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The source of bacteria in fresh cream, and the methylene blue reduction test as a guide to hygienic quality

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Attention has been focused recently on fresh cream (Colenso, Court & Henderson, 1966; Gerken, Coleman & Winner, 1968; Barrow, Miller, Johnson & Hingston, 1968; Hutchison, Barrow, Henderson & Wright, 1968) and it has been shown that a large proportion of samples examined had high bacterial counts (more than 100,000 bacteria/ml.); many contained coliform bacteria and some contained so-called faecal coliform strains. Since most of the samples had been pasteurized or manufactured from pasteurized milk, it has been assumed that the bacterial content of these creams is largely due to contamination after pasteurization. It has also been shown in these investigations that the methylene blue reduction test served as a reasonably reliable guide to the hygienic quality of fresh cream despite occasional anomalous results. The purpose of this investigation was first to examine a number of creams, identify as many as possible of the bacterial strains present and arrive at some conclusion about their source of origin. Next it was hoped to compare the results of the methylene blue test with the bacteria in the creams and study any anomalous results that might occur.

MATERIALS AND METHODS

One hundred and twenty-nine samples of fresh cream from sources in Worcestershire were brought to the laboratory by Public Health Inspectors or the County Council Milk Sampling Officer. The samples had either been heat-treated as cream or, if not, had been manufactured from heat-treated milk and were usually submitted within 2 hr. of purchase. They were examined as soon after arrival in the laboratory as possible. Viable counts were carried out by the pour-plate method using decimal dilutions of cream in $\frac{1}{4}$-strength Ringer's solution. Nutrient agar and McConkey agar incubated aerobically at 37°C. were used for the total bacterial count and coliform count. McConkey agar plates were incubated in a water-tight brass canister (Burman 1967; Barrow & Miller, 1967) immersed in a water bath at 44°C. for the so-called faecal coliform count. Plates were examined after 24 and 48 hr. incubation. Where coliform bacteria and especially *Escherichia coli* were suspected confirmatory biochemical tests (indole production test, methyl-red test and citrate utilization) were carried out. Also a loopful of undiluted cream was spread on a blood agar plate and the plate examined after 24 and 48 hr. incubation. Colonies were picked on to other blood agar plates to obtain pure cultures. Gram-staining, motility, oxidase production, catalase production and glucose fermenta-

tion/oxidation were then tested for in all strains isolated. These tests are those of the first-stage examination (Cowan & Steel, 1966). Second-stage tests (Cowan & Steel, 1966) were then used for full identification, which was possible with most of the strains examined.

For the differentiation of the coli-aerogenes type of bacteria the method recommended and described by the coli-aerogenes Sub-Committee of the Society of Applied Bacteriology (Report, 1956) was adopted. This differentiation differs from that used by Cowan & Steel (1966) in that Cowan and Steel label motile members of the genus *Klebsiella* as *Enterobacter*. A number of coli-aerogenes type bacteria remained unidentified. Some of these would have been classified as irregular types if the old nomenclature of Wilson *et al.* (1935) had been adhered to. Some difficulty was experienced in identifying saprophytes.

The methylene blue reduction test was carried out after the manner of the P.H.L.S. working party (Report, 1958), and the volumetric method used as follows. To 1 ml. of methylene blue solution prepared as for the examination of milk (The Milk (Special Designation) Regulations 1963) and 7 ml. of $\frac{1}{4}$-strength Ringer's solution in a reductase tube, cream was added to the 10 ml. mark with a wide-tipped pipette. A sterile rubber bung was then inserted, the tube was inverted once and was incubated for 17 hr. in a water bath at $20 \pm 0{\cdot}5°$C. If the mixture was still blue after this time the tube was incubated at $37 \pm 0{\cdot}5°$C. for a further 4 hr. Every half hour the tube was removed, inverted and replaced for further incubation if the blue colour had not disappeared.

RESULTS

Table 1 shows the Gram-negative bacteria isolated from the 129 samples of fresh cream, and Table 2 the Gram-positive bacteria. The remainder of the tables deal with the results of the methylene blue reduction test. Table 3 shows how the creams fell into the four grades and tables 4, 5 and 6 show the bacteria present in the samples in the various grades. Generally there was a relation between total counts of bacteria and the results of the dye-reduction test. Those creams reducing the dye during overnight incubation at $20 \pm 0{\cdot}5°$C. or this plus a short period (less than 4 hr.) at $37 + 0{\cdot}5°$C. generally had high counts; thus, of 71 creams reducing the dye overnight 53 had counts of over 100,000 bacteria/ml. (Table 6)* and of 21 creams reducing the dye in $\frac{1}{2}$–4 hr. at $37 \pm 0{\cdot}5°$C. six had counts of over 100,000 bacteria/ml. (Table 5). On the other hand, of 37 creams not reducing the dye after 4 hr. incubation at $37 \pm 0{\cdot}5°$C. only one sample had a count of over 100,000 bacteria/ml. (Table 4). There were some anomalies which will be discussed later.

* Although the upper limit of 100,000 bacteria/ml. was selected for constructing the tables, counting of bacteria was actually taken to 4 millions. Of the 53 creams in table 6 with counts of over 100,000 bacteria/ml., 45 had counts of more than 4 million bacteria/ml., and of the 6 in table 5, three had counts of more than 4 million bacteria/ml.

DISCUSSION

The origin of the bacteria isolated from the creams

Though the cream had either been pasteurized as cream or had been made from pasteurized milk many bacteria were found in the samples examined; it is therefore difficult to believe that the conditions of manufacture were hygienic. If the creams had been prepared from untreated milks the presence of most of the Gram-negative bacteria could have been explained. Most of these can usually be found in milk that has been collected in an unhygienic manner and are believed to come from the outside of the udder, the interior of the milk vessels and from dust in the milking parlour. These bacteria, however, are usually killed during pasteurization.

Table 1. *Gram-negative organisms from* 129 *samples of fresh cream*

Escherichia	
E. coli I	7
E. coli II	11
E. coli III	1
Citrobacter	
Cit. freundii I	24
Cit. freundii II	5
Klebsiella	
K. aerogenes I	22
K. aerogenes II	3
K. cloacae	12
Unidentified *Klebsiella* spp.	9
Hafnia	
Hafnia alvei	1
Alkalescens dispar group	
Alkalescens dispar	1
Unidentified coliform spp.	16
Aeromonas	
A. formicans	11
A. liquefaciens	5
Unidentified	4
Neisseria	
N. catarrhalis	9
N. pharyngis	2
N. flavescens	2
Unidentified	1
Acinetobacter	
Unidentified spp.	2
Chromobacterium	
Ch. violaceum	1
Pseudomonas	
Unidentified spp.	5
Alkaligenes	
Alkaligenes facecalis	1

Table 2. *Gram-positive organisms isolated from* 129 *samples of fresh cream*

Bacillus	
B. cereus	7
B. megaterium	5
B. subtilis	4
B. licheniformis	3
B. brevis	2
B. badius	2
B. pantothenticus	1
B. pulvifaciens	1
B. firmus	1
B. macerans	1
B. pumilans	1
B. coagulans	1
Unidentified	7
Streptococcus	
Strep. mitis	7
Strep. durans	2
Strep. bovis	4
Strep. dysgalactiae	2
Strep. faecalis	1
Unidentified streptococci	1
Aerococcus	
A. viridans	3
Corynebacterium	
Corynebacterium spp.	1
Micrococcus	
Various spp.	12
Staphylococcus	
Various spp. (non coagulase producing)	20
Staph. aureus	1

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Gram-negative bacilli

When bacilli of this description, e.g. coliforms or those of the genera *Aeromonas*, *Acinetobacter*, *Chromobacter* and *Pseudomonas*, are found in cream manufactured from pasteurized milk or cream that has itself been pasteurized, the conclusion

Table 3. *Decolorization of methylene blue by* 129 *samples of fresh cream*

Time taken to decolorize methylene *blue (hr.)*	Number of *samples examined*	*Grade*	Number of samples with: Coliform *organisms in 0·1 ml.*	Number of samples with: *E. coli in 0·1 ml.*	Number of samples with plate count of (thousands per ml.): *0–1*	*>1–100*	*>100*
0	71 (55)	IV	50 (39)	7 (5)	3 (2)	15 (12)	53 (41)
½–2	13 (10)	III	9 (7)	1	1	7 (5)	5 (4)
2½–4	8 (6)	II	2	0	4 (3)	3 (2)	1
>4	37 (29)	I	4 (3)	0	31 (24)	5 (4)	1
Totals	129		65 (50)	8 (6)	39 (30)	30 (23)	60 (47)

Figures in parentheses are percentages of the total samples.

Table 4. *Bacteria in* 37 *fresh creams*

(These creams did not decolorize methylene blue in 4 h. at 37°C. after overnight incubation at 20 ± 0·5°C., i.e. 'passed' the test and were classed as Grade I creams.)

Bacterial count 0–1000 bacteria/ml.	Bacterial count 1001–100,000 bacteria/ml.	Bacterial count >100,000 bacteria/ml.
No. of samples = 31	No. of samples = 5	No. of samples = 1
B. brevis	*B. subtilis*	*B. badius*
B. megaterium	*B. licheniformis*	*Micrococcus citreus*
B. pumilis	*B. megaterium*	*Klebsiella* spp.
B. subtilis (2)	*Staph. epidermidis* (3)	
Bacillus spp. (5)	*Micrococcus* spp.	
Staph. epidermidis (2)	*Strep. bovis*	
Strep. durans	*Strep. mitis*	
Strep. bovis	*N. pharyngis*	
Aerococcus viridans	*Pseudomonas* spp.	
Mould	Coliform spp.	
M. catarrhalis	*Cit. freundii I*	
Aeromonas formicans	*Aeromonas* spp.	
Aeromonas liquifaciens		
E. coli II		
Cit. freundii I		
K. aerogenes		
Eighteen of the 31 samples did not yield growth of any bacteria.		

must be that they have been introduced after pasteurization is complete, e.g. during the handling that accompanies 'ageing', i.e. that part of the processing of cream when it is stored for at least 24 hr. at or below 40°F. to increase the viscosity, or during the filling of the cartons or bottles, and must have been derived from the lids of churns, the containers, cartons, bottles, table tops, cloths or the hands or persons of the workers.

The presence of *E. coli I* deserves special consideration. The habitat of this bacterium is the gut of humans or animals and while it is true that an accumulation of subsequent generations of *E. coli I* can be found in pipes, machinery and in or

Table 5. *Bacteria in* 21 *samples of fresh cream*

(The samples decolorized methylene blue in ½–4 hr. at 37°C. after overnight incubation at 20 ± 0·5°C., i.e. were in the intermediate position between 'failing' and 'passing' the test, and were classed as Grades II and III or 'fairly satisfactory'.)

Time taken to decolorize methylene blue (hr.)	Bacterial count 0–1000 bacteria/ml.	Bacterial count 1001–100,000 bacteria/ml.	Bacterial count > 100,000 bacteria/ml.
		Grade III	
	No. of samples = 1	No. of samples = 7	No. of samples = 5
½–2	Coliform spp.	*B. cereus* (2) *B. licheniformis* *Staph. epidermidis* (3) *E. coli I* Coliform spp. (4) *K. aerogenes I* *K. cloacae* *Aeromonas formicans* (2) *Klebsiella* spp. *Acinetobacter*	*B. megaterium* *B. pulvifaciens* *B. pantothenticus* *Staph. citreus* *Micrococcus* spp. *Strep. faecalis* *Strep. bovis* (2) *Strep. dysgalactiae* *N. catarrhalis* *E. coli II* *Cit. freundii I* *K. cloacae* *Aeromonas* spp.
		Grade II	
	No. of samples = 4	No. of samples = 3	No. of samples = 1
2½–4	*B. megaterium* *Cit. freundii I* *Klebsiella* spp. *Aeromonas* spp.	*Staph. epidermidis* *Micrococcus* spp. Coliform spp. (2) *Pseudomonas* spp. *K. ozaenae* *Aeromonas formicans*	*B. licheniformis* *Alkaligenes faecalis*

on utensils so that such contamination can hardly be said to be due to direct excretal contamination, there is no doubt at all that the presence of this bacterium in a dairy or cream manufacturing plant must have been due to excretal contamination in the first instance. Unfortunately in a number of dairies, especially those in rural areas, there is much manual filling of containers. After 'ageing', the cream is carried in vessels to a table where it is poured from a jug into cartons or bottles which are capped by hand. In some dairies those who perform these tasks are unskilled and untrained; their knowledge and understanding of hygiene is poor. Occasionally the filling room adjoins the yard; farm workers, farm vehicles and even animals pass close by; where this is so it is possible, indeed likely, that contamination with *E. coli I* is of direct excretal origin.

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Gram-negative cocci

These consist of *Neisseria catarrhalis*, *N. pharyngis*, *N. flavescens* and one unidentified *Neisseria*. Usually one would label these bacteria as part of the flora

Table 6. *Bacteria in* 71 *samples of fresh cream*

(These samples decolorized methylene blue after overnight incubation at 20 ± 0·5°C., i.e. 'failed' the test and were therefore classified as Grade IV creams.)

Bacterial count 0–1000 bacterial/ml.	Bacterial count 1001–100,000 bacteria/ml.	Bacterial count >100,000 bacteria/ml.	
No. of samples = 3	No. of samples = 15	No. of samples = 53	
B. badius	*B. cereus*	*B. coagulans*	*Cit. freundii I* (13)
Micrococcus	*B. pumilis*	*B. megaterium*	*Cit. freundii II* (7)
Cit. freundii I	*B. macerans*	*B. subtilis*	*E. coli III*
K. cloacae	*Strep. bovis*	*B. cereus* (4)	Coliform spp. (12)
	A. viridans	*B. brevis*	*K. aerogenes I* (12)
	S. epidermidis (2)	*Bacillus* spp. (3)	*K. aerogenes II* (3)
	Micrococcus spp.	*S. epidermidis* (18)	*Hafnia*
	N. catarrhalis	*S. aureus*	*K. cloacae* (7)
	Cit. freundii I	*Micrococcus* spp. (11)	*K. ozaenae*
	K. aerogenes I	*Strep. mitis*	*K. pneumoniae* (2)
	K. aerogenes II	*Strep. durans*	*Klebsiella* spp.
	E. coli II	*Strep. dysgalactiae*	*Alkalescens dispar*
	K. cloacae (3)	*Strep.* species	*Pseudomonas* spp.
	Citrobacter spp. (4)	*A. viridans*	*A. formicans* (5)
	Coliform spp. (2)	*N. catarrhalis* (5)	*A. liquifaciens* (4)
	A. formicans	*N. pharyngis*	*Aeromonas* spp.
	Klebsiella spp. (unidentified)	*N. flavescens*	
		Neisseria spp. (2)	
		Corynebacterium	
		Acinetobacter	
		Ch. violaceum	
		E. coli I (7)	
		E. coli II (7)	

of the naso-pharyngeal passages. They have also been isolated rarely from the conjunctiva of animals. According to Wilson & Miles (1964) 'The habitat of those Gram-negative cocci that have been adequately described, with the exception of *N. gonorrhoeae* and *N. meningitidis*, is almost exclusively the nasopharynx of healthy and diseased persons and animals'. According to Breed, Murray & Smith (1957) 'All known species are parasitic'. While one would hesitate to use *Neisseria* as an absolute indicator of contamination from human or animal respiratory sources it is difficult to avoid the inference that these bacteria were present in the creams as a result of contamination by saliva and droplets from those engaged in manual filling of the containers in the course of talking, coughing and throat clearing.

Cream of course is not the only food that is liable to contamination during preparation and packing but it is a highly nutrient fluid for bacteria, often prepared and packaged under poor conditions and consumed unheated.

Gram-positive bacteria

These included a large number of the bacillus species (aerobic spore-bearers). These, of course, might have been present in the milk and survived pasteurization. They might also have been derived from the dust and air of the dairy and therefore while harmless in themselves give some indication of the degree of cleanliness of the premises.

Anomalous results

Among the 129 creams subjected to the methylene blue test were seven (5·4 %) that gave anomalous results. Three creams that did not yield a growth of bacteria at 37 ± 0·5° C. decolorized methylene blue in 2, 3 and 3½ hr. at 37 ± 0·5° C. respectively. In addition to these, three creams 'failed' the test by decolorizing methylene blue after overnight incubation at 20 ± 0·5° C. but the bacterial count in each was low. The type of organism isolated did not suggest any reason for failure. In one cream it was *Klebsiella cloacae* (300 bacteria/ml.), in another the bacteria isolated were *Bacillus badius* and *Aerococcus viridans* (700 bacteria/ml.) and in the third *Citrobacter freundii I* and *Micrococcus* spp. (100 bacteria/ml.). The type of bacteria present and the low counts would usually entitle these samples to be considered as satisfactory, yet they 'failed' the test. The seventh sample to yield an anomalous result did not decolorize methylene blue in 4 hr. at 37° C. yet *B. badius* and *Micrococcus citreus* were present in a count of more than 4 million bacteria/ml.

It was disappointing to find that the methylene blue test did not invariably pick out those creams which contained bacteria suspected of being of human or of animal origin. Thus five creams 'passed' the test although they contained bacterial species such as *Streptococcus bovis*, *Strep. durans*, *E. coli II*, *Cit. freundii I* and *K. aerogenes I*, all strains of possible intestinal origin. One cream graded as 'fairly satisfactory' yielded *Cit. freundii I*.

Value of the methylene blue test

There were seven (5·4 %) anomalous results to the methylene blue reduction test. This is a fairly high percentage and alone could possibly exclude the use of the dye test as a statutory test for fresh cream. The test also failed to identify creams that contained bacteria of possible human or animal origin. Nevertheless it served very well as a guide to the numbers of bacteria in cream and it follows therefore that it could serve as a guide to its keeping quality. It seems that it could be turned to good practical use in this respect especially if samples were examined regularly. A series of 'failures' by the test would justify examination of premises, equipment and methods to discover the sources of contamination.

SUMMARY

One hundred and twenty-nine samples of fresh cream collected in Worcestershire were examined bacteriologically. Sixty (46·5 %) creams had counts of over 100,000 bacteria/ml. The bacteria present were of many varieties, the commonest being *Bacillus* spp. (aerobic spore formers), Gram-negative bacilli, staphylococci

and micrococci. Since most of the creams had been either pasteurized as cream or manufactured from pasteurized milk it was thought that the many bacteria were present because of contamination after pasteurization due to three main causes; unsatisfactory or unhygienic premises, unsuitable equipment, manual handling during the filling and capping process.

The methylene blue test results related well with bacterial counts but there were seven (5·4 %) anomalous results. Although the methylene blue reduction test therefore could serve as a simple and reasonable guide to the hygienic quality of fresh cream, 5·4 % of anomalous results would perhaps make it unsuitable as a statutory test.

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REFERENCES

BARROW, G. I. & MILLER, D. C. (1967). A bacteriological study of fresh cream and cream products in Cornwall. *Mon. Bull. Minist. Hlth* **26**, 254.

BARROW, G. I., MILLER, D. C., JOHNSON, D. L. & HINGSTON, C. W. J. (1968). *Brucella abortus* in fresh cream and cream products. *Br. med. J.* ii, 596.

BREED, R. S., MURRAY, E. G. D. & SMITH, N. R. (1957). *Bergey's Manual of Determinative Bacteriology*, 7th ed. London: Baillière, Tindall & Cox.

BURMAN, N. P. (1967). Development of membrane filter techniques. II. Adaptation to routine and special requirements. *Proc. Soc. Wat. Treat. Exam.* **16**, 40.

COLENSO, R., COURT, G. & HENDERSON, R. J. (1966), Fresh cream in Worcestershire: A bacteriological study. *Mon. Bull. Minist. Hlth* **25**, 153.

COWAN S. T. & STEEL, K. J. (1966). *Manual for the Identification of Medical Bacteria*. Cambridge University Press.

GERKEN, A., COLEMAN, J. C. & WINNER, H. I. (1968). Bacteriological impurity of dairy-cream samples in London. *Lancet* i, 634.

HUTCHISON, J. G. P., BARROW, G. I., HENDERSON, R. J. & WRIGHT, A. E. (1968). The bacteriological quality of dairy creams. *Lancet* ii, 99.

Milk (Special Designation) Regulations, 1963. Statutory Instrument 1963, No. 1571. London: H.M.S.O.

REPORT (1956). The nomenclature of coli-aerogenes bacteria. Report of the Coli-Aerogenes Sub-Committee of the Society for Applied Bacteriology. *J. appl. Bact.* **19**, 108.

REPORT (1958). The bacteriological examination and grading of fresh cream. Report of a working party of the Public Health Laboratory Service. *Mon. Bull. Minist. Hlth* **17**, 77.

WILSON, G. S. & MILES, A. A. (1964). *Topley and Wilson's Principles of Bacteriology and Immunity*. 5th ed. London: Edward Arnold.

WILSON, G. S., TWIGG, R. S., WRIGHT, R. D., HENDREY, C. G., COLWELL, M. P. & MAIER, I. (1935). The bacteriological grading of milk. *Spec. Rep. Ser. med. Res. Coun.* No. 206.