ON CERTAIN FACTORS INFLUENCING THE SURVIVAL OF BACTERIA IN WATER AND IN SALINE SOLUTIONS

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Received for publication December 3, 1929

This paper deals with the survival of bacteria in the apparent absence of nutrient material (unwashed bacteria in 0.85 per cent NaCl solution and in distilled water); in the actual absence of nutrient material (washed bacteria in the same suspending fluids); and in the presence of varying amounts of nutrient material (with addition of broth to the above suspending fluids). It also contrasts bacterial survival in distilled water with survival in 0.85 per cent NaCl solution and deals with the influence of bacterial concentration on survival in the above fluids at various temperatures.

LITERATURE

Prolonged survival in water. Most of the observations on survival of bacteria in water have been made upon bacterial concentrations insufficient to allow survival longer than 2 or 3 months (Strauss and Dubarry, 1889; Frankland, 1895; Wheeler, 1906; Livingstone, 1921). A few instances of much more prolonged survival in water have been recorded (*S. cholerae*, 7 months, Ficker, 1898; *B. typhosus*, 490 days, Konrádi, 1904). I have been unable to find any record of prolonged survival of bacteria in NaCl solution.

Factors influencing survival in water and in saline solutions. Among the factors which have been found to influence the survival of bacteria in aqueous suspensions, the following may be noted: the character of the water and particularly its content of organic matter (Bolton, 1886; Wheeler), temperature (Frankland;

Wheeler), diffuse light (Wheeler), sterilization of the water (Frankland; Wheeler), the amount of inoculum (Ficker; Livingstone), the age of the culture used in making the suspension (Ficker), the addition of minute amounts of culture media (Bolton), and previous prolonged contact of the water with metal (Ficker). In addition, Cohen (1922) and Winslow and Falk (1923) have extensively investigated the influence of the pH of water and of saline solutions on the survival of bacteria. Winslow and Falk (1918 and 1923), Panisset, Verge and Carneiro (1925), and Shaughnessy and Criswell (1925), and Duthóit (1923) have compared the viability of bacteria in distilled water with that in saline solutions of varying concentration. Ficker and Cohen have made some observations on the influence of substances absorbed into the water from glass containers. Whipple and Mayer (1906) have shown that lack of oxygen unfavorably influences longevity of *B. typhosus* in water. The viability of bacteria in water in nature, or under conditions intended to simulate those in nature has been studied by Houston (1912 and 1913–1914), Jordan, Russell and Zeit (1904), Russell and Fuller (1906), and others. The comparative viability of washed and unwashed bacteria in aqueous suspensions has been investigated by Winslow and Brooke (1927). The organisms whose survival in saline solutions has been most frequently studied are *B. typhosus*, *B. coli*, and *S. cholerae* while the survival of others such as *Strep. haemolyticus* (Livingstone), and *Staph. aureus* (Bolton; Panniset, Verge and Carneiro, and others) has also been investigated to some extent.

MATERIAL AND METHODS

The organisms employed in the following studies were laboratory stock cultures, most of which had been grown for prolonged periods on artificial media. Acid-cleaned, sterile, ordinary glass test tubes were used for storing bacterial emulsions to be tested for survival, the tubes being plugged with cotton stoppers, but not sealed. A mark was made to indicate the height of the fluid in the tubes, and loss by evaporation was subsequently made up to the mark from time to time, by the addition of sterile distilled

water. Chemically pure sodium chloride was used in making NaCl solutions, and in some of the later experiments Merck's blue label quality was employed. Both the saline solutions and the distilled water were sterilized in pyrex flasks, in the autoclave, at 15 pounds pressure, for 15 minutes.

Colony counts were made by pipetting and suitably diluting 0.01 cc. of the bacterial emulsion, and sowing, on nutrient agar pour plates, a quantity likely to yield 100 to 200 colonies. In all cases bacterial emulsions to be tested were first thoroughly agitated. Sodium chloride solution 0.85 per cent was used as the diluting fluid.

Washing was performed by centrifuging bacterial emulsions, twice resuspending and centrifuging them in distilled water or in 0.85 per cent NaCl solution, under aseptic precautions. When supernatant fluid was to be used, it was always obtained after the first centrifuging.

Purity of emulsions of bacteria of the colon-typhoid group was controlled by obtaining typical reactions on Russell media, from time to time. Gram stains were also made at various intervals from bacterial emulsions used in the experiments.

PROLONGED SURVIVAL

In this experiment bacterial emulsions were made by washing the growth off twenty-four-hour (*B. tuberculosis*, forty-eight-hour) agar slant cultures, making 10 cc. quantities of emulsion containing about 2 to 3 billion colony producers per cubic centimeter. These emulsions were kept in the dark and were tested for viability, from time to time, by sowing one loopful of emulsion on an agar slant.

1. Prolonged survival in 0.85 per cent NaCl solution. (*a*) At 37°C.: *B. pyocyaneus*[3] remained viable $30\frac{3}{4}$ months. *B. paratyphosus* A[2] and B,[2] *B. entertidis*,[2] *B. coli-communis*,[2] *B. dysenteriae* Shiga,[2] *B. tuberculosis*,[2] and *Strep. viridans*[2] (*ignavus*) remained viable for $13\frac{1}{2}$ months. *B. typhosus*[1] (strain 1) remained viable

[1] Found to be non-viable a few months later.
[2] Not tested later.
[3] Still under observation.

for 8 months, while *B. typhosus*[1] (strain 2, Rawlings) remained viable for 5 months. *B. mucosus-capsulatus*[1] survived 3½ months. (After 30¾ months *B. pyocyaneus* still showed pigment production. After 13½ months *Strep. viridans* showed methemoglobin production.) (*b*) At room temperature: *B. typhosus*[3] survived 32 months. *B. pyocyaneus*[3] and *B. coli*[3] survived 31¼ months, and *B. paratyphosus* B[1] survived 21¾ months. (*c*) At 0° to 8°C.: *B. typhosus*[1] remained viable 25¾ months; *B. pyocyaneus*,[1] 25 months; and *B. tuberculosis*,[1] 16 months.

2. Prolonged survival in distilled water. (*a*) At 37°C.: *B. pyocyaneus*[3] survived 30¾ months; *Strep. viridans*[1] (*ignavus*), 21¾ months; *B. tuberculosis*,[1] 16 months; and *B. typhosus*[1] (Rawlings), 14 months. (*b*) At room temperature: *B. typhosus*[3] (Rawlings) remained viable 32 months; *B. pyocyaneus*,[3] *B. coli*,[3] *B. mucosus-capsulatus*,[3] and *B. tuberculosis*,[3] 31¼ months; and *B. paratyphosus* B[1], 25 months. (*c*) At 0° to 8°C.: *B. pyocyaneus*[1] and *Strep. viridans*[1] (*ignavus*) survived 25 months; *B. typhosus*[1] (Rawlings), 22½ months; *B. tuberculosis*[1], 21¾ months; and *B. coli*[1] and *B. paratyphosus* B[1], 16 months.

In my experience, survival of the organisms mentioned above has been prolonged much more uniformly in distilled water and in 0.85 per cent NaCl solution, than on solid media. Cultures of the same strain made at different times on solid media but stored under the same conditions may either die within 2 months or survive more than 6 months.

EFFECT OF BACTERIAL CONCENTRATION ON SURVIVAL AT VARIOUS TEMPERATURES, (1) IN 0.85 PER CENT NaCl SOLUTION, (2) IN DISTILLED WATER

In this series of experiments eighteen- to twenty-hour agar slant cultures of *B. typhosus* were washed off and emulsified; some, in distilled water, and others, in 0.85 per cent NaCl solution. From emulsions made in this way, a set of 10 dilutions was prepared for each experiment, with a bacterial concentration in each dilution 50 per cent of that in the preceding one, the first dilution in each case containing approximately 3 to 4 billion colony producers per cubic centimeter (10 cc. quantities in 18

mm. test tubes). One of these sets of emulsions in 0.85 per cent NaCl solution was stored at 37°C., another at 15° to 20°C., and

TABLE 1

Colony count per cubic centimeter of emulsion of B. typhosus (Rawlings) after time indicated in 0.85 per cent NaCl solution at 37°C.

DILUTION NUMBER	1 HOUR	24 HOURS	42 HOURS	4 DAYS	9 DAYS	15 DAYS	22 DAYS	29 DAYS	43 DAYS	56 DAYS
1	3075M	2700M	2260M	500M	76M	6.4M	1.6M	1.8M	1.3M	670T
2	1540M			1400M	10M	2.4M	580T	420T	410T	430T
3	870M	837M	250M	30M		1M	142T	155T	244T	96T
4	440M			1M	750T	74T	100			
5	220M	56M	7.5M	300T	200	0				
6	110M				10−					
7	60M	4M	266T	100−	0					
8	30M				1					
9	15M	400T	5T−	5−	1					
10	8.9M			0	0					

TABLE 2

Colony count per cubic centimeter of emulsion of B. typhosus (Rawlings) after time indicated in 0.85 per cent NaCl solution at room temperature (15° to 20°C.)

DILUTION NUMBER	5 HOURS	36 HOURS	8 DAYS	11 DAYS	15 DAYS	25 DAYS	36 DAYS	49 DAYS	88 DAYS	128 DAYS
1	4530M	2950M		2489M	1670M	410M	620M	35M	67M	55M
2	2260M									
3	1130M	1410M								
4	566M			405M	386M	185M	7M			1M
5	283M	277M								
6	141M			40M	48M	4M	180T	277T	240T	150T
7	70M	23M			6.4M		14T	18T	0	
8	35M				380T	14T	10−			
9	17M				6T	100−				
10	8.8M		800	320	30	0				

M = millions; T = thousands; − = less than.

N.B.: Figures given in 1 hour and 5 hour columns are based on actual colony counts of dilutions 1 and 10.

a third at 0° to 8°C. Likewise one set of these emulsions in distilled water was stored at each of the above temperatures. Two sets (two different strains) of *B. typhosus* were placed in 0.85

per cent NaCl solution at 37°C. Colony counts were made at intervals by the method indicated above under "methods." The results are given in tables 1 to 6.

1. Survival of B. typhosus in 0.85 per cent NaCl solution (tables 1 to 4). Only 4 dilutions survived 15 days in 0.85 per cent NaCl solution at 37°C. (i.e., only those containing 440 million colony producers or more per cubic centimeter).[4] At room temperature all 10 dilutions survived for this period (the tenth containing 8.8 million colony producers per cubic centimeter); and at 0° to 8°C. 6 dilutions survived the 15 days

TABLE 3

Colony count per cubic centimeter of emulsion of B. typhosus (Rawlings) after time indicated in 0.85 per cent NaCl solution in cold room (0° to 8°C.)

DILUTION NUMBER	5 HOURS	36 HOURS	8 DAYS	11 DAYS	15 DAYS	25 DAYS	36 DAYS	49 DAYS	88 DAYS	128 DAYS
1	2944M	3650M	3220M	940M	3000M	2880M	1260M	1770M	129M	54M
2	1472M									
3	736M	860M								
4	368M			220M	312M	385M	50M			1.2M
5	184M	167M								400T
6	92M			40M	6.2M	2.2M	180T	547T	120	
7	46M	20M		100−						
8	23M			10−						
9	11M	612T		0						
10	5.7M		0	0						

M = millions; T = thousands; − = less than.

N.B.: Figures given in 1 hour and 5 hour columns are based on actual colony counts of dilutions 1 and 10.

(i.e., those containing 92 million colony producers or more per cubic centimeter). Room temperature appears to be more favorable for the survival of *B. typhosus* in 0.85 per cent NaCl solution than 0° to 8°C., and much more favorable than 37°C.

[4] Evidence of the great resistance of *B. typhosus* in high bacterial concentration to relatively strong NaCl solutions was found in an experiment, in which *B. typhosus* (Rawlings) in concentrations of about 3 billion colony producers per cubic centimeter survived 2 weeks in both 8 and 4 per cent NaCl solution when kept in 10 cc. quantities at 37°C., and *B. typhosus* (strain 1) survived 2 weeks in 8 per cent and 2 months in 4 per cent NaCl solution.

An initial sowing of more than 220 million colony producers per cubic centimeter was required for survival beyond 9 days at 37°C.,

TABLE 4

Colony count per cubic centimeter of emulsion of B. typhosus (strain I) after time indicated in 0.85 per cent NaCl solution at 37°C.

DILUTION NUMBER	1 HOUR	24 HOURS	42 HOURS	4 DAYS	9 DAYS	15 DAYS	22 DAYS	29 DAYS	43 DAYS	56 DAYS
1	3025M	1450M	2120M	160M	20M	6.7M	2.6M	1.1M	778T	415T
2	1500M			210M	8M	4.2M	2M	650T	124T	182T
3	750M	450M	310M	20M		354T	756T	70T	103T	480
4	375M			5M	470T	55	0			
5	188M	33M	14M	1.1M	14T	0				
6	94M				10−					
7	47M	1.6M	66T	100−	1					
8	23M				1					
9	11M	200T−	5T−	5−	0					
10	5.1M			2	0					

TABLE 5

Colony count per cubic centimeter of emulsion of B. typhosus (Rawlings) after time indicated in distilled water at 37°C.

DILUTION NUMBER	5 HOURS	36 HOURS	8 DAYS	11 DAYS	15 DAYS	25 DAYS	29 DAYS	36 DAYS	49 DAYS	128 DAYS
1	3072M	3100M	100M	14M	4.3M	13M		8.2M	8.7M	185T
2	1536M									4T
3	768M	750M						360T		10−
4	384M		23M	1M	1.9M	195T		233T	78T	10−
5	192M	182M					6T	10−		
6	96M			310T	62T	750	5−			
7	48M	31M					0			
8	24M					100−	0			
9	12M			2.6T	110	0				
10	6M		500	100	4					

M = millions; T = thousands; − = less than.

N.B.: Figures given in 1 hour and 5 hour columns are based on actual colony counts of dilution 1 and 10.

and more than 46 million for survival beyond 11 days at 0° to 8°C., while only 8 million were required for 15 days survival at 15° to 20°C.

An early period of high death rate of *B. typhosus* in 0.85 per cent NaCl solution was observed, as compared with a later period of comparatively low death rate. For example, in dilution 1 at 37°C. (table 1) at the end of the first 3 weeks only 1.6 million colony producers per cubic centimeter survived out of an original 3 billion (approximately 0.05 per cent). However, of this 1.6 million colony producers per cubic centimeter which survived 3 weeks, 1.3 million per cubic centimeter or approximately 80 per cent survived another 3 weeks. Also, in dilution 1 at room temperature (table 2) at the end of the first 88 days only 67 million

TABLE 6

Colony count per cubic centimeter of emulsion of B. typhosus (Rawlings) after time indicated in distilled water at room temperature (15° to 20°C.)

DILUTION NUMBER	5 HOURS	8 DAYS	11 DAYS	21 DAYS	28 DAYS	42 DAYS	60 DAYS	79 DAYS	100 DAYS
1	2682M	2200M			900M	205M	65M		62M
2	1350M								
3	680M								
4	345M	220M							3.6M
5	173M								
6	86M	90M							210T
7	43M								
8	21M								800
9	10M								7
10	5.4M		2.7M	2.1M	570T	33T	740	50	10−

M = millions; T = thousands; − = less than.

N.B.: Figures given in 1 hour and 5 hour columns are based on actual colony counts of dilutions 1 and 10.

colony producers per cubic centimeter survived out of an original 4.5 billion (approximately 1.5 per cent). On the other hand, of this 67 million colony producers per cubic centimeter remaining after 88 days, 55 million or approximately 82 per cent survived another 40 days. Similarly, in the case of dilution 1 at 0° to 8°C. (table 3) at the end of the first 88 days 129 million colony producers per cubic centimeter survived out of an original 3 billion (approximately 4.5 per cent), but, of this 129 million colony producers per cubic centimeter remaining after 88 days, 54 million or approximately 41 per cent survived for another 40 days.

Comparison of table 4 with table 1 shows that the results with the two strains of *B. typhosus* in 0.85 per cent NaCl solution at 37°C. were approximately the same.[5]

2. Survival of B. typhosus in distilled water (tables 5 and 6). Survival of *B. typhosus* (Rawlings) in distilled water at 37°C. (table 5) was very much more prolonged in the weaker bacterial concentrations, than in bacterial concentrations of approximately equal strength in 0.85 per cent NaCl solution at the same temperature (table 1). All 10 dilutions remained viable in distilled water for 15 days, while only 4 survived for this period in 0.85 per cent NaCl solution, although each dilution in 0.85 per cent NaCl solution was of slightly higher bacterial concentration than the correspondingly numbered dilution in distilled water. Dilution 1 in distilled water remained viable for 14 months, while dilution 1 in 0.85 per cent NaCl solution remained viable for only 5 months.

Survival was still more prolonged in distilled water at room temperature (table 6) than in distilled water at 37°C. Dilution 10 at room temperature yielding 740 colony producers per cubic centimeter after 60 days, out of about 6 million per cubic centimeter at the commencement of the experiment, while at 37°C. all dilutions containing less than 190 million colony producers per cubic centimeter at the commencement of the experiment were non-viable after 36 days. Survival in distilled water at room temperature was also much more prolonged than in 0.85 per cent NaCl solution at room temperature (cf. table 6 with table 2).

The table showing the results with *B. typhosus* in distilled water at 0° to 8°C. has been omitted to save space; however, 2 million colony producers per cubic centimeter remained in dilution 10 after 30 days, out of an original 6 million colony producers per cubic centimeter at the commencement of the experiment. This dilution was still viable after 94 days. This is a much more pro-

[5] A similar experiment was conducted with *B. coli* and *B. paratyphosus* B. These organisms showed ability to survive in much lower bacterial concentration than *B. typhosus* under similar circumstances. With an initial colony count of 52 million per cubic centimeter *B. patatyphosus* B survived 28 days and *B. coli-communis* survived 21 days in 0.85 per cent NaCl solution at 37°C.

longed survival than was obtained in 0.85 per cent NaCl solution at 0° to 8°C. (see table 3). Still weaker concentrations of *B. typhosus* in distilled water were tested later at 0° to 8°C., one set made with washed, the other with unwashed bacteria. Concentrations of about 2 million colony producers per cubic centimeter (whether washed or unwashed) remained viable for 50 days, those of about 2000 to 200,000 (washed or unwashed) remained viable for about 3 weeks, and those of 50 to 1200 remained viable for 4 to 13 days. No effect of washing was apparent in this experiment. (See discussion.)

An early period of high death rate succeeded by a later period of comparatively low death rate was also observed in the case of *B. typhosus* in distilled water. Dilution 1 in distilled water at room temperature (table 6) furnishes an example of this phenomenon. In this case the number of colony producers surviving after 42 days was approximately 8 per cent of the number present at the commencement of the experiment, while, of the 205 million which survived for 42 days, 62 million or approximately 30 per cent survived for another 58 days.

SURVIVAL OF WASHED BACTERIA, EFFECT OF MATERIAL REMOVED IN WASHING

In these experiments emulsions of *B. typhosus* were made by washing off eighteen- to twenty-hour agar slant cultures. These emulsions were centrifuged, the supernatant fluid was removed, and saved where required. The bacterial sediment was resuspended and recentrifuged, and the supernatant fluid discarded. Finally, the sediment was resuspended to make an emulsion of washed bacteria. Either distilled water or 0.85 per cent NaCl solution was used exclusively throughout each experiment.

Table 7 shows that the washed bacteria (both strain 1 and strain 2) were all dead in 42 days, while both the unwashed controls still showed more than 50 thousand colony producers per cubic centimeter after 80 days. The experiment with emulsion WSF, table 8 shows that when the material washed from the bacterial emulsions is restored to them, they will survive as well as the unwashed controls.

TABLE 7

Wash experiment 1. Colony count per cubic centimeter of washed and of unwashed emulsion of B. typhosus (strain 1) and (strain 2, Rawlings) after time indicated in 0.85 per cent NaCl solution at 37°C.

		5 HOURS	7 DAYS	13 DAYS	35 DAYS	38 DAYS	42 DAYS	80 DAYS
Strain 1	U	2500M	46M	28M	1.8M		830T	57T
	W	2200M	16M	9M	2T−	10	0	
Strain 2	U	1700M	106M	47M	14M		9M	827T
	W	2800M	8M	2.7M	1T		10−	

TABLE 8

Wash experiment 2. Colony count per cubic centimeter of emulsion of B. typhosus (strain 2, Rawlings) in 0.85 per cent NaCl solution at 37°C. after time and preparation indicated

		5 HOURS	2 DAYS	15 DAYS	18 DAYS	22 DAYS	42 DAYS	49 DAYS	56 DAYS
Strain 2	U	4000M	1500M	11M		7M	2.7M	510T	2.5M
	WS	3300M	100M	10T−	10−	0			
	WSF	6400M	350M	1.7M		5M	3.8M	5.2M	2.6M
	SF	40M	20M	1.9M		1M	800T	2M	1.6M

TABLE 9

Wash experiment 3. Colony count per cubic centimeter of washed and of unwashed emulsions of B. typhosus (Rawlings) after time indicated in distilled water at 37°C.

DILUTION NUMBER		3 HOURS	6 DAYS	13 DAYS	16 DAYS	26 DAYS	33 DAYS	42 DAYS	85 DAYS	119 DAYS
U	1	2600M			36M	2.9M	1.6M	1.5M	265T	70T
	2	236M			1.6M	56T	45	0		
	3	20M		2T	109					
	4	1.9M	2T	0						
W	1	2850M			650T	700	36	0		
	2	258M			2.7T	0				
	3	22M		260	1					
	4	2.1M	6T	47	0					

U = unwashed; W = washed; WS = washed bacterial sediment resuspended in 0.85 per cent NaCl solution; WSF = washed bacterial sediment resuspended in supernatant fluid removed after first centrifuging; SF = supernatant fluid removed after first centrifuging; M = millions; T = thousands; − = less than.

Supernatant fluid removed during washing of an emulsion is really a very dilute emulsion of bacteria in the undiluted soluble constituents of the original emulsion. It is not surprising, therefore, that the bacteria present in supernatant fluid in comparatively small numbers should survive in such a favorable medium for a period out of all proportion to their numbers. This was what actually happened (emulsion SF, table 8). Similar results were obtained when another similar supernatant fluid was set up in a series of 10 dilutions. The protocol of the latter has been omitted to save space, but a summary of the result follows. With an initial 82 million colony producers per cubic centimeter in dilution 1 and 41 million in dilution 2, dilutions 1 and 2 both showed a colony count of over 40 thousand after 14 days, and 10 colonies each after 29 days. This is more than 3 times as long as the period for which dilutions of similar bacterial concentration survived when the soluble constituents of the emulsion were diluted to the same extent as the bacteria (dilutions 6 and 7 of tables 1 and 4).

The effect of washing is equally manifest when distilled water is used as the washing and suspending fluid instead of 0.85 per cent NaCl solution (cf. table 9 with tables 7 and 8). Practically no difference in duration of survival of washed and unwashed bacterial emulsions in distilled water was noted when the bacterial concentration was not greater than 22 million colony producers per cubic centimeter, but a decided difference was noted when the bacterial concentration reached 236 million. This corresponds with the results of a previous experiment (see above) in which no effect of washing was obtained using low bacterial concentrations in distilled water at 0° to 8°C. (See discussion.)

From these washing experiments, two facts are clearly demonstrated: first, the removal from a bacterial emulsion, of its soluble constituents results in relatively early death of the resuspended bacteria; and, second, a much reduced bacterial population, in the undiluted soluble constituents of an emulsion results in relatively long survival. However, the presence of the soluble constituents of an emulsion in an undiluted or relatively undiluted state is insufficient altogether to compensate for a great reduction in bacterial population.

ADDITION OF BROTH TO EMULSIONS OF B. TYPHOSUS, EFFECT ON SURVIVAL

In one of these experiments varying amounts of broth from 0.0005 to 1 cc. were added to 10 cc. emulsions of *B. typhosus* in 0.85 per cent NaCl solution containing about 300 million colony producers per cubic centimeter, and the emulsions were then placed at 37°C. Another similar experiment was conducted using larger proportions of broth from 10 to 100 per cent.

It was found that plain nutrient beef heart infusion broth in the proportion of one in a thousand was sufficient definitely to prolong the survival of *B. typhosus* in emulsion in 0.85 per cent NaCl solution at 37°C., and that 10 to 20 per cent plain nutrient broth was the optimum concentration for prolonged survival. Emulsions containing more or less than this proportion died out much more quickly.

SURVIVAL OF VARIOUS ORGANISMS IN DISTILLED WATER, AND IN NaCl SOLUTIONS

In these experiments emulsions of various organisms containing about 300 million colony producers per cubic centimeter in distilled water, and in 0.4, 0.85 and 1.5 per cent NaCl solution were tested for survival at room temperature, at 0° to 8° and at 37°C.

The results indicated that most organisms tested will live as long or longer in distilled water and in 0.4 per cent NaCl solution than in 0.85 per cent NaCl solution, and usually longest in distilled water; such organisms included: *B. paratyphosus* B, *B. coli*, *B. mucosus-capsulatus*, *B. tuberculosis*, *B. diphtheriae*, *B. pertussis*, 2 strains of *Strep. hemolyticus* (*pyogenes*, one freshly isolated), *Strep. viridans* (*ignavus*), *Strep. viridans* (*salivarius*, freshly isolated), and 2 strains of *Staph. aureus* (one freshly isolated). Survival was uniformly shorter in 1.5 per cent than in 0.85 per cent NaCl solution at all 3 temperatures for all organisms tested. The only solution in which *B. pyocyaneus* failed to survive for 25 months at all 3 temperatures was 1.5 per cent NaCl solution. Most organisms survived longer at room temperature than at 0° to 8°C., although the streptococci, *B. diphtheriae* and a recently isolated strain of *B. mucosus-capsulatus* were exceptions to this rule.

IS 0.85 PER CENT NaCl TOXIC TO ORGANISMS STORED IN NUTRIENT MEDIA?

Various organisms were grown in plain nutrient heart infusion broth containing respectively: no NaCl, 0.4 per cent NaCl and 0.85 per cent NaCl. These cultures were stored at 37°C. *B. typhosus* survived more than 3 times and *B. coli* more than twice as long in the broth containing no salt as in the broth containing 0.85 per cent NaCl. Survival in broth containing 0.4 per cent NaCl was of intermediate duration. *B. pertussis* and *B. tuberculosis* also survived definitely longer in the salt free broth than in that containing 0.85 per cent NaCl. Other organisms tested showed irregularly variable results.

DISCUSSION

Effect of glassware on reaction. Probably the fact that pyrex glassware was used entirely for sterilizing distilled water and saline solutions in the above experiments has been of some importance, since Esty and Cathcart (1921) have shown that when such liquids are sterilized by steam in ordinary soft glassware they become alkaline, but when heated in pyrex glassware they become acid; and slight acidity favors survival according to Winslow and Falk (1923).

Buffer effect of bacteria. Probably of even greater importance has been the buffering action exerted by bacteria in water and in NaCl solutions, which tends to keep the reaction in a zone favorable for survival, as shown by Shaughnessy and Falk (1924) and Shaughnessy and Winslow (1927). The former authors found that this buffering action was greater in distilled water than in NaCl solutions. This corresponds with my finding that survival of bacteria is more prolonged in distilled water than in NaCl solutions, and offers a probable explanation of that result.

Protective substances and effect of washing. The so-called water of condensation on agar slant media is undoubtedly an important factor in survival of unwashed bacteria in emulsions either in water or in NaCl solutions, since it is carried over in all cases where emulsions are made by washing the growth off agar slants, and since Healy (1926) has shown that it is richer in albumen and

total solids than nutrient broth. The water of condensation may indeed be the most important factor removed by washing. In this connection it is interesting to note that Winslow and Brooke have demonstrated the protective influence of broth as well as peptone or meat extract upon washed emulsions of *B. cereus*. As they contend the effect is probably not a nutritive one. The results of Winslow and Brooke differ from those here reported in that they found the survival of *B. coli* to be uninfluenced by washing. This is readily explained by the fact that their experiment was of short duration and bacterial concentrations used were comparatively low, whereas I found the result of washing manifesting itself with *B. typhosus* after days rather than hours and not manifesting itself at all in low bacterial concentrations. (One would expect *B. coli* to be influenced by washing in a manner somewhat similar to *B. typhosus*.) Great dilution of the soluble products of an emulsion of bacteria may be almost equivalent to their total removal. This probably explains why no effect of washing was noted at low bacterial concentration (see experiment conducted at 0° to 8°C. and also see dilutions 3 and 4, table 9).

Factors favoring survival of bacteria. Low temperature, a minimum of nutrient material, a minimum or absence of sodium chloride, and a very high bacterial concentration are factors which favor survival of bacteria, but are unfavorable for bacterial growth. It is therefore necessary to differentiate clearly between those conditions which are favorable for growth and those which are favorable for survival.

The marked protective influence of low temperature (room temperature or lower) upon *B. typhosus* in low concentration in distilled water as demonstrated by the results of this investigation indicates that low temperature is probably of very great importance in the production of the winter river typhoid in temperate climates referred to by Hill (1911), although probably, interference with sunlight and diffuse light (Wheeler) by snow covered ice is also a factor. Similarly, my results add further support to the conclusion of Hinds (1917), that low temperature is to be considered an important factor in the results of Ruediger (1911) on summer and winter differences in polluted river water.

My finding that distilled water is a more favorable medium for the survival of *B. typhosus* and *B. coli* than 0.85 per cent NaCl solution corresponds with the results of Panisset, Verge and Carneiro and of Shaughnessy and Criswell with *B. coli*, but not with those of Winslow and Falk (1923) with *B. coli*, nor with those of Duthóit with *B. coli* and *B. typhosus*. It is worthy of note that the experiments of the authors quoted were all of short duration (only a few days at most), while mine were of long duration (from several days to many months). Consequently different factors might come into operation.

SUMMARY AND CONCLUSIONS

1. Various bacterial emulsions have remained viable for periods ranging from 5 to 32 months in 0.85 per cent NaCl solution at various temperatures, and from 14 to 32 months in distilled water.

2. The bacterial concentration required for survival of *B. typhosus* in emulsion in distilled water or in 0.85 per cent NaCl solution for a given time at a given temperature can be approximately determined. This varies greatly with the temperature and emulsifying fluid employed.

3. Distilled water is a more favorable medium for the survival of *B. typhosus* than 0.85 per cent NaCl solution. The same thing is true for a number of other organisms, including: *B. coli*, *B. tuberculosis*, *B. diphtheriae*, *Strep. hemolyticus* and *Strep. viridans*.

4. The prolonged survival of *B. typhosus* in distilled water and in 0.85 per cent NaCl solution is associated with a late period of comparatively low death rate. In the case of *B. typhosus* at 37°C. the onset of a period of low death rate may be detected after about 3 weeks.

5. The fact that *B. typhosus* in distilled water is able to survive so much longer (5 times as long in my experiment) at room temperature than at 37°C. points to low temperature as a factor of considerable importance in the production of winter river typhoid in temperate climates.

6. Washing *B. typhosus*, either in 0.85 per cent NaCl solution,

or in distilled water shortens its survival period when resuspended in these fluids. The supernatant fluid removed in washing prolongs the survival period of the relatively few bacteria which are inevitably present in it. Great dilution of an emulsion of bacteria appears to be almost equivalent to washing in its effect.

7. Ten to 20 per cent plain nutrient broth diluted with 0.85 per cent NaCl solution is the optimum concentration of broth for prolonged survival of *B. typhosus* at 37°C.; 0.1 per cent is sufficient definitely to prolong survival.

8. Sodium chloride in 0.85 per cent concentration appears to be toxic to certain organisms when stored in nutrient media at 37°C. Reduction in the concentration of sodium chloride in culture media might assist in the preservation of cultures of certain delicate organisms.

9. Room temperature appears to be more favorable for the survival of most organisms in distilled water and in 0.85 per cent NaCl solution than 0° to 8°C. The streptococci and *B. diphtheriae* are exceptions.

10. The duration of survival of several of the Gram negative bacilli appears to be more uniformly long in distilled water and in NaCl solutions than it is on solid media.

The author wishes to express appreciation for helpful advice and criticism offered by Dr. H. H. Bullard and Dr. J. H. Fisher.

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