A BACTERIOLOGIC STUDY OF BAKERY PRODUCTS WITH SPECIAL REFERENCE TO FOOD POISONING ORGANISMS

by

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I

INTRODUCTION

The common occurrence of food poisoning outbreaks which have been definitely traced to bakery products, especially those of the custard or cream-filled variety, has led us to the isolation and study of bacteria which may be commonly found in such products. Special emphasis has been given to the staphylococci, since they seem at the present time to be the group of organisms most commonly associated with food poisoning in the United States.

Bacterial intoxications occurring through the ingestion of food may include a host of various diseases. Such specific diseases as tuberculosis, undulant fever, cholera, typhoid, dysentery, and septic sore throat are commonly transmitted by food. In these infections, however, the food is merely the carrier of the organism, and does not furnish a medium in which the organism grows and forms its toxic products, as do those organisms commonly associated with food poisoning. Clostridium botulinum, several members of the Salmonella group, and certain staphylococci comprise the food poisoning group. All of these organisms are capable of producing, as a product of their metabolism, a substance which is toxic to a greater or less degree when in contact with the human gastro-intestinal tract. Certain other organisms have been reported as etiological

agents in food poisoning outbreaks, but are relatively uncommon, and have often not definitely been proven the cause.

The relatively uncommon occurrence of botulism, its anaerobic nature, and its high fatality distinctly separate it from the more common forms of bacterial food poisoning. The symptoms of botulism are related to the central nervous system and are not gastro-intestinal in nature--- a fact which further differentiates it. A consideration of botulism will be eliminated from this work because no attempt was made to isolate and study anaerobic organisms.

The symptoms of staphylococcic and Salmonella food poisoning are very similar, being manifested in the form of a gastro-enteritis. Paroxysms of vomiting and diarrhea accompanied by nausea, dizziness, cramps, and considerable prostration are common. These acute symptoms usually pass off within a few hours, and though anorexia and general weakness may persist for several days, it appears not unusual for the patient to feel normal within twenty-four hours after the onset. These types of food poisoning differ in that the incubation period of the staphylococci is usually somewhat shorter than that of the Salmonella. As yet, there have been no deaths traced to staphylococcic food poisoning, while the Salmonella type is said to have a mortality rate of from 1 to 2 per cent. Innumerable references to food poisoning outbreaks caused by one or the

other of these types may be found in the literature. It is interesting to note, however, that most of the symptoms are sub-clinical in nature, and thus a far greater number of cases undoubtedly are never reported. These types of food poisoning, then, are more than likely responsible for much undiagnosed human suffering and, because of the short duration, are commonly disregarded.

Jordan,(1931) has observed that the incidence of staphylococcic food poisoning is much higher than that of Salmonella in the United States. European reports, on the other hand, would indicate that staphylococcic food poisoning is relatively rare. They attribute 90 per cent of the food poisoning they have observed to members of the Salmonella group. Staphylococcic food poisoning may well be called "American", while that of the Salmonella, "British".

Table 1 is a chronological tabulation of food poisoning outbreaks traced to staphylococci. In some of these outbreaks the staphylococcus in question has definitely been proven enterotoxic in nature by the ability of its sterile filtrate to reproduce in human volunteers or monkeys characteristic symptoms when taken by mouth. In other cases the intraperitoneal injection of the sterile filtrate into kittens with the production of characteristic gastrointestinal symptoms has been used as the criterion of its enterotoxic nature. In the remaining cases the food in question has merely been found contaminated with great

TABLE 1

Food Poisoning Outbreaks Traced to Staphylococci

Year of out-break	Place	Incrimin-ated food	By whom reported & year of publication	Number of persons involved
1907*		dried beef	Owen (1907)	19
1914	Phillipines	milk	Barker (1914)	several
1927*	Pasadena	French va-nilla ice cream	C. W. Arthur (Jordan 1931)	--
1927*	Pasadena	custard-filled cream puffs	C. W. Arthur (Jordan (1931)	--
1928*	Boston	chocolate cream pie	Nelson (1928)	150
1929*	Chicago	wedding cake	Jordan (1931)	20
1929*	Chicago	custard-filled cream puffs	Jordan (1931)	17
1929	1929	Christmas cake	Dack, Cary, Wool-pert, Wiggens (1930)	11
1930	Puerto Rico	cheese	Jordan (1930)	4
1930	Milwaukie	devil's food cake	Jordan (1931)	3
1930*	N. Y. State	church fair cake	N. Y. State Dept. Health (1930)	4
1930*	N. Y. State	cream puffs & chocolate eclairs	N. Y. State Dept. Health (1930)	130

Year of out-break	Place	Incrimin-ated food	By whom reported & year of publication	Number of persons involved
1931	Panama Can-al Zone	chicken gravy	Jordan and Hall (1931)	2
1931	--	milk	Ramsey and Tracy (1931)	1
1931*	Jersey City New Jersey	layer cake fil-ling	Jordan (1930)	16
1932	Milwaukie	custard-filled ec-lairs, cof-fee cakes, poppy seed rolls	Jordan and Bur-rows (1934)	54
1932	Chicago	eclairs, cream-fil-led cake, strawberry tarts, cus-tard slice	Jordan and Bur-rows (1934)	31
1932	Milwaukie	coffee cake	Jordan and Bur-rows (1934)	7
1932	Chicago	custard-filled doughnuts	Jordan and Bur-rows (1934)	2
1932	Cleveland Ohio	custard-filled pie	Buchanan and E-cker	several hundred
1933	Winona Minnesota	chocolate eclairs	Jordan and Bur-rows (1934)	several
1933	Puerto Rico	sweet po-tato candy, ham	Costa Mandry (1933)	
1933	Southern education-al institu-tion	filling of chocolate eclair	McBurney (1933)	150

Year of out-break	Place	Incrimin-ated food	By whom reported & year of publication	Number of persons involved
1933	Pleasant Hill, Tenn.	milk	Crabtree and Letter (1934)	242
1933*	Shoreham Vermont	home made ice cream	DeWitt (1935)	43
1934	Ann Arbor Michigan	chicken salad	Haynes (1935)	231
1934*	Veteran's Administration Institution	cold sliced tongue	Matz (1935)	18
1934	Virginia	cake with custard filling	Corpening and Fo hall (1935)	12
1934*	London	butter	Fanning (1935)	70
1935	Indianapolis	tongue sandwiches	Dack, Bowman and Harger (1935)	187
1935	Southern Illinois	milk	Shaughnessy and Grubb (1936)	25
1936*	North Albany New York	chocolate eclairs	New York State Dept. of Health	6
1936	Vancouver, B. C.	custard-filled vanilla slices	Dolman (1936)	3
1936	San Francisco, Cal.	custard cakes	Geiger (1937)	110
1937*	Troy, N. Y.	strawberry cream pie	New York State Dept. of Health (1937)	5
1937*	Renssler, N. Y.	cocoanut cream pie	New York State Dept. of Health (1937)	4

Year of out-break	Place	Incrimin-ated food	By whom reported & year of publication	Number of persons involved
1937*	Troy, N. Y.	strawber-ry cream pie	New York State Dept. of Health (1937)	12

*No further proof given that a staphylococcus was the etiological agent than that large numbers of staphylococci were found to be present in the incriminated food.

numbers of staphylococci. A study of the incriminated foods listed in Table 1 will readily impress one with the fact that custard-filled bakery products are by far the most common offenders. A realization of the fact has led us to this bacteriologic study of bakery goods.

II

EXPERIMENTAL WORK

Collection and Study of Samples

In this investigation, the bakery products were purchased on the open market, having been selected at random and brought in their usual bakery packages immediately to the laboratory. Each sample was removed as aseptically as possible from its package and one gram portions taken from various parts of the sample by use of a sterile knife, forceps, and stirring rod. Each one gram portion was placed in a 9 cc. sterile water blank and thoroughly emulsified by use of a sterile glass stirring rod. The one gram portion in 9 cc. of water was considered a 1/10 dilution, and from it 1/100, 1/1000, and 1/10,000 dilutions were made. The 1/100 and 1/10,000 dilutions were plated out using horse or rabbit blood-infusion agar, incubated 48 hours at 37°C. and counted. After the counts were made all organisms present in significant numbers were picked and transferred to blood-infusion agar slants, incubated 24 hours at 37°C., and stained by Gram's method. It is of interest to note here that on most plates one type of organism was present in far greater numbers than any other. In several instances, to all appearances, a pure culture of the organism existed.

As a routine measure, 1/2 cc. of the 1/100 and 1/10,000

dilutions of the samples were placed in lactose fermentation tubes as a presumptive test for Escherichia coli. Whenever the lactose tubes after 24 hour incubation at 37°C. showed acid production with 10 per cent or more gas, a loopful from the fermentation tube was streaked on Endo's agar to confirm the presence of Escherichia coli.

In several instances, a second bacterial count was made on samples which had been kept at room temperature for 24 hours after the first count had been made. The results of these counts are given in Table 2, from which it can be readily seen that in most of the samples, the prolonged incubation was responsible for a considerable rise in count.

A part of the samples used were obtained during March and April, which were characteristically cool months, and the remainder were taken during July, which was characteristically hot. From Tables 3 and 4 a comparison of the counts of the groups of samples may be made, which leads to a consideration of the effect of weather conditions on the bacterial content of these bakery products. The results placed in these tables seem to show consistently higher counts for the samples taken during July than for the March and April samples, being especially true of the custard-filled products. In July it was difficult to obtain custard-filled products from several bakeries, due to the fact that their production was discontinued during

TABLE 2

Bacterial Counts Per Gram Before and After Bakery Products Have Stood Twenty-four Hours at Room Temperature

Product	Initial Count	Count After Standing 24 Hours
custard puff (10)		
custard filling	500,000	1,280,000
crust	200	20,000
chocolate eclair (19)		
custard filling	3,000,000	1,500,000
pineapple pastry (24)		
cake substance	1,000	16,500
chocolate pastry (26)		
cream filling	200	5,000
cake substance	400	5,000
walnut cream pie (30)		
filling	30,000	2,500,000 (all staph.)
custard puff (36)		
filling	2,500,000	4,000,000
chocolate eclair (38)		
custard filling	1,200	50,000
raspberry cream pie (39)		
whip cream topping	50,000	50,000

the summer. Upon inquiry as to the reason for this practice, we found the common answer to be that "they didn't keep in hot weather". Evidently complaints had arisen which had caused them to change their procedure as regards this type of product. In some other bakeries it was interesting to note that their custard and cream-filled goods were kept refrigerated during the warmer weather. Immediate refrigeration of custard-filled products is actually necessary regardless of weather conditions, for the usual temperature of bakeries, being well above ordinary temperatures at any time of the year, offers excellent incubation temperatures for bacteria. Stritar, Dack, and Jungewaelter (1936) state that the control of staphylococci in custard-filled bakery goods is a serious health problem. They feel, however, that refrigeration in itself is not a complete solution to the problem. The products may become contaminated during manufacture and the organisms lie dormant during refrigeration. Then, unless the custard-filled goods are refrigerated after purchase by the housewife, incubation for a few hours at room temperature is sufficient time for the organisms to multiply and elaborate their toxin. They suggest a heat-treating method which should reduce the danger of food-poisoning from custard-filled puffs and eclairs, whereby these products are reheated for a time and temperature sufficient to kill the staphylococci without impairing the flavor or appearance of the product. The

recommendation for routine use is 30 minutes at temperatures ranging from 190.6°C. (375°F.) to 218.3°C. (425°F.). This principle of heating food may as well be made use of in this connection as in the pasteurization of milk.

In Tables 3 and 4 are listed the kinds of bacteria isolated from the bakery samples analyzed. After morphological identification by Gram stain, all cultures of staphylococci, streptococci, and coliform organisms were retained for further study. Due to the fact that staphylococci were present more frequently and in greater numbers than any other bacteria, and because of their relationship to food poisoning, primary consideration has been given to them.

TABLE 3

Bacterial Counts and Organisms Present in Bakery Products Examined in March and April

Sample	Count per Gram	Organisms Present
1. custard filled doughnut		
a. custard filling	--	staphylococci and spore forming rods
b. dough	--	staphylococci and spore forming rods
2. chocolate eclair		
a. custard filling	--	E. coli
b. dough and frosting	--	E. coli
3. custard filled doughnut		
a. filling	325,000	streptococci
b. dough	5,700	streptococci and spore forming rods
4. chocolate eclair		
a. custard filling	1,000	staphylococci and streptococci spore forming rods
b. dough and frosting		
5. glazed doughnut	500	spore forming rods
6. sugar cookie	0	
7. apple pie		
a. apple filling		
b. top crust	500	staphylococci
8. lemon cream pie		
a. lemon custard filling	20	
b. meringe	800	streptococci
9. custard snail		
a. custard	200,000	streptococci
b. custard and jelly topping	200,000	staphylococci and streptococci

Sample	Count per Gram	Organisms Present
c. dough	1,000	
10. custard puff		
a. custard filling	500,000	staphylococci, E. coli, spore forming rods
b. crust	200	staphylococci and E. coli, spore forming rods
11. chocolate eclair		
a. custard filling	2,500,000	spore forming rods
b. dough and frosting	410,000	spore forming rods
12. custard puff		
a. custard filling	2,500,000	spore forming rods
b. dough and frosting	20,000	spore forming rods
13. lemon custard pastry		
a. lemon custard	260,000	staphylococci and sarcina
b. pastry shell	6,500	staphylococci and sarina
14. custard puff		
a. custard filling	2,500,000	small gram neg. bacilli
b. frosting and dough	1,000,000	small gram neg. bacilli
15. chocolate whip cream cake		
a. cake substance	2,500,000	small gram neg. bacilli
b. cream filling	2,500,000	spore forming rods
16. custard puff		
a. custard filling	2,500,000	spore forming rods
b. crust	720,000	small gram neg. bacilli
17. hazelnut cream pie		

Sample	Count per Gram	Organisms Present
a. meringe	2,500,000	spore forming rods
b. filling	520,000	Salmonella
c. crust	2,500,000	Salmonella
18. custard pastry		
a. custard	2,000,000	E. coli, large spore forming rods
b. frosting	20,000	E. coli, large spore forming rods
c. crust	25,000	E. coli
19. chocolate eclair		
a. custard filling	3,000,000	E. coli, large spore forming rods
b. crust	500,000	E. coli
20. chocolate eclair		
a. custard filling	100	
b. frosting	800	
c. crust	100	
21. custard filled doughnut		
a. custard	300	
b. dough	0	
22. almond tart		
a. filling	0	
b. crust	500	staphylococci and spore forming rods
23. chocolate eclair		
a. custard filling	150,000	spore forming rods
b. frosting and crust	3,000	staphylococci
24. pineapple pastry		
a. frosting	300	
b. cake substance	1,000	staphylococci and streptococci
c. nut frosting	5,000	staphylococci
d. pineapple filling	400	
25. chocolate cream pastry		

Samples	Count per Gram	Organisms Present
a. cream filling	20,000	staphylococci, sarcini
b. cake substance	3,900	staphylococci
c. frosting	mold	streptococci and staphylococci
26. chocolate pastry		
a. filling	200	
b. cake substance	400	
27. carmel custard pie		
a. cream topping	2,500,000	streptococci and staphylococci
b. carmel filling	10,000	staphylococci
28. chocolate eclair		
a. custard filling	2,500	staphylococci, small gram neg. bacilli
b. frosting and crust	2,500,000	
29. custard filled doughnut		
a. filling	1,200	staphylococci and small gram neg. bacilli
b. dough	0	
30. walnut cream pie		
a. meringe and cream topping	17,500	staphylococci and streptococci
b. filling	30,000	staphylococci
31. butterscotch pie		
a. cream topping	460,000	staphylococci
b. filling	210,000	staphylococci
32. Boston cream pie		
a. whip cream topping	460,000	streptococci
b. filling	210,000	
c. cake substance	2,000	staphylococci
d. raspberry topping	mold	
33. devil's food cake		

Samples	Count per Gram	Organisms Present
a. frosting	10,000	staphylococci
b. cake substance	1,200	staphylococci
34. jelly roll		
a. frosting	400	molds
b. cake		
35. cocoanut cream pie		
a. filling	70,000	
b. meringe		
36. custard puff		
a. frosting and crust	100,000	staphylococci, E. coli
b. custard filling	2,500,000	staphylococci, E. coli and Salmonella
37. custard filled doughnut		
a. filling	1,000,000	staphylococci, small gram neg. bacilli
b. dough	13,000	Neisseria, staphylocci
38. chocolate eclair		
a. custard filling	1,200	Neisseria
b. dough	2,000	
39. raspberry cream pie		
a. whip cream topping	50,000	streptococci
b. raspberry filling	0	
40. custard pie		
a. custard	molds	
41. Boston cream pie		
a. whip cream top-	70,000	staphylococci
b. cake substance	15,000	staphylococci
c. custard filling	5,000,000	staphylococci

TABLE 4

Bacterial Counts and Organisms Present in Bakery Products Examined in July

Sample	Count per Gram	Organisms Present
42. chocolate nut cake		
a. frosting	500,000	streptococci
b. cake substance	3,800	streptococci
43. chocolate eclair		
a. custard filling	--	molds
b. frosting and crust	--	molds
44. cocoanut white cake		
a. cocoanut	2,600	staphylococci
b. boiled marshmellow frosting	5,800	streptococci
c. cake substance	9,800	streptococci
45. decorated white cake		
a. decorative frosting	2,200	staphylococci
b. boiled marshmellow frosting	210,000	staphylococci
c. cake substance	11,500	staphylococci
46. chocolate eclair		
a. custard filling	300,000	molds and large gram pos. rods
b. frosting and crust	3,000	molds and large gram pos. rods
47. chocolate eclair		
a. custard filling	1,500	staphylococci
b. frosting and crust	1,300	staphylococci
48. marshmellow roll		
a. marshmellow	0	

Samples	Count per Gram	Organisms Present
filling		
b. crust	600	streptococci
49. chocolate eclair		
a. custard filling	5,000,000	large grams pos. spore forming rods (positive)
b. frosting and crust	10,000	molds
50. custard filled doughnuts		
a. filling	5,000,000	large gram pos. spore forming rods
b. doughnut dough	5,000,000	molds
51. custard puff		
a. custard filling	5,000,000	gram pos. spore formers and coliform organisms
b. frosting and dough	3,000,000	gram pos. spore formers and coliform organisms
52. chocolate eclair		
a. custard filling	5,000,000	large gram pos. rods
b. frosting and crust	5,000,000	large gram pos. rods
53. chocolate cup cake		
a. frosting	500	--
b. cake substance	100	--
54. vanilla cup cake		
a. frosting	200	--
b. cake substance	100	--
55. custard filled doughnut		
a. filling	120,000	large gram pos. spore forming rods
b. dough	3,400	sarcina (pure culture)

Sample	Count per Gram	Organisms Present
56. custard filled cupcake		
a. filling	1,400	staphylococci (pure culture)
b. cake substance	0	
57. chocolate cake		
a. frosting	600	spreaders and molds
b. cake substance	0	
58. chocolate eclair		
a. custard filling	5,000,000	molds and spore formers
b. frosting and crust	50,000	molds and spore formers
59. maple cupcake		
a. frosting	6,500	staphylococci
b. cake substance	400	staphylococci and streptococci

Study of Organisms Isolated

Staphylococci

The history of staphlococcic food poisoning in this country dates back to December, 1929, when Dack and co-workers (1930) traced an outbreak of food poisoning to two three-layer sponge cakes decorated ornately for Christmas, which proved to be heavily contaminated with yellow staphylococci. In 1914 Barber reported that a staphylococcus of the albus variety was apparently the cause of several attacks of acute gastro-enteritis on a farm in the Philippines. Milk that was inoculated with the strain isolated, and then incubated for about eight hours, caused similar illness when drunk by the experimenter. Jordan and Burrows (1935) give reference to two even earlier observations of food poisoning which pointed to staphylococci as the cause. Since Dack's report in 1929, the literature contains many reports of such outbreaks, as Table 1 would indicate. A considerable amount of work has been stimulated in an attempt to determine the nature of the toxic principle and to develop some suitable means of detecting its presence.

It is now an established fact that the ability of the staphylococcus to produce food poisoning symptoms is inherent in the exotoxin which it produces. Dack and co-workers (1930) fed sterile filtrates of two different

strains isolated from the Christmas cake in amounts of from 2 to 10 cc. to human volunteers, and produced in them severe gastro-intestinal symptoms similar to those experienced by the persons involved in the outbreak. Jordan, Dack, and Woolpert (1931), in making the first attempt to determine the nature of the enterotoxic factor in staphylococcic filtrates, found that the substance was not completely destroyed by exposure for 30 minutes to the temperature of boiling water, but that some diminution in toxic power may be caused by heating even at temperatures below 100°C. They also showed that the toxic quality did not disappear after storage at low temperatures for as long as 67 days, but is perhaps somewhat weakened. A strong dose of chlorine did not destroy the toxic quality when in contact for 3 minutes with it. Woolpert and Dack (1933), using the method of production of staphylococcus toxin on semisolid medium under partial carbon dioxide atmosphere (Burnet, 1929, 1930), produced powerful staphylococcic gastro-intestinal poisons which, when fed to rhesus monkeys, consistently caused symptoms of food poisoning similar to those seen in man. They found this enterotoxic substance to be distinct from the hemolysin, dermotoxin, and killing toxin found in such preparations. They also found that the gastro-intestinal poison was more resistant to heat and to adsorption than the other toxins, and was not neutralized by antiserum effective against the others.

In no case, however, was the food poison produced without the production of these other toxins, and, whenever food poison was formed, there seemed to be a rough correlation between the amount of food poison and the amounts of these other toxins. They were able to actively immunize the rhesus monkey against the effects of this poison, but attempts at passive immunization failed. Jordan and Burrows (1935) found that cultivation over long periods of time tends to decrease the ability of the strains to produce demonstrably enterotoxic filtrates, even in an atmosphere containing 25 per cent carbon dioxide. By successive transfers on starch medium they were able to restore the property of producing enterotoxic substance to a number of strains that were originally positive but had long given negative results. Stritar and Jordan (1935) using 94 strains of staphylococci of diverse origin--local skin suppurations, normal human throats, surgical cases such as osteomyelitis, etc., foodstuffs implicated in food poisoning--were very inclined to agree with other investigators who have found that there are no good criteria for the differentiation of various types of staphylococci. They, too, were impressed with the fact that biochemical, hemolytic, and agglutinative characters are not closely correlated. The "food-poisoning" strains agree with the other members of the group in not constituting a clearly marked division. Thus the power to provoke food poisoning

is not limited to any recognizable variety of staphylococci.

With the above facts well in mind, as to the possibility of classifying the staphylococci, especially as regards their power to cause food poisoning, a general study of the staphylococci isolated from the bakery products was undertaken.* Table 5 includes, for the strains isolated, the culture number, a reference to the bakery product from which the strain was isolated, the pigment, the hemolytic titre of its toxin, the power of its toxin to produce gastro-intestinal symptoms when injected intraperitoneally into kittens, its power to produce an enzyme capable of causing the coagulation of oxalated rabbit blood-plasma, its fibrinolytic power, and the results of Stone's method for detecting enterotoxic staphylococci.

In the preparation of the staphylococcus toxins, the method suggested by Dolman (1932) and Dolman and Kitching (1936) was followed. A semi-solid agar is prepared from

*In addition to those strains of staphylococci isolated from the bakery products, six other strains have been included in the study. Two strains, designated as "24 MA" and "Wood 46" were obtained from C. E. Dolman. The other strains designated as A, B, C, and D were obtained from D. B. Charlton. Strain A was isolated from a pie incriminated in a food poisoning outbreak. Strain D was also isolated from an incriminated pie, but had originally been obtained from the laboratory of C. E. Dolman. Strain B was isolated from a piece of chocolate cake known to have been the cause of an outbreak of food poisoning. The cake had been obtained in August and had been kept until June in a refrigerator, at which time this strain was again isolated from the sample. Strain C was isolated from a staphylococcus infection on the arms of workers in a cannery.

TABLE 5

Results of Studies on Staphylococci Isolated From Bakery Products

Culture No.	Sample No.	Isolated from	Pigment	Hemolysin	Enterotoxin	Plasma Coag.	Fibrinolysis	Stone's Method
46	1a	custard-filled doughnut filling	cream	0	-	0	0	0
47	1b	custard-filled doughnut dough	orange	0	0	0	0	0
48	2a	chocolate eclair custard filling	orange	0	-	0	0	0
49	2a	chocolate eclair custard filling	orange	1/200	-	+	0	0
52	2a	chocolate eclair custard filling	orange	1/800	+	+	0	0
70	10a	custard puff custard filling	albus	0	-	0	0	0
122	22b	almond tart crust	albus	0	0	0	0	0
125	23b	chocolate eclair frosting	albus	0	0	0	0	0
127	24b	pineapple pastry cake substance	yellow	0	0	0	0	0
128	24b	pineapple pastry cake substance	cream	0	-	0	0	0
134	25a	chocolate pastry cream filling	cream	0	-	0	0	0

Culture No.	Sample No.	Isolated from	Pigment	Hemolysin	Enterotoxin	Plasma Coag.	Fibrinolysis	Stone's Method
135	25a	chocolate pastry cream filling	albus	0	-	0	0	0
136	25a	chocolate pastry cream filling	orange	0	0	0	0	0
139	25a	chocolate pastry cream filling	albus	1/800	0	0	0	0
142	25b	chocolate pastry cake substance	cream	0	0	0	0	0
143	25b	chocolate pastry cake substance	albus	0	-	0	0	0
148	27a	carmel custard pie cream topping	orange	0	-	0	0	0
150	27b	carmel custard pie filling	orange	1/100	0	+	0	0
151	27b	carmel custard pie filling	orange	1/200	-	+	0	0
152	27b	carmel custard pie filling	orange	1/200	-	+	0	0
156	25a	chocolate pastry cream filling	albus	0	-	0	0	0
159	28a	chololate eclair custard filling	orange	0	0	0	0	0
164	29a	custard filled doughnut filling	orange	0	-	0	0	+
165b	30a	walnut cream pie meringe	orange	1/200	0	+	0	+

Culture No.	Sample No.	Isolated from	Pigment	Hemolysin	Enterotoxin	Plasma Coag.	Fibrinolysis	Stone's Method
167	30b	walnut cream pie filling	cream	1/800	-	+	0	0
168	30b	walnut cream pie filling	cream	1/800	0	+	0	0
169	31a	butterscotch pie cream topping	albus	0	-	0	0	0
170	31b	butterscotch pie filling	albus	0	-	0	0	0
171	31b	butterscotch pie filling	cream	1/800	0	+	0	0
178	32c	Boston cream pie cake substance	cream	0	-	0	0	0
179	30b	walnut cream pie filling	cream	1/200	0	+	0	0
180	30b	walnut cream pie filling	cream	0	-	0	0	0
181	30b	walnut cream pie filling	cream	1/200	-	+	0	0
182	30b	walnut cream pie filling	orange	1/200	-	+	0	0
183	33a	devil's food cake filling	albus	0	-	0	0	0
185	36a	custard puff frosting	albus	1/1600	-	0	0	+
192	37a	custard-filled doughnut filling	orange	0	-	0	0	0
194	37b	custard-filled doughnut dough	yellow	0	-	0	0	0

Culture No.	Sample No.	Isolated from	Pigment	Hemolysin	Enterotoxin	Plasma Coag.	Fibrinolysis	Stone's Method
203	41a	Boston cream pie whip-cream topping	albus	0	-	0	0	0
205	41b	Boston cream pie cake substance	albus	0	-	0	0	0
218	44a	white cake cocoanut frosting	cream	0	0	0	0	0
221	45a	cake colored decorative frosting	albus	0	0	0	0	0
222	45b	white cake boiled frosting	orange	0	0	0	0	0
223	45b	white cake boiled frosting	orange	0	0	0	0	0
224	45b	white cake boiled frosting	orange	0	0	0	0	0
226	47a	chocolate eclair custard filling	cream	0	0	0	0	0
235	56a	custard-filled cake filling	albus	0	-	0	0	0
236	59a	maple cup cake frosting	orange	0	-	0	0	0
238	59b	maple cup cake cake substance	cream	0	-	0	0	0
24 MA			cream	1/800	0	+	0	0
Wood 46			albus	1/800	0	+	0	0
A			orange	0	-	+	0	0
B			orange	1/400	0	+	0	-
C			orange	1/200	-	+	0	-
D			orange	0	-	+	0	0

beef infusion broth with 1 per cent peptone, adjusted to pH 7.2, adding sufficient agar to give 0.3 per cent agar concentration. The surface of Petri dishes containing this medium is inoculated with small amounts (0.5 cc.) of a young broth culture of the staphylococcus in question and incubated for 40 hours in an atmosphere of 30 per cent carbon dioxide and 70 per cent oxygen at 37°C. At the end of the incubation period, a peculiar odor, not usually associated with staphylococcus cultures, is evident. The suspension is freed from agar by passage through cheese cloth, after which it is Seitz filtered or passed through a Berkefeld candle.

Dolman and Kitching (1936) state that, when the toxin is so prepared, the dermonecrotic and hemolytic properties appear to be closely allied, at least quantitatively, and for practical purposes the in vitro assay of the homolysin content of staphylococcal filtrates may be taken as a sufficiently accurate index of their exotoxin content. The hemolytic titre of the toxin was determined immediately after its preparation, eliminating the necessity of adjusting the pH, so as to prevent any deterioration or diminution of hemolytic power. The titre of the hemolysin was determined by noting the highest dilution of the toxin which would produce an arbitrary degree of hemolysis of 1 per cent suspension of washed rabbit erythrocytes in isotonic saline. The tubes were incubated for one hour at 37°C. and then placed in the ice box over night before being

read. Dolman (1932) has shown that rabbit corpuscles should be used in preferance to corpuscles from other species of laboratory animals because, first, they are more susceptible to staphylolysin, and secondly, the lytic titre obtained by using them corresponds to the in vivo toxicity. Table 5 includes the hemolytic titre determined for each of the strains of staphylococci isolated.

A suitable method for the detection of any enterotoxic properties which the staphylococcus filtrates might possess is difficult to determine. When all lower animals were found to be not visably affected when fed these filtrates, human volunteers seemed the only possibility. The extensive use of human volunteers, however, provides a tremendous problem and in most cases is an impossibility. The susceptibility of M. rhesus monkeys was first reported by Jordan and McBroom (1931) and has since been verified by several investigators. The success of this method, however, is limited by the difficulty in procuring and handling these monkeys. Borthwick (1933) has reported the use of guinea pigs and rabbits for the detection of the presence of the food-poisoning substance in staphylococcal filtrates. He has obtained uniformly positive results by injecting the toxin directly into the stomach of the animal, and, at the same time, adjusting the reaction of the stomach to pH 7.3. The animals die within five days and post mortem examinations show signs of acute gastro-enteritis and

marked congestion of internal organs associated with hemorrhage in the stomach and kidneys. His reason for changing the reaction of the stomach is based on experimental work which indicated that upon the addition of staphylococcus toxin to gastric contents in vitro, a slightly acid (pH 6.8) or a slightly alkaline (pH 7.8) reaction impaired its activity, whereas at pH 7.3 no inactivation occurred. Dolman, Wilson, and Cockcroft (1936) report that their attempts to reproduce Borthwick's work have been entirely unsuccessful, and that they have found no reference to confirmation of this work by anyone else.

These investigators have set forth a method involving the use of kittens as a means of determining the enterotoxic power of staphylococcus filtrates. They found that the intraperitoneal injection of about 2 cc. of formalinized filtrate, prepared from an enterotoxin-producing strain of staphylococcus, will cause a very characteristic syndrome in kittens. Marked lassitude and weakness with unsteadiness comes on shortly after the injection and culminates (often in from 15 to 30 minutes) in the first of a series of intermittent paroxysms of vomiting associated with diarrhoea. The kitten may appear extremely ill for several hours, but recovery, when it sets in, is usually rapid and complete. They recommend the use of kittens weighing from 350 to 550 grams and aged about 6 to 8 weeks, for they are much easier to handle and seem more sensitive to the

enterotoxic substance than adult cats.

An intraperitoneal injection of the enterotoxin gives rise in the kitten to the same type of symptoms as the feeding experiments in man and monkeys, which have been thought to be the result of a direct gastro-intestinal irritant. The gastro-intestinal tract of the kitten at autopsy, however, shows no evidence of having been subject to the action of an acute irritant (Dolman, Wilson, and Cockcroft--1936). This surely leads to an interesting pharmacological problem.

In an attempt to determine whether or not any of the strains of staphylococci, isolated from the various bakery products in this investigation, might be capable of producing the food poisoning substance, the toxins prepared from each of the strains were inoculated intraperitoneally in 3 cc. amounts into young kittens. Before testing on kittens for the presence of enterotoxin, however, it is necessary to free the filtrate from any other toxic elements which it might possess. The results of injecting filtrates containing potent hemolytic, necrotizing, and lethal exotoxins would undoubtedly be very harmful. To destroy these other toxic elements, Dolman, Wilson, and Cockcroft (1936) added a 0.3 per cent solution of formaldehyde to the filtrates and kept them in the incubator at 37°C. until rabbit and sheep cell hemolysins were no longer detectable. In more recent experiments they have taken

advantage of the greater heat stability of the enterotoxin as compared to the hemolysin, and have placed the filtrates in a boiling water bath for 30 minutes just prior to the injection. They found that only negligible amounts of rabbit and sheep cell hemolysins survived this treatment, but that enterotoxin, where present, proved heat-stable.

In the preparation of filtrates for kitten inoculation we have made use of this latter method. Those filtrates which proved to be non-hemolytic in the hemolysin titration did not require this treatment, of course. Non-hemolytic as well as hemolytic strains were tested on the kittens, for, as stated before, no definite correlation seems to exist between hemolytic ability and enterotoxin production.

In a preliminary experiment using Strain #52, complete hemolysis of the rabbit cells took place at a 1/200 dilution of the filtrate, and yet, 3 cc. of the filtrate after being left for 30 minutes in a boiling water bath, when injected intraperitoneally into a kitten, proved to be enterotoxic. The heat killing method, which seems to be just as effective in destroying the hemolytic properties of the filtrates, proves to be much simpler than formalinization.

The results of the kitten inoculations are included in Table 5.

Strain #52, an orange colored staphylococcus, which was isolated from the custard filling of a chocolate eclair,

produced a filtrate which was capable of causing the typical food poisoning symptoms when injected intraperitoneally into kittens. On one occasion, 2 cc. of the heat-treated filtrate was injected subcutaneously into a 467 gram kitten, being followed in one hour with a 2 cc. intraperitoneal injection. Ten minutes following the second injection the kitten showed signs of sleepiness, in fifty-five minutes passed a loose stool, and in seventy minutes vomited in a projectile, spasmic manner. For four or five hours following the injection of the filtrate the kitten was weak and sleepy. Upon another occasion, 3 cc. of another preparation of the toxin of strain #52, after being heat-treated, was injected intraperitoneally into a 750 gram kitten. The kitten had several loose stools and vomited one hour after the injection. This kitten recovered from the effects of the enterotoxin more rapidly than the other, for two hours after the injection it appeared to be perfectly normal.

Woolpert and Dack (1933) found that they could successfully actively immunize monkeys against the enterotoxin. Dolman, Wilson, and Cockcroft (1936) also found that kittens given several spaced injections of enterotoxic filtrates became resistent, for blood serum from one such kitten neutralized the enterotoxic properties of an equal volume of a potent filtrate, so that the mixture proved innocuous when injected into a normal kitten.

In our work the same kitten was used as many as three or four times to test the enterotoxic properties of the filtrates. Had more of the strains been found enterotoxic, an immunity could have been developed in certain of these kittens. Immunity can be no factor here, however, in an attempt to explain the lack of enterotoxicity in so many of the strains.

Of the twenty-three strains of staphylococci tested for their enterotoxic qualities, strain #52 was the only one which definitely proved to be enterotoxic. Some of those which didn't produce the symptoms in kittens may have lost their power to produce an enterotoxin because of the fact that they had been grown for some time on laboratory media. Some of the strains were kept for nearly six months, which necessitated several transfers before a filtrate was prepared from them and inoculated into the kittens. Jordan and Burrows (1935) observed that certain strains of staphylococci, which, when freshly isolated from food poisoning outbreaks, had manifested definite toxigenic powers, but after periods of two or three months, and in one case a year the strains lapsed, as it were, and by use of the usual methods of producing enterotoxic filtrates, these strains seemed to have lost their toxigenic power. They found that, by successive transfers on starch medium, the property of producing enterotoxic substance could be restored to a number of these strains. They also found that certain bacteria that had

never yielded enterotoxic filtrates in previous laboratory tests gave positive results after they had been transferred on starch medium.

One enterotoxic strain out of the twenty-three tested is significant, however, for if 4 per cent of all staphylococci which are found to contaminate bakery products were enterotoxic, a considerable amount of work could be well afforded the control of this 4 per cent.

Pathogenic staphylococci have been shown to have the power of producing an enzyme capable of causing the coagulation of citrated or exalted blood plasma (Fisher, 1936). This enzyme is usually absent in saprophytic strains and usually present in pathogenic strains. The formation of coagulase, however, does not parallel hemolysin production. Some investigators claim that the coagulase production correlates better with pathogenicity than does the hemolysin production, but the most widely accepted view is that the two properties, when taken together, show a high degree of correlation with pathogenicity. In carrying out this test, rabbit blood plasma is prepared by drawing fresh blood into a flask containing sufficient potassium oxalate to give approximately 0.5 per cent solution of salt in the blood. The oxalated blood is then centrifuged, and the supernatant plasma used for the test. 0.5 cc. of plasma is placed in a tube and a large loopful of the growth from a 24 hour extract agar culture of the strain to be tested is emulsified well

with the plasma. A similar tube is left uninoculated as a control. The tubes are incubated in a 37°C. water bath for two hours, being examined at one-half hour intervals for coagulation. Table 5 includes the results of this test as carried out on our staphylococci.

Staphylococci are also known to have fibrinolytic properties, that is, some of them have the power of dissolving self-produced fibrinogen clots, while others dissolve plasma clots and destroy fibrinogen in solution. No non-coagulase producing staphylococci are capable of fibrinolysis. Fisher (1936) states that fibrinolytic strains may be more dangerous in human infections since the destruction of the fibrin barrier facilitates spread of infection, and the release of emboli into the circulation. None of our staphylococci exhibited this fibrinolytic property.

Stone (1935) has described a cultural method for the detection of staphylococci of "food-poisoning" type, which makes use of their power to liquify gelatin. His medium consisted of 15 grams of dried gelatin (Difco) and 3.3 grams of beef extract (Difco) per liter, tubed in 4 cc. amounts and plugged with cotton, cork-stoppered, and paraffined to prevent drying. Planting was by stab to the bottom of the medium. After incubation at 37°C. for 24 hours, the medium was placed in a water bath at 21°C. until the control tube became solid. Liquifaction in any degree was considered positive. Dolman, Wilson, and Cockcroft (1936) report that in their

hands this method yielded results out of conformity with those given by the kitten test. Chinn (1936) found no value whatsoever to this method in his study of the reactions of food-poisoning types in gelatin. During the time Chinn was carrying on his investigation, Stone announced a change in his original medium. It now consists of 3 per cent gelatin, 1.5 per cent agar, and 3 per cent beef extract. The medium is poured into plates, and material from the suspected food or pure cultures may be streaked on them. After incubation at 37°C. for 24 hours a developer is poured upon the surface of the medium so that all colonies are completely covered. The developer is made by dissolving one pound of ammonium sulfate in a liter of distilled water. A positive reaction is indicated by a clear zone around the colonies of the food-poisoning type. Chinn tested his known enterotoxic strains on this medium also and found it to give about 61 per cent positive reactions, which is the same value he obtained using the original medium.

Five of our strains gave a clear-cut positive reaction when tested by Stone's revised method. It is most interesting to note the way in which this saturated solution of ammonium sulfate precipitates the albumin in the medium, leaving a clear zone around the colony of the organism which, in the course of its metabolism, apparently has changed the protein content of the medium. These five strains do not correlate in any of their other properties.

A study of these staphylococci isolated from the bakery products has shown (1) that both aureus and albus varieties are present; (2) that the filtrates of some strains are not hemolytic, but that some are capable of causing the hemolysis of 1 per cent washed rabbit erythrocytes in dilutions as high as 1/1600; (3) that one strain of the t wenty-three tested produced a filtrate, 3 cc. of which when injected intraperitoneally into a young kitten produced the typical food poisoning symptoms on two occasions; (4) that eighteen of the strains were capable of causing the coagulation of oxalated rabbit blood plasma; (5) that none of these strains had fibrinolytic properties; and (6) that five of the strains were of the food poisoning type as determined by Stone's revised method. It is apparent that these properties do not correlate in any way, which only further substantiates the observation that staphylococci are not easily classified. Staphylococci of all kinds are present in bakery products.

Streptococci

Streptococci have, in several instances, been found in foods implicated in food poisoning. Table 6 is a summary of the outbreaks reported in the literature in which streptococci have been isolated from the suspected foods. Jordan and Burrows (1934) report that in their laboratory they have repeatedly tested cultures of streptococci from various sources, including suspected foods, and until recently had never obtained results from feeding sterile filtrates of the cultures similar to those manifested in staphylococcic food poisoning. Linden, Turner, and Thom (1926) fed cats with a culture of streptococci from suspected cheese and produced illness, while Cary, Dack, and Myers (1931) fed 40 cc. of a filtrate, which contained living streptococci, of a culture from suspected sausage, to a human volunteer producing signs of illness. In both of these cases, though, the living streptococci as well as their products of metabolism were swallowed. Jordan and Burrows (1934) report an outbreak of food poisoning traced to a cocoanut cream pie, which was found to yield a pure culture of a green-producing streptococcus. They fed 25 cc. amounts of a sterile filtrate of the culture to monkeys and produced symptoms identical with those repeatedly observed after feeding staphylococcus filtrates. To follow up these results they prepared filtrates from nine strains of green-

TABLE 6

Food Poisoning Outbreaks Traced to Streptococci

Year of Out-break	Place	Incrimin-ated food	By whom reported & Year of publication	Number of Persons Involved
1924	New York State	cheese	New York State Health News (1924)	--
1925	Beddeford, Me.	Albanian cheese	Linden, Turner, and Thom (1926)	9
1926	Kansas City, Kansas	American cheese	Linden, Turner, and Thom (1926)	22
1926	Sendai Silk factory	cooked cuttle-fish	Aoki and Sakai (1926)	50
1928	---	---	New York State Health News (1928)	--
1928	Boston	chocolate cream pie	Nelson (1928)	151
1931	Chicago	Vienna sausage	Cary, Dack, and Myers (1931)	75
1933	Winona, Minn.	Cocoanut cream pie	Jordan and Bur-rows (1934)	--

producing streptococci isolated from various sources, and found one from an infected tooth and one from feces yielding the enterotoxic substance. They tested similarly six hemolytic strains of streptococci also isolated from diverse sources, and found one from the throat of a patient with scarlet fever and one from heart blood obtained at autopsy capable of yielding enterotoxic filtrates. This work clearly indicates that streptococci of both alpha and beta types and of diverse origin and character are capable of generating, in their cultures, a substance which when fed to monkeys in amounts of 25 to 50 cc. produces in a few hours vomiting and lassitude. The physical and chemical properties of this substance, so far as they have been studied, are similar to those previously observed to characterize the toxic substance present in certain staphylococcus filtrates.

In studying the strains of streptococci isolated from the bakery products, no effort was made to determine the enterotoxic nature of their filtrates.

Owing to the fact that practically all samples used in this investigation were of a custard-filled nature and consequently required milk in their preparation, the streptococci isolated were studied in an attempt to determine their origin. If the streptococci had gained entrance by way of the milk, a bovine strain probably would be the contaminating organism. On the other hand, the occurrence of a human strain would more than likely indicate human contamination during

the preparation.

A scheme for the identification of strains commonly encountered in human infections which is presented in the American Public Health Association Year Book (1935-1936) has been followed in this study. Their final hydrogen ion concentration, ability to hydrolyze sodium hippurate, and trehalose and sorbitol fermentations have been determined. Table 7, in addition to the data from the differential tests mentioned, includes the culture numbers of the streptococci and the type of hemolysis they produce.

In determining the hemolysis of twenty-four of the strains, a comparison was made as to their ability to hemolyze human, rabbit, and horse blood. For the most part, very little difference could be noted, but in those instances where any difference was apparent, a smaller zone of hemolysis occurred on the human blood plates, and a larger zone on the horse blood plates. The type of hemolysis produced by the various strains is recorded in Table 7 as alpha or the viridans type, beta or the hemolytic type, and gamma or the non-hemolytic type.

The numerous efforts to discover a biochemical method for differentiating streptococci of human and animal origins have only in part been successful. No single differential test in itself has proved to be sufficient. Several investigators (Ayers, 1916, Ayers, Johnson, and Davis, 1918, and Avery and Cullen, 1919) have shown that certain hemolytic

TABLE 7

Results of Studies on Streptococci Isolated From Bakery Products

Culture No.	Sample No.	Isolated from	Hemolysis	Final H ion Conc. (pH)	Na hipp. Hydrolysis	Trehalose	Sorbitol
53	3a	custard-filled doughnut filling	γ	5	0	+	+
57	4a	chocolate eclair custard filling	β	5	0	+	+
58	4a	chocolate eclair custard filling	δ	5	0	+	+
60	4a	chocolate eclair custard filling	β	5	0	+	+
62	4a	chocolate eclair custard filling	β	5	0	+	+
63	3b	custard-filled doughnut dough	β	5	0	+	+
74	8b	lemon-cream pie filling	α	5	0	0	0
76	8b	lemon-cream pie filling	α	6	0	0	0
129	24b	pineapple pastry cake substance	α	6	0	0	0
146	27a	caramel custard pie cream topping	α	5	+	+	0
147	27a	caramel custard pie cream topping	α	6	0	0	0
149	27a	caramel custard pie cream topping	α	6	0	+	0
154	24b	pineapple pastry cake substance	α	6	0	0	0
166	30a	walnut cream pie meringe	α	5	+	+	0

Culture No.	Sample No.	Isolated from	Hemolysis	Final H ion Conc. (pH)	Na hipp. Hydrolysis	Trehalose	Sorbitol
172	32a	Boston cream pie whip-cream topping	α	6	0	0	0
173	32a	Boston cream pie whip-cream topping	α	6	0	0	0
174	32a	Boston cream pie whip-cream topping	β	5	0	+	+
199	39a	raspberry cream pie whip-cream topping	α	5	+	+	0
200	39a	raspberry cream pie whip-cream topping	α	5	+	+	0
201	39a	raspberry cream pie whip-cream topping	α	5	+	+	0
206	41b	Boston cream pie cake substance	α	5	+	+	0
208	41c	Boston cream pie custard filling	α	5	+	+	0
210	39a	raspberry cream pie whip-cream topping	α	5	+	+	0
211	39a	raspberry cream pie whip-cream topping	α	5	+	+	0
213	42a	chocolate nut cake frosting	α	-	0	0	0
216	42a	chocolate nut cake frosting	α	-	0	0	0
219	44b	cocoanut white cake frosting	α	-	0	0	0

Culture No.	Sample No.	Isolated from	Hemolysis	Final H ion Conc. (pH)	Na hipp. Hydrolysis	Trehalose	Sorbitol
220	44c	cocoanut white cake substance	∝	-	0	0	0
227	48b	marshmellow roll pastry shell	∝	-	0	0	0
237	59a	maple cup cake frosting	∝	-	0	+	+

streptococci of bovine origin can be differentiated from human streptococci by the final hydrogen-ion concentration produced when they are grown in glucose broth. In the determination of the final hydrogen-ion concentration produced by our streptococci a dextrose beef-infusion broth was prepared and its reaction adjusted to pH 8.2. Broth cultures of the streptococci in 2 cc. portions were inoculated into tubes containing 5 cc. of the dextrose beef-infusion broth. After 96 hours incubation, the hydrogen-ion concentration was determined colorimetrically using methyl red as the indicator. As a rule, hemolytic bovine strains produce a higher acidity than do hemolytic human strains, those of bovine origin usually resulting in a pH lower than 5 and those of human origin above pH 5.

Ayers and Rupp (1922) were able to separate bovine streptococci from those of human origin by their ability to hydrolyze sodium hippurate. For our purposes, a sodium hippurate pepsin broth was prepared according to a formula given in the APHA Yearbook (1935-1936), which consisted of:

Distilled water	1000 cc.
Peptone	10 gm.
Pepsin	5 gm.
Calcium chloride	0.3 gm.
Ferric chloride (1% solution)	1 drop
Sodium hippurate	5 gm.

The sodium hippurate is added to only one-half the amount prepared, the other half being used for control purposes. The reaction is adjusted to pH 7.1. Broth cultures of the

streptococci in 0.2 cc. amounts added to 5 cc. of both the sodium hippurate pepsin broth and the broth not containing sodium hippurate. After 48 hours incubation 0.5 cc. of a 12 per cent ferric chloride solution is added to 2 cc. amounts of the cultures to test for the occurrence of hydrolysis. After thoroughly shaking the tube, the hydrolysis of the sodium hippurate is noted by an insoluble precipitate of ferric benzoate, which remains in the tube. The hydrolysis of sodium hippurate results in the formation of benzoic acid and glycocoll. The useful ness of this method has thus far only been proven for the differentiation between beta hemolytic streptococci of human and bovine origin. Ayres and Rupp in their investigation found sodium hippurate to be hydrolyzed by 44 strains of hemolytic streptococci from the udders of cows, but not by the 33 strains of hemolytic streptococci of human origin which were in their culture collection. They found that some alpha types from the udder of the cow did not produce the hydrolysis, while the hydrolyzing property, on the other hand, is common among the lactic type of streptococci. Furthermore, their studies did not show that the ability to split sodium hippurate was limited to hemolytic types. This test, therefore, cannot be applied indiscriminately to all types of streptococci.

Edwards (1932) working with a group of hemolytic streptococci derived from bovine and other domestic animals which had not successfully been differentiated from human strep-

tococci, carried out a series of fermentation reactions. These strains were characterized by low acid production, inability to hydrolyze sodium hippurate, and active hemolysis of blood cells in a fluid medium---characteristics, all of which, are possessed by streptococci of human origin. He found that 94 per cent of these strains could be clearly differentiated from human strains by their ability to produce acid from sorbitol and their failure to attack trehalose. Accordingly, trehalose and sorbitol fermentations were carried out on all of our streptococci. Tubes of 3 per cent chlor-phenol-red beef-infusion broth containing 1 per cent trehalose or 1 per cent sorbitol were inoculated with 0.1 cc. of broth culture of the organism in question. As a control on the amount of natural sugar in the medium, a tube of the medium to which no carbohydrate had been added was similarly inoculated. The tubes are incubated for one week, being examined for presence of fermentation after one, two, and seven days. This test, as well as the other differential tests, proves to be valuable only for a certain group of streptococci, and can only be used to substantiate the data acquired from the other tests.

The results of these tests carried out to differentiate between human and bovine streptococci, do not present a clear-cut division by any means. As can be gathered from the discussion of the tests, new means of differentiation have been devised only to provide a way of distinguishing

between human and bovine strains which could not be separated by other methods. This leads to a lack of correlation among the tests.

By their hemolytic ability and failure to hydrolyze sodium hippurate, five of the strains can be called human in origin. No strains having these properties were found to produce acid from sorbitol and not attack trehalose. Thus the sorbitol and trehalose fermentations were of no value in the differentiation. These five strains, however, produce a higher acidity than other of the strains, which would not substantiate their human origin.

Since the usefulness of sodium hippurate hydrolysis has only been proven for the differentiation between beta hemolytic streptococci of human and bovine origin, it was of no value in the differentiation of our alpha strains. None of our beta hemolytic strains hydrolyzed sodium hippurate, so no further bovine strains could be recognized in that way.

Of the twenty-three alpha hemolytic strains, on the basis of their acid production, only ten of them would be bovine and seven of them human.

Although a very poor differentiation can be noted as a result of these tests, it is evident that strains of both human and bovine origin undoubtedly are present. Thus the contamination of these products may be the result both of contaminated dairy products and human contact.

Intestinal Organisms

The intestinal organisms isolated in this study include eight strains of *Escherichia coli* (confirmed after typical lactose fermentation reaction by characteristic colony formation on Endo's agar), two strains of *Aerobacter aerogenes*, (recognized by typical lactose fermentation reaction), and five strains of organisms of the Salmonella group (determined by their failure to ferment lactose and their fermentation of glucose with acid and gas). Gilbert, Coleman, and Laviano (1932) attribute certain food poisoning outbreaks to toxic substances formed by strains of *Aerobacter cloacae* and members of the Proteus group. Reports of this kind, to our knowledge, are exceedingly rare, and hence no effort has been made in this study to determine the presence of these organisms. Jordan and Burrows (1935) give reference to instances in which *Serratia marcescens* has been the suspected organism in food poisoning outbreaks, and also instances in which the ubiquitous hay bacillus was suspected. These, however, are also rare.

Jordan and Burrows (1935) in their study of enterotoxic bacterial filtrates, obtained evidence that a typical strain of *Escherichia coli* grown under certain conditions, can produce an enterotoxic substance, for a strain which they had isolated from cow dung and kept as a stock culture in

the laboratory, after six transfers on a custard medium, yielded a filtrate, 25 cc. of which produced vomiting in a susceptible monkey. This enterotoxic property was lost until again induced by transplantation on starch agar or the custard medium. Aside from the sanitary significance, then, which the occurrence of these strains of Escherichia coli isolated from the bakery products might have, they are of some consequence as a possible source of food poisoning. A number of food poisoning outbreaks have been recorded in which Escherichia coli has been found in the incriminated food in enormous numbers. Cheese has been the most common offender in these instances.

Jordan and Burrows (1935) succeeded also in producing an enterotoxic filtrate from a strain of Aerobacter aerogenes, which was originally isolated from an egg salad sandwich, one of a lot suspected to be epidemiologically implicated in an extensive food poisoning outbreak effecting 150 or more persons. They were able to again induce the toxigenic property of the strain by transfer on starch-containing medium.

The rare occurrence of organisms of the Salmonella group as compared with the frequent isolation of staphylococci in our investigation is only in accordance with the fact that Salmonella food poisoning outbreaks are rarely reported in this country. Savage and White (1925) in a study of one hundred outbreaks occurring in Great Britan listed

Salmonella aertrycke as their greatest offender, with Salmonella enteriditis next in order. Dack, Cary, and Harmon (1928) attempted to demonstrate the toxicity of sterile filtrates of Salmonella aertrycke and Salmonella enteriditis, but met with no success. Jordan and Burrows (1935) in attempting to parallel their work on staphylococci made another attempt to obtain a toxic Salmonella filtrate. At this time they succeeded in causing illness in monkeys with a filtrate of one strain of Salmonella aertrycke which had been transferred six times on the starch agar medium. Another strain of Salmonella aertrycke (typhi murium), which they were fortunately able to obtain soon after isolation, gave positive results when freshly tested, but promptly lost its enterotoxic power. However, it regained its toxigenic qualities after starch agar transfer. These results confirmed the contention of Savage (1932) that the power of producing an enterotoxic substance is possessed by some freshly isolated strains of Salmonella, but that this toxigenicity is lost on cultivation, so that the property is not manifested by old stock strains. No effort was made to determine the toxicity of filtrates of the strains isolated in this study.

Salmonella food infection has been traced in a few instances to custard-filled bakery products, although staphylococci are more commonly found to be contaminants of such foods. Staff and Grover (1936) report an outbreak of food infection

producing the classical gastroenteric symptoms in a reported total of 208 persons and fatal terminations in 3 of the cases. A cooked-cream filling contaminated with Salmonella enteritidis seemed to be the common factor in all cases. Bacteriologic and epidemiological evidence suggested the possibility of rodents as the contaminating agents. The more widespread occurrence of staphylococci in general probably accounts for the higher incidence of staphylococcic food poisoning in this type of food, for it is evident that the rich custard, because of the nature of its ingredients--sugar, milk, eggs, and starch--would offer an unexcelled culture medium for either type of organism.

Possible Sources of Contamination

In an effort to trace the possible sources of contamination of these bakery products, some consideration may well be given to those constituents which are frequently added to such products after the cooking or baking process is completed. The banana is often used in cream-filled pies, whip cream cakes, or various fancy pastries, all of which are not, as a rule, cooked or baked after the banana is added. It occurred to us, therefore, that the banana might well be a source of contamination in the many food products in which it is found.

Does the banana pulp actually reach the consumer in a "germ-proof package sealed by nature", as is so frequently referred to? It seems possible that such a porous skin which allows gaseous exchange in controlling the ripening process might be penetrable to bacteria. Tellarico (1908) while studying the enzymatic changes taking place during maturation of bananas, attempted to determine the bacteriologic condition of the normal fruit. His efforts to cultivate bacteria in sterile boullion, agar, and gelatin with banana pulp as the source of infection were so generally negative in result as to lead him to conclude that normally the fruit is sterile. Bailey (1912), in making bacteriologic and biochemical studies on the banana, found the inner portion of the pulp of sound bananas to be

practically sterile, but in the regions of the inner coats of the peel he found a few bacteria, which he felt were held in check during normal ripening. He found the banana peel to be a means of protection against bacterial invasion through experimental work consisting of immersing bananas in tap water and in distilled water to which an active culture of the bacillus of malignant edema (Clostridium oedematis-maligni) had been poured.

Some experimental work on banana pulp and the penetrability of the skin led us to agree that the pulp of a normal banana with unbroken skin is sterile. The pulp from five normal bananas was removed aseptically, sliced with a sterile knife into Petri dishes, and incubated at 37°C. for one week. The plates remained absolutely sterile with the exception of one, which contained a section from the stem end of one of the bananas. The organism present was a gram-positive, spore-forming rod. Further proof of the sterility of the pulp was gained by macerating sections of pulp from various portions of five bananas in sterile saline and plating out the suspension in different dilutions. After incubation at 37°C. for four days all of the plates were sterile.

No relationship seemed to exist between the spots on the skin of the banana and bacterial growth. No bacteria could be isolated from scrapings under the spots on the skin; no difference could be noted between the bacteria which

could be isolated from the spots on the skin and those from any other portion of the skin; no spots could be produced by swabbing pure cultures of various organisms on the skin and allowing the banana to incubate for periods as long as one week.

A study of the bacteria which we isolated from the skin of the banana, showed that staphylococci are present in greater numbers than any other single morphological group of bacteria. Thus, if the pulp is sterile in itself, the possibility that it may act as a carrier still exists. The presence of so many staphylococci on the skin of the banana and the fact that a certain amount of manipulation by hand is indispensable in peeling and slicing it, point to the banana as a carrier in the problem. We found banana pulp, in itself, to be an excellent medium for supporting bacterial growth. Staphylococci, streptococci, organisms of the Salmonella group, the diphtheria bacillus, Escherichia coli, and the typhoid bacillus remained alive on sections of banana pulp left in Petri dishes at room temperature for as long as twelve days. Multiplication was evident in the case of the staphylococci, the typhoid bacillus, and Escherichia coli. It is not amiss, then, to conclude that organisms which find their way to the banana pulp, may well remain alive, even multiplying in some cases, until they are placed in some bakery product, especially those of a cream or custard-filled nature, where they have excellent opportunities

for growth and to produce their toxic products when they are of a nature to do so.

Visits to twelve restaurants where bananas are used extensively, and to two pie factories, which bake banana cream pies daily, revealed the fact that many times a secondary grade of bananas are used. These, often being torn from the hand, have exposed ends and are frequently cut and badly bruised. These more incriminating factors, however, are counteracted by the fact that no banana cream pie is left from one day to the next to be served, and fruit salads are consistently made just before use. Thus little time is given for the bacteria to multiply and produce enterotoxic substances.

Nuts of all kinds, raisins, cocoanut, chocolate flakes, and other substances which are commonly added to custard-filled pastries may also be considered as sources of contamination. They are frequently added by hand to these products and may well be carriers of contaminating organisms.

Since milk is an essential constituent of all custards, we have also considered the possibility that it might be responsible for carrying bacteria into these bakery products. The occurrence in bakery products of streptococci, seemingly of bovine origin, lends further support to this theory. A study of thirty milk samples obtained through the courtesy of the City Health Department laboratory revealed that, of the twenty-three organisms isolated, eight of them were

staphylococci. Very frequently in the preparation of custards, a temperature corresponding to that of the thermal death point of most staphylococci is not reached. The high percentage of staphylococci found to be present in these samples, which might be a fair example of the bacterial content of milk used by bakeries in general, surely is worthy of some consideration in the problem of tracing the source of contamination of these products.

SUMMARY

The isolation and study of organisms which are found to be present in bakery products obtained on the open market indicate that considerable numbers are present especially in those products of a custard or cream-filled nature. Staphylococci are present in the greatest numbers, although streptococci and intestinal organisms are also found frequently. Allowing the products to stand from one day to the next at room temperature is responsible for a considerable rise in the bacterial count. Consistently higher bacterial counts are obtained for bakery products obtained in July than for those obtained in March and April.

Staphylococci of both albus and aureus varieties, which do not correlate in hemolytic titre, plasma coagulative ability, or fibrinolytic power, or by Stone's method of detecting food poisoning types, were isolated. One strain out of twenty-three tested produced a filtrate, 3 cc. of which produced typical food poisoning symptoms upon intraperitoneal injection into a kitten. Streptococci of alpha, beta, and gamma types were isolated, some of which appear to be bovine and others human in origin, as determined by their acid production, ability to hydrolyze sodium hippurate, and trehalose and sorbitol fermentation. Eight strains of Escherichia coli, two of Aerobacter aerogenes, and five of the Salmonella group were isolated in this in-

vestigation.

The banana is a possible carrier of contaminating organisms to these bakery products, for it, as well as such substances as raisins, nuts, or cocoanut, is commonly added to custard-filled bakery goods after the product has been cooked or baked. The pulp of a normal banana with unbroken skin is sterile, but it was found to be an excellent medium for supporting bacterial growth. A significant number of staphylococci were isolated from representative milk samples, which indicates that milk may also be a contaminating factor for bakery products.

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