled water had to be made.

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 30°—37° C. for 48 hours.

Test 3. Deci-multiple quantities were put into Bile-Salt 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, a looplet was plated after appropriate dilution on Bile-Salt Lactose Agar and an endeavour made to determine the identity of the colonies by 16 different tests.

Test 4. An estimation of the number of spores of the *Bacillus enteritidis sporogenes* present.

Test 5. An estimation of the number of streptococci present by incubating deci-multiple quantities of the ice-cream in neutral Red Glucose Broth at 35° to 37° C. and examining microscopically the sediment. The character of the chains of streptococci, when present, were noted. Where these organisms as seen by the microscope appeared to be present in large numbers, attempts were made to isolate the microbe 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 series of samples:

TABLE I.

Shewing quantities examined for the five primary tests.

Tests	"*a*" Samples taken immediately after heating	"*b*" Samples taken after cooling	"*c*" Samples taken after freezing
1 and 2	·01 and ·0001 c.c.	·0001 and ·000001 c.c.	Same as "*b*".
3 and 5	10, 1, ·1 and ·01 c.c.	·10, 1, ·1, ·01, ·001 and ·0001 c.c.	Same as "*b*".
4	100, 10 and 1 c.c.	100, 10 and 1 c.c.	Same as "*b*".

Test 1. *The number of colonies per cubic centimetre capable of growing on nutrient gelatine (reaction* + 1 %) *at* 20° *to* 22° C. *in* 3 *days.*

"a" samples. With regard to the samples taken immediately after heating it may be said that in 14 cases total liquefaction occurred within the three days' incubation. 1 was liquefied on the 1st day, 7 on the 2nd day, and 6 on the 3rd day. These samples are excluded therefore from the averages. In three instances no growth occurred with 0·01 cubic centimetre, and in these cases the results have been entered as less than 100 colonies per cubic centimetre. For the purposes of calculation they have been assumed to contain 100 colonies per cubic centimetre, and these were the lowest results recorded. In three cases the results were high, viz. 846,000 colonies, 10,000,000 colonies and 20,000,000 colonies per cubic centimetre respectively. Including all these results for the 36 samples which did not liquefy within three days, the average count was found to be 867,319 colonies per cubic centimetre. Excluding the three high counts already referred to, and taking the average of the remaining samples, the count becomes 11,439.

The preliminary heating is obviously therefore not a process which is conducted so as to secure a sterile product although sterilization of the ice-cream mixture can be accomplished at the first stage. The three samples which shewed less than 100 colonies per cubic centimetre may have been sterile. One of these was heated for an hour and a half in a water-bath amidst most filthy surroundings; another was heated directly over the fire under quite the best conditions discovered during the inspections, while the third was heated in the same manner as the first under fair sanitary conditions. It is clear therefore that the sterility of the product at this stage depends on the heating alone.

The following laboratory experiments were conducted to ascertain how long it was necessary to heat the mixture of milk, sugar and cornflour by the methods in vogue before it became sterile.

Experiment 1. *Heating by "water-bath" method.* Ice-cream mixture was heated in a double saucepan, the inner vessel containing the milk, sugar and cornflour being placed in the outer containing cold water which was gradually brought to the boil. A drop of the ice-cream mixture was put into nutrient broth with sterile precautions every 5 minutes for 40 minutes after the water had begun to boil. No growth resulted after 25 minutes.

The maximum temperature attained by the ice-cream mixture heated under these conditions was 92° C. It rose to this temperature after the water had boiled 10 minutes, and remained constant throughout the remainder of the experiment.

Experiment 2. *Heating directly over flame.* A similar experiment was conducted in which the ice-cream ingredients were boiled directly over the flame with constant stirring to prevent burning of the product. Drops of the mixture were put into nutrient broth at intervals of 2 minutes after boiling had begun and no growth was obtained after 8 minutes.

It is therefore fair to conclude that with the possible exception of the three samples shewing less than 100 microbes per cubic centimetre none had been heated at a sufficient temperature for a sufficiently long period to secure sterility, as heating by means of the water-bath for 30 minutes or directly over the flame for 10 minutes positively ensures a sterile article.

"b" samples. The second series of samples were taken at varying intervals after cooling had begun. One sample was taken only $1\frac{1}{2}$ hours after it had been put out to cool, while the maximum period of cooling before taking the second sample was, in two instances, 28 hours. The average number of hours which these samples had been cooling was $15\frac{1}{2}$. Seven gelatine plates of the "b" samples were liquefied completely in two days, and 8 in 3 days, *i.e.* 15 in all, and these plates are excluded from further consideration. The lowest count recorded was 20,000 organisms per cubic centimetre in each of two cases, and the highest 102,240,000. The average for all the 35 enumerated samples was 13,042,857, an enormous increase over the average for the samples taken immediately after heating, viz. 867,319. This is what is to be expected when the material in question is a nutrient fluid such as ice-cream exposed to contaminating conditions, and shews how important it is to have rapid cooling under clean conditions.

"*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 on 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. 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. 12 samples liquefied during the three days' incubation and these are excluded from further consideration. The lowest count recorded was 50,000, the highest 3,800,000,000, and the average for the 38 enumerated samples 372,213,421 colonies per cubic centimetre. 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 (five in number), excluding which gives an average of 16,470,515 organisms per cubic centimetre—a number in accord with other workers.

Test 2. *Number of organisms capable of growing on nutrient agar* (*reaction* + 1 %) *at* 35°—37° C. *in* 2 *days.*

The agar plates were all counted after two days' incubation. Speaking generally, the number of colonies growing at 35° to 37° C. was found to be less than the corresponding number growing at 20° to 22° C., but the same gradual increase in the number of microbes during the manufacture of ice-cream is to be noted with both counts as the following summary shews (Table II).

Causes of the increase in the number of organisms in commercial ice-cream.

1. *Multiplication of organisms during cooling.* The average period of cooling before freezing was about 20¾ hours for the 50 samples under review and during this time the organisms not destroyed by the initial heating grow and multiply.

2. *The addition of organisms during the freezing process.* The high average count amongst the samples taken after freezing is also to be

TABLE II.

Classifying the results of Tests 1 *and* 2.

	Test 1. (Gelatine counts)			Test 2. (Agar counts)		
	"*a*"	"*b*"	"*c*"	"*a*"	"*b*"	"*c*"
	Samples taken immediately after heating	Samples taken after cooling	Samples taken after freezing	Samples taken immediately after heating	Samples taken after cooling	Samples taken after freezing
No. of samples shewing less than 100 organisms per c.c.	3	—	—	1	—	—
No. of samples shewing between 100 and 1000 organisms per c.c.	4	—	—	4	—	—
No. of samples shewing between 1000 and 10,000 organisms per c.c.	17	—	—	18	1	—
No. of samples shewing between 10,000 and 100,000 organisms per c.c.	9	10	2	15	15	6
No. of samples shewing between 100,000 and 1,000,000 organisms per c.c.	1	9	13	7	12	14
No. of samples shewing between 1,000,000 and 10,000,000 organisms per c.c.... ...	2	5	5	5	11	10
No. of samples shewing between 10,000,000 and 100,000,000 organisms per c.c. ...	—	9	11	—	11	16
No. of samples shewing over 100,000,000 organisms per c.c.	—	2	7	—	—	4
No. of samples liquefied	14	15	12	—	—	—
Average no. of organisms per c.c. in samples taken immediately after heating ...	867,319			379,010		
Average no. of organisms per c.c. in samples taken immediately after cooling ...	13,042,857			6,861,600		
Average no. of organisms per c.c. in samples taken immediately after freezing ...	372,213,421			34,467,000		

TABLE III.

Shewing number of organisms in ice-cream immediately before and after freezing.

Manufacturer	Date of examination 1909	No. of organisms per c.c. capable of growing on nutrient gelatine (reaction +1 %) at 20° to 22° C. in 3 days		No. of organisms per c.c. capable of growing on nutrient agar (reaction +1 %) at 35° to 37° C. in 2 days	
		Immediately before freezing	Immediately after freezing	Immediately before freezing	Immediately after freezing
*No. 6	Feb. 28	10,000	30,000	230,000	470,000
,, 7	April 30	3,000	14,000	5,000	12,000
,, 26	May 1	2,300	36,000	18,000	29,000

* The nos. relate to laboratory references.

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explained 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 Table III, giving the results of the examination of samples taken from vendors immediately before and after freezing.

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 on, and the ice-cream mixture was frozen by rotation in a 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.

3. *Multiplication of organisms during frozen period.* The increase in the number of organisms in commercial ice-cream 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 arrival 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 459,000, after 9 hours 603,000, and after 12 hours 611,000 colonies per cubic centimetre. Similarly the "agar count" of the ice-cream on its arrival at the laboratory was 93,000 colonies per cubic centimetre. After 3 hours the "agar count" was 112,000, after 6 hours 126,000, after 9 hours 130,000 and after 12 hours 139,000 colonies per cubic centimetre. During this experiment the temperature of the ice-cream varied between 28° F. and 28·8° F.

Test 3. *The Bile-Salt Glucose Broth test.*

All samples were submitted to this test, and the reaction produced within 48 hours noted. The quantities examined have already been given (vide Table I), the total for each of the "*a*" samples being 11·11

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cubic centimetres, and for each of the "*b*" and "*c*" samples 11·1111 cubic centimetres. Only two samples gave a negative result. This is not as it should be, as 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.

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

TABLE IV.

Shewing the results given by the three sets of samples with the Bile-Salt Glucose Broth test.

	"*a*" Samples taken immediately after heating	"*b*" Samples taken after cooling	"*c*" Samples taken after freezing
Producing no change in 11·11 c.c.	2	—	—
,, acid in 10 but not in 1 c.c.	3	—	—
,, ,, 1 ,, ,, ·1 c.c.	16	—	—
,, ,, ·1 ,, ,, ·01 c.c.	20	10	1
,, ,, ·01 ,, ,, ·001 c.c.	—	8	1
,, ,, ·001 ,, ,, ·0001 c.c.	—	—	—
,, ,, ·0001 c.c.	—	1	1
,, acid and gas in 10 but not in 1 c.c.	1	2	—
,, ,, ,, 1 ,, ,, ·1 c.c.	2	3	5
,, ,, ,, ·1 ,, ,, ·01 c.c.	5	7	5
,, ,, ,, ·01 ,, ,, ·001 c.c.	1	10	19
,, ,, ,, ·001 ,, ,, ·0001 c.c.	—	7	7
,, ,, ,, ·0001 c.c.	—	2	11
Total	50	50	50

From Table IV it will be seen that nine of the samples taken immediately after heating 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. ·01 cubic centimetre. Thirty-one of the samples taken after cooling produced this reaction, two giving it in ·0001 cubic centimetre. In the third series of samples, *i.e.* those taken after freezing, 47 out of the 50 samples examined shewed fermentation, no less than 11 producing acid and gas in ·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 supports the previous results of the gelatine and agar counts (vide Table II).

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 Bile-Salt Glucose Broth. From the smallest quantity in each series shewing this change a plate was made on Bile-Salt Lactose Agar and the nature of the colonies developing in 24 hours at 35° to 37° C. noted. The colonies varied from red colonies with production of haze in the surrounding medium to red without haze, pink and lastly white. 95 such plates were made.

In six instances plates were made from different dilutions of the same sample, and in these six with one exception 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 three 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 all 258 colonies were taken for examination from the 95 plates, 108 being red colonies with haze, 69 red, 22 pink and 59 white.

The differential reagents.

The reagents employed were:

For fermentation, glucose, lactose, mannite, maltose, galactose, laevulose, adonite, saccharose, raffinose, inulin, salicin, dulcite.

For fluorescence—neutral red.

For indol—peptone water.

For Voges and Proskauer's reaction—glucose broth.

For liquefaction—gelatine.

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 %	tinted with litmus.
Peptone	2 %	
Lemco	1 %	
Gelatine	10 %	
5 % KHO Solution	1 %	
Distilled water	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 colour 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 (1907, January, June).

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

Three of the following:

A. One 3″ × 1″ tube (white wool) containing five 2″ × ¼″ tubes with the following media:

1. Litmus glucose gelatine—white bead
2. „ lactose „ —brown „
3. „ mannite „ —blue „
4. „ maltose „ —green „
5. „ galactose „ —black „

Three of the following:

B. One 3″ × 1″ tube (blue wool) containing four 2″ × ¼″ tubes with the following media:

1. Litmus laevulose gelatine—no bead
2. „ adonite „ —red bead
3. „ saccharose „ —yellow bead
4. „ raffinose „ —indigo bead.

Three of the following:

C. One 3″ × 1″ tube (brown wool) containing four 2″ × ¼″ tubes with the following media:

1. Litmus inulin gelatine—brown bead
2. „ salicin „ —blue „
3. „ dulcite „ —green „
4. Neutral red „ —no „

One of the following:

D. One 3″ × 1″ tube (white wool) containing six 2″ × ¼″ tubes with the following media:

3 with glucose broth (blue bead)
3 with peptone water (no bead).

After these tubes suitably marked were inoculated with the colonies to be tested they were allowed to incubate at 35° to 37° C., but at the end of three hours the gelatine media (A, B and C) were removed and placed for half an hour in an ice-chest and afterwards incubated 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 colour of the litmus or the presence of a bubble of gas in the medium.

The gelatine tubes were looked at daily for fermentation up to seven days. It was not found to be practicable on account of the numbers in use to keep them longer than that time. Definite production of acid and gas within that period was duly noted. 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 the reagents employed. Houston (1907, December, pp. 23—25) has pointed out the extreme delicacy of gelatine media for fermentation tests and this statement was amply supported during the present inquiry, some organisms producing neither acid nor gas in seven days, others giving only a slight production of acid without gas, and others again a 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 seven days allotted for the completion of these results it may be said that the intensity of the coloration depended upon the period of growth. But from the experience of a few instances specially tested it may be said that the indol reaction if not detectable at the end of 24 hours' 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 there was added a quantity of a 2 % solution of caustic soda equal in volume to about 25 % of the volume of the broth. The tubes were then allowed to remain at room temperature for four days longer, daily observations being made during this time. The colour 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 four days.

The value of the various reagents employed for differentiation.

258 colonies were examined, and from the results obtained 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 those media. MacConkey (1905) has already made the same statement.

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

(1) All colonies definitely red in colour on Bile-Salt Lactose 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 (1908, p. 324) has referred to this fermentation of lactose by colonies which are colourless on Bile-Salt Lactose Agar.

Fluorescence was produced by 233 out of 258 colonies examined, or by 90·3 %. This is a higher percentage than obtained by the writer (1908, p. 16) 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 (1907, January, p. 47) 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 have not yet been definitely decided. It is generally accepted as a reaction given only by bacilli of the *Bacillus lactis aerogenes* and *Bacillus 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 (1906, p. 250) has stated and MacConkey (1908) has already controverted. But of the 54 positive organisms only two could be placed in the lactis aerogenes group by their other reactions and only two 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 were all found to be more or less of diagnostic value. No great importance was attached to the test for liquefaction of gelatine, where the results were negative, as the time given (7 days) is short for this test. So far as it goes however it is interesting to note that 12 out of the 13 liquefiers were white colonies on Bile-Salt Lactose Agar.

The nature of the glucose fermenters *isolated.*

In only three of the ice-creams was it possible to isolate an organism giving the same reactions in two successive stages. This fact indicates the great multiplicity of *glucose fermenters* which abound in ice-cream and lends strong presumption to the view that fresh organisms are added by contamination during manufacture.

For the 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.

TABLE V.

Shewing the reactions of known organisms with the differential media employed in the present investigation.

Organism	Obtained from	Lactose	Fluorescence	Indol	Voges and Proskauer's reaction	Adonite	Saccharose	Raffinose	Inulin	Salicin	Dulcite	Liquefaction of gelatine
B. coli communis (Escherich)	Dr A. T. MacConkey	+	+	+	−	−	−	−	−	−	+	−
B. lactis aerogenes ...	,, ...	+	+	−	+	+	+	+	−	+	−	−
B. acidi lactici (Hüppe)	1. Kral through Dr R. M. Buchanan 2. Dr A. C. Houston 3. Dr A. T. MacConkey	+	+	+	−	+	−	−	−	−	−	−
B. cloacae	1. Kral through Dr R. M. Buchanan 2. Dr A. T. MacConkey	−	+	−	+	−	+	+	−	−	−	+
	3. Dr A. C. Houston	−	+	−	+	+	+	+	−	−	−	+
B. paracoli (Widal) ...	Kral through Dr R. M. Buchanan											
B. enteritidis (Gaertner)	1. Kral through Dr R. M. Buchanan 2. Dr A. C. Houston 3. Original strain through Dr W. G. Savage											
B. paratyphosus B (Schottmüller) ...	1. Kral through Dr R. M. Buchanan 2. Dr A. C. Houston 3. Dr W. G. Savage											
B. paratyphosus B (Achard) ...	Kral through Dr. R. M. Buchanan											
B. paratyphosus B (McWeeney)	Prof. E. J. McWeeney	−	+	−	−	−	−	−	−	−	+	−
B. paratyphosus A (Schottmüller) ...	1. Kral through Dr R. M. Buchanan 2. Dr A. C. Houston 3. Dr A. T. MacConkey 4. Dr W. G. Savage											
B. paratyphosus A (Brian and Kayser)	Kral through Dr R. M. Buchanan											
B. aertrycke ...	Dr A. T. MacConkey											
B. sinpestifer ...	Prof. Uhlenhuth through Dr W. G. Savage											
B. psittacosis ...	Dr A. T. MacConkey											
B. Friedlander ...	Dr A. C. Houston	+	+	−	−	+	+	+	−	+	+	−
,, (Nicolle)	Dr A. T. MacConkey											
B. levans	,, ...	−	+	−	+	−	+	−	+	+	−	+
B. oxytocus perniciosus	,, ...	+	+	−	−	+	+	+	+	+	+	−
B. rhinoscleromatis ...	,, ...											
B. Grünthal ...	,, ...	+	+	+	−	−	+	−	−	−	−	−
B. coscoroba ...	,, ...	+	+	+	−	−	+	A	−	−	−	−
B. cavicida (Brieger)	,, ...	+	+	+	−	−	−	−	−	A	−	−
B. typhosus ...	Prof. R. F. C. Leith ...	−	−	−	−	−	−	A	−	−	−	−

+ = 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.

A = production of acid.

With the exception of the *Bacillus typhosus*, which produced merely acid, all the above organisms produced acid and gas in glucose, mannite, maltose, galactose and laevulose.

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Comparing the reactions of the 258 *glucose fermenters* isolated from the samples of ice-cream examined with those given in Table V it was found that 66 of them could be classified as follows:

TABLE VI.

Classifying 66 *of the* 258 *glucose fermenters isolated from the "a" "b" and "c" samples examined.*

	No. of times recognised
Bacillus oxytocus perniciosus (V & P +)	5
,, ,, ,, (V & P −) or ,, *rhinoscleromatis* (indol − inulin +)	5
,, *coli* (sacc. + raff. + dulc. +)	8
,, ,, (,, − ,, ,, ,,)	4
,, *cloacae* (lact. + liquefaction −)	2
,, *coli* (sacc. + raff. − dulc. −) or ,, *Grünthal* or *Bacillus coscoroba*	3
,, *coli* (sacc. − raff. − dulc. −) or ,, *cavicida* (dulc. −)	18
,, *coli* (sacc. + raff. − dulc. +)	3
,, *acidi lactici* (raff. −)	4
,, *coli communis* (sacc. − raff. − dulc. +)	11
,, *Friedländer* (indol −)	1
,, *lactis aerogenes* (indol −)	2

Coli Group.

In Table VI 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 three instances did these members of the coli group not yield red colonies on Bile-Salt 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 Bile-Salt 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.

Bacillus coli. Bacillus Grünthal. Bacillus coscoroba. Bacillus cavicida.

So far as these tests go it is impossible to distinguish between certain varieties of the *Bacillus coli*, *Bacillus Grünthal*, *Bacillus coscoroba* and *Bacillus cavicida* as seen on Tables V and VI. The *Bacillus Grünthal* has been placed in the coli group by Morgan (1905, p. 1258). *Bacillus coscoroba* is stated to have been the cause of an epidemic in swans. This was studied by Tritrop (1900, *Ann. Inst. Pasteur*, p. 224) and his results were published attributing the outbreak to this bacillus. The *Bacillus cavicida* has been isolated from faeces by Brieger (1884). He states that this organism is pathogenic for guinea-pigs when inoculated subcutaneously, but is without effect when taken with food. According to this observer *Bacillus cavicida* is allied to *Bacillus coli communis* and *Bacillus lactis aerogenes*.

Bacillus oxytocus perniciosus *and* **Bacillus rhinoscleromatis.**

Next to the coli group of organisms the organism found in most abundance was the *Bacillus oxytocus perniciosus*. This is an organism which was first described by Wyssokowitsch (Macé, 1904), who isolated it from old milk. It is pathogenic for mice and rabbits when large doses are used.

MacConkey (1906, p. 403) identified this organism 16 times in 170 organisms isolated from milk.

A glance at Table V 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.

Bacillus cloacae.

The *Bacillus cloacae* has been isolated twice. A good account of this organism is given by MacConkey (1905, p. 348).

Bacillus lactis aerogenes *and* **Bacillus pneumoniae** (*Friedländer*).

The *Bacillus lactis aerogenes* has been identified twice and the *Bacillus pneumoniae* (Friedländer) once.

Authorities are not agreed as to the relationship between these organisms. Some consider them identical. Others consider the *Bacillus lactis aerogenes* as identical with the *Bacillus coli communis*. The presence of Voges and Proskauer's reaction is the most important test yet put forward for the identification of the *Bacillus lactis aerogenes*. MacConkey (1906, p. 403) isolated the latter twice from various samples of milk, but was unable to identify the *Bacillus pneumoniae* (Friedländer) during the investigation. He considered that the *Bacillus cloacae* and *Bacillus lactis aerogenes* could be found in milk in greater abundance after it had been kept some time.

Bacillus acidi lactici.

The *Bacillus acidi lactici* has also been recognised. MacConkey (1905, p. 378) has suggested that this organism while occurring in faeces sometimes disappears so quickly that, if found to do so constantly, 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 *Bacillus 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.

The reactions of the remaining 192 isolated organisms are shewn in Table VII.

In this table it is to be noted that 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 74 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 (1905, 1906) lays considerable stress on the value of 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 being extensively used. It is equally true that in the present state of our

TABLE VII.

Classifying the 192 *unrecognised glucose fermenters of the* 258 *isolated from the "a" "b" and "c" samples examined.*

Total no. of organisms	Lactose	Fluorescence	Indol	Voges and Proskauer's reaction	Adonite	Saccharose	Raffinose	Inulin	Salicin	Dulcite	Liquefaction of gelatine
2	+	+	+	+	+	+	+	+	+	+	–
3	+	+	–	+	+	+	+	+	+	+	+
3	+	+	+	+	+	+	+	–	+	+	–
5	+	+	+	–	+	+	+	+	+	+	–
1	+	+	+	–	+	+	+	+	+	–	–
2	+	+	+	–	–	+	+	+	+	+	–
2	+	+	–	+	–	+	+	+	+	+	–
1	–	+	–	+	+	+	+	+	+	–	+
1	+	+	+	+	+	–	+	–	+	–	–
6	+	+	+	–	+	+	+	–	+	–	–
1	+	+	+	–	+	+	+	–	–	+	–
1	+	+	+	–	+	+	–	–	+	+	–
1	+	+	+	–	–	+	+	–	+	+	–
1	+	+	–	+	+	–	+	–	+	+	–
6	+	+	–	–	+	+	+	+	+	–	–
3	+	+	–	–	+	+	+	–	+	–	+
7	+	+	–	–	–	+	+	+	+	+	–
3	+	–	–	+	+	+	+	–	+	+	–
4	+	–	–	–	+	+	+	+	+	+	–
2	+	+	+	–	+	+	–	+	–	–	–
2	+	+	+	–	–	+	+	–	+	–	–
3	+	+	–	+	+	+	–	+	–	–	–
2	+	+	–	+	–	+	+	–	+	–	–
4	+	+	–	–	+	+	+	–	+	–	–
2	+	+	–	–	+	+	–	+	+	–	–
1	+	+	–	–	–	+	+	+	+	–	–
3	+	+	–	–	–	+	+	–	+	+	–
1	+	–	+	+	–	+	+	–	+	–	–
1	+	–	+	–	+	+	+	–	+	–	–
1	–	+	–	+	+	+	+	–	+	–	–
1	–	+	–	+	–	+	+	–	+	–	+
1	–	–	–	+	+	+	+	+	+	–	–
1	–	–	–	+	–	+	+	+	+	+	–
4	+	+	+	–	–	+	+	–	–	–	–
1	+	+	–	+	+	+	–	–	–	–	–
2	+	+	–	+	–	–	+	–	+	–	–
1	+	+	–	–	+	–	+	–	+	–	–
15	+	+	–	–	–	+	+	–	+	–	–

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TABLE VII (*continued*).

Total no. of organisms	Lactose	Fluorescence	Indol	Voges and Proskauer's reaction	Adonite	Saccharose	Raffinose	Inulin	Salicin	Dulcite	Liquefaction of gelatine
3	+	+	−	−	−	+	+	−	−	+	−
1	+	+	−	−	−	+	+	−	−	−	+
1	+	−	+	−	+	+	+	−	−	−	−
3	+	−	+	−	−	+	+	−	−	+	−
1	+	−	−	−	+	+	+	−	+	−	−
4	−	+	−	+	−	+	+	−	+	−	−
4	−	+	−	−	−	+	+	+	+	−	−
3	+	+	+	−	−	−	+	−	−	−	−
1	+	+	+	−	−	−	−	−	−	−	+
4	+	+	−	+	−	+	−	−	−	−	−
2	+	+	−	+	−	−	+	−	−	−	−
8	+	+	−	−	−	+	+	−	−	−	−
3	+	+	−	−	−	+	−	−	−	+	−
3	+	+	−	−	−	−	+	−	+	−	−
6	+	+	−	−	−	−	+	−	−	+	−
1	+	+	−	−	−	−	−	+	+	−	−
2	+	−	+	−	−	+	+	−	−	−	−
1	+	−	+	−	−	−	+	−	−	+	−
1	+	−	−	−	+	+	+	−	−	−	−
1	+	−	−	−	+	+	−	−	+	−	−
1	−	+	+	+	−	+	−	−	−	−	−
1	−	+	−	−	+	+	+	−	−	−	−
1	−	+	−	−	−	−	+	−	−	+	+
1	−	−	−	−	−	+	+	−	+	−	+
1	+	+	−	+	−	−	−	−	−	−	−
2	+	+	−	−	−	−	+	−	−	−	−
4	+	+	−	−	−	−	−	−	−	+	−
1	−	+	+	+	−	−	−	−	−	−	−
2	−	+	−	+	−	+	−	−	−	−	−
1	−	+	−	+	−	−	+	−	−	−	−
4	−	+	−	−	−	−	+	−	+	−	−
1	−	−	−	−	−	−	+	−	+	+	−
13	+	+	−	−	−	−	−	−	−	−	−
1	+	−	−	−	+	−	−	−	−	−	−
1	+	−	−	−	−	−	−	−	−	−	+
3	−	+	+	−	−	−	−	−	−	−	−

+ = 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.

All the above organisms produced acid and gas in glucose, mannite, maltose, galactose and laevulose.

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knowledge no organism can be absolutely recognised without such tests. It would serve a useful purpose if some definite method for their application and the classification of results was put forward for general adoption.

Test 4. *The test for the* **Bacillus enteritidis sporogenes.**

The next step in the inquiry was to ascertain in what numbers the spores of the *Bacillus enteritidis sporogenes* existed in the samples examined. The method of conducting this test was as follows. Fifty cubic centimetres of sterile water were put into sterile long necked flasks 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 sterile 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 7 days were 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 vaseline, no longer anaerobic.

In no case was a smaller quantity of ice-cream than 1 cubic centimetre used, and the results are given in Table VIII.

This table shews that in only 9 of the 50 samples taken immediately after boiling was no reaction produced in all three quantities examined, 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 those quantities. It has already been pointed out that the ice-cream product should be a sterile fluid immediately after heating,

and in point of fact it was found that 100 cubic centimetres of milk, sugar and cornflour when heated and stored under laboratory conditions (vide p. 105) remained free from *Bacillus enteritidis sporogenes* for 72 hours.

TABLE VIII.

Classifying the results given by the three sets of samples with the Bacillus enteritidis sporogenes test.

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

The presence of the **Bacillus enteritidis sporogenes** *in the ingredients of ice-cream.*

So far as the ingredients of ice-cream are concerned the *Bacillus enteritidis sporogenes* was found in samples of milk and cornflour, but not in sugar. Milk, however, contains the organism in by far the highest numbers, for while the *enteritidis change* was given with as little as 0·1 cubic centimetre of milk, this reaction was not produced with less than 10 grammes of cornflour in the present investigation.

A consideration of the increase in numbers of the **Bacillus enteritidis sporogenes.**

(*a*) *By multiplication.* This is possible as the boiling and the layer of cream at the top render the conditions sufficiently anaerobic for the growth 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 (1897—1900) has shewn that this organism is to be found in relatively great numbers in soil, and other observers have supported his conclusions, while Hewlett (1899) and Klein (1897—1899) have shewn that this organism is wide spread in its distribution and especially prevalent in dust.

Test 5. *Streptococci.*

Streptococci are considered by some bacteriologists to be indicators of recent pollution but the isolation of these organisms is not easy as is shewn by Gordon (1902—06), Houston (1903—05), Andrewes and Horder (1906), Andrewes (1906—07), Savage (1907—07), and my own experience (1908) confirms this. In one case in the present investigation I examined 67 minute colonies for streptococci without a single positive result. We have therefore in routine work to rely on the microscopical examination of a culture made from the material under investigation.

The results of such a microscopical examination are set out in the following table:

TABLE IX.

Classifying the results given by the three sets of samples with the streptococci test.

	"*a*" Samples taken immediately after heating	"*b*" Samples taken after cooling	"*c*" Samples taken after freezing
Shewing no streptococci in 11·11 c.c.	31	23	7
Shewing streptococci in 10 but not in 1 c.c.	—	1	2
,, ,, ,, 1 ,, ,, ·1 c.c.	3	—	1
,, ,, ,, ·1 ,, ,, ·01 c.c.	13	8	6
,, ,, ,, ·01 ,, ,, ·001 c.c.	3	15	7
,, ,, ,, ·001 ,, ·0001 c.c.	—	3	15
,, ,, ,, ·0001 c.c.	—	—	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 more of these sources of contamination, to which ice-cream is exposed, 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 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 many diseases. Unfortunately only in the outbreak investigated by Robertson (1906) 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, valuable data on which to base the consideration of standards would have been in our possession. 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 Table II) in all these samples was 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 samples taken immediately after boiling and found to contain less than 1000 organisms per cubic centimetre capable of growing at 20°—22° C., the initial heating could not have been satisfactory. If, on the other hand, these 7 samples be carefully considered at the three 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. Table X shews the results of enumeration so far as these seven samples are concerned.

It will be noted that the best of these samples are Nos. 42 and 46, with No. 41 not 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 No. 6 require explanation. This manufacturer shewed special care in the making of his ice-cream. He reserved buckets and an out-

house 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 X, it seems reasonable to assume that frozen ice-cream prepared under the various conditions laid down in the text should not contain more than 1,000,000 organisms per cubic centimetre capable of growing at 20° to 22° C. or 35° to 37° C.

TABLE X.

Shewing the number of organisms per c.c. in seven sets of samples, the "a" sets of which yielded less than 1000 *organisms per c.c. capable of growing on nutrient gelatine at* 20°—22° *C. in* 3 *days.*

Gelatine Counts.

	Sample no. 2*	Sample no. 6	Sample no. 41	Sample no. 42	Sample no. 46	Sample no. 49	Sample no. 50
"*a*"—after heating	less than 100	less than 100	200	600	less than 100	600	600
"*b*"—after cooling	180,000	liquefied	70,000	20,000	50,000	200,000	1,280,000
"*c*"—after freezing	7,000,000	12,000,000	100,000	50,000	100,000	2,720,000	4,600,000

Agar Counts.

	Sample no. 2*	Sample no. 6	Sample no. 41	Sample no. 42	Sample no. 46	Sample no. 49	Sample no. 50
"*a*"—after heating	200	2,000	900	200	less than 100	6,600	3,000
"*b*"—after cooling	20,000	100,000	70,000	10,000	,, 10,000	80,000	880,000
"*c*"—after freezing	5,000,000	20,000,000	600,000	30,000	20,000	140,000	2,000,000

* The numbers of the samples relate to laboratory references.

The experiment which was carried out (vide p. 105) 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 by manufacturers under my instructions as follows:

Method of preparation. The ice-cream mixture was boiled in a pot directly over the fire for 15 minutes, in the case of manufacturers Nos. 6, 7 and 26 in Table XI, and afterwards poured into the freezer through a metal sieve. In the case of the other manufacturers, Nos. 1, 11 and 12 in Table XI, 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 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:

TABLE XI.

Manufacturer	Time of taking sample	No. of colonies per c.c. capable of growing on nutrient gelatine (reaction +1 %) at 20° to 22° C. in 3 days	No. of colonies per c.c. capable of growing on nutrient agar (reaction +1 %) at 35° to 37° C. in 2 days
*No. 6	Immediately after heating	1,200	10,000
	,, ,, freezing	3,300	16,000
	22 hours ,, ,,	27,000	41,000
	92 ,, ,, ,,	119,000	127,000
No. 7	Immediately after heating	2,000	11,000
	,, ,, freezing	2,000	16,000
	22 hours ,, ,,	14,000	48,000
	70 ,, ,, ,,	77,000	110,000
No. 26	Immediately after heating	less than 1,000	less than 1,000
	,, ,, freezing	,, ,,	,, ,,
	20 hours ,, ,,	4,500	6,000
	44 ,, ,, ,,	41,000	35,000
No. 1	Immediately after heating	less than 1,000	3,000
	,, ,, freezing	1,100	4,000
	48 hours ,, ,,	63,000	71,000
	62 ,, ,, ,,	112,000	125,000
No. 11	Immediately after heating	sterile	sterile
	,, ,, freezing	200	400
	26 hours ,, ,,	9,000	16,000
	46 ,, ,, ,,	30,000	26,000
No. 12	Immediately after heating	sterile	sterile
	,, ,, freezing	200	400
	20 hours ,, ,,	7,000	10,000
	44 ,, ,, ,,	35,000	17,000

* The nos. relate to laboratory references.

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From Table XI it will be seen that 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 (1906) require that ice-cream shall not contain more that 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 (1907, p. 1016), 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 35,120,000 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 XI.

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 1000 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 by any of the other samples prepared as described by these six manufacturers.

Ice-cream prepared in the laboratory (vide p. 106) 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 cubic centimetre of the finished product. In fact this is a 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 as described by the six manufacturers, Nos. 6, 7, 26, 1, 11 and 12, the *Bacillus enteritidis sporogenes* was found after the mixture had stood frozen in all cases in 100 cubic centimetres and in three instances in 10 cubic centimetres as well. In no case was it found in 1 cubic centimetre.

Ice-cream which was prepared in the laboratory (vide p. 119) 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 fifty frozen samples originally examined 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 and properly stored ice-cream should not shew the presence of this bacillus in less than 10 cubic centimetres. Houston (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 (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* nor the *Bacillus enteritidis sporogeñes* could be demonstrated in the same amount.

In the ice-cream specially prepared by manufacturers, Nos. 6, 7, 26, 1, 11, 12, streptococci were present in ·01 cubic centimetre in the frozen samples of four of these makers and absent from 10 c.c. in the samples of the two remaining manufacturers. In no case were they present in ·001 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 cubic centimetre. On this standard 38 or 76 % of the samples examined pass. Houston (1905) for like reasons suggests a lenient standard as regards streptococci in milk. 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;
(*B*) Contamination during cooling and freezing from

(*a*) unclean vessels and covers,
(*b*) the addition of unclean ice to hasten freezing,
(*c*) the unclean hands of the manufacturer,
(*d*) dirty surroundings.

The scientific method of the manufacture of ice-cream.

(4) To secure a pure ice-cream:

(*a*) 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.

(*b*) 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.

(*c*) Fresh milk should be used in its manufacture.

(*d*) 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.

(*e*) 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.

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

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

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.

(*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.

(*c*) Should not produce acid and gas in Bile-Salt Glucose Broth with a less quantity than 0·1 cubic centimetre.

(*d*) Should not contain the *Bacillus enteritidis sporogenes* in less than 10 cubic centimetres.

(*e*) Should not contain streptococci in less than ·001 cubic centimetre of ice-cream.

The isolated organisms.

(6) Two hundred and fifty-eight *glucose fermenters* were isolated and studied.

(*a*) 66 of these were recognised as follows:

One or other variety of *Bacillus coli* was recognised 47 times.

The *Bacillus oxytocus perniciosus*, or the *Bacillus rhinoscleromatis* was recognised ten times.

The *Bacillus acidi lactici* was identified four times.

The *Bacillus cloacae* was isolated twice.

The *Bacillus lactis aerogenes* was identified twice.

The *Bacillus pneumoniae* (Friedländer) was isolated once.

(*b*) It was possible to arrange the remaining 192 organisms in 74 different groups.

The work in this paper was done while I acted as Assistant Medical Officer of Health to the City of Birmingham, and I have to thank Dr John Robertson, who suggested the subject to me, for his help and guidance during the inquiry and for permission to publish this paper.

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

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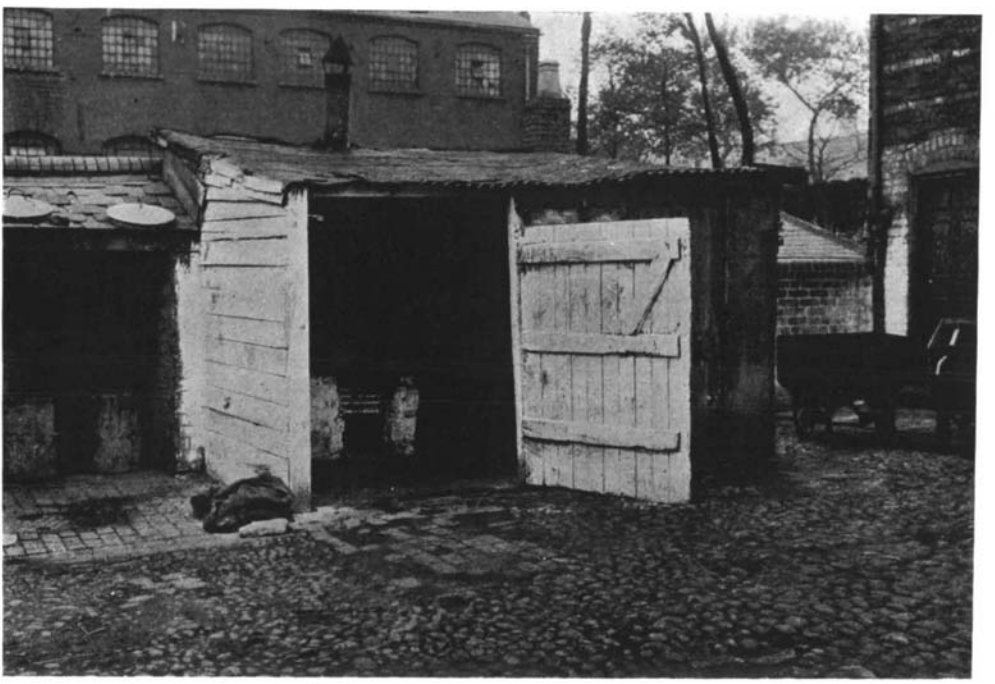
PLATE VII.

Photograph. A common type of premises. Classified as "dirty" and used for the preparation of ice-cream by eight manufacturers. Note the roof, ill-fitting boards, unpaved floor, roughly paved yard and uncovered dustbins close by.



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