THE SURVIVAL OF *SHIGELLA SONNEI* ON COTTON THREADS

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(With 3 Figures in the Text)

INTRODUCTION

The experiments described here were undertaken to provide some controlled and quantitative information on the resistance of *Shigella sonnei* to drying.

MATERIALS AND METHODS

The organism used in all the experiments was a stock strain of *Sh. sonnei* which had been kept in the laboratory by repeated subculture for some years. It was morphologically unstable and in any plating an appreciable number of rough variants could be seen. A single smooth colony was cultured on Dorset egg medium at the beginning of the work and all subsequent cultures were made from this stock culture.

The method of drying the organisms and investigating their subsequent survival was adopted with minor modifications from one developed by The Committee on Formaldehyde Disinfection, P.H.L.S. (1958).

Ordinary cotton thread (No. 36) boiled for ½ hr. in distilled water to remove the size, was wound on glass formers to make hanks 4 in. long containing twenty threads. The hanks were then attached to lengths of wire by which they could be suspended in closed screw-capped jars. The relative humidity within the jar could be controlled by putting saturated solutions of various salts in it.

After soaking in a suitable suspension of the organism, excess fluid was drained off the hanks and they were then transferred to the jars. At any desired interval after this, the bundles were removed and, after discarding the first ½ in., ¼ in. lengths were cut off and the individual threads teased out and plated on nutrient agar. After 24 hr. incubation counts were made of the numbers of colonies developing from each thread. It was found necessary to examine bundles dipped in suspensions of different bacterial concentrations to make sure that at least one countable plate was available. The counts obtained in this way are, however, liable to error as it is not always possible to distinguish individual colonies clearly, and this may introduce a systematic error tending to underestimate the numbers surviving.

To arrive at an estimate of the fraction of organisms surviving after various periods of drying it is necessary to know how much fluid is absorbed by a single thread. Two series of experiments were done to determine this quantity. For the first method threads were prepared in the usual way and soaked in a suspension

of *Sh. sonnei* in broth. After draining the hank, the last $\frac{1}{2}$ in. was cut off and then the next $\frac{1}{4}$ in. was snipped off into 5 ml. of broth in a screw-capped bottle. After vigorous agitation to break up the bundle of threads and to shake the organisms out of them, counts were made of the organisms in the bottle. Knowing the concentration of organisms in the original suspension it was then possible to calculate how much fluid was absorbed by a single thread. The average quantity on a single thread was estimated by this method to be 0·53 ml. The technical difficulties associated with direct estimations resulted in rather erratic figures for the series and it was decided to check these by the second method.

Bundles of thread prepared in the usual way were soaked in a solution of potassium iodate in these experiments. After soaking and draining, the bundles were dried overnight and the individual threads were extracted in distilled water, the iodate content then being measured colorimetrically. The volume of fluid absorbed was estimated in this way to be about 0·6 ml. per thread which agrees well with the direct estimate within the experimental errors. As the chemical method was more accurate the value 0·6 ml. was used in estimating the number of organisms on a thread at the outset of each experiment.

The saturated solutions used to produce different relative humidities (R.H.), selected from those given by Lang (1951), were as follows:

Saturated solution	R.H. % (15° C.)
$CaCl_2$ (fused)	0
$CaCl_2.6H_2O$	35
NaBr	58
NaCl	78
KBr	84

At lower temperatures, the R.H.'s are slightly lower than those given and at higher temperatures rather higher, but the deviations are not large. The temperature was not strictly controlled during the experiments, which were carried out at two temperature ranges: 15–20° C. (room temperature) and 5–10° C. (cold). A maximum–minimum thermometer was left by the jars during each experiment and read every 24 hr. as a check on any gross fluctuations in temperature. The results at room temperature were obtained by leaving the jars in a cupboard in the laboratory. In this position they were never exposed to direct sunlight and were usually in the dark. Experiments in the cold were carried out in the air-lock leading to a 4° C. cold room. The temperature here remained within narrower limits and the threads were almost always in the dark, only being exposed for short periods to artificial light.

RESULTS

Two separate series of experiments were carried out: one on suspensions of organisms dried from nutrient broth, and the other on suspensions in distilled water. Attention was mainly concentrated on the fraction surviving after 24 hr. drying, but some observations were made on the long-term survival of the organism in broth suspensions; these are liable to cumulative errors and would need extensive

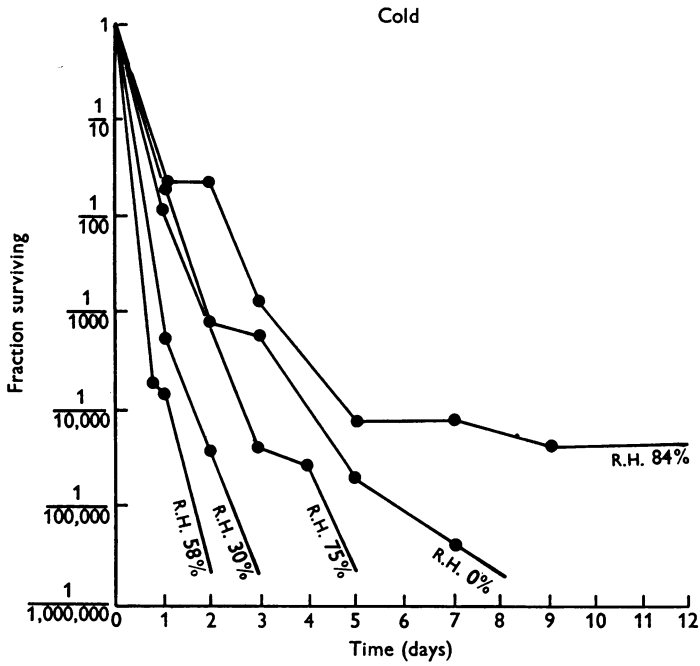

Fig. 1. Survival curves for *Sh. sonnei* on cotton threads at 5–10° C. and various R.H.'s. Threads were impregnated with a broth suspension.

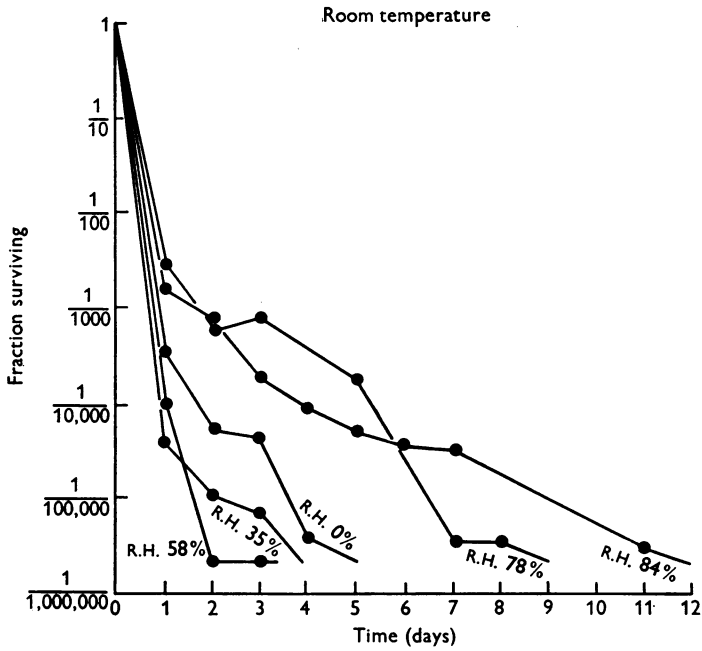

Fig. 2. Survival curves for *Sh. sonnei* on cotton threads at 15–20° C. and various R.H.'s. Threads were impregnated with a broth suspension.

replication to give very accurate results. The main results on the long-term survival of broth suspensions are summarized in Figs. 1 and 2. It will be seen that survival is, on the whole, better at low temperatures, and better at high and low than at intermediate humidities.

Table 1. *Logarithm of fraction of* Shigella sonnei *surviving on cotton threads after 24 hr. drying at various relative humidities and temperatures*

	Logarithm fraction surviving after 24 hr.	
R.H.	Room temp. (15–20° C.)	Cold (5–10° C.)
0	−3·44	−1·70
35	−4·40	−3·28
58	−3·99	−3·83
78	−2·55	−1·96
84	−2·81	−1·66

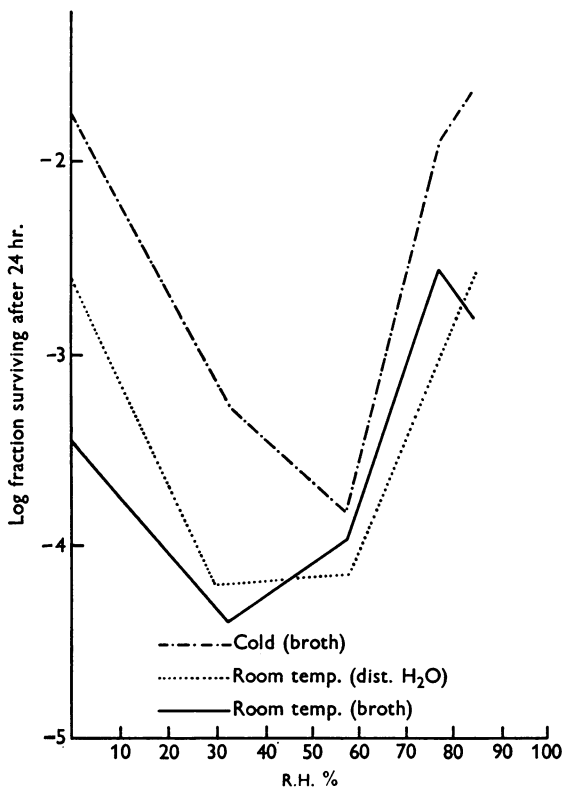

Fig. 3. Relative survival of *Sh. sonnei* on cotton threads after 24 hr. drying at various temperatures and R.H.'s.

The results for survival of broth suspensions after 24 hr. drying at various R.H.'s and temperatures are given in Table 1 and Fig. 3. There is a well-marked minimum of survival at a R.H. of about 50 % and survival is better at low temperatures,

though the curve relating survival and R.H. is of the same general shape in both temperature ranges.

When the experiments with suspensions in broth had been completed it was seen that the results were very similar to those of Dunklin & Puck (1948) on survival of airborne suspensions of pneumococci in droplets, which also revealed a minimum of survival at intermediate R.H.'s. As they found that this minimum was abolished when the suspensions were made in distilled water instead of broth, a series of experiments were set up with *Sh. sonnei* to see whether this organism, when dried on threads, showed the same effect. The results at first, when using ordinary distilled water from a Manesty still, were very irregular. However, when double-distilled water from an all-glass still was used, consistent results could be obtained and these are represented graphically in Fig. 3, which shows the fraction surviving after 24 hr. drying at room temperature and at various R.H.'s. The curve is very similar to that for broth suspensions, and there is a well-marked minimum at about the same range of R.H. as for broth suspensions. The distilled water suspensions were made up by harvesting a 16 hr. nutrient agar slope of the organism into distilled water, washing once, and resuspending in distilled water.

DISCUSSION

The experimental findings reported here, showing that there is a minimum of survival at intermediate R.H.'s, are quite clear-cut, but the estimated values of the fractions surviving at various periods may be somewhat underestimated owing to difficulties in counting. For this reason, and because conditions of survival in faeces are so different, it would be unwise to draw any epidemiological conclusions from the survival curves. When these experiments were originally undertaken it was felt that results obtained with broth suspensions would provide an upper limit for survival since, on the whole, conditions in faeces would be more unfavourable to the organism. This may be true in the faeces of a chronic carrier of *Sh. sonnei*, but could be quite untrue in the faeces of an acute case where the presence of quantities of mucus and the comparative absence of other organisms would considerably improve survival.

The existence of a range of R.H.'s at which survival is comparatively poor seems to be fairly well established, and is not readily accounted for by the explanation advanced for a similar minimum in the case of pneumococci, by Dunklin & Puck (1948). These authors suggest that at very low humidities the organism is so dehydrated that it is not susceptible to toxic agents, while at high R.H.'s enough water is bound to protect the organism from the effects of the solution in which it lies. If this explanation is to hold for the results given here, then it must be assumed that toxic solutes enter the distilled water from the cotton threads. While this is possible it seems unlikely, since the threads are all boiled for $\frac{1}{2}$ hr. in a large volume of distilled water before use. Even if this process did not extract all solutes from the threads one would not expect that enough remained to make the distilled water as toxic as nutrient broth.

SUMMARY

1. Experiments have been carried out to estimate quantitatively the survival of *Sh. sonnei* at various R.H.'s and two different temperature ranges.

2. A fraction may survive for 7–10 days under favourable conditions.

3. At R.H.'s of 40–60 % the organism survives less well than at higher or lower values.

4. Survival is better at temperatures of 5–10° C. than at 20–30° C.

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