TRANSMISSION OF GROUP A STREPTOCOCCI

III. THE EFFECT OF DRYING ON THE INFECTIVITY OF THE ORGANISM FOR MAN*

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(With 3 Figures in the Text)

INTRODUCTION

The sources of airborne bacteria within living quarters are minute droplet nuclei which remain suspended in the air following expulsion from a carrier and con-taminated particles which have become suspended from dried environmental deposits. Since group A streptococci may be isolated from environmental deposits in populations experiencing an epidemic of streptococcal respiratory disease (Loosli, Lemon, Wise & Robertson, 1952), it has been assumed that contaminated particles of dust serve as an important reservoir of infection. Indeed, many of our measures employed to control respiratory infection in recent years have been directed against this reservoir.

Recently, it has been demonstrated that group A streptococci naturally deposited on blankets or in particles of dust do not appear to play an important role in the spread of streptococcal respiratory infections (Perry, Siegel, Rammelkamp, Wanna-maker & Marple, 1957; Perry, Siegel & Rammelkamp, 1957). In fact, the evidence indicates that group A streptococci deposited in the environment rarely, if ever, cause respiratory disease. The present study was designed to obtain further information on the factors responsible for the loss of infectivity of streptococci after expulsion from the respiratory tract. These studies also presented an oppor-tunity to make observations on the time of development of clinical symptoms and the bacteriology of the oro-pharynx following inoculation of group A organisms.

METHODS

A total of three sets of experiments was performed utilizing as a source of group A streptococci the contaminated secretions of patients with classical symptoms and signs of acute streptococcal respiratory disease. The initial inocula were prepared from secretions obtained early in the illness and were dried at room temperature

for 4–8 hr. Subsequent inocula were prepared from secretions obtained from each donor later in the course of the illness, but the periods of drying were decreased. No specific therapy was administered to the donor patient until after the last inoculum was prepared.

Dust was obtained from floor sweepings and, after sifting through triple mesh (14 gauge) wire screen, was sterilized by dry heat. The donor patient expectorated into a sterile Petri dish containing the sterile dust. After thorough mixing the contaminated dust was exposed to the air at room temperature for as long as 8 hr. Throughout this period the dust was agitated at regular intervals and aliquots were removed and diluted in dextrose-phosphate broth. Pour plates of the various dilutions were made in agar containing 5 % sheep blood and 1 : 1,000,000 gentian violet. Following incubation for 18 hr. at 37° C., the β-haemolytic colonies were enumerated and an estimate was made of the number of streptococcal chains contained in the specimen of dust employed for inoculation.

Inoculations were performed by one of three methods. If the specimen of dust was dry or only slightly damp, 0·1 g. was placed in a small sterile plastic tube which was bent at an 80-degree angle at the distal end. The proximal end was attached to a large rubber bulb. The distal end was inserted through the mouth up and behind the soft palate, and the dust was then forcibly expelled into the naso-pharynx by sudden pressure on the bulb. Wet dust was placed directly on the posterio oro-pharynx with a bacteriological loop. Later, inoculation of fresh, wet secretions was accomplished by rubbing a swab obtained from the patient directly on the oro-pharyngeal tissues of the volunteer. Similarly, transfer of contaminated secretions was made from the nose of the donor to the nose of the recipient.

All volunteer subjects were healthy male members of the laboratory staff. None harboured group A streptococci in the oro-pharynx prior to inoculation. Following inoculation, cultures of the oro-pharynx and the anterior 2–3 cm. of both nares were obtained at regular intervals. One swab was streaked directly on to sheep blood agar plates and another was placed in Pike's (1945) selective media. In the latter instance, the Pike cultures were incubated at 37° C. overnight and subcultured on sheep blood agar. The agar plates were inspected after overnight incubation and the number of β-haemolytic colonies recorded. Selected colonies from each plate were identified serologically by standard procedures (Swift, Wilson & Lancefield, 1943).

Blood from each volunteer was obtained prior to inoculation, and again approximately 3 weeks after the last exposure. Bacteriostatic antibody tests were performed by a modification (Denny, Perry & Wannamaker, 1957) of the Rothbard (1945) technique. Prior to and following inoculation, oral temperatures were taken at regular intervals and all symptoms recorded. Regular observations of the oro-pharynx and cervical lymph nodes were made at the time the cultures were obtained. Total leucocyte counts were performed at frequent intervals.

RESULTS

The donor of the secretions for the first study developed a sore throat and fever on 25 May and entered the hospital on the following day. On admission, the oro-pharyngeal tissues were red, swollen and the tonsils exhibited discrete areas of

exudate. The cerival nodes were neither enlarged nor tender. The leucocyte count was 11,000 and cultures from both the nose and throat showed a predominant growth of β-haemolytic streptococci of *Type 14*. The antistreptolysin titre on admission was 200 and 20 days later was 500.

Dust contaminated with fresh secretions from this patient was prepared on 27 May and allowed to dry at room temperature for 8 hr., at which time no moisture could be detected in the sample by gross inspection. According to estimates, the numbers of viable streptococci present in the dust initially and after 4 and 8 hr. of drying did not change. One-tenth gramme of dust dried for 8 hr. was introduced into the posterior naso-pharynx of three volunteers as described. The inoculum contained 10,500,000 streptococcal chains. The volunteers were presumably susceptible to *Type 14* infections, since sera obtained prior to inoculation contained no type-specific antibody.

An example of the method of observation and results in one of these volunteers is shown in Fig. 1. Cultures of the oro-pharynx were obtained at 2-hourly intervals for 94 hr. No group A, *Type 14* organisms were isolated. Two of the recipients showed single β-haemolytic colonies on one and two occasions, respectively. These organisms were classified as group B and *Type 4* streptococci. No signs of an illness developed and the total leucocyte count remained in the normal range in all three subjects.

Ninety-four hours after the inoculation a fresh specimen of secretions was obtained from the same donor who was now in the seventh day of illness. A wet inoculum of dust containing approximately 32,500 *Type 14* streptococcal chains was prepared and placed with a bacteriological loop on the posterior pharyngeal wall. It was observed that the dust particles immediately disappeared, presumably by swallowing. Two of the volunteers were followed for 40 hr. and no group A streptococci were recovered from the throat culture. The remaining voluteer was cultured for 4 successive days and no infection was observed.

Since infection did not result from these inoculations, two of the original recipients and a new volunteer were inoculated directly with a swab obtained from the patient. The patient was now in the ninth day of illness and an oro-pharyngeal culture plated directly on a blood agar showed less than fifty β-haemolytic colonies.

Of the three volunteers so inoculated, one developed classical symptoms and signs of a streptococcal infection (Fig. 1). In this patient, who was one of the original subjects, a few *Type 14* colonies were isolated 2 hr. after the procedure and again at 14 hr. Large numbers of colonies on the blood agar plate were first observed 24 hr. after inoculation and 4 hr. prior to the first symptom. Fever did not develop until about 42 hr. after exposure to the organism. The illness in this individual was severe and was followed by peritonsillar cellulitis. No illness developed in the other two volunteers and, other than a single colony of *Type 14* streptococci isolated from one volunteer 24 hr. after inoculation, all cultures were negative. The antistreptolysin titre did not increase in these two subjects.

In the second study the donor was a patient exhibiting exudative tonsillitis of 6 days duration. The total leucocyte count on the third day of illness was 19,300 and *Type 19* streptococci were identified from several oro-pharyngeal cultures.

A small amount of secretions obtained from the mouth and oro-pharynx was mixed with sterile dust and dried for 4 hr. at room temperature in a sterile Petri dish. It was noted that at 2 hr. the dust was slightly moist, but at 4 hr. the dust resembled dry powder.

Fig. 1. Results of inoculation of a volunteer with contaminated dust in the first study.

A total of 0·1 g. of dust dried for 4 hr. containing 3050 colonies of streptococci was blown into the posterior naso-pharynx of two volunteers. The observations made on one of these subjects is shown in Fig. 2. During the 64 hr. following inoculation, no streptococci were isolated from either subject and no symptoms developed. At this time a second inoculation was performed employing the same donor, who was now in the ninth day of illness and exhibited no symptoms. A swab was rubbed over the oro-pharyngeal tissues of the recipient. Likewise, a nasal swab was transferred directly to the nares of the volunteer. The culture of the throat of the donor showed only a few β-haemolytic colonies, whereas the nasal culture showed no such colonies on the blood agar plate.

Following the inoculation one recipient remained well, showed no group A streptococci on culture, and developed no increase in titre of antistreptolysin. As shown in Fig. 2, the other recipient developed a sore throat 44 hr. after inoculation. The first positive culture was obtained 38 hr. after inoculation, but large numbers of these colonies were not observed until after symptoms developed.

The donor for the third study was observed 2 days after the onset of a sore throat. Examination showed exudative tonsillitis; the leucocyte count was 12,200 and the culture showed *Type 14* streptococci. On the fifth day of illness a large

amount of pharyngeal secretions was mixed with sterile dust and allowed to dry at room temperature. Two hours later the sample was wet, and at 4 hr. the particles were still adherent and appeared to be slightly damp.
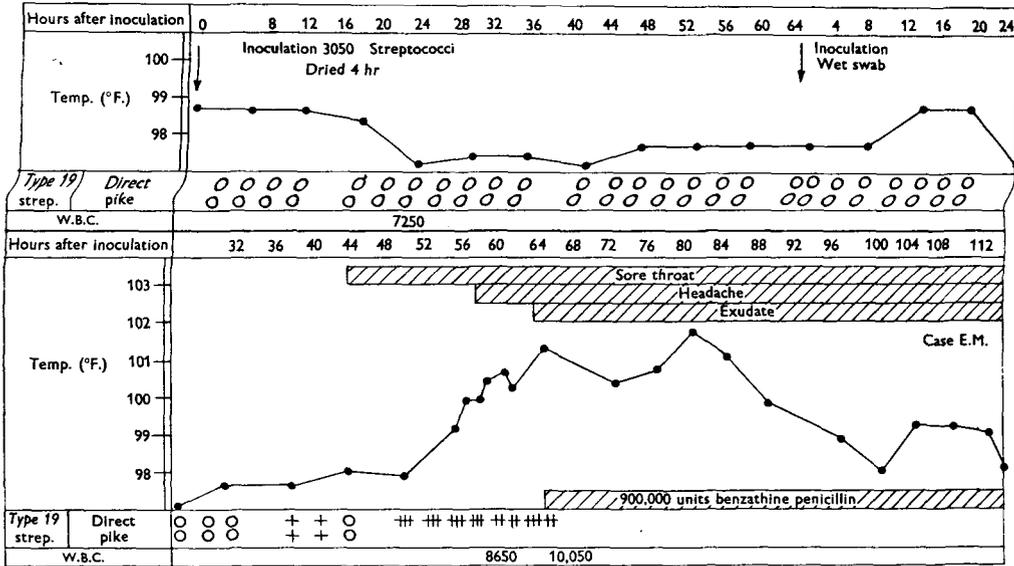

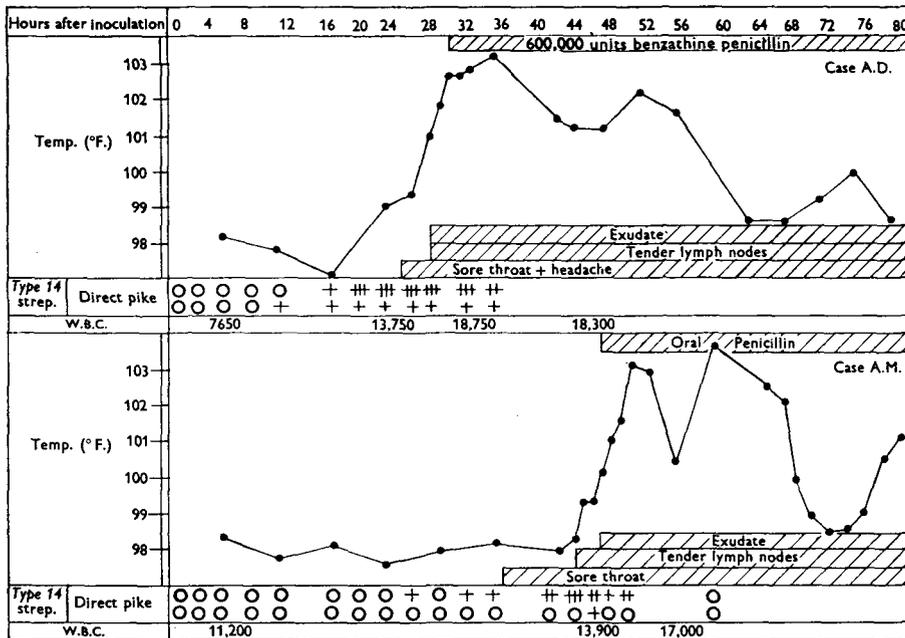Fig. 2. Results of inoculation of a volunteer with contaminated dust in the second study.

Fig. 3. Infection developing in two volunteers after inoculation of damp contaminated dust in the third study.

One-tenth gramme of the dust dried 4 hr., containing an estimated 22,000,000 streptococci, was forcibly blown into the posterior naso-pharynx of three volunteers. One recipient showed no signs of infection, and all subsequent cultures were negative. The results obtained in the other two subjects are shown in Fig. 3. Symptoms of a streptococcal infection developed 24 and 35 hr. after exposure and cultures of the throat were first positive at 10 and 26 hr., respectively, after the inoculation.

DISCUSSION

The practice of destroying certain articles and sterilizing others which have been used by patients with scarlet fever, has resulted from the general concept that environmental deposits of streptococci are important in the spread of disease. In more recent years, those interested in the spread of disease by airborne routes have emphasized the high degree of contamination of air, clothing, bedding and dust. Various procedures have been devised to control or to eliminate the bacteria in the environmental reservoirs, but it has been difficult to demonstrate that such methods are followed by a reduction in illness (Wright, Cruickshank & Gunn, 1944; Begg, Smellie & Wright, 1947; Willmon, Hollaender & Langmuir, 1948).

An indirect approach to the problem of the role of environmental contamination in the spread of streptococcal disease was recently reported (Perry *et al.* 1957). In these studies individuals were exposed to blankets containing large numbers of group A streptococci. Men so exposed did not exhibit an increased risk to infection, and indeed, it is doubtful whether any subject acquired an infection from the organisms deposited on the blankets. The failure of infection to occur in these studies can be interpreted in two ways. Either such deposits are not infectious or they did not find access to the mucous membranes of the recipients of the blankets. Further studies involving direct inoculations were necessary to separate these two possibilities.

In the initial studies (Perry, Siegel & Rammelkamp, 1957) dust collected from the floors of military units experiencing epidemics of streptococcal disease was used as the inoculum. A total of seventeen inoculations were made with dust containing four different serological types of streptococci, and in no instance did infection result. Furthermore, individuals who were known to harbour no group A streptococci failed to acquire an infection when residing in contaminated living quarters, or when exposed to contaminated dust forcibly blown into a small enclosure. These studies showed in a direct fashion that group A streptococci in dry dust do not produce respiratory disease when deposited on the respiratory mucous membranes.

In the above studies where dust was employed, inoculation of the recipients was accomplished many hours after the dust was contaminated. This interval could not be determined accurately, and, in addition, it could not be established that the organisms in the dust were actually deposited by individuals with active disease. In the present study the group A streptococci were obtained directly from patients with streptococcal respiratory disease. Therefore, the strains employed can be assumed to be capable of producing disease in man. Although the number of experiments is small, the data suggest that group A streptococci deposited in dust lose their ability to infect man once they are dried. However, drying under natural

conditions does not appear to reduce appreciably the number of streptococci in the dust samples which will grow out on artificial media. The results of these and previous studies (Perry *et al.* 1957; Perry, Siegel & Rammelkamp, 1957) strongly *suggest that dried streptococci in the environment, although viable, are not an* important source of respiratory disease. If these observations employing dried dust are valid, then it would appear doubtful that droplet nuclei containing viable group A streptococci serve as an important source of infection.

In the present studies, as well as those reported in the past (Perry *et al.* 1957), the volunteers were considered susceptible to infection on the basis that serum from the recipients contained no measurable antibody against the specific types contained in the inoculum. In the work reported here susceptibility was further defined by the demonstration of infection following direct inoculation of secretions from the patient.

Few bacteriological observations have been made during the incubation period of streptococcal infections. For a time following inoculation it is difficult or impossible to recover the organism, and it is possible that they are temporarily lost by dispersion or that only a few organisms survive. Subsequently, they invade the tissue and rapidly spread. Large numbers of organisms are obtained on culture only a few hours before the onset of symptoms. Thus, it would appear that the patient with streptococcal respiratory disease is not likely to serve as an important source of infection until a few hours before symptoms develop.

CONCLUSION

Oro-pharyngeal secretions from patients with streptococcal infections were placed in sterile dust and dried for 4–8 hr. without appreciable decrease in number of organisms recoverable on artificial media. When dry, such contaminated dust did not cause respiratory disease upon direct inoculation into volunteers, whereas infections resulted following inoculations with wet dust or with direct transfer of secretions to volunteers.

In successful inoculations, the inoculated organisms could rarely be recovered until a few hours before the onset of symptoms, at which time they appeared in large numbers.

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