DISSEMINATION OF STREPTOCOCCI THROUGH INVISIBLE SPUTUM.

IN RELATION TO SCARLET FEVER AND SEPSIS.

ALICE HAMILTON, M.D.
(From the Memorial Institute for Infectious Diseases.)
CHICAGO.

INTRODUCTION.

Since the completion of this investigation an article has appeared in the *Archiv für klinische Chirurgie,* by Mendes de Leon, describing experiments conducted along the same lines as my own, and having practically the same results. Dr. de Leon calls attention to the work done in Flügge's laboratory which proved the importance of droplets of sputum in the dissemination of tuberculous infection, and comments on the fact that the sputum as a source of infection has apparently been overlooked by surgeons. He instituted experiments similar to those of Flügge, but modified so as to reproduce the conditions of the operating room, and succeeded in proving that the mouth of the operating surgeon is a fruitful source of streptococcal infection. During an ordinary operation, not performed before a class, a surgeon speaks from 150 to 500 words. Plates laid on the table before a number of surgeons while they spoke the above number of words were found to contain about 75 colonies, the larger number of which were streptococci; the colonies of diplococci, staphylococci, sarcinæ following in this order of frequency. Tested on rabbits and on guinea-pigs, five out of ten of the strains of streptococci proved virulent, six out of ten of the diplococci and two out of three staphylococci. Antiseptic mouth washes were found to be of no value; the number of colonies on the plates was slightly diminished, but the proportion of streptococci increased, for the effect of the wash was to increase the amount of thin watery saliva, and it is this kind of saliva which seems to play the chief part in the spread of streptococci.

Dr. de Leon recommends a mouth guard of special construction, which, on test, was found to hold back almost completely the droplets of sputum.

It will be found that my experiments add little that is new to those of Dr. de Leon. They will serve, however, to confirm his statement as to the dissemination of streptococci by the sputum expelled in talking, and also to show that forcibly expelled breath, even in the absence of speech, is a source of infection.

SCARLET FEVER.

These experiments were undertaken primarily in connection with scarlet fever. In the Memorial Institute for Infectious Diseases one or two occurrences had suggested the possibility that the streptococcus might pass from one patient to another, and that a scarlet-fever patient with severe streptococcal complications might become a source of danger to the uncomplicated cases in the same ward.

The theory that the streptococcus is the causative agent in scarlet fever is finding daily fewer supporters and bids fair to be abandoned entirely, yet the importance of this organism as the cause of most of the complications of scarlet fever has suffered no diminution. Streptococcal infection is responsible for most of the cases of severe necrotic angina, for the otitis media and its consequent mastoiditis and meningitis, for the purulent rhinitis and conjunctivitis, for the suppurating glands, the purulent synovitis, many of the cases of bronchopneumonia, and probably for all cases of true nephritis, while many deaths in the course of this disease are deaths from sepsis and not from scarlet fever proper. Rumpl, indeed, believes that in the majority of cases this complication is more important than the disease, and that, from the fourth day on, the symptoms caused by the streptococcus usually predominate and may completely mask the scarlet fever proper.

Recent bacteriologic studies in scarlet fever show that the streptococcus is present in the large majority of cases. It was found in 40 out of 41 throats (Weaver), 4 being pure cultures. It was found in pure culture in 38.8 per cent. of cases of pseudomembranous pharyngitis (v. Ranke). Babes found it in the internal organs of 17 fatal cases, and Baginsky and Sommerfeld found it in the blood and internal organs of 82 fatal cases.[1] In many instances it is apparently of a low virulence, in others it seems to be non-virulent, for scarlet fever may run its course without any streptococcal complications, even when the streptococcus is present on the tonsils. The appearance of such complications, therefore, must mean invasion not only by the streptococcus, but by a virulent strain. It is a common laboratory procedure to change a non-virulent into a virulent strain or to heighten the virulence for the lower animals. Sometimes it can be effected simply by transplantation to a more favorable nutrient medium; e. g., blood serum or ascites fluid, or in other cases it is done by passing the germ successively through susceptible animals. In this way an enormous increase of virulence can be attained. Experiments in heightening virulence for human beings are, of course, out of the question, yet there seems no reason to doubt that the same thing may be true of them also, and that a given organism, only slightly virulent for man, may have its virulence raised by similar methods; e. g., by changing its habitat from a normal pharynx to one altered by catarrhal inflammation, or by passing successively from patient to patient. We may imagine a streptococcus of low virulence in the throat of a scarlet-fever patient being disseminated into the air of a hospital ward and, when inhaled by a second patient, increasing in virulence and setting up a necrotic pharyngitis in its second victim, a general sepsis in the third, and so on until it reaches the point when it is capable of causing the fulminating type of sepsis.

In an epidemic of scarlet fever it is sometimes noted that while many of the cases are mild, all the children in a certain family are taken with a malignant, fatal form of the disease. Usually we explain such occurrences as instances of a family predisposition to or unusually low resistance to the scarlatinal infection. Possibly, however, such cases may be due to successive infection with an increasingly virulent strain of streptococcus.

That cases of scarlet fever with septic complications may be a source of danger to others is no new idea. In the hospital of the Pasteur Institute in Paris all scarlet-fever patients are kept strictly isolated in separate rooms up to the period of convalescence. I was told when visiting this institution that the result had been a very low average of cases with complications and sequelæ. The same statement is made by R. E. Lauder, medical superintendent of the Borough and Port Fever Hospital, Southampton, England, who not only separates

1. A full review of the literature of this subject is given in an article by Ludvig Hektoen, "Streptococcemia," THE JOURNAL A. M. A., March 14, 1903.

the acute cases from the convalescents, but isolates all those with discharges from nose, ears or eyes, and all with swollen tonsils or lymphatic glands. As a result he claims a decided diminution in the number of patients with such complications.

At the Memorial Institute for Infectious Diseases, Chicago, several occurrences have pointed to the transmission of virulent streptococci from scarlet-fever patients to normal persons through the respiratory tract. One of the staff has twice contracted severe purulent rhinitis and pharyngitis when in attendance on cases of scarlet fever with unusually extensive streptococcal complications. Another developed a severe tonsillitis with chills and fever the first time he visited the scarlet-fever ward, and streptococci were found in his throat and blood. Still another developed a streptococcal tonsillitis after his first visit to the ward, and again after performing an autopsy on the body of a child dying of scarlatinal sepsis. In this hospital, as in others, it has been observed that the nurses are apt to have sore throat of varying severity during the first days of their service.

During April and May, 1904, an effort was made to prove whether or not scarlet-fever patients actually disseminate streptococci into the surrounding air. The method employed was the following: Sterile Petri dishes were filled with rabbit's blood agar (one part of defibrinated rabbit's blood to eight parts of glucose-peptone agar), and were used to catch the invisible droplets of sputum expelled from the mouths of scarlet-fever patients when coughing or crying, or when breathing heavily with the mouth open. Fifty cases were examined from the Memorial Institute, from the Cook County Hospital and from the Scarlet Fever Hospital of the New York Department of Health. At the same time that the plates were collected, cultures on glucose agar were made from the tonsils and examined for the presence of the streptococcus.

The method does not give very uniform results. It is impossible to regulate the amount of sputum which will reach the plate, for a vigorous cough may not produce as many colonies as a more feeble one, and crying may not produce as many as simple breathing, the variations depending on the dryness of the mouth and the thickness of the sputum. It was found impossible to make any absolute rule as to the time of exposure of the plates, or the distance at which they were held. For the majority of cases two plates were used; one held at from 12 to 15 centimeters from the mouth, the other at from 18 to 24. The patient was asked to cough twice while each plate was so held. If the cough was feeble the plate was held nearer, if very vigorous, farther away. In the case of crying babies the plates were held for one-half minute in front of the mouth. Some of the patients were too ill or too young to cough, and then the plate was simply held before the mouth for almost a minute, provided the patient was breathing rather forcibly and with the mouth open. In spite of every effort to attain uniform results the colonies on the plates varied in number from nothing to 175.

On blood agar the colonies of streptococcus are usually easily distinguishable, for the hemolytic action of this organism on rabbit's corpuscles results in the formation around the colony of a ring or halo which is either clear or greenish, according to the hemolytic power of the individual streptococcus. The halo around the pneumococcus colonies is more decidedly green than that around the streptococcus (Schottmüller), and the staphylococcus colonies, which also have a clear halo, can usually be distinguished easily by their greater size. In all cases of doubt the colonies were transferred to bouillon and examined the next day.

As a result of the study of these 50 cases, it was found that streptococci were present on the tonsils of 47, as shown by the cultures from swabs. It was expelled by coughing or crying in 20 out of the 34 patients who could either cough or cry, and by expiration in 13 out of the 16 patients who were too ill to be made to do so. A greater proportion of positive cases were obtained from the patients over 10 years of age than from those under; the feeble attempts at coughing made by the younger children and the feeble expiration often resulted in plates with very few colonies. Seventeen of the 23 cases over 10 years of age had streptococci on their plates, only 16 of the 27 under this age.

The proportion of streptococcus colonies to those of other organisms varied from five out of nine colonies to one out of ninety-eight. The proportion also varied in the two plates taken from the same individual. Thus in one case the first plate had 23 colonies, with no streptococci; the second—held farther away—had 43, of which 12 were streptococcus colonies. The plates collected from the 16 patients who were too ill or too young to cough contained colonies ranging from 3 up to 100. There were streptococcus colonies in 13 of those 16 cases; in 7 of the 13 this organism formed over 25 per cent. of all the colonies present.

These 50 cases represented almost all varieties of scarlet fever, and were taken during the first three weeks of the disease. Fifteen had severe pharyngitis, but the number of streptococci expelled by these cases did not average higher than the number expelled by the others.

These experiments prove that living streptococci are expelled into the air by scarlet-fever patients, and this not exceptionally, but in as much as 66 per cent. of all cases. In all probability some are non-virulent—those from the cases in which the angina is mild in character. In others there is reason to believe that they are moderately virulent, and in some the virulence may be of a very high grade. Animal experiments to determine the degree of virulence of a given streptococcus are notoriously unsatisfactory, as an organism which has been rendered highly virulent for one species of animal may be only slightly so for another, and an organism isolated from a case of scarlatinal septicemia may not prove at all virulent for the lower animals. We must, therefore, be satisfied with probabilities, but these are certainly strong enough for us to proceed on the assumption that a strain of streptococcus which is virulent for one human being is virulent for others also. Since, then, it has been shown that the streptococcus is expelled into the air surrounding the patient, we are justified in urging that those patients who are suffering from severe streptococcal complications should be isolated from uncomplicated patients, or that in hospitals where isolation is impossible a gauze mask should be used to cover the patient's mouth.

SURGICAL SEPSIS.

The theory of the "unity of the streptococcus" has gained ground of late years, although Schottmüller still distinguishes two species on the ground of cultural variations and different degrees of virulence. Schottmüller himself, however, states that the streptococci which cause erysipelas, phlegmon, septicemia, puerperal sepsis, the complications of scarlet fever, diphtheria, etc., are indistinguishable from each other. The same species

apparently may cause processes as different as a paranitium and a case of fulminating puerperal sepsis, according to the degree of the virulence of the strain, the character of the tissue invaded and the resistance of the individual. It is easy to show experimentally that the same organism can be used to produce different pathologic processes. The streptococcus isolated from a case of angina in man may set up either abscesses, erysipelas or general sepsis in a rabbit. The streptococcus from the body of a child dying of scarlatinal sepsis at the Memorial Institute for Infectious Diseases caused not only a local suppurative process in the finger of an interne who assisted at the autopsy, but also a severe tonsillitis with albuminuria, and it is certainly probable that the converse might also occur; that the organism which has caused a tonsillitis in one person might, if inoculated in a denuded surface of a second, set up suppuration in the latter. If streptococci are expelled from the throat of the nurse or of the surgeon at the time of an operation or of a surgical dressing, there seems to be no reason why they should not gain lodgment in the tissues of the patient and be responsible for a local suppuration or a general sepsis.

Every hospital with a surgical or maternity service has histories of mysterious epidemics of septic infection which could not be explained satisfactorily—which occurred apparently spontaneously and could not be traced to any neglect of ordinary precautions on the part of surgeons or nurses. I have tried to discover whether in any hospital such accidents were coincident with epidemics of sore throat among the nurses, internes or attending surgeons, but apparently the idea is a new one, and no definite information can be obtained as to such a coincidence. Slight sore throat is so common in our climate in winter that it occasions little remark, and even when it attacks a large number of nurses at once it may not actually incapacitate any of them and is quickly forgotten. In one hospital I find that this was suggested as a possible cause for a succession of cases of streptococcal infection in a surgical service. After all possible causes had been inquired into in vain, one of the house physicians called attention to the fact that several nurses on surgical duty at the time had been suffering from a form of tonsillitis caused, apparently, by the streptococcus.

In order to discover whether the air expelled by healthy adults may actually be a source of streptococcal infection, a bacteriologic examination was undertaken, during the months of April and May, of the invisible sputum of 50 persons, 40 of whom were hospital nurses, and 10, physicians connected with hospitals. We know that streptococci are present on the tonsils in normal throats in the great majority of cases, if not invariably. Hilbert and Ruth Tunnicliff each examined the tonsils of 100 normal throats and found it in all. The bacteriology of the saliva has been studied by Netter, Hübener, Barthel and v. Dungern, all of whom found streptococci, the last as a constant inhabitant of the saliva. The technic used in this study was the same as in the former, except that in addition to the invisible sputum expelled in coughing, that expelled in speaking also was examined.

A culture was made from the throat, and plates were held before the mouth at a distance of from 12 to 36 centimeters while the individual coughed twice, and at a distance of from 9 to 24 centimeters while he counted aloud up to 100. The 50 persons were all in good health, although a few, who will be referred to later on, had slight sore throat, but not enough to keep them from their usual work.

The following results were obtained: Streptococci were found in 46 of the 50 cultures taken from the tonsils. Colonies were found on 33 of the plates held before the mouth while coughing, and on 26 of those held while speaking. In several cases they were found on one set of plates, but not on the other, so that the number of those who expelled streptococci, either in coughing or in speaking, or in both ways, rose to 42, almost as many as those whose throat cultures contained streptococci. It was found that the streptococci were expelled to a distance of at least 24 centimeters from the mouth in speaking, to a distance of at least 36 centimeters in coughing, and that whispering, with its forced expiration, produced more sputum than speaking in an ordinary voice. The percentage of positive cases in this series of experiments is greater than in the first series, owing to the fact that the persons examined were adults, and the expulsion of sputum much more vigorous than in the case of the scarlet-fever children.

As in the plates from the scarlet-fever cases, the number of colonies varied greatly—from nothing to 225—and also the proportion of streptococcus colonies—from one in 30 to 30 out of 39. The plates held before the mouth while speaking had about the same number of colonies and the same proportion of streptococcus colonies as the plates held while coughing. The plate from one nurse contained 35 colonies, 28 of them streptococcus; that from another 58 colonies, 39 of them streptococcus; that from a physician had 74 colonies, 34 of them streptococcus.

Among the 50 patients described above were 9, of whom 5 had, at the time the examination was made, a mild angina, the other 4 had had tonsillitis of moderate severity within the preceding three weeks. All of these 9, with one exception, expelled streptococci in speaking or in coughing, the one negative case being a staphylococcus infection. In three of these cases the streptococcus formed 50 per cent. or more of all colonies on the plates.

It has already been stated that animal experiments are unsatisfactory, so far as the determination of the virulence of an organism is concerned; nevertheless, as a matter of curiosity, seven of the organisms isolated from these cases were injected into as many guinea-pigs in the dose of 1 per cent. of the body weight, for as the guinea-pig is not very susceptible to the streptococcus, a relatively large dose must be used. Four of the seven strains thus tested proved pathogenic, the animals dying after from one to three days and the streptococcus being recovered from the peritoneal cavity and organs. It is not probable that the streptococci from the throats of these people engaged in hospital work were more virulent than would be those from the throats of persons engaged in other work, for Hilbert tested ten organisms isolated from the throats of healthy school children, and found them all pathogenic to white mice.

The conclusions to be drawn from this second series of observations are obvious. Surgeons and nurses may themselves be the source of septic infection in their patients even after all the usual precautions as to disinfection have been observed. The surgical dressings are prepared beforehand and handled during the operation by nurses with unprotected mouths, who necessarily speak or whisper to each other while so engaged, and who can hardly guard against an unexpected cough.

The surgeon is, however, a far greater source of danger than the nurse. His head is close to the wound, sometimes within a few inches of it, he must give orders to his assistants, and even while he is silent, the strain incident on a difficult operation is liable to make him breathe harder with his lips apart; the short forcible expirations carrying—as shown above—numerous droplets of germ-laden sputum. Especially is the danger great when the operation is performed before a class, and the surgeon talks continuously, explaining his procedure as he works.

It is a matter of ordinary observation that there is a great difference in persons as regards the amount of saliva in the mouth. We have all talked to individuals who expel droplets of saliva into the air to a decided distance from their mouths. I was told by a student in a large medical college in Chicago that he had often noticed at the clinics of a certain surgeon that, when the light was from a certain direction, he could see, from his seat in the amphitheater, a continuous spray of saliva coming from the mouth of the surgeon while he discoursed to the class and conducted his operation. Obviously, protection to the mouth, of such sort as to catch and imprison the droplets of sputum, should be a routine precaution for surgeons and for surgical nurses during operations.

The danger of transmitting streptococcal infection seems to be increased during and for at least three weeks after an attack of sore throat, and it would seem advisable for surgeons to take special precautions during that time and for nurses to be assigned to duties other than surgical.

SUMMARY.

Streptococci are expelled from the mouth in the invisible droplets of sputum by coughing, speaking, whispering, crying or breathing forcibly through the mouth. They are expelled to a distance of at least 36 centimeters.

Thirty-three out of 50 scarlet-fever patients, most of them children, were found to expel streptococci in coughing, crying or breathing; 42 out of 50 normal adults were found to expel streptococci in coughing or in speaking.

The streptococci thus disseminated may be inhaled by others, and may set up streptococcal complications or may fall on the tissues exposed at an operation and cause suppuration.

Just as the virulence of an individual strain of streptococcus may be raised by planting on certain nutrient media or by passing through susceptible animals, so, in all probability, it may be raised by passage from one human being to another.

In this way may be explained the conversion of a case of simple scarlet fever into one of scarlatinal sepsis, and in the same way may be explained the cases of surgical sepsis which occur after all usual precautions have been taken.

Cases of scarlet fever with streptococcal complications should be isolated from cases without such complications.

Surgeons and nurses should have their mouths protected during the time of an operation.

My thanks are due to Dr. Hektoen, who suggested this investigation, and to Dr. W. M. Park of the New York Department of Health, and Dr. Baum of the Cook County Hospital, Chicago, for courtesies shown me in the collection of the material.

Special Article

IMMUNITY.

CHAPTER XI.

THE NATURE OF THE SUBSTANCES CONCERNED IN AGGLUTINATION.

Two substances are necessary for the phenomenon of agglutination; one, the active or agglutinating substance, exists in the serum, while the other, the substance acted on or the agglutinable substance, is presented in the bacteria. The agglutinable substance is generally supposed to be passive in the reaction, while the agglutinating property seems to possess a ferment-like element, which acts on the agglutinable substance. Agglutinin, the term used in the preceding chapter, is now generally applied to the substance in the serum. Recently the bacterial constituent has been called agglutinogen, because of the belief that the agglutinable substance, when introduced into the animal body, stimulates the latter to the formation of agglutinin; hence agglutinogen means, not agglutination producing, but agglutinin producing. These shorter terms will be used for the sake of brevity.

Terms.

The tendency to consider agglutination a vital phenomenon has been shown to be incorrect; for bacteria which have been killed may be agglutinated by serums and even by some purely chemical substances.

The presence of agglutinogen in an organism may be demonstrated in three ways: 1. The mere fact of its agglutinability by a serum is evidence of the presence of an agglutinable substance. 2. If during infection or immunization the serum acquires agglutinating properties, the organism possesses an agglutinogenic substance. 3. If an organism is mixed with a serum containing the specific agglutinin, and later is removed by centrifugation, the resultant disappearance of agglutinin from the serum, which may be demonstrated, shows that something in the bacteria (agglutinogen) has combined with the agglutinin.

Agglutinogen.

The location of agglutinogen in the bacterial cells has received some discussion. There is a tendency to believe that it exists in the cell envelope or perhaps on its surface. It appears to be formed in the cell, and, in some cases, it may be excreted into a surrounding medium; certainly when bacteria die and disintegrate agglutinogen is liberated. Filtrates of the cultures of certain organisms (entirely free from bacterial cells), when injected into animals, will cause the formation of agglutinins. Also, just as a micro-organism is able to absorb the agglutinin from its specific antiserum by a process of chemical union, so a filtrate of the type mentioned is able to neutralize the agglutinating power of the serum; here, too, a chemical union is assumed between the free agglutinogen and the agglutinin.

Distribution of Agglutinogen.

The filtrates of certain cultures exhibit another phenomenon when they are mixed with their specific antiserums; this has to do with the bacterial precipitins of Kraus. If, for example, the filtrate of an old typhoid bouillon culture is mixed with antityphoid serum, a distinct precipitate is formed which eventually settles to the bottom of the tube. This is a specific reaction, and does not occur if the filtrate is mixed with some other immune serum. It is thought by some that this so-called precipitable substance in the filtrate is identical with the agglutinable substance (agglutinogen), but this point is still the subject of investigation.

The Precipitation Reaction.

Agglutinogen may also be extracted from micro-organisms by chemical processes. The presence of the substance in the extracts becomes manifest when immunization with them causes the formation of an agglutinating serum. This, again, is the "test of immunization."

The agglutinogen of one bacterium is not identical with that of any other. If they were identical immunization with the