THE CONTROL OF INFECTION SPREAD BY SYNTHETIC CREAM

By BETTY C. HOBBS and MURIEL E. SMITH

The Food Hygiene Laboratory, Central Public Health Laboratory, Colindale, N.W.9

INTRODUCTION

Since October 1940, when the sale of natural cream was forbidden, synthetic cream has been proved or suspected to be responsible for nearly every major outbreak of paratyphoid fever in England and Wales, as well as for numerous outbreaks of food poisoning caused by staphylococci or organisms of the salmonella group.

At present there is no accepted definition of synthetic cream, but in this article we shall consider it to be an emulsion of vegetable oils or fat with water, with or without the addition of other substances whether dairy products or not. For our experimental work a few creams were made up with butter fat, and we shall refer to them for convenience of comparison as 'synthetic' creams, although it would be justifiable to regard them as 'artificial' (Food and Drugs (Milk, Dairies and Artificial Cream) Act, 1950).

Baker's filling, a semi-solid substance of butter, or margarine, and sugar, which does not support the growth of micro-organisms because of its high sugar and fat content, is outside the scope of this paper.

There are no bacteriological standards laid down for synthetic creams, nor is there any standard method for their examination. The synthetic creams produced by large manufacturers are so treated that, in general, it may be assumed they are safe at the time of leaving the place of manufacture, in so far as they are free from organisms likely to cause food poisoning or food-borne infection. However, their treatment thereafter gives no assurance of their continued safety, for in the bakeries they are exposed to numerous sources of contamination.

A code of practice which insisted on measures such as heat treatment during preparation, sterile sealed containers for transport and storage at temperatures not exceeding 45° F. would reduce the risks of contamination, but it would not necessarily protect the cream during manipulation in the bakery. Even the war-time restriction of ingredients to those which produced a synthetic cream which was unable to support the growth of micro-organisms would be ineffectual once the cream came into contact with confectionery from which essential growth factors were absorbed (Thomson, 1953). The suggestion that all types of synthetic cream should be protected by the addition of a harmless substance able to inhibit the growth of pathogenic bacteria likely to cause food poisoning would not be readily acceptable, and yet it seems to be the only solution to the difficulty. The practical implications of this suggestion are considered in the following work.

PRELIMINARY OBSERVATIONS

Observations on the 'sterility' of certain makes of synthetic cream were begun in 1949, when routine tests on samples of synthetic cream 'A' gave results which indicated that little or no growth occurred in this product. Furthermore, when coliform bacilli or staphylococci were added to it there was no evidence of multiplication, and it was impossible to recover the original inoculum after 2–24 hr.

The routine examination of other makes of synthetic cream showed that there was a considerable variation in the numbers and types of organisms found. Some creams encouraged the rapid growth of micro-organisms, while others inhibited or failed completely to support bacterial growth. There seemed to be two possible explanations for this phenomenon: either there was a nutritional deficiency in the cream which discouraged growth or there was some inhibitory agent present. Both possibilities were investigated.

The makers of cream 'A' supplied cream of two consistencies: *liquid*, the constituents of which were said to be palm-kernel oil, ground-nut oil, methyl cellulose (vegetable colloid), soluble saccharin and water; and *solid*, composed of powdered egg yolk, hardened sweet coconut fat, sugar, margarine, maltose and sweetened ground-nut fat.

From 1950 to 1952, sixty samples of the liquid cream 'A' were examined which had been collected at the depot by sanitary inspectors, from pipelines, from churns and also from containers ready for retail sale. Organisms were not seen in direct films, while plate counts at 37° C. were less than 10, 100 or 500 colonies per ml., according to the method used for counting. Coliform bacilli were not found in 0·1 ml. Direct cultures were sterile, but from enrichment cultures aerobic sporing bacilli were sometimes isolated and Gram-negative coccobacilli, probably members of the *Achromobacterium* group, were occasionally found.

Each ingredient of the liquid cream was tested for its ability to support the growth of the test cultures.

Dilutions of methyl cellulose were made in both distilled water and broth at 0·5, 5 and 10% concentrations; each dilution was inoculated with approximately 500,000 per ml. of *Bacterium coli*. Counts were made immediately and after 24 hr. at 37° C. The results showed that the vegetable colloid neither encouraged nor inhibited the growth of *Bact. coli*.

Ten per cent suspensions of fats and soluble saccharin were tested. The fats, but not the saccharin, allowed the survival of the test organisms. Nevertheless, saccharin in the finished product was unlikely to be responsible for the bactericidal effect, and it was more probable that the particular mixture of ingredients lacked essential growth factors.

The solid cream, containing egg yolk and margarine amongst its ingredients, when diluted 50% encouraged the growth of the test organisms, coliform bacilli and staphylococci. Likewise other samples of synthetic cream also containing powdered egg as well as butter, sugar, arrowroot, gum and water, which were submitted from another firm, gave high counts and readily supported bacterial growth. The results of bacteriological examination of samples of synthetic cream

Table 1. *Results of bacteriological examination of various samples of synthetic cream (commercial).* *Received May–August* 1952

Source (producer)	Direct microscopic smear	Colony count per ml. Yeastrel milk agar at 37° C.	Colony count per ml. Miles & Misra (surface)	Coliform bacilli	Predominant organisms (direct and enrichment cultures)
A	No organisms seen	<10	<500	Absent in 0·1 ml.	Sterile
B	No organisms seen	—	48,000	Present in 0·1 ml. non-faecal	Coliform bacilli
B	A few Gram-positive cocci	<10	<500	Absent in 0·1 ml.	Sterile
C	Not done	$>10\times10^6$	24×10^6	Present in 0·01 ml. non-faecal Present in 0·1 ml. faecal	Coliform bacilli
D	Numerous Gram-negative bacilli	140,000	$1{\cdot}7\times10^6$	Present in 0·01 ml. non-faecal	Coliform bacilli
E	Occasional Gram-positive bacilli	1,200	7,500	Absent in 0·1 ml.	Organisms of the *Chromobacterium* group
F	No organisms seen	360	<500	Absent in 0·1 ml.	Sterile
G	Occasional Gram-positive bacilli	1,900	2,200	Absent in 0·1 ml.	Organisms of the *Achromobacterium* group
G	Occasional Gram-positive bacilli	<100	<500	Absent in 0·1 ml.	*Staphylococcus albus* only
H	No organisms seen	<100	<500	Absent in 0·1 ml.	Sterile
I	Numerous Gram-negative bacilli	$6{\cdot}7\times10^6$	$3{\cdot}8\times10^6$	Present in 0·01 ml. non-faecal	Coliform bacilli
J	No organisms seen	390,000	400,000	Absent in 0·1 ml.	Organisms of the *Achromobacterium* group

from ten different manufacturers is shown in Table 1. The samples were sent to us by post, together with a completed form indicating briefly the distribution, process of manufacture and ingredients of the creams. The counts at 37° C. ranged from less than 10 to more than 10 million colonies per gram.

The growth of Salmonella paratyphi B *in manufactured synthetic cream*

The creams 'A' and 'B', samples of which showed counts of less than 500 colonies per ml., were inoculated with *Salmonella paratyphi B* and incubated at 30° C. for 7 days. Both samples of cream were then reinoculated with *Salm. paratyphi B* and incubated for a further 7 days. The results are given in Table 2. Cream 'A' became sterile after 1 day and remained so in both experiments. In the first half of the experiment no paratyphoid bacilli could be isolated from cream 'B' after the first day, but a contaminant, which was probably an organism of the *Pseudomonas* group, increased steadily up to 7 days. In the second part of the experiment both the paratyphoid bacilli and the pseudomonas were recoverable by direct culture after 5 days. After 7 days the pseudomonas persisted, but the paratyphoid bacilli were found only after liquid enrichment. These results indicated that an inoculum of approximately 280,000–440,000 paratyphoid bacilli would not survive in cream 'A', and that in cream 'B' the organisms would slowly die out over a period of days.

Table 2. *Results of surface plate counts on samples of cream 'A' and cream 'B' inoculated with* Salmonella paratyphi B *and incubated at* 30° C.

		Colony count per ml.	
Date	Time	Cream 'A'	Cream 'B'
29. viii. 52	0 hr.	440,000	360,000
	1 day	< 500	180,000 (contaminants)
	3 days	< 500	$6{\cdot}3 \times 10^6$ (contaminants)
	5 days	< 500	$3{\cdot}7 \times 10^6$ (contaminants)
	7 days	< 500	$4{\cdot}5 \times 10^6$ (contaminants)
Reinoculated	0 hr.	350,000	280,000
5. ix. 52	1 day	< 500	180,000 (*Salm. paratyphi B*)
			380,000 (contaminants)
	3 days	< 500	25,000 (*Salm. paratyphi B*)
			$4{\cdot}5 \times 10^6$ (contaminants)
	5 days	< 500	750 (*Salm. paratyphi B*)
			6×10^6 (contaminants)
	7 days	< 500	160,000 (contaminants)*

* *Salm. paratyphi B* was isolated through selenite.

The striking difference between the two creams with regard to the growth of contaminants may also be observed. From these and other results it would seem that organisms which are able to break down fat by means of the enzyme lipase metabolize the constituents of cream containing neither margarine nor butter, nor other protein material and multiply readily, but that organisms such as coagulase-positive staphylococci, *Salm. paratyphi B*, and *Bact. coli*, which are not lipolytic, cannot survive in creams made from fat other than margarine or butter and without added protein material.

To extend these observations it was decided to prepare creams of varying composition in the laboratory and to test their ability to encourage the growth of pure cultures of bacteria; similar experiments were carried out by Baillie & Freeman (1952). At the same time a method for controlling the growth of potentially pathogenic bacteria was also investigated.

METHODS

The ingredients of the synthetic creams made in the laboratory were warmed to 44° C., passed through a small domestic cream machine and pasteurized at 60° C. for ½ hr. The recipe used was based on that for cream 'A' and consisted of 30 g. fat, 20 g. vegetable colloid (methyl cellulose), 0·2 g. salt and 2·5 g. sugar, made up to 100 ml. with water.

The 'sweetfat' used in some experiments was obtained commercially, and it was said to contain approximately 5 % sugar. Various kinds of fat were used in different tests, and to encourage bacterial growth 6 ml. milk and 1 ml. egg yolk were added per 100 ml. of cream in later experiments.

The test organisms used for growth curves were washed from overnight agar slopes with quarter-strength Ringer solution, and standardized to the required density by means of opacity tubes. After inoculation the creams were well shaken.

Colony counts on routine samples of synthetic cream submitted for examination were obtained from yeastrel milk-agar pour plates incubated for 2 days at 37° C. and also by surface counts on MacConkey agar, nutrient agar, or blood agar, depending on the test organism, using the Miles & Misra technique (1938).

Growth of staphylococci and Bacterium coli *in laboratory-made synthetic cream*

Table 3 shows the results of counts on batches of synthetic cream made up in the laboratory with different fats and either with or without milk, sugar and other ingredients. The concentration of fat was varied in some experiments from 15 to 60 % (w/v), but there was no corresponding variation in the counts; it was therefore decided to use a standard concentration of 30 % fat. The temperature of incubation varied, and in different experiments either 37, 30 or 20° C. were used. Counts were made at 0, 18–24 hr., and usually at 42 hr.

In all the creams made with 'sweetfat' and in most of those made with retail cooking fat, with and without sugar, and without the addition of protein material the colony count was reduced to less than 500 per ml. of cream in 18–24 hr. When 1 % (w/v) peptone or 6 % (v/v) milk was added to the cream growth occurred.

Creams made with margarine encouraged growth, particularly of *Bact. coli*, and when milk and sugar were added the growth was further enhanced. When butter was used instead of margarine the counts were still higher, particularly in the presence of milk and sugar. The addition of 1 % egg yolk to cream made with butter provided the most nutritive medium of all. Similar results were obtained when *Salm. paratyphi B* was added to the various types of laboratory synthetic cream.

Table 3. *Growth of staphylococci and* Bacterium coli *in synthetic cream*

Incubation temperature (°C.)	Variation in ingredients	No. of experiments	Average colony count per ml. of inoculated creams: Staphylococci 0 hr.	Staphylococci 18–24 hr.	Staphylococci 42 hr.	*Bacterium coli* 0 hr.	*Bacterium coli* 18–24 hr.	*Bacterium coli* 42 hr.
37	Sweetfat	7	240,000	< 500	—	200,000	< 500	—
37	Cooking fat, no sugar	3	130,000	< 500	< 500	220,000	56,000	8,000
37	Cooking fat, no sugar	1	180,000	2×10^6	580,000	300,000	1×10^6	410,000
37	Cooking fat plus sugar 2·5 % (w/v)	4	220,000	< 500	< 500	220,000	2,000	< 500
37	Cooking fat plus sugar 2·5 % (w/v)	2	420,000	380,000	1,000	240,000	$1{\cdot}5 \times 10^6$	2×10^6
37	Sweetfat plus 1 % peptone (w/v)		280,000	63×10^6	—	200,000	63×10^6	—
37	Sweetfat plus 6 % milk (v/v)	4	200,000	20×10^6	—	190,000	32×10^6	—
37	Margarine without milk or sugar	5	170,000	$1{\cdot}5 \times 10^6$	$2{\cdot}5 \times 10^6$	170,000	22×10^6	20×10^6
37	Margarine with milk and 2·5 % sugar	1	140,000	10×10^6	30×10^6	180,000	86×10^6	100×10^6
30	Margarine with 6 % milk and 2·5 % sugar	5	290,000	6×10^6	13×10^6	300,000	88×10^6	97×10^6
20	Margarine with 6 % milk and 2·5 % sugar	2	220,000	780,000	2×10^6	230,000	$3{\cdot}5 \times 10^6$	76×10^6
37	Butter without milk or sugar	4	230,000	29×10^6	20×10^6	210,000	41×10^6	24×10^6
30	Butter with 6 % milk and 2·5 % sugar	5	300,000	26×10^6	31×10^6	210,000	94×10^6	76×10^6
30	Butter with 6 % milk, 2·5 % sugar and 1 % egg yolk (v/v)	6	340,000	110×10^6	100×10^6	290,000	200×10^6	190×10^6

Base used in all creams: colloid 20 g., salt 0·2 g., fat 15–60 g., generally 30 g. made up to 100 ml. with water. —= Not done.

Further experiments with synthetic cream

A few experiments were made to find out if possible whether *Bact. coli* and staphylococci failed to grow in cooking fat cream, either because the cream lacked an essential growth factor or because an unknown bactericidal substance was present.

Various substances were added to synthetic cream composed of cooking fat, colloid, salt and sucrose to find out whether they would encourage the growth of the test organisms at 30° C.

Gelatin, milk-casein hydrolysate, egg yolk and soyabean lecithin were found to encourage growth, whereas sources of nitrogen such as ammonium sulphate, urea and glutamine did not. 0·2% charcoal improved growth, although it was impossible to say why this was so. The charcoal may have served as a growth factor, as an absorbent for some inhibitory substance, or its action may have been a mechanical one such as breaking up the fat.

The results of comparative counts on samples of each of the ingredients and of the whole mix of cooking-fat synthetic cream inoculated with *Bact. aerogenes* and *Bact. coli* showed that *Bact. aerogenes* could grow in a medium which could not support the growth of *Bact. coli*.

In a further experiment cooking-fat creams inoculated with the test organisms were incubated either at 4, 20 or at 30° C. and the results compared with Ringer solutions inoculated with the same organisms and incubated at the same three temperatures. The results showed that there was a variation in the rate at which the test organisms died out in different batches of cream, and that at +4° C. the survival of the test organisms was slightly greater in Ringer solution than in the batches of cream. These results, together with those on the effect of the addition of charcoal, indicated that the inhibition of the test organism in fat-emulsion synthetic cream could be due to the presence of an inhibitory agent.

The experiments of Chapman & McFarlane (1943) showed that the peroxide value of acetone extracts of dried milk powder increased on storage of the powder in open tins at room temperatures. A similar increase of fat peroxides may possibly occur in fats after prolonged storage. Whether this causes the variation in inhibitory power of certain synthetic creams has not been ascertained.

The observations so far made indicate that the growth of pathogenic organisms such as coagulase-positive staphylococci and *Salm. paratyphi B* in synthetic cream can be partially controlled if the composition of the mix is limited to certain ingredients. Though the reason for the inhibitory nature of such creams of poor nutritional quality has not been conclusively established, it is clear that when protein is added to them the normal growth conditions are restored.

Thomson (1953) has established that inhibitory creams in contact with bread or cake will take up from the flour protein material of nutritional value for bacteria and thereby lose their inhibitory nature and become actively encouraging to bacterial growth.

Further proof of this was provided by recent investigations undertaken by this laboratory into the excessively high bacterial counts, including coagulase-positive

staphylococci, in synthetic cream from buns and cakes on sale to the public. A demonstration given at the large bakery in which they were made showed clearly that, while the manufactured cream was being prepared for the confectionery, the hands of the operative were used continually to assist in mixing the cream, sugar and water.

The cream reached the bakery from the manufacturers in tin containers closed by tightly fitting lids. Examined immediately on arrival the cream showed counts of less than 10,000 colonies per gram of which none were of pathogenic significance. The count did not increase after overnight storage at cool atmospheric temperature. After admixture with sugar and water, when it was much handled, the cream was added to cakes, buns, tarts and pastry, and the finished confectionery was stored overnight in the bakery ready for distribution the following day.

Cream from buns collected from the retail shops 24 h. after preparation showed bacterial counts greater than 100 million organisms per gram, including coagulase-positive staphylococci and faecal coliform bacilli. Thus, however pure the synthetic cream may be when it leaves the manufacturers, there is no guarantee that it will not become contaminated in the bakery. Furthermore, the warmth, moisture and nutriment inside the pastry casing of, for example, a cream bun provide ideal conditions for bacterial multiplication.

Efforts to convince bakery workers of the potential dangers of their practices have not always been successful, and the introduction of a code of practice into a bakery would raise tremendous difficulties for those faced with the responsibility of enforcement.

It was decided, therefore, to test the control of pathogens in synthetic cream of varying ingredients by the addition of a bacteriostatic substance and in particular of an oxidizing agent such as hydrogen peroxide, so that if possible the safety of the substance would be independent of the faults in handling.

Most of the experiments to be described were carried out with laboratory-made cream containing margarine or butter, milk and egg, that is, of a high nutritional quality to ensure maximum growth conditions.

A 20-volume solution of H_2O_2 was kept in a dark bottle at 4° C. and titrated against standard potassium permanganate before each experiment. The concentration is expressed throughout as the percentage of pure H_2O_2 (w/v).

Effect of varying amounts of hydrogen peroxide on the growth of staphylococci, Bacterium coli *and* Salmonella paratyphi B *in laboratory-made synthetic cream*

Varying amounts of H_2O_2 were added, after pasteurization, to synthetic creams made in the laboratory. The samples of cream were inoculated with staphylococci and *Bact. coli* and incubated at 30° C. and sometimes also at 20° C. At these temperatures the peroxide was effective at certain concentrations, whereas when cream with added peroxide was incubated at 37° C. similar concentrations of peroxide failed to inhibit growth.

Experiments showed that a concentration of 0·003 % H_2O_2 inhibited the test organisms in synthetic creams containing cooking fat, colloid, salt, sucrose and

Table 4. *Effect of varying concentrations of* H_2O_2 *on the growth of staphylococci and* Bacterium coli *in synthetic cream made with butter* (30 *g.*), *colloid* (20 *g*), *salt* (0·2 *g.*), *sucrose* (2·5 *g.*), *and milk* (6 *ml.*) *at* 30° C. *with and without egg yolk* (1 *ml.*) *and water to* 100 *ml.*

			Colony count per gram or ml. (average or range)			
			Staphylococci		*Bact. coli*	
No. of experiments	% of H_2O_2	Time (hr.)	Control (without H_2O_2)	Test (with H_2O_2)	Control (without H_2O_2)	Test (with H_2O_2)
1 (no egg)	0·00015	0	230,000	350,000	140,000	160,000
		18–24	$7{\cdot}3 \times 10^6$	$4{\cdot}8 \times 10^6$	50×10^6	55×10^6
		42–48	11×10^6	$9{\cdot}8 \times 10^6$	Not done	Not done
3 (no egg)	0·00042–0·0012	0	580,000	700,000	450,000	530,000
		18–24	11×10^6	39,000	100×10^6	44×10^6
		42–48	20×10^6	77,000	78×10^6	21×10^6
1 (no egg)	0·00154	0	240,000	170,000	210,000	340,000
		18–24	$3{\cdot}5 \times 10^6$	< 500	88×10^6	270,000
		42–48	19×10^6	< 500	150×10^6	290,000
6 (no egg)	0·00211–0·00361	0	190,000–750,000	230,000–1×10^6	240,000–600,000	150,000–750,000
		18–24	$8{\cdot}6 \times 10^6$–28×10^6	< 500–30,000	71×10^6–130×10^6	< 500–51,000
		42–48	11×10^6–58×10^6	< 500–19×10^6	78×10^6–100×10^6	< 500–$8{\cdot}5 \times 10^6$
3 (no egg)	0·00421–0·00542	0	270,000	130,000	120,000	120,000
		18–24	$4{\cdot}6 \times 10^6$	< 500	37×10^6	< 500
		42–48	$8{\cdot}5 \times 10^6$	< 500	37×10^6	< 500
3 (plus egg)	0·003–0·0046	0	350,000	290,000	300,000	290,000
		18–24	110×10^6	37×10^6	220×10^6	200×10^6
		42–48	86×10^6	120×10^6	260×10^6	250×10^6
2 (plus egg)	0·0062–0·0077	0	250,000	260,000	230,000	150,000
		18–24	140×10^6	3,100	180×10^6	330,000
		42–48	9×10^6	< 500	190×10^6	190×10^6
5 (plus egg)	0·0092–0·0154	0	200,000	220,000	300,000	230,000
		18–24	140×10^6	< 500	190×10^6	< 500
		42–48	25×10^6	< 500	150×10^6	< 500

milk. Table 4 shows the results obtained from experiments carried out at 30° C. on margarine or butter creams. They indicate that, in creams without egg yolk, concentrations of H_2O_2 below 0·00154 % failed to inhibit growth completely; between 0·00154 and 0·00361 % there were variable results; and with concentrations above 0·0042 % there was a bactericidal effect.

In order to test the effect of H_2O_2 on organisms growing under the most favourable conditions of nutrition, 1 % egg yolk was added to the ingredients of the cream described in the previous experiment. The results of adding varying dilutions of H_2O_2 to this cream are also shown in Table 4. A concentration of 0·0077 % H_2O_2 was necessary to control the growth of staphylococci, and a higher concentration, approximately 0·01 %, was necessary to control the growth of coliform bacilli.

A few experiments were carried out on the effect of long incubation at 20 and 30° C. on the inhibition by H_2O_2 of staphylococci and *Bact. coli* in margarine cream without egg. The results indicated that, when the dose of H_2O_2 was sufficient to suppress the growth of the test organisms after 1 day, there was no further growth up to a period of 7 days at either 20 or 30° C., though certain contaminants could grow in the cream even in the presence of H_2O_2.

Repeat experiments with cream containing butter, milk and egg showed that in the absence of H_2O_2 there was a rapid multiplication of the test organisms in the first 24 hr. followed by a steady decline in numbers. In the presence of 0·0077 % H_2O_2 the growth of both staphylococci and coliform bacilli was inhibited, but other contaminating organisms multiplied profusely. No contaminants grew when the concentration of H_2O_2 was raised to 0·01082 %.

The effect of H_2O_2 on *Salm. paratyphi B* in cream made with butter, colloid, salt, sucrose and milk, with and without egg yolk, at 30° C. is shown in Table 5. These results show that with 0·003 % H_2O_2 the growth of paratyphoid bacilli was reduced in 1 day, but that the count rose again after 2 days' incubation. A concentration of 0·0123 % H_2O_2 inhibited paratyphoid bacilli after 1 day in cream with added egg yolk.

Table 5. *Effect of varying concentrations of* H_2O_2 *on* Salmonella paratyphi B *in synthetic cream (butter, colloid, salt, sucrose, milk, with and without* 1 % *egg yolk) at* 30° C.

		Colony count per ml.	
% of H_2O_2	Time	Control (without H_2O_2)	Test (with H_2O_2)
0·0015	0 hr.	290,000	230,000
	1 day	140×10^6	19,000
	2 days	98×10^6	160×10^6
0·0030	0 hr.	280,000	250,000
	1 day	200×10^6	< 500
	2 days	160×10^6	550,000
0·0123 and 0·0138 (1 % egg yolk)	0 hr.	68,000	40,000
	1 day	250×10^6	< 500
	2 days	380×10^6	< 500
	3 days	250×10^6	< 500
	5 days	110×10^6	< 500
	7 days	11×10^6	< 500

https://doi.org/10.1017/S0022172400027431 Published online by Cambridge University Press

The results so far indicate that a concentration of 0·003 % H_2O_2 inhibits the multiplication of the three test organisms, staphylococci, *Bact. coli* and *Salm. paratyphi B*, in cooking fat, margarine and butter creams containing milk but no egg. When a 1 % concentration of egg yolk is added to the mixtures approximately three times the concentration of H_2O_2, namely, 0·0077–0·0123 %, is necessary to inhibit the growth of the test organisms.

Effect of H_2O_2 *on samples of synthetic cream from various manufacturers*

Manufactured synthetic creams were used in experiments to test the effectiveness of different concentrations of H_2O_2 in the sterilization of creams already heavily contaminated or in maintaining the sterility of those in which no organisms could be found. For the first three samples examined 0·006, 0·012, 0·018, 0·024 and 0·048 % concentrations of H_2O_2 were used, but for later samples the concentrations of 0·012 and 0·024 % were omitted. Counts were made on the surface of blood-agar plates at 0, 1, 3 and 6, 7 or 8 days. The results shown in Table 6 indicated that in creams with an initial count of less than 500 per ml. the addition of 0·006 % H_2O_2 would maintain sterility for 7 days at 30° C. This was also true for two creams ('E' and 'B') showing initial counts of 7,500 and 48,000 per ml. respectively.

In cream 'D' the initial count of approximately 1·7 million colonies per ml. was reduced to less than 500 per ml. by the addition of 0·018 % H_2O_2. The organisms in cream 'C' which showed an initially high count of 24 million colonies per ml. were not controlled by 0·048 % of H_2O_2.

The tentative assumption may be made that the addition of 0·006 % of H_2O_2 will prevent the multiplication of organisms in synthetic creams of certain makes for 7 days if the initial count is less than 50,000 colonies per gram. Laboratory experiments showed, however, that higher concentrations of peroxide were required in the presence of proteins, for example, egg and milk proteins.

With larger numbers of organisms it is probable that the amount of H_2O_2 required to maintain sterility is dependent on the size of the initial bacterial population. The conclusion to be drawn from these results is that the addition of H_2O_2 should be made as soon as possible after pasteurization.

Effect of varying concentrations of H_2O_2 *on a large inoculum of* Salmonella paratyphi B *in manufactured creams 'A' and 'B'*

The results of the first part of this experiment (Table 7) showed that in the samples of cream without added H_2O_2 the organisms in the inoculum of approximately 8 million *Salm. paratyphi B* per ml. gradually died; in the sample of cream 'B' no organisms were found after 3 days. The presence of 0·006 % H_2O_2 reduced the count to less than 500 in 1 day for cream 'A' and in 3 days for cream 'B'. A concentration of 0·018 % effectively reduced the count to less than 500 colonies per ml. in 1 day for both creams. In the second part of the experiment the same samples of cream, after incubation at 30° C. for 1 week, were reinoculated with a large number of *Salm. paratyphi B*, 16–19 million per ml. The results indicated that the H_2O_2 was no longer effective, and the gradual reduction in the

numbers of organisms recovered was similar in all samples whether they contained H_2O_2 or not. The results for the samples of cream 'A' containing 0·006 % H_2O_2 were anomalous.

Effect of storage for three weeks at three different temperatures on the inhibition by H_2O_2 *of* Salmonella paratyphi B *in synthetic cream*

Cream 'B' was chosen for the experiment, and two concentrations of H_2O_2 were used, 0·006 and 0·012 %. The temperatures of storage were 30, 20 and +4° C., and the size of the inoculum was varied from approximately 1 to 12 million organisms per gram.

H_2O_2 was added to the samples, which were then left at the three temperatures. At the end of 1- 2- and 3-week storage periods portions of each sample were removed and inoculated with *Salm. paratyphi B*. The inoculated samples were incubated at 30° C. and counts were made after 1, 3, 5 and 7 days' incubation. The results showed that after a period of 3 weeks' storage at 4° C. both the 0·006 and the 0·012 % concentrations of H_2O_2 were still able to inhibit the growth of *Salm. paratyphi B*. After 3 weeks storage at 20° C. only the 0·012 % concentration of H_2O_2 was able to inhibit the growth of *Salm. paratyphi B*, while at 30° C. it suppressed the growth of this organism after 1 week but only partly controlled it after 2 and 3 weeks storage.

The degree of suppression of growth with the 0·006 % concentration of H_2O_2 was variable after storage at 20 and 30° C., depending on the size of the inoculum. After storage for 2 and 3 weeks at 30° C. the counts increased appreciably; apparently not only had the H_2O_2 failed to retain its power of inhibiting growth but some other factor related to the inhibitory nature of the creams, irrespective of added H_2O_2, had become inactivated.

The experiment was repeated using a laboratory-made synthetic cream containing 30 g. margarine, 20 g. colloid, 0·2 g. salt, 2·5 g. sucrose, 1 ml. of egg yolk and 6 ml. of milk made up to 100 ml. with distilled water. With an inoculum of half to 1 million *Salm. paratyphi B* per gram, 0·012 % H_2O_2 prevented growth in samples of cream when incubated at 30° C. after preliminary storage for 3 weeks, in the presence of H_2O_2 at 4 or 20° C. After storage at 30° C., 0·012 % H_2O_2 prevented growth for two weeks only. 0·006 % H_2O_2 was unable to prevent growth.

These experiments showed that 0·012 % H_2O_2, when added either to a fat emulsion cream or to a margarine, egg and milk cream, inhibited the growth of a moderately large number of *Salm. paratyphi B* after storage of the cream at 4 or 20° C. for at least 3 weeks or at 30° C. for 1 or 2 weeks.

Control experiments using the commercial synthetic cream 'B' were carried out on the effect of H_2O_2 on *Salm. paratyphi B* after storage for 3 weeks at the three different temperatures of 30, 20 and +4° C. The results showed that although the number of *Salm. paratyphi B* slowly decreased over the period of examination the organisms could still be recovered after storage of the cream for 3 weeks.

Table 6. *Effect of addition of varying concentrations of* H_2O_2 *to samples of commercial synthetic cream at* 30° C.

Source (producer)	Time	Colony count per ml. Control (without H_2O_2)	% H_2O_2 in test 0·004	0·006	0·012	0·018	0·048
B	0 hr.	48,000	—	—	—	—	—
	1 day	30×10^6	—	< 500	—	< 500	< 500
	3 days	23×10^6	—	< 500	—	< 500	< 500
	7 days	18×10^6	—	< 500	—	< 500	< 500
B	0 hr.	< 500	—	—	—	—	—
	1 day	$3{\cdot}3 \times 10^6$	—	< 500	—	< 500	< 500
	4 days	$1{\cdot}3 \times 10^6$	—	< 500	—	< 500	< 500
C	0 hr.	24×10^6	—	—	—	—	—
	1 day	33×10^6	—	53×10^6	60×10^6	28×10^6	23,000
	3 days	98×10^6	—	40×10^6	50×10^6	88×10^6	75×10^6
	8 days	170×10^6	—	120×10^6	140×10^6	130×10^6	48×10^6
D	0 hr.	$1{\cdot}7 \times 10^6$	—	—	—	—	—
	1 day	6×10^6	—	5×10^6	< 500	< 500	< 500
	3 days	10×10^6	—	20×10^6	25,000	< 500	< 500
	7 days	$6{\cdot}3 \times 10^6$	—	$3{\cdot}8 \times 10^6$	9×10^6	< 500	< 500
E	0 hr.	7,500	—	—	—	—	—
	1 day	50×10^6	—	< 500	< 500	< 500	< 500
	4 days	60×10^6	—	< 500	< 500	< 500	< 500
F	0 hr.	< 500	—	—	—	—	—
	1 day	25×10^6	—	< 500	—	< 500	< 500
	3 days	13×10^6	—	< 500	—	< 500	< 500
	7 days	15×10^6	—	< 500	—	< 500	< 500
G	0 hr.	< 500	—	—	—	—	—
	1 day	$1{\cdot}8 \times 10^6$	—	< 500	—	< 500	< 500
	3 days	33×10^6	—	< 500	—	< 500	< 500
	7 days	$9{\cdot}8 \times 10^6$	—	< 500	—	< 500	< 500
H	0 hr.	< 500	—	—	—	—	—
	1 day	150×10^6	—	< 500	—	< 500	< 500
	3 days	73×10^6	—	< 500	—	< 500	< 500
	7 days	110×10^6	—	< 500	—	< 500	< 500

TABLE 6 (*continued*)

I	0 hr.	$3{\cdot}8 \times 10^6$	—	—	—	—	—
	1 day	12×10^6	—	5×10^6	—	750	200
	6 days	18×10^6	—	13×10^6	—	3×10^6	330,000
J	0 hr. 20° C.	$> 2{\cdot}5 \times 10^6$	—	—	—	—	—
	3 days	$2{\cdot}4 \times 10^6$	32×10^6	—	—	—	—
	7 days	5×10^6	22×10^6	—	—	—	—
J	0 hr. 20° C.	7×10^6	0·0048	—	—	—	—
	3 days	930,000	13×10^6	24×10^6	—	—	—
	7 days	$1{\cdot}8 \times 10^6$	$6{\cdot}5 \times 10^6$	$6{\cdot}8 \times 10^6$	—	—	—
K	0 hr.	10×10^6	—	—	—	—	—
	3 days	$3{\cdot}7 \times 10^6$	22×10^6	—	—	—	—
	7 days	$8{\cdot}8 \times 10^6$	$1{\cdot}4 \times 10^6$	—	—	—	—

Table. 7. *Effect of varying concentrations of* H_2O_2 *on a large inoculum of* Salmonella paratyphi B *in two synthetic creams 'A' and 'B' incubated at* 30° C.

	Colony count per ml.							
	Cream 'A'				Cream 'B'			
		% H_2O_2 in test				% H_2O_2 in test		
Time	Control (without H_2O_2)	0·003	0·006	0·018	Control (without H_2O_2)	0·003	0·006	0·018
15. ix. 52: 0 hr.	$8{\cdot}1 \times 10^6$	$8{\cdot}2 \times 10^6$	$7{\cdot}9 \times 10^6$	$7{\cdot}2 \times 10^6$	$8{\cdot}3 \times 10^6$	$8{\cdot}2 \times 10^6$	$6{\cdot}1 \times 10^6$	$4{\cdot}3 \times 10^6$
1 day	$5{\cdot}8 \times 10^6$	$1{\cdot}7 \times 10^6$	< 500	< 500	56,000	61,000	24,000	< 500
3 days	560,000	240,000	< 500	< 500	< 500	< 500	< 500	< 500
5 days	160,000	$6{\cdot}8 \times 10^6$	< 500	< 500	< 500	< 500	< 500	< 500
Reinoculated								
22. ix. 52: 0 hr.	17×10^6	19×10^6	17×10^6	19×10^6	18×10^6	16×10^6	19×10^6	18×10^6
2 days	$5{\cdot}4 \times 10^6$	$8{\cdot}8 \times 10^6$	$7{\cdot}0 \times 10^6$	$7{\cdot}0 \times 10^6$	$4{\cdot}6 \times 10^6$	$6{\cdot}6 \times 10^6$	$6{\cdot}6 \times 10^6$	$3{\cdot}9 \times 10^6$
4 days	$3{\cdot}1 \times 10^6$	$5{\cdot}9 \times 10^6$	16×10^6	100,000	$2{\cdot}1 \times 10^6$	$4{\cdot}0 \times 10^6$	$5{\cdot}1 \times 10^6$	$1{\cdot}6 \times 10^6$
7 days	$1{\cdot}3 \times 10^6$	$1{\cdot}9 \times 10^6$	29×10^6	260,000	900,000	$1{\cdot}7 \times 10^6$	$2{\cdot}5 \times 10^6$	$1{\cdot}2 \times 10^6$

DISCUSSION

Synthetic creams which contain protein are almost unique among foodstuffs, not because, like many other foods, they are excellent media for bacterial proliferation, but because they are sold to the public without any attempt to restrict the dangers of bacterial contamination. Cream confectionery, for example, may be left for many hours at atmospheric temperature before sale to the consumer. A large proportion of the population is thus exposed to a vehicle of infection not only of food poisoning but also of paratyphoid fever.

Synthetic cream containing protein substances is equivalent or superior to milk and liquid ice-cream mix in its ability to encourage bacterial growth. It is often much handled before storage for indefinite periods under varying temperature conditions. Finally, it is eaten without further heat treatment.

The suggestion has been made that hygienic codes of practice to be followed in the preparation of synthetic cream would adequately protect the public against eating contaminated cream. While acknowledging the fact that recommendations about heat treatment of the manufactured product, methods of transport in sealed containers and storage times and temperatures may lessen the risks of contamination, during and immediately after manufacture, yet they cannot abolish the potential dangers of contamination in the bakehouse. These arise from members of the staff either harbouring pathogenic staphylococci in septic lesions on the hands or in the nose, or excreting organisms of the enteric group in their faeces or urine, or from the faeces of rats and mice infected with organisms of the salmonella group, or from impure water or milk supplies, or possibly from substances used in the preparation of the confectionery.

The application of Codes of Practice in the bakery is considered to be impracticable. Consequently the suggestion is made, that as a precaution against food poisoning the only alternative is to protect synthetic creams of whatever constitution by the addition of a harmless substance inhibitory to the growth of intestinal pathogens and of staphylococci.

The objection may be made that the addition of a protective bacteriostatic substance to cream may provide a convenient cover for dirty methods. The same objection was raised over the pasteurization of milk and proved to be unfounded in practice. With manufactured cream cleanliness alone is unlikely to ensure a safe product, and the addition of a bacteriostatic substance is essential.

Hydrogen peroxide is volatile, it has never been known to have given rise to toxic symptoms, and the Public Health (Milk and Cream) Regulations, 1912, allowed its use for the preservation of milk; the use of H_2O_2 was also allowed for the preservation of natural creams. Similarly, in the earlier years of the century, H_2O_2 combined with mild heat was regularly used in the Budd method for controlling the growth of bacteria in infants' feeds.

There seems no reason, therefore, why H_2O_2 should not be used to ensure the safety of synthetic cream, though its use would contravene the present Regulations relating to the addition of preservatives to foodstuffs. Nevertheless, the suggestion

that it should be used is put forward in the interest of all those who take part in the preparation, serving or eating of synthetic cream.

SUMMARY AND CONCLUSIONS

1. The growth in synthetic cream of certain pathogenic bacteria may be inhibited by two methods:

(*a*) By limiting the ingredients to cooking fat or other fatty material, excluding margarine or butter, emulsifying agent such as methyl cellulose, sugar and salt. The cream must not then be left in contact with confectionery from which nutritive material may be absorbed. This last condition renders the method impracticable as a means of control.

(*b*) By the addition to synthetic cream, including those containing milk and egg, of a bacteriostatic agent such as hydrogen peroxide.

2. Contaminants may grow in emulsified fats in the absence of protein. These are probably lipolytic organisms which are able to utilize the products split from the fat. Organisms such as coagulase-positive staphylococci, *Salmonella paratyphi B*, and *Bacterium coli*, tend to die out within 24 hr. or after a few days in creams lacking protein.

3. The concentration of H_2O_2 necessary to inhibit the growth of test organisms is dependent on the ingredients of the cream. In the presence of butter, milk and egg yolk at least three times the concentration of H_2O_2 is necessary as when emulsified fat without added protein is used. The suggestion is made that concentrations of H_2O_2 from 0·005 to 0·02% should be added to all commercially produced synthetic cream, the actual concentration used depending on the constituents of the cream.

4. To prevent the growth of contaminating organisms in commercially produced synthetic cream, H_2O_2 should be added immediately after pasteurization and cooling when the bacterial count is still low. Even comparatively high concentrations of H_2O_2 will not inhibit or control the growth of bacteria already present in large numbers.

5. The inhibiting power of 0·012% of H_2O_2 remained effective in a fat-emulsion cream and also in a laboratory cream containing margarine, egg and milk for 3 weeks at +4 and 20° C.; at higher temperatures of storage the stability of the oxidizing agent is doubtful.

6. Since synthetic cream is exposed to so many different sources of contamination, particularly in the bakery, and since there is no method of controlling the growth of pathogenic organisms that have gained access to it other than by the addition of a bacteriostatic agent, it is strongly recommended that the present regulations on preservatives should be altered to allow the addition of H_2O_2 in suitable concentration to synthetic cream.

We are grateful to all those who have helped in the supplies of material, and in particular we wish to thank the Public Health Department, St Pancras Town Hall; Mr E. Capstick, United Dairies Ltd.; Mr J. Valentine Backes, President, Bakery Allied Traders' Association; and also Messrs Bakcos Catering Supplies Ltd.,

A. Bellamy and Co. Ltd., Farma Cream Products Ltd., Krema Ltd., Malga Products Ltd., Ramsay, Braddon and Co. Ltd., Quality Foods Ltd., Sunnyside Products Ltd., Unicream Ltd., and Vitacream Ltd.

We are indebted to Mr L. Bailey for his technical assistance.

REFERENCES

BAILLIE, A. J. & FREEMAN, G. G. (1952). The rate of growth of Salmonellas and other bacteria in some synthetic creams. *J. Sci. Fd Agric.* **3**, 616.

CHAPMAN, R. A. & MCFARLANE, W. D. (1943). A colorimetric method for the determination of fat peroxides and its application in the study of the keeping quality of milk powders. *Canad. J. Res.* **21**, 133.

MILES, A. A. & MISRA, S. S. (1938). The estimation of the bactericidal power of the blood. *J. Hyg., Camb.*, **38**, 732.

THOMSON, S. (1953). Paratyphoid fever and baker's confectionery: an analysis of an epidemic in South Wales in 1952. *Mon. Bull. Minist. Hlth Lab. Serv.* **12**, 187.

(*MS. received for publication* 30. xii. 53)