THE VIABILITY OF VARIOUS SPECIES OF BACTERIA IN AQUEOUS SUSPENSIONS

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INTRODUCTION

During the past ten years numerous investigations have been reported, from this and other laboratories, in regard to the viability of bacteria in aqueous suspensions. The majority of these studies (Jordan, Russell and Zeit, 1904; Konradi, 1904; Russell and Fuller, 1906; Wheeler, 1906; Whipple and Mayer, 1906; Houston, 1908, 1909, 1911 and 1914; Ruediger, 1911; Clemesha, 1912; Hinds, 1916; Rector and Daube, 1917; Winslow and Cohen, 1918; Winslow and Falk, 1919; Winslow and Falk, 1923 a and b; Meier, 1924; Shaughnessy and Criswell, 1925) have dealt with *Bact. coli* and with forms closely related to *Bact. coli*,—organisms which possess a relatively high degree of viability in water, subsisting in approximately undiminished numbers for twenty-four hours even when suspended in distilled water.

In the course of recent investigations carried out in this laboratory we became interested in the behavior of certain other types of bacteria which appeared to function very differently when suspended in dilute aqueous solutions. The aerobic spore-former, *B. cereus* in particular, seemed to suffer a very rapid mortality in such menstrua and the object of the present study was to investigate this phenomenon and the factors governing it somewhat more closely.

TECHNIQUE

The organisms studied have been four in number, *B. cereus*, *B. megatherium*, *Bact. coli* and *E. prodigiosus*. All were stock

laboratory strains. Cultures were carried on standard nutrient agar and the growth from an ordinary slant was suspended in 100 cc. of sterile distilled water and washed over the surface of standard nutrient agar in a Kolle flask. The Kolle flasks were incubated at 37°C. for eighteen to twenty hours in the case of *Bact. coli* and *E. prodigiosus* and at 20°C. for twelve to fourteen hours in the case of the spore formers, a condition which ensures an abundant development of vegetative cells free from spores. The growth from the flasks was then washed off in 100 cc. of the menstruum to be tested and the suspension filtered through sterile absorbent cotton to remove clumps. Three or

TABLE 1

Viability of B. cereus after centrifuging in distilled water

Bacteria per cubic centimeter

SERIES	PERIOD	pH 6	pH 7	pH 8	pH 9
	minutes				
A	0	12,000,000	171,000,000	242,000,000	271,000,000
	30	13,700	6,600	17,800	9,900
	60	9,000	3,000	12,800	10,200
B	0	7,400	2,500	3,300	14,700
	15	1,600	5,800	1,700	0
	30	0	500	100	100
	60	0	0	0	0

4 cc. of this heavy suspension were then transferred to 100 cc. of the same menstruum to give us the final test suspension and platings were made on agar at appropriate intervals.

VIABILITY IN DISTILLED WATER, WITH AND WITHOUT CENTRIFUGATION

In our first studies *B. cereus* was washed off from the Kolle flasks in distilled water, filtered and diluted as described above, and then centrifuged twice in sterile centrifuge tubes for twenty minutes. The first centrifuging, the first re-suspension and the second centrifuging were performed in ordinary sterile distilled water (slightly acid) in series A and in distilled water adjusted

to pH 7.2 in series B; while the second re-suspension was made in distilled water adjusted to the four different hydrogen-ion concentrations indicated in table 1.

The original number of bacteria present in the centrifuged suspension is indicated opposite the 0 period in the table. Its absolute value has no particular significance since the amount of heavy suspension transferred to the final suspension was not controlled. It is evident, however, that in all instances the bacteria died out very rapidly when suspended in the aqueous menstrua. With the initially high numbers of series A less

TABLE 2

Viability of bacteria suspended in distilled water without centrifuging

Per cent surviving

ORGANISM	PERIOD	pH 6	pH 7	pH 8	pH 9
	minutes				
B. cereus	0	100	100	100	100
	15	79	113	72	58
	45	36	55	281	47
	75	8	160	295	80
	90	104	11	23	13
	120	2	40	23	49
B. megatherium	0	100	100	100	100
	30	24	16	69	11
	60	78	46	86	80
	90	61	132	71	82

than 0.1 per cent of the initial numbers survived at any pH value after thirty minutes while with the small numbers of series B the bacteria had disappeared after one hour.

We next thought it would be of interest to see what would happen if the centrifugal treatment were omitted. In these tests the bacteria were washed off and filtered through cotton as before and then diluted in the distilled water adjusted to the desired pH values. The results in terms of per cent surviving are presented in table 2.

Here we find a wholly different situation. Since the centrifugal treatment has been omitted the 0 period in table 2 cor-

responds to the forty-five-minute period in table 1 and the seventy-five-minute or ninety-minute period in table 2 to the thirty-minute period in table 1,—measuring back to the original washing off of the bacteria. Yet making full allowance for this difference it is evident that in the uncentrifuged suspensions protective substances of some sort were carried over from the agar culture which prevented the sudden mortality which occurs in the centrifuged suspensions. This conclusion was further checked by the tests presented in table 3. In this series all our four test organisms were used and control tests were also made in ordinary nutrient broth,—suspension, filtering, centrifugal treatment and re-suspension being carried out in each case

TABLE 3

Viability in broth and water before and after centrifuging

Per cent surviving

	B. CEREUS		B. MEGATHERIUM		E. PRODIGIOSUS		BACT. COLI	
	Broth	Water	Broth	Water	Broth	Water	Broth	Water
Before centrifuging	100	100	100	100	100	100	100	100
After centrifuging	88	0	197	0	44	0	70	280
One hour later	43	0	300	0	37	0	70	131

in the appropriate menstruum (broth or distilled water respectively).

The 0 values in this table do not indicate absolute sterility but in all cases they do indicate that less than 0.01 per cent of the original numbers were surviving.

These data show very clearly that the bacteria studied do not change materially in numbers when centrifuged and suspended in broth for one hour and that *Bact. coli*, as shown by all previous work, maintains itself or even increases in numbers under similar conditions in distilled water. All of the three other organisms tested on the other hand died out almost completely when suspended in distilled water and washed free from protective substances by centrifugal treatment.

EFFECT OF NUTRIENT BROTH UPON VIABILITY

Our next experiments were conducted to determine the concentration of nutrient broth necessary to protect *B. cereus* against the harmful effect of aqueous suspension. Six different dilutions of broth were therefore made in distilled water and these dilute broths were used for washing, centrifuging and re-suspension in the usual manner. The results presented in table 4 indicate that one part of broth in 100 completely protects and one part in 1000 exerts a very considerable favorable influence. It is thus clear why the bacteria survive in uncentrifuged suspensions.

TABLE 4

Viability of B. cereus in dilute broth suspensions

Per cent surviving

PERIOD	BROTH					
	Undiluted	1:10	1:100	1:1000	1:100,000	1:1,000,000
Before centrifuging	100	100	100	100	100	100
After centrifuging	52	37	208	44	0	0
One hour later	52	45	196	12	0	0

VIABILITY IN SALT SOLUTIONS

We next sought to determine the particular factors involved in the protective action of nutrient broth, and first studied the effect of salt concentration with the possibility in mind that osmotic pressure might be the factor involved. Sodium chloride solutions and Ringer-Locke solutions of various strength were used for this purpose. In NaCl of 0.145 M, 0.145 M and 1.450 M strength less than 0.1 per cent of the bacteria (*B. cereus*) survived one hour after centrifuging and in Ringer-Locke solution of double strength, normal strength and one-half strength less than 1 per cent survived at a corresponding period. Clearly the presence of electrolytes will not counteract the harmful effects of suspension in distilled water.

VIABILITY IN SUGAR SOLUTIONS

Although there is of course no sugar entering as a factor into the protective effect of nutrient broth it seemed of some general interest to determine whether the bacteria would survive better in the presence of saccharine substances than in solutions of electrolytes. Tests were therefore made in double isotonic, isotonic and half-isotonic strength of ordinary commercial glucose, but again with wholly negative results. Only one per cent of the organism present survived centrifugal treatment in double isotonic sugar solution and less than 0.5 per cent survived in the lower concentrations.

TABLE 5

Viability of B. cereus in broth, pepton, meat extract and half-strength Ringer-Locke solution

Per cent surviving

	BROTH	PEPTON	MEAT EXTRACT	RINGER-LOCKE
Before centrifuging	100	100	100	100
After centrifuging	56	43	51	3
One hour later	71	53	57	1

VIABILITY IN DILUTE SOLUTIONS OF PEPTON AND MEAT EXTRACT

There remained for consideration as possible protective factors the meat extract and the pepton contained in our nutrient broth. We next proceeded to study the effect of these substances by themselves.

In table 5 are presented data for the viability of *B. cereus* in broth, pepton and meat extract solution and in half-strength Ringer-Locke solution as a control. The broth solution, as usual, contained 5 grams Difco pepton and 3 grams Liebig's meat extract to one liter of water. The pepton solution contained 5 grams of pepton to the liter and the meat extract solution 3 grams of meat extract to the liter. In each case all washing, centrifuging and re-suspension was performed in the particular menstruum studied.

As before the presence of electrolytes (Ringer-Locke solution)

failed to protect; but the bacteria survived in either pepton or meat extract solution about as well as in nutrient broth.

Several series of control tests indicated that *B. cereus* would survive in essentially undiminished numbers in either pepton solution (0.5 per cent) or meat extract solution (0.3 per cent). In a one-hundredth dilution of these same solutions (0.005 per cent pepton and 0.003 per cent meat extract) or in a similar dilution of either pepton or meat extract alone they survive equally well, but in a one-thousandth dilution the protective effect disappears. (See table 6.)

TABLE 6

Viability of B. cereus in pepton, and meat extract solutions

Per cent surviving

	PEPTON		MEAT EXTRACT	
	0.005 per cent	0.0005 per cent	0.003 per cent	0.0003 per cent
Before centrifuging	100	100	100	100
After centrifuging	55	10	62	50
One hour later	59	2	57	0

SUMMARY OF CONCLUSIONS

1. Certain strains of *Bact. coli* survive for several hours in practically undiminished numbers when suspended in distilled water and centrifuged twice to remove substances carried over from the agar slope on which they have previously been grown.

2. The other types of bacteria studied,—*B. cereus*, *B. megatherium*, and *E. prodigiosus*,—when treated in the same way die off almost immediately so that less than one per cent of the organisms originally present are alive one hour after the completion of centrifugal treatment.

3. When the bacteria are suspended in distilled water without centrifuging, the bacteria do not die off in the manner described being protected by substances carried over from the original agar cultures.

4. Salt and sugar solutions are no more favorable than distilled water, to the bacteria studied.

5. On the other hand a menstruum containing one part of nutrient broth in one hundred parts of water completely abolishes the lethal effect and still higher dilutions reduce it materially.

6. The same protective effect is exerted by a similar dilution of either of the two ingredients of nutrient broth (0.005 per cent pepton or 0.003 per cent meat extract) but not by a dilution ten times as great.

7. The influence of meat extract and of pepton in thus promoting the viability of bacteria cannot presumably be due to their use as nutrients since the effect is too rapid and too far-reaching; it cannot be due to osmotic effects since salt and sugar solutions fail to exert any such influence; it would seem probable that the phenomenon is analogous to the operation of a protective colloid.

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