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The survival of *Shigella sonnei* on cotton, glass, wood, paper, and metal at various temperatures*

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INTRODUCTION

Recently Spicer (1959) found that *Shigella sonnei* survived for 7–10 days on cotton threads in the dessicated state. This poses quite a serious problem to workers in laboratories who accidentally may touch cotton plugs or sides of glass test-tubes with cultures of *Sh. sonnei*, since the organism is able to survive the usual incubation periods of 2–7 days. The present investigation deals with the survival of 10 strains of *Sh. sonnei* grown upon nutrient broth, the usual laboratory medium for the cultivation of this organism, when placed upon glass, cotton, wood, paper, and metal and stored at $-20°$, 4°, 15°, 37° and 45° C. The purpose of this study was to determine the length of time cells of *Sh. sonnei* remained viable under these experimental conditions.

MATERIALS AND METHODS

The 10 strains of *Sh. sonnei* used in these experiments were repeatedly checked morphologically, biochemically, and serologically (Lederle Laboratories Division, Group D Shigella grouping sera) for purity. The cultures were grown at 37° C. on nutrient agar (Difco) slants for 48 hr.; these were retained as the stock strains and were stored at 4° C. The cultures used for the experimental work were transferred daily in nutrient broth at 37° C. Twenty-four hour cultures of *Sh. sonnei* had a population of approximately 1×10^9 cells per ml.

The 24 hr. cultures were placed upon sterile cotton (small balls), wood (pieces of wood applicator sticks, 1 cm. × 1 cm.), paper (pieces of Whatman No. 1 filter paper, 1 cm. × 1 cm.), glass (microscope cover slides, approximately 1 cm. × 1 cm.), and metal (small aluminum tags for marking animals) with a capillary pipette. One drop of the culture material was placed on these various items. The pieces of wood, cotton, paper, glass, and metal were placed aseptically into sterile Petri dishes and stored at the various temperatures, i.e., $-20°$, 4°, 15°, 37° and 45° C. At daily intervals, using a sterile forcep, the various pieces were carefully removed and placed in test-tubes containing 10 ml. of nutrient broth. The tubes were

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gently rotated between the palms of the hands, incubated at 37° C. for 24, 48 and 72 hr., and read visually by observing whether the tubes were turbid or clear. If the tubes appeared turbid, indicating growth, this was an indication that the cells of *Sh. sonnei* had survived the treatment. Gram-staining was performed on the organisms which grew to check against possible contamination during the various handling procedures.

The following strains of *Sh. sonnei* were employed throughout these studies: Strains F-6, F-141, and F-264 obtained from Dr Maxwell Finland, Boston City Hospital, Boston, Massachusetts; strain 13-3 which was isolated overseas in 1943 and sent to the Walter Reed Army Hospital was obtained from Dr Arthur Abrams; strain CH which was isolated in April 1960 from a man suffering from a severe diarrheal infection was obtained from Dr H. M. Gezon, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania; strains B-2569-2, B-2547-3, B-2501-3, B-2506-3, and B-2504-3 were sent to us by Commander T. M. Floyd, Naval Medical Center, Bethesda, Maryland.

All survival experiments were performed under conditions of humidity obtained in the laboratory at the time. The mean humidity during the period of 9 a.m. to 5 p.m. was 29 % (the range was 19–33 %); the mean humidity during the period of 6 p.m. to 6 a.m. was 26 % (the range was 17–29 %). The relative humidity was determined by using the wet and dry bulb measurement method employing the Taylor (Mason's form) Hygrometer. Although we were quite aware of the importance of relative humidity as was pointed out by Spicer, we were unable to carry out controlled experiments where humidity was varied or regulated to the desired level due to lack of adequate facilities.

RESULTS

The results of the survival of *Sh. sonnei* on glass are presented in Table 1. There appears to be only a slight variation in the survival times of the different strains of *Sh. sonnei*. There was a large drop in the survival time when the organisms were held at 4° C. compared to the cells held at −20° C. Viable cells could not be recovered when the cells were held at 45° C. suggestive of a possible killing action at this higher temperature. At 37° C. the bacteria survived from 1 to 6 days on glass.

Sh. sonnei survived on cotton (at −20° C.) for 26–33 days (Table 2). Only a slight variation was observed in the survival of the 10 strains studied. There was considerably longer survival of the cells on cotton than on glass (19–32 days on cotton compared to 4–10 days on glass) when the cells were held at 4° C. This was also true of the cells kept at 15° and 37° C.

Sh. sonnei survived the longest period at −20° C. when placed upon wood (36–44 days) (Table 3). Survival at 37° C. was also more extensive than cells placed upon paper, glass, metal or cotton. Furthermore, a few strains also survived at 45° C. Survival times at 37° C. ranged from 3 to 13 days.

Survival time on metal was the shortest of the materials tested at all temperatures tested. Considerably more strain variation was observed when the cells were placed on metal (see Table 4).

Strain variation in terms of survival was quite extensive at −20° C. for cells placed upon paper (Table 5). Strain 13-3 survived 10 days at this temperature while strain B-2547-3 survived for 49 days. Survival at the other temperatures, namely, 4°, 15°, 37° and 45° C., also varied from strain to strain.

Table 1. *Survival of* Shigella sonnei *upon glass at various temperatures*

Holding temperature (°C.)	Strain of *Sh. sonnei*									
	F-6	F-141	F-264	13-3	CH	B-2569-2	B-2547-3	B-2501-3	B-2506-3	B-2504-3
	Survival time in days*									
−20	30	35	30	30	28	28	24	28	28	25
4	6	5	7	7	4	4	5	10	4	5
15	8	7	10	9	6	5	10	10	9	8
37	3	3	2	4	1	1	3	6	3	1
45	0	0	0	0	0	0	0	0	0	0

* Survival time is the number of days the preparation contained viable cells as measured by growth when inoculated into nutrient broth tubes.

Table 2. *Survival of* Shigella sonnei *on cotton at various temperatures*

Holding temperature (°C.)	Strain of *Sh. sonnei*									
	F-6	F-141	F-264	13-3	CH	B-2569-2	B-2547-3	B-2501-3	B-2506-3	B-2504-3
	Survival time in days									
−20	30	26	28	30	27	36	33	27	29	31
4	27	30	26	26	31	32	29	19	24	25
15	25	27	22	24	20	19	22	20	21	23
37	7	6	8	7	5	10	11	9	7	12
45	0	0	0	0	0	0	0	0	0	0

Table 3. *Survival of* Shigella sonnei *on wood at various temperatures*

Holding temperature (°C.)	Strain of *Sh. sonnei*									
	F-6	F-141	F-264	13-3	CH	B-2569-2	B-2547-3	B-2501-3	B-2506-3	B-2504-3
	Survival time in days									
−20	39	40	44	36	46	44	47	38	41	42
4	18	17	21	20	22	16	15	20	21	24
15	12	15	16	17	15	13	11	18	20	23
37	3	11	6	10	9	6	5	11	13	12
45	0	0	0	0	1	0	1	0	0	0

The variation in survival may have been due to slight variations in the initial populations of the cultures exposed to dessication. However, in order to minimize this factor, the cultures were adjusted to yield approximately 1–2 × 10^9 cells per ml.

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Table 4. *Survival of* Shigella sonnei *on metal at various temperatures*

Holding temperature (°C.)	Strain of *Sh. sonnei*: F-6	F-141	F-264	13-3	CH	B-2569-2	B-2547-3	B-2501-3	B-2506-3	B-2504-3
	Survival time in days									
−20	20	21	28	19	16	23	17	24	16	29
4	10	9	11	8	8	12	7	13	11	14
15	6	4	3	5	2	7	2	6	4	9
37	3	0	1	1	0	3	0	2	0	4
45	0	0	0	0	0	0	0	0	0	0

Table 5. *Survival of* Shigella sonnei *on paper at various temperatures*

Holding temperature (°C.)	Strain of *Sh. sonnei*: F-6	F-141	F-264	13-3	CH	B-2569-2	B-2547-3	B-2501-3	B-2506-3	B-2504-3
	Survival time in days									
−20	28	14	36	10	27	34	49	57	19	34
4	18	10	22	10	21	24	40	39	21	29
15	3	8	16	4	9	15	28	21	13	10
37	0	1	5	0	0	3	10	8	6	2
45	0	0	0	0	0	0	2	1	0	0

DISCUSSION

Sh. sonnei cells were quite resistant to dessication when placed on common materials found in bacteriological laboratories, namely, cotton, glass, wood, paper, and metal. It appears that laboratory apparatus and glassware accidentally contaminated with cultures of *Sh. sonnei* should be promptly disinfected in order to avoid laboratory infections since the organisms retain viability for considerable periods.

These results confirm those of Spicer (1959) who found that *Sh. sonnei* survived better at 5–10° C. than at 20–30° C. One reason that might explain better survival at 4–15° C. compared to 37° C. is that the cells kept at 37° C. might undergo more rapid endogenous metabolism during the holding period leading to death of the cells possibly by starvation. Cells held at −20° C. were viable for longer periods of time; this could be explained by the preservative action of freezing temperatures upon bacteria. Although it is generally known that *Sh. sonnei* grows well at a temperature of 45° C. (Stuart, Zimmerman, Baker & Rustigian, 1942; Stuart & Rustigian, 1943) in the present studies the cells did not survive even for a short period of one day. A few strains did survive a day or so at 45° C., but the survival rate was considerably lower than at lower temperatures. Several mechanisms could account for this: (1) increased metabolism of the cells resulting in death due to either starvation or catabolism of essential enzyme(s), and (2) direct killing action of this high temperature upon the cells.

SUMMARY

1. Ten strains of *Sh. sonnei* were placed upon metal, wood, cotton, paper, and glass and held at −20°, 4°, 15°, 37° and 45° C. Survival of the cells was most extensive when placed at −20° C. and practically none of the strains survived when held at 45° C.

2. Slight variations in the survival of different strains was observed when the cells were placed upon glass and cotton. Strain variation was greater when survival time was measured by placing *Sh. sonnei* on wood, paper, and metal.

REFERENCES

Spicer, C. C. (1959). The survival of *Shigella sonnei* on cotton threads. *J. Hyg., Camb.*, **57**, 210.

Stuart, C. A. & Rustigian, R. (1943). Further studies on the Eijkman reactions of *Shigella* cultures. *J. Bacteriol.* **46**, 105.

Stuart, C. A., Zimmerman, A., Baker, M. & Rustigian, R. (1942). Eijkman relationships of the coliform and related bacteria. *J. Bacteriol.* **43**, 557.