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A NOTE ON THE VIABILITY OF BACILLUS DYSENTERIÆ [1]

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The present study was initiated for two reasons: First, to obtain information as to the viability of *Bacillus dysenteriæ* outside the human body; second, to search for a medium by means of which the stools of dysentery patients or carriers could be preserved and the detection of bacillary dysentery be made easier. Kolle and Hetsch [2] state that the resistance of *B. dysenteriæ* outside the body is not very great. Dried on various objects, the dysentery bacilli do not survive longer than eight to ten days; in a wet medium they remain viable for several months. Direct sunshine kills them in thirty minutes. The nontoxic strains, being more resistant than the toxic strains, are viable for several months in culture media. Winter [2] found *B. dysenteriæ Y*, dried on cloth, viable for one hundred fifty days. In symbiosis with *B. dysenteriæ* Shiga the nontoxic types outlive the Shiga type.

The study of the viability of the intestinal pathogenic bacteria supplies information necessary to the epidemiologist for the study of outbreaks under local conditions; it may help to explain certain regularity or periodicity of outbreaks and enable the sanitarian to take rational measures to prevent an outbreak from spreading beyond his control.

[1] The experiments were conducted in the Serum Laboratory, Bureau of Science, Manila, P. I.

[2] Die Experimentelle Bakteriologie und die Infektions-Krankheiten, Urban & Schwarzenberg, Berlin and Vienna, 6th ed. 1 (1922) 376.

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As far as the bacteriologic diagnosis of bacillary dysentery is concerned we find in Teague's methylene blue-eosin lactose agar a differential medium of great help, but we lack an enrichment process which would enable us to detect with comparative ease small numbers of *B. dysenteriæ*. We find, therefore, that in a fresh stool of an early case there is little difficulty in isolating the microbe, whereas stools that are examined some time after they have been passed or stools from convalescents rarely give satisfactory results. This is due not only to the great bacteriophage susceptibility of *B. dysenteriæ*, but also, frequently, to the effect of symbiosis with other intestinal bacteria, particularly *B. coli*.

Owing to the great variety of the representatives of the *B. dysenteriæ* group and to the constant association in stools of *B. dysenteriæ* with *B. coli* (which apparently is more resistant to all adverse conditions than *B. dysenteriæ*), the realization of our hope of possessing as simple and reliable a bacteriologic diagnostic method for the detection of bacillary dysentery as that for the detection of cholera is very remote and the search for dysentery carriers, both convalescent and contact, is still fraught with the greatest difficulties.

EXPERIMENTS ON THE VIABILITY OF BACILLUS DYSENTERIÆ

The arrangement of the viability experiments of *B. dysenteriæ* was as follows:

- Viability of culture:
  - In water.
  - In salt solution.
  - In bile.
  - In glycerine solution.
  - In acid and alkaline bouillon.
  - Resistance to drying.
- Viability of *B. dysenteriæ* in stool:
  - Normal artificial stool.
  - Sterilized artificial stool.
  - Resistance to drying in stool.
  - Natural dysenteric stool.

CULTURE

In water.—The culture used in these experiments was one that had been isolated recently from a case of acute bacillary dysentery and corresponded to the Shiga type. Two large sterile test tubes, one containing 10 cubic centimeters of sterile distilled water and the other 10 cubic centimeters of sterile tap water, were inoculated with one loopful of twenty-four-hour-old agar

culture of the above-mentioned strain. The contents were then well stirred, and transplants from the tubes onto acid agar slants were made immediately and every third day thereafter. The tubes containing *B. dysenteriæ* suspended in water were allowed to stand at room temperature, protected from direct sunshine. The results of this experiment are shown in Table 1.

In salt solution.—The culture used in this experiment was the same as the one used in the previous one. In a series of test tubes decreasing dilutions of concentrated sodium chloride solution were placed. The total volume of each tube was 1 cubic centimeter. One loopful of a twenty-four-hour-old agar culture was emulsified in each of the tubes, stirred well, and allowed to stand at room temperature, protected from direct sunshine. Subcultures were made immediately and every three to four days thereafter. The results of this experiment are presented in Table 1.

In bile.—The same culture and arrangement were used as in the previous experiments except that bile was used instead of the salt solution. Due to evaporation the bile solution dried completely in seventy-seven days and *B. dysenteriæ* could not be isolated after that date. (See Table 1.)

In glycerine.—The same technic and culture were used in this experiment as in the previous one. The details and results are shown in Table 1.

In acid and alkaline bouillon.—Two tubes containing 10 cubic centimeters of acid bouillon +1, two tubes containing 10 cubic centimeters of +0.3 bouillon, and two tubes containing neutral bouillon were inoculated with the above-mentioned culture of *B. dysenteriæ* (Shiga type) and allowed to stand in the incubator at 37° C. Transplants were made immediately and at definite intervals. Similarly, a series of alkaline bouillon was planted. One tube of +0.3 bouillon, one tube of neutral bouillon, and one tube of −0.3 bouillon were planted with *B. dysenteriæ* (Flexner type) and included in this experiment. The details and results are to be seen in Table 1.

Resistance to drying.—Strips of filter paper (3 centimeters by 1 centimeter) were placed in sterile Petri dishes, soaked well with an emulsion of *B. dysenteriæ* (Shiga type), and dried in vacuo at room temperature over calcium chloride. As soon as the paper strips were dried, and every third day thereafter, a small square of the paper was cut off by means of sterile scissors and forceps and placed in a tube containing meat broth and incubated twenty-four to forty-eight hours. The growth that

took place in the tube was identified by the addition of 0.1 cubic centimeter of antidysenteric serum. For the results, see Table 1.

As far as viability of *B. dysenteriæ* in pure cultures is concerned, we find that—

1. *Bacillus dysenteriæ* survives longer in tap water than in distilled water, but neither in distilled water nor in tap water does it survive as long as *B. coli.*

2. Glycerine has a pronounced antiseptic effect on *B. dysenteriæ* and *B. coli* in high concentration. In higher dilutions glycerine is about equally effective a preservative for *B. coli* as for *B. dysenteriæ.*

3. Bile proved to be an excellent preservative for *B. dysenteriæ* and *B. coli* and very useful in preservation of stock cultures of *B. dysenteriæ.*

4. Dried in vacuo, *B. coli* proved to be more resistant than *B. dysenteriæ.*

5. *Bacillus coli* proved to be more resistant to alkaline reaction than *B. dysenteriæ*, which survived a considerable time in acid bouillon but died quickly in alkaline medium.

VIABILITY OF BACILLUS DYSENTERIÆ IN STOOL

Normal artificial stool.—About 2 grams of fresh normal fæces were emulsified in 10 cubic centimeters of normal salt solution, stirred well, and filtered through cotton to remove the large particles. One loopful of a twenty-four-hour-old agar culture of *B. dysenteriæ* was emulsified in this suspension. Transplants were made at intervals; the details and results are evident from Table 2.

Sterilized artificial stool.—A stool emulsion was prepared in the same way as described in the preceding experiment, with the exception that the stool emulsion was heated for thirty minutes at 100° C. and then cooled and inoculated. The results can be seen from Table 2.

Resistance to drying in stool.—Fresh normal and fresh sterilized stool emulsions were prepared in the same manner as described above. *Bacillus dysenteriæ* (Shiga) and *B. coli* emulsions were prepared also, in the same way as mentioned above. Mixtures of bacterial emulsions and of stool emulsions were made and strips of filter paper were impregnated with these mixtures. The saturated strips of paper were then dried rapidly in vacuum over calcium chloride. Immediately, and every two days thereafter, small pieces of the impregnated and dried papers were placed in tubes of bouillon and incubated at 37° C.

At the end of twenty-four hours incubation Teague's methylene blue-eosin plate was inoculated from each of the bouillon cultures. Suspicious colonies were fished out, transplanted, and identified by means of serum and sugar reactions. Further details and the results are evident from Table 2.

Natural dysenteric stool.—A series of stools from early suspected cases of dysentery was used in the following experiments. The stools were received partly in the natural state and partly diluted with alkaline peptone water because examination for cholera was also requested. This fact accounts for the alkaline reactions registered in the brief protocols given below of stools 2, 8, and 9.

Stool No. 2.—Liquid stool containing mucus and blood. Reaction, alkaline to litmus. *Bacillus dysenteriæ* (Flexner type) was isolated from this stool. Suspension of this stool was made and the viability of *B. dysenteriæ* studied in the following media: Physiologic salt solution, distilled water, tap water, +1 per cent bouillon, −1 per cent bouillon, and 50 per cent bile salt solution. About 2 grams of the stool sample were emulsified in 10 cubic centimeters of each medium mentioned. The stool sample as received was included for control. Furthermore, part of the stool suspension in distilled water was dried on filter paper in vacuo over calcium chloride. Immediately, and on alternate days thereafter, a loopful of the various emulsions was streaked on Teague's medium and a small strip of the dried stool on filter paper was planted in bouillon and plated on Teague's medium. Further identification of *B. dysenteriæ* was carried out in the same manner as described above. The details and results are evident from Table 3.

Stool No. 8.—Liquid stool containing mucus and blood. Reaction, acid to litmus. Specimen more than twenty-four hours old when received. *Bacillus dysenteriæ* (Shiga type) was isolated from this stool.

Stool No. 9.—Liquid stool containing mucus. Reaction, acid to litmus. *Bacillus dysenteriæ* (Shiga type) was isolated from this specimen. (See Table 3.)

From the experiments on the viability of *B. dysenteriæ* in artificial and natural dysenteric stools the following conclusions can be drawn:

1. Unlike the vibrio of cholera, *B. dysenteriæ* is fairly resistant to drying. It will survive longer in a sterile than in a normal fresh stool.

2. *Bacillus dysenteriæ* will survive a considerable length of time in water. In salt solution, and consequently in sea water, it will survive longer than in tap or distilled water.

3. Physiological salt solution and bile are favorable media for the survival of *B. dysenteriæ* in the stool and the combination of the two may help to preserve *B. dysenteriæ* in the stools of dysentery patients and carriers, in at least a certain percentage of cases.

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TABLE 1.—*Results of viability tests of Bacillus dysenteriæ and B. coli, separately, in various media.*

Medium.	*B. dysenteriæ* survived—	*B. coli* survived—
	Days.	*Days.*
Distilled water	18	69
Tap water	27	61
Glycerine:		
100 per cent	(a)	7
50 per cent	4	6
25 per cent	10	20
12.5 per cent	30	27
6.25 per cent	42	42
Sodium chloride solution:		
100 per cent	(a)	8
50 per cent	(a)	3
25 per cent	4	10
12.5 per cent	10	17
6.25 to 0.1 per cent	40	27–36
Ox bile in salt solution: [b]		
50 per cent	70	70
25 per cent	70	70
12.5 per cent	70	70
6.25 per cent	70	70
3.125 per cent	70	70
Bouillon:		
+1 (37° C.) Shiga	70	0
—1 (37° C.) Shiga	4	0
+0.3 (37° C.) Shiga	63	0
—0.3 (37° C.) Shiga	2	0
Flexner dysentery:		
+0.3	63	0
—0.3	1	0
Bacillus dysenteriæ + dried on paper (28° C.) Shiga	16	77

[a] Not found. [b] Present until it dried completely.

TABLE 2.—*Results of viability experiments with Bacillus dysenteriæ and B. coli in normal fresh stool and normal sterilized stool.*

Stool.	Salt solution.	Bile, 50 per cent.	Stool inoculated with—		*B. dysenteriæ* survived—	*B. coli* survived—
			B. dysenteriæ.	*B. coli.*		
					Days.	*Days.*
Normal fresh	+	0	+	0	3	>6
Do	+	+	+	0	5	>6
Normal sterilized	+	0	+	0	66	0
Do	+	+	+	0	75	0
Do	+	0	+	+	15	>21
Do	+	+	+	+	17	>21
Normal fresh inoculated and dried in desiccator	0	0	+	0	3	21
Normal sterilized inoculated and dried in desiccator	0	0	+	0	12	0

TABLE 3.—*Viability of Bacillus dysenteriæ and B. coli in dysenteric stools placed in various media.*

Stool No.	Reaction to litmus.	Type of *Bacillus dysenteriæ* isolated.	Stool.		Salt solution.		Water.	
			B. dysenteriæ.	*B. coli.*	*B. dysenteriæ.*	*B. coli.*	*B. dysenteriæ.*	*B. coli.*
2	—	Flexner	1	>16	9	>16	7	>16
8	+	Shiga	4	>6	3	>6	0	0
9	+	do	2	>6	4	>6	0	0

Stool No.	Tap water.		Bouillon.				Bile, 50 per cent.		Dried in desiccator.	
			+1		—1					
	B. dysenteriæ.	*B. coli.*	*B. dysenteriæ.*	*B. coli.*	*B. dysenteriæ.*	*B. coli.*	*B. dysenteriæ.*	*B. coli.*	*B. dysenteriæ.*	*B. coli.*
2	7	>16	9	>16	7	>16	7	>16	7	11
8	0	0	0	0	0	0	4	>6	------	------
9	0	0	0	0	0	0	6	>6	------	------