THE RESISTANCE OF MENINGOCOCCI TO DRYING[1]

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INTRODUCTION

Ever since the observation of Albrecht and Ghon (1901) that meningococci died within 24 hours after drying on glass cover slips, these microörganisms have been regarded as highly sensitive to dehydration. This opinion was supported by the experiments of Bettencourt and Franca (1904) in which meningococci were dried on glass and also on cotton cloth, and kept at room temperature (20–25°C) and at 37°C. In some instances they were alive at the end of three hours but never at six. Lingelsheim (1905) also observed that meningococci died as soon as they were dried on such objects as glass cover slips. If dried on porous material such as cloth or filter paper they survived longer, but never more than 6 hours at 37° or over 12 hours at room temperature in the dark or in diffuse daylight.

Flügge (1905) mentions some experiments made in his laboratory by Kache who dried meningococci on garnets and glass and found them to be dead after 24 hours in the dark. After drying from fluid containing protein or on linen instead of glass, they sometimes survived as long as 24 or 36 hours, but were almost always dead at 48 hours. Foster and Gaskell (1916) found that meningococci dried on cover slips in a sulphuric acid desiccator were not viable after 5 or 10 minutes. Elser and Huntoon (1909), however, found that while the majority of their strains died in 24 hours, some could survive as long as 72 hours after drying on glass. Their rather elaborate series of experiments suggested that the media on which the organisms had been cultivated, as well as the duration of growth, affected their ability to survive in the dried state.

Jungeblut (1935) states that meningococci dried on cover slips, paper or linen, rarely live longer than 24 hours, although the survival time may be lengthened to several days if they have been dried in human secretions.

The tests of viability employed in the experiments cited above are open to criticism, now that better methods of cultivation are available. Re-investigation of the survival of dried meningococci seemed justified, not only for its bearing on the epidemiology of meningococcal meningitis, but also because bacteriologists working with this microörganism are prone to regard it as a delicate one which perishes rapidly on drying.

The present study concerns only the duration of viability of meningococci dried on films on various objects under ordinary atmospheric conditions and at room temperature in the dark. The effects of light on the survival time is the subject of the succeeding communication.

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SURVIVAL OF DRIED MENINGOCOCCI IN THE DARK

Live, virulent meningococci were dried on glass, wood, and cloth, kept in the dark at various temperatures under atmospheric conditions and cultured from time to time to test their viability.

Methods. The glass surfaces were furnished by the small glass balls of about 4 mm. diameter, which are in common use in bacteriological laboratories for the defibrination of sterile blood, usually called "beads" although they are not perforated. A number of these were poured onto a surface culture of meningococci grown overnight on a solid medium contained in an ordinary pint medicine bottle and were rolled around by gentle shaking of the bottle. Care was exercised that their surfaces were coated as nearly equally as possible. They were then poured out into several sterile Petri dishes which were placed in a large desiccator containing concentrated sulphuric acid. The cover of each Petri dish was propped up about a quarter of an inch by means of a small cork, to permit escape of water vapor without exposing the contents to contamination from the air. During the process of drying, the desiccator was covered by a black rubber apron to protect its contents from the daylight.

The method of drying in a desiccator was adopted for the sake of convenience rather than for any effect on the survival of the dried microörganisms. Drying was usually complete within an hour or two whereas a much longer time was required if the Petri dishes (with their covers raised a quarter of an inch) were placed on a laboratory table during any but dry winter weather. This latter procedure sometimes permitted a certain amount of contamination from the air. The duration of viability was not affected by the rate of dehydration unless it was prolonged to many hours, as it was when done outside of the desiccator during very humid weather. Under those circumstances, the survival time was appreciably shortened.

As soon as drying was complete—indicated by the failure of the beads to stick to each other or to the bottom of the Petri dish—the dish was closed, removed from the desiccator and put into a dark cupboard at room temperature (18–24°C). The dishes were not sealed and were opened each day to remove one bead for culture.

The viability of the dried meningococci was tested at intervals by removing one of the glass beads with sterile forceps and dropping it onto the surface of a blood agar plate poured less than an hour before. The bead was rolled around over the whole surface by gentle sidewise shaking of the Petri dish in several directions and the bead was then removed. The culture was incubated and examined on each of the three succeeding days.

For the other drying surfaces, pieces of wood about 1 cm. square were cut from ordinary tongue depressors and pieces of cloth from various kinds of cotton fabric. After careful rinsing in distilled water, they were sterilized by dry heat and then infected by touching them to surface cultures of meningococci, dried in the same way as the glass beads and kept in a dark cupboard at room temperature. Viability of the meningococci on each of these materials was determined by moistening a piece with a drop of sterile water and rubbing it gently over the surface of a blood agar plate.

Strains employed. In most of the experiments a Type I strain of meningococcus was used. It had maximal virulence for mice, i.e. less than 10 organisms suspended in 4 per cent mucin, sufficed to initiate a lethal infection in a mouse by intraperitoneal inoculation (Miller and Castles, 1936). A few experiments in which both Type I and Type II strains were run in parallel showed no significant difference between them in survival time.

Virulence of the surviving meningococci. On several occasions the last surviving meningococci were tested for virulence in the following way: when the number of colonies per plate was reduced to a very few, transplants were made to agar slants which were grown for 5 hours and then suspended in mucin and injected into mice according to the method customarily employed (Miller and Castles, 1936). The mice dying within 48 hours were autopsied and cultures made of heart's blood to prove that death resulted from meningococcus sepsis. The results indicated that the meningococci were fully virulent.

TABLE 1

Viability of meningococci dried on glass beads

DAYS AFTER DRYING	GROWTH
1 day	++++
2 days	++++
3 days	++++
4 days	+++±
5 days	+++
7 days	++
8 days	+
9 days	+
10 days	22 colonies
11 days	0
12 days	0

++++ = confluent growth.
+++ = innumerable colonies.
++ = 100–400 colonies.
+ = 25–100 colonies.
0 = no growth.

Results. The results of a typical experiment are presented in table 1 and show that as late as ten days after drying, a few viable meningococci could be recovered from the surface of glass beads. It was not until the 11th day that the microörganisms were all dead. The reduction in the numbers of viable organisms during the first three days could not be detected by the method employed.

The progressive diminution of the numbers of viable meningococci dried on pieces of wood or cloth was not as regularly demonstrable, presumably because the surface and texture of such materials prevented as complete contact with the surface of the culture medium as was possible with glass beads. The decline in survival rate, therefore, showed greater irregularity. Nevertheless, it was frequently possible to recover viable meningococci from wood and cotton cloth 8 and 7 days, respectively, after drying.

The results indicate clearly that meningococci are much more resistant to drying than is generally supposed. Living, virulent organisms were recovered with regularity from the surfaces of glass, wood, and cotton cloth a week (in the case of glass beads, as long as 10 days) after drying and storing in a dark cupboard at room temperature (18–24°).

The effect of temperature was not systematically investigated, but a few observations showed that survival was prolonged in the icebox and shortened in the incubator.

The procedure described above is not to be confused with the method of drying meningococci in the frozen state, described by Rake (1935), a modification of Swift's (1921), for purposes of preserving strains in storage.

SURVIVAL OF MENINGOCOCCI IN SATURATED SALT SOLUTIONS

Additional information concerning the resistance of meningococci to dehydration was obtained by suspending them in saturated aqueous solutions of three salts of fairly high solubility: potassium chloride, sodium chloride and ammonium chloride.

Method. A sterile saturated solution of each salt was made by adding enough of the solid to 50 ml. of water in a flask to leave a considerable excess of the salt undissolved throughout the course of the experiment. The flasks were then autoclaved.

Meningococci grown as described above were recovered from an overnight culture in a few milliliters of water and an equal portion added to each flask. The suspensions were mixed by gentle agitation from time to time to make certain that saturation of the salt was maintained.

In most experiments in this series the flasks were kept at room temperature. At various intervals thereafter a 4 or 5 ml. portion of the supernatant (containing the suspended microörganisms but not the solid salt) was removed, centrifuged, and a loopful of the sediment cultured by streaking it onto the surface of a freshly prepared blood agar plate. These cultures were examined for growth on each of the following three days.

The effect of hydrogen ion concentration on the survival time was determined by adjusting a series of saturated salt solutions between the extremes of pH 5.5 and 8.0 by the addition of small quantities of the appropriate acid or base. The final pH was tested colorimetrically.

Results. The results of a typical experiment are presented in table 2 and indicate that at room temperature a certain number of meningococci are able to survive in saturated solutions of potassium chloride and sodium chloride for 30 and 24 hours respectively and in ammonium chloride for only 3 hours. Among a number of similar experiments the maximum survival time in potassium chloride was 51 hours and in sodium chloride 30 hours. In ammonium chloride the microörganisms died regularly within 4 hours at room temperature. Survival was prolonged to a maximum of 4 days at icebox temperature and shortened at 37°.

The molar concentrations of these saturated solutions were not equal. At

24°C they were calculated to be as follows: KCl = 4.69 M, NaCl = 6.15 M, NH_4Cl = 7.29 M. Nevertheless, the differences noted above cannot be explained on the basis of relative molar concentrations, because meningococci suspended in equimolar solutions of the three salts, all at 4.69 M (which is saturated for KCl, the least soluble), died off in the same order, though not at exactly the same rate as in the experiments described above. It is clear, therefore, that those differences are due to the relative toxicity of the three anions.

Experiments in which the hydrogen ion concentrations of the saturated salt solutions were varied between pH 5.5 and 8.0 showed that in the case of both potassium chloride and sodium chloride the optimum for the prolongation of survival time lay between pH 7.3 and 7.6. Deviation in either direction from this optimum range affected the survival time less in the case of potassium chloride than in the case of sodium chloride.

TABLE 2

Viability of meningococci in saturated salt solutions

Growth from a loopful of sediment recovered by centrifugation from 4.0 ml. samples removed at various times

TIME	SATURATED SOLUTION OF:		
	KCl	NaCl	NH_4Cl
hours			
1	++++	++++	+++
3	++++	+++	++
6	+++	++	0
24	+++	+	0
30	++	0	0
53	0	0	0

DISCUSSION

The meningococcus has generally been regarded as a sensitive organism unable to resist such adverse changes as are brought about by drying. The earlier experiments on which this opinion was originally based, date back to a time when cultivation of meningococci on artificial media was less satisfactory than it is today. We expected, therefore, that our tests for viability would show some lengthening of the period during which positive cultures could be obtained, but we were quite unprepared for the difference between our results and those of earlier investigators.

In the present study the microörganisms were subjected to dehydration by two means: (*a*) drying on the surfaces of objects and (*b*) suspension in saturated salt solutions. For the former the surfaces we used were those of glass, wood, and cotton cloth. Drying was accomplished at room temperature and atmospheric pressure, but was hastened by enclosure for a few hours in a desiccator containing concentrated sulfuric acid or anhydrous calcium chloride. When the films of microörganisms were dry by ordinary standards, not thoroughly desic-

cated, the objects were removed and placed in a dark cupboard. In this state and under these conditions the meningococci remained alive for a number of days. It was not possible to determine the proportion of the original bacterial population on a given surface which survived to any given time, because the method employed gave no information on their rate of death during the whole period of observation, but only the duration of survival of the most resistant individuals.

The effect of daylight of various intensities on the survival time of meningococci is the subject of a succeeding communication.

No observations were made on the survival of meningococci derived directly from patients or carriers, as contained for example in infected discharges from the upper respiratory tract. Our results do suggest the possibility, however, that fomites may be able to play a rôle in the spread of meningococcal meningitis, a possibility usually overlooked in considering the epidemiology of that disease.

The other method of subjecting meningococci to dehydration was suspension in saturated solutions of potassium, sodium, and ammonium chlorides. The differences in survival times were clearly due to the relative toxicity of the anions, that of potassium being least toxic; sodium only slightly more and ammonium much more so. In KCl viable meningococci persisted for 30 to 51 hours.

The study of the duration of viability of meningococci suspended in saturated salt solutions contributes nothing of practical applicability. It was included because such treatment subjects the microörganisms to dehydration by another means and supplies additional evidence of the ability of meningococcus to survive exposure to environmental changes commonly regarded as rapidly lethal.

SUMMARY

Meningococci were dried on glass beads, pieces of wood, and cotton fabrics, which were kept at room temperature in the dark and cultured each day. Viable meningococci were recovered from glass as long as 10 days and from wood and cotton cloth, 8 and 7 days thereafter. Survival was shortened at 37° and prolonged in the ice box (6–10°). The virulence of the viable meningococci for mice was not lost. The possible bearing of this observation on the epidemiology of meningococcic meningitis is discussed.

Meningococci were also subjected to dehydration by suspending them in saturated solutions of three salts of high solubility, KCl, NaCl, and NH_4Cl. Aliquot portions were removed at intervals and the microörganisms sedimented by centrifugation and cultured. Viable meningococci were recovered from saturated KCl after 30 hours and from NaCl after 24 hours, but never after more than 3 hours in NH_4Cl. The differences in survival time were due to the relative toxicity of the anions of the solutions rather than to differences in their molar concentration. The meningococci surviving such treatment were still virulent for mice.

CONCLUSIONS

Meningococci are much more resistant than is generally supposed to dehydration either in films dried on the surface of objects protected from light, or suspended in saturated solutions of non-toxic salts such as sodium or potassium chloride.

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