Certain Factors Affecting the Growth of Food Poisoning Micrococci*

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THE increasing number of food poisoning epidemics which are traceable directly to preformed toxins produced by certain species of micrococci (staphylococci) have caused health officers to take notice of this type of gastrointestinal upset. Little information is available on the general characteristics of these organisms other than the work of Dack *et al.*[1] and others who have studied in part the cultural characteristics of the micrococci responsible as well as the factors associated with their toxin production.

Stone[2] has reported that all of the micrococci associated with food poisoning epidemics have one cultural characteristic in common, namely, gelatin liquefaction. It has been known for some time that a large percentage[3] of micrococci liquefy gelatin and particularly the *Micrococcus aureus* types which are usually encountered in gastrointestinal upsets traceable to toxin producing micrococci. Stone has reported that a special medium prepared by drying the gelatin before making up and increasing the amount of beef extract from 0.3 per cent to 3 per cent, will produce a medium which micrococci will more frequently liquefy than the commonly used medium with 12 per cent gelatin and 0.3 per cent beef extract.

A study (Table I) of the relative rapidity of gelatin liquefaction as compared in ordinary nutrient gelatin and Stone gelatin indicates that toxin producing micrococci are somewhat more active from the standpoint of gelatin liquefaction in Stone's gelatin than in the more commonly used nutrient gelatin. Certain strains, particularly Nos. 8, 42, and 135, originally received from Jordan, were very slow liquefiers of ordinary nutrient gelatin but in Stone's gelatin liquefaction was much more rapid, while there were a few strains of Stone's gelatin in which liquefaction was slower. Repetition of these tests indicated that the rapidity of gelatin liquefaction is a very valuable character of the toxin producing micrococci. Certain other factors are involved in controlling the rapidity of gelatin liquefaction and as a cultural character it is not particularly suitable for differentiating the toxin producing type as a group.

The drying of the gelatin probably plays little part in increasing the efficiency of Stone's medium, but an increase (Table II) in the percentage of meat extract was found to increase the rate of gelatin liquefaction. Experiments in which varying amounts of beef

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TABLE I

Relation of Standard Gelatin and Stone's Gelatin to Time of Liquefaction

Culture Number	Number of Hours First Liquefaction Was Noted: A.P.H.A. Standard Gelatin (1907)	Stone's Gelatin	History of Cultures	Source
B2	24	24	Illinois State Department of Health	Milk
*3	..	..	Lister Institute	Cake
*8	2,080	48	University of Chicago	Cake
*9	24	24	Lister Institute	Chicken gravy
*42	2,080	48	Lister Institute	
*95A	48	24	University of Chicago	Cream filled cake
*95B	24	48	National Institute of Health	Cream filled cake
*95C	24	24	Lister Institute	Cream filled cake
*135	2,080	912	Lister Institute	Infected compound fracture
*137	48	912	National Institute of Health	Abscess on thigh
173E	24	24	U.S.D.A.	Cream pie filling
35,556–B7	48	48	U.S.D.A.	Pickled tongue
35,556–B9	24	48	U.S.D.A.	Pickled tongue
35,556–B13	24	48	U.S.D.A.	Pickled tongue
35,556–B14	24	24	U.S.D.A.	Pickled tongue
49,711–B1	24	912	U.S.D.A.	Cream filled pastries
49,711–B2	24	48	U.S.D.A.	Cream filled pastries
49,711–3P7	24	48	U.S.D.A.	Cream filled pastries
49,711–3P12	24	..	U.S.D.A.	Cream filled pastries

* Strains originally obtained from Dr. E. O. Jordan

extract from 0.7 per cent to 3.0 per cent were used has shown that in most cases a gelatin which contains 2 per cent or more beef extract was more susceptible to liquefaction by the toxin producing micrococci than one with smaller amounts. These observations could not be confirmed in all cases, as the organisms varied somewhat in their rate of gelatin liquefaction with increased amount of meat extract.

It will be noted that the onset of liquefaction was exceedingly slow for many of the strains. It is debatable whether an organism which takes nearly 3 months to begin liquefaction should be considered as a liquefier in the same sense as other strains which produce demonstrable liquefaction in relatively few hours.

The toxin producing micrococci appear to be ubiquitous in nature. They are found in many sources and may be prevalent in many types of food. A study of the udder flora of over 7,000 cows shows that over 20 per cent discharge an orange liquefying micrococcus similar in cultural characteristics to the toxin producing types. However, there was no evidence of toxin production judged by effects on the consumers of their milk. A study was made of 32 chicken pies served at public suppers, in which relatively accurate information was available as to any effects of preformed toxins. In all relatively large numbers of orange liquefying micrococci were found but no evidences of gastrointestinal disturbances were noted in any of the consum-

TABLE II

Effect of Per cent of Beef Extract Upon Liquefaction of Gelatin by Food Poisoning Micrococci

	Number of Hours First Liquefaction Was Noted in Increased Percentages of Beef Extract			
Culture No.	*0.7*	*1.0*	*2.0*	*3.0*
B2	48	24	24	24
3	..	..	..	..
8	1,400	1,400	144	144
9	168	168	48	48
42	1,400	1,400	240	144
95C	24	24	24	24
95A	168	48	24	48
95B	336	168	168	48
135	2,030	2,080	2,080	720
137	336	336	144	240
173E	168	48	24	48
35,556–B7	1,200	720	48	48
35,556–B9	168	48	24	24
35,556–B13	168	168	144	48
35,556–B14	336	168	144	144
49,711–1	1,200	720	144	144
49,711–2	960	48	144	48
49,711–3P7	1,400	720	144	48
49,711–3P12	168	168	48	48

ers. These micrococci were identical with the toxin producing types with the exception of the toxin producing property. It is apparent either that the toxin producing types are widely distributed in nature requiring conditions favorable to toxin production, or our present cultural reactions are inadequate for their identification.

If these types are so apparently widespread and in most instances retarded in their ability to produce toxin, information is necessary as to the factors which might inhibit their development in food when present. Certain ingredients in prepared foods are known to have an inhibiting effect on the growth of organisms. Information is lacking on the effect of these substances on the growth of toxin producing micrococci. A study was made (Table III) of the effect of sucrose upon these organisms and the minimum amount which must be present to inhibit their growth. Increasing amounts from 20 to 50 per cent sucrose were added to veal broth which was subsequently heavily inoculated with 2 standard strains of toxin producing micrococci then incubated at 37° C. for 18 days. The toxin producing micrococci developed in relatively high concentrations of sucrose. For the first 24 hours. growth was vigorous in concentrations

TABLE III

Effect of Sucrose Upon Growth of Food Poisoning Micrococci

		Number (million) Organisms per c.c. in Increasing Percentages of Sucrose							*Number of Organisms (millions) in Inoculum*
Culture	*Time of Incubation*	*20*	*25*	*30*	*35*	*40*	*45*	*50*	
95	24 hours	1,790	5,190	2,500	1,570	1,300	2,340	3,090	28
	48 hours	3,070	2,420	3,180	1,890	1,780	2,530	2,890	
	5 days	3,920	6,400	3,120	52	601	410	348	
	14 days	15	970	1,930	....	....	....	....	
	18 days	....	....	....	....	....	....	....	
42	24 hours	1,920	2,570	2,850	2,160	3,950	3,030	2,930	476
	48 hours	3,570	3,580	3,100	3,290	1,780	2,640	2,280	
	5 days	5,000	6,000	255	2,640	3,100	14	113	
	14 days	5,200	710	16	91	1,090	....	....	
	18 days	....	....	93	....	....	....	....	

as high as 50 per cent. During the second 24 hours, growth was materially checked in the higher concentrations although no decided reduction in number was apparent. At the end of 5 days, the numbers of micrococci were materially reduced, probably not through the action of the high concentrations of sugar alone, but due in part to the presence of the acid developed in the medium. Repeated observations showed that from 5 until 18 days the number of organisms in the higher percentage of sucrose rapidly decreased. In a medium containing 35 per cent of sucrose or more the organisms were practically all inhibited or killed at the end of 18 days. At the end of 14 days it was found that 20 per cent of sucrose broth was sufficient to reduce materially the number of organisms. The acid produced by the organisms in the medium is not an important factor in itself, in this case, as an increased amount of sucrose did not increase the amount of acid formed. The amount of acid produced was as great in the lower as in the higher concentrations. The combined action of the acid and sucrose may have been responsible for the reduction in number of viable micrococci in the flasks containing the larger amounts of sucrose.

Similar experiments were carried out with various concentrations of sodium chloride. It was noted that the toxin producing micrococci are somewhat resistant to all concentrations of sodium chloride up to and including 12 per cent, although in concentrations as low as 6 per cent growth was materially inhibited as compared with the controls containing no sodium chloride. It is no doubt difficult to obtain sufficient practical concentration of sodium chloride foods to inhibit definitely the growth of all the cells contained in cultures of toxin producing micrococci. Sodium chloride appeared to have no killing effect, as the numbers after 48 hours' incubation remained approximately constant through the 18 day incubation.

Similar experiments were done using various amounts of acetic acid as the inhibiting agent. Veal infusion containing as a minimum 0.05 per cent and increasing to 0.25 per cent was prepared and inoculated with 2 strains of toxin producing micrococci. It was found (Table V) that acetic acid has a very definite inhibiting effect. Concentrations as low as 0.05 per cent materially reduced the growth rate below that found in the control containing no acetic acid. Concentrations as low as 0.15 per cent at the end of 48 hours brought about a decided reduction in the number of organisms, while at the end of 7 days, media containing 0.2 to 0.25 per cent were practically free of organisms. In contrast to the effect of sucrose and sodium chloride, acetic acid had an inhibiting effect in low dilutions and a definite killing effect in higher concentrations.

TABLE IV

Effect of Sodium Chloride Upon Growth of Food Poisoning Micrococci

Culture	Time of Incubation	Number (million) of Organisms per c.c. Found in Increasing Percentages of Sodium Chloride							Number of Organisms (millions) in Control
		6.0	7.0	8.0	9.0	10.0	11.0	12.0	
95	24	520	410	340	420	310	140	50	1,040
95	48	400	320	380	268	270	186	136	1,430
42	24	540	260	250	260	220	270	160	550
42	48	320	213	210	258	195	140	125	1,000

TABLE V

Effect of Acetic Acid Upon Growth of Food Poisoning Micrococci

Culture No.	Time of Incubation	Number of Organisms (million) per c.c. in Increasing Percentages of Acetic Acid					Number of Organisms (millions) per c.c. In	
		0.05	0.10	0.15	0.20	0.25	Inoculum	Control
95	24 hours	1,470	170	3,960	7,200	0.2	7	2,400
	48 hours	1,450	590	0.7	....	....		2,090
	4 days	2,900	890	....	....	....		3,490
	7 days	1,870	1,350	....	....	....		2,680
42	24 hours	350	179	59	2	0.1	27	1,270
	48 hours	265	330	153	....	....		1,400

TABLE VI

Associative Action of Acetic Acid, Sucrose, and Sodium Chloride on the Growth of Food Poisoning Micrococci

Culture No	Time of Incubation	Number (million) Organisms per c.c. In			
		Sucrose 30% Sodium Chloride 10% Acetic Acid 0.10%	Sucrose 35% Sodium Chloride 11% Acetic Acid 0.05%	Inoculum	Control
95	24 hours	40	35	15	960
	48 hours	100	150		1,220
42	24 hours	35	34	19	750
	48 hours	58	48		1,250

Work of Pederson and Breed [4] has indicated that a combination or associated action of various inhibiting agents might take place when each agent in this combination is used in a per cent which when used alone was not sufficient to inhibit growth. With this in mind 2 media were prepared, one which contained 30 per cent sucrose, 10 per cent sodium chloride, and 0.1 per cent acetic acid, and the other 35 per cent sucrose, 11 per cent sodium chloride, and 0.05 acetic acid. Any one of these ingredients in the percentage used, if used alone would not materially inhibit growth. However, when used in combination (Table VI) a decided inhibitive action was apparent. In this particular experiment, 15,000,000 organisms were inoculated into these 2 media as well as the control. At the end of 24 hours, although a small amount of growth had taken place, it was materially less than that in the control.

It is apparent that types indistinguishable culturally from the toxin producing micrococci are widespread in milk and food products. It is also indicated that sucrose and sodium chloride have very definite inhibiting action upon the growth of the toxin producing micrococci, while small percentages of acetic acid not only inhibit but reduce the number of these organisms in veal infusion broth media. These ingredients were found to act in lower concentrations as inhibiting agents when used together than alone.

REFERENCES

1. Dack, G. M., *et al.* An Outbreak of Food Poisoning Proved to be Due to a Yellow Hemolytic Staphylococcus. *J. Prev. Med.*, 4:167, 1930.

2. Stone. A Cultural Method for Classifying Staphylococci as of the "Food Poisoning" Type. *Proc. Soc. Biol. & Med.*, 33:185–187, 1936.

3. Hucker, G. J. Studies on the Coccaceae. II. A Study of the General Characters of the Micrococci. *New York State Agri. Exper. Sta. Bull. 100*, 1924, 83 pp.

4. Pederson, C. S., and Breed, R. S. Preservative Action in Catsup of Salt, Sugar, Benzoate and Acid. *New York State Agri. Exper. Sta. Bull. 538*, 1926, 15 pp.

5. Wolpert, O. C., and Dack, G. M. Relation to Gastro-intestinal Poison to Other Toxic Substances Produced by Staphylococci. *J. Infect. Dis.*, 52, 6, 1930.