MENINGITIS CAUSED BY ATYPICAL GRAM-NEGATIVE COCCI

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Gram-negative cocci found in samples of purulent spinal fluid during routine laboratory examinations are apt to be reported as meningococci without further confirmation. While the meningococcus no doubt is the most common gram-negative coccus associated with acute meningitis, there are many others which play a rôle, particularly in sporadic cases. Error in diagnosis is also apt to occur in certain cases of meningitis caused by metachromatic cocci or by gram-positive cocci if the Gram stain is improperly applied, and also as a result of the fact that cocci, ordinarily gram-positive, may be decolorized when they are dead or phagocyted. Recognition of these problems and greater care in laboratory diagnosis will prevent wasting antimeningococcus serum in disease not caused by typical meningococci and may help to explain the failure of specific antimeningococcus serum in certain instances.

Branham, Mitchell and Brainin, (1938) recently pointed out the difficulties attending the differentiation of even such well-known bacteria as gonococci from the meningococcus in cases of meningitis. Many of the strains of gonococci which they studied were agglutinated by polyvalent antimeningococcus serum. Sugar fermentation reactions were somewhat more reliable in differentiation but the most fundamental differences appeared in cultural studies pertaining to colony morphology and growth requirements. Even more confusion in diagnosis is apt to arise in dealing with other gram-negative cocci such as Micrococcus catarrhalis, Micrococcus florens, Micrococcus crassus,
Micrococcus flavus and other still unnamed varieties, or when multiple infection with two or more strains occurs. German bacteriologists thirty or forty years ago were especially concerned with the problem and many of their publications are referred to in the papers of Forbes (1920) and others. A prolonged controversy arose as to whether the different atypical microorganisms encountered were (a) separate and distinct types of bacteria, (b) variant or mutant forms of the meningococcus, (c) secondary invaders, or (d) contaminants. The problem is illustrated in the following two case reports and studies, the first of which apparently involved an atypical gram-negative coccus; and the second, a type I meningococcus with unusual growth characteristics, together with an atypical staphylococcus.

Accurate knowledge of a complex problem is always desirable.

Case 1 (by courtesy of Dr. I. McQuarrie). A girl, aged 7, suddenly vomited and had diarrhea and headache. She was admitted to the pediatric department of the University of Minnesota Hospital four days later with a diagnosis of meningitis. Rigidity of the neck, Kernig’s sign and other disturbed reflexes were noted. A blood culture was negative. The spinal fluid was under normal pressure, but was turbid, contained 68 leukocytes per cu. mm. (56 per cent polymorphonuclear cells) and numerous extracellular and intracellular gram-negative diplococci, which were reported to be meningococci. Antimeningococcus serum was promptly injected intraspinously and subsequent doses were given daily for 6 days. The child improved and her temperature returned to normal about 10 days after admission to the hospital.

Bacteriologic studies. Gram-negative cocci were seen in smears from the spinal fluid before serum injection and on the day after. They were cultivated from the same samples of fluid and also from spinal fluid taken on the fourth day of treatment. Growth was obtained from the spinal fluid only in liver-peptone-broth tubes. The broth became turbid after 24 hours at 37°C. and no sediment or pellicle formed. Subcultures grew on ordinary laboratory media at temperatures between 10° and 37°C., better toward the higher level. On plain agar and blood agar plates the colonies after 24 hours at 25°C. were about a millimeter
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in diameter, dull, grayish and translucent. Several days later, the colonies measured 3 or 4 millimeters in diameter and opaque yellowish-gray sectors and wedges formed in many of the colonies. Subcultures of the translucent portions reproduced translucent colonies with later opaque sector development. Subcultures of the opaque area produced similar opaque colonies (fig. 1) in which translucent sectors and wedges appeared after a few days. In one opaque point colony a large translucent wedge formed and at the edge of the translucent portion an opaque wedge developed. The cocci from both forms were identical in morphology and in biological behavior. They were usually

gram-negative but, unless thoroughly decolorized, many retained the purple color, especially in old cultures. The cocci were oval or spherical, usually in pairs with adjacent sides flattened, occasionally single or in clusters. The long axis of the diploforms was at right angle to the line of union. There was marked variation in size. Cocci from both opaque and translucent colonies caused hemolysis on blood agar, formed an acid coagulum in milk after 7 days at 37°C, slowly liquefied gelatin, caused no change in maltose, mannitol, or sucrose broth, but formed acid after 7 days in glucose and less in lactose broth. No agglutination occurred in various types of antimeningococceus serum

Fig. 1. Colonies About Two Weeks Old from Culture Obtained in Case 1
Magnified about × 6. Several translucent sectors are shown in the opaque colonies.
obtained from Dr. C. P. Miller of Chicago, from Dr. S. Branham of Washington and from commercial sources. Broth cultures were avirulent when injected intraperitoneally into white mice, guinea pigs and rabbits.

It is obvious that the bacterium is not a meningococcus nor does its behavior coincide in all respects with the criteria laid down for Micrococcus catarrhalis, Micrococcus florens (Davison, Davison, and Miller, 1917), Micrococcus crassus (Elser and Hunter, 1909), Micrococcus flavus and others (Branham, 1930). It has many, but not all of the characteristics of each of the varieties named, but for reasons given in the discussion it does not seem desirable to give the strain a new name.

The second case apparently involves the problem of dual infection and of type transformation which confronted Von Hibler (1907), Stoevesandt (1908), Forbes (1920), Köhlisch, (1915), McDonald (1908), Kempf, Gilman and Zerfas (1933) and others (Sen, 1936).

**Case 2.** A young man, age 23, was said to have had meningitis following sinusitis in 1932. On April 10, 1936 he noted sore throat and awakened the next day with severe headache followed by vomiting and chills. He was then admitted to the Student Health Service of the University of Minnesota Hospital. His throat was inflamed and a shallow ulcer was seen in the right tonsillar fossa. His neck was slightly rigid; there was general hyperesthesia of the skin, but no disturbance of reflexes until later in the day when Kernig's sign appeared. The leukocytes numbered 27,000 and a gram-positive coccus was cultivated from the blood. The spinal fluid was under 24 mm. of mercury pressure and contained 8000 cells of which 70 per cent were polymorphonuclear. A direct smear of the spinal fluid examined in the Minnesota State Board of Health laboratory showed numerous intracellular gram-negative diplococci. The patient was then given antimeningococcus serum intraspinally and intramuscularly. There was considerable improvement in his condition on the following day, but the neck was still rigid. His temperature declined and became normal after several days.

**Bacteriologic studies.** As a part of a routine procedure, portions of the same specimen of spinal fluid were sent to the Min-
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Meningitis caused by Gram-negative cocci was reported to the Minnesota State Board of Health laboratory and to the University Hospital laboratory. The former reported the presence and subsequent cultivation of typical meningococci and the hospital laboratory reported the presence of gram-positive intracellular diplococci. The gram stain of the latter was retained even when decolorization was carried to the point at which all color was gone from the nuclei of the leukocytes in the smear.

The meningococcus. Because of the different reports from the two laboratories, cultures of the meningococcus first isolated were sent to Dr. C. P. Miller and to Dr. Sara Branham both of whom confirmed its identity as type I meningococcus. The strain was subsequently tested in our laboratory and found to have certain unusual characteristics. The culture from a Loeffler medium slant was seeded in 9 stab punctures in a deep (10 mm. thick) plain agar plate. After 48 hours at room temperature growth appeared at 3 of the 9 points of inoculation. The colonies were translucent and composed mostly of gram-negative diplococci, but, here and there, were found distinctly gram-positive cocci, even after thorough decolorization. Another culture was made in a 100 cc. flask of broth and incubated 6 days at 37°C. Subcultures on plain agar showed two forms of colony, large and small, composed of similar cocci most of which were gram-negative, but a few were gram-positive. Numerous tetrads were present and the cocci were pleomorphic and metachromatic.

The Gram-positive cocci isolated in the hospital laboratory. Gram-positive cocci, which were cultivated from the spinal fluid and from the blood before serum treatment, and from the spinal fluid on the day after, were apparently identical. Subsequent cultures from the blood and spinal fluid were sterile. Fluid from the second spinal fluid specimen was inoculated in numerous stabs in a plain agar plate and growth occurred at each site, the colonies measuring 2 mm. after 6 days at 25°C.

A culture in liver-peptone-broth from the first specimen was plated on plain agar. Three forms of colonies appeared (1) many small translucent ones, (2) several larger, thicker, opaque brownish ones and (3) a few opaque white ones. The appearance
of a variety of colonies was similar to the experience reported in a case of meningitis caused by Micrococcus tetragenus, and was regarded as evidence of variation in a single strain of bacteria. Subcultures from each of the colony types bred true. The brown colonies on occasion developed both translucent and white forms as wedges or daughter colonies. The cocci isolated from the blood first produced colonies of the white type, and in later subcultures similar translucent, and the brownish, forms appeared.

Cocci from all three colony types were gram-positive and were arranged singly, in pairs, in tetrads and in clusters like staphylococci. Certain characteristics of the three types are shown in table 1.

<table>
<thead>
<tr>
<th>TABLE 1</th>
<th>Maltose mannitol lactose glucose sucrose milk gelatin hemolysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>White</td>
<td>++</td>
</tr>
<tr>
<td>Brown</td>
<td>+</td>
</tr>
<tr>
<td>Translucent</td>
<td>+</td>
</tr>
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Tests made by Dr. W. A. Kreidler.

Cocci from all three forms were avirulent for white mice after intraperitoneal inoculation. Agglutination tests were negative in serum obtained from the patient, but were irregularly positive in low dilutions of several samples of antimenengococcus serum, regardless of source or type.

We believe that the patient had primary meningococcus meningitis and was host to a staphylococcus which became a secondary invader. The three colony forms were regarded as variants of the strain of staphylococcus in question. It is too remote a possibility to suggest that the staphylococcus was a mutant form of the meningococcus. A few experiments were made to determine whether or not growth of meningococci in the liver-peptone-broth used in our laboratory tended to transform them into gram-positive cocci. Nine strains of meningococci were grown for various periods up to 6 months in liver-peptone-broth. Transplants were made once or twice a week.
Plate cultures from these tubes at various times revealed the usual type of colony which was smooth and transparent, and two variant forms, one, rough and the other, moist and sticky. No gram-positive cocci were encountered from any of the colonies examined.

It is probable that under ordinary routine diagnostic conditions the gram-positive cocci isolated from the patient’s spinal fluid in our laboratory would be disregarded or dismissed as contaminants, yet the presence of identical cocci in the blood and similar experiences of other observers, notably Köhlish (1915) and Kempf and his associates (1933), show that the occurrence of more than one species of bacteria in cases of meningitis is not uncommon.

DISCUSSION

The problem in both cases appears to involve the question of bacterial variation or type transformation as described previously (Reimann, 1935) in a case of meningitis caused by *Micrococcus tetragenus* in which three colony forms of *Micrococcus tetragenus* were immediately detected and later 13 additional variant forms of the original strain were isolated. These various types derived from each other differed considerably in several of the biological tests commonly used. Certain of the differences were as great or greater than those which are customarily used to separate and identify the closely related strains of the *Neisseria* group and the various atypical meningococcus strains (Reimann, 1937). In case 1, the gram-negative bacterium showed two colony forms neither of which had characteristics to identify it with previously established varieties. It was not determined however, whether they were variant forms or types of certain known varieties or a separate variety. It is obvious, however, that not all gram-negative cocci found in the spinal fluid in cases of meningitis are meningococci.

The problem in case 2 was still more complicated. The type I meningococcus recovered grew on plain agar at room temperature, which is at variance with all but a few descriptions of meningococci, and further, many of the cocci retained the
gram stain. The latter observation was similar to that of McDonald (1908) and others, yet Murray (1929) voices the general opinion that meningococci are never gram-positive. Two colony types were observed in old cultures as noted also by McDonald (1908) and Canti (1918).

The gram-positive cocci isolated from the spinal fluid and from the blood, might have been looked upon either as mutation forms of the meningococcus as suggested by Köhlisch (1915), or as staphylococci and secondary invaders. Here again evidence of type transformation was present among the three colony forms derived. There were therefore obtained from the same spinal fluid sample five different colony types, namely, two forms of the meningococcus and three of the staphylococcus. Under the old monomorphic conception of classification these may have been mistaken for five separate strains, or as evidence of contamination, but in reality they represent the variant forms or types of a member of the Neisseria group and those of a staphylococcus which we believe was a secondary invader. Similar circumstances may have caused the polemic of thirty years ago about the relationship between the meningococcus, the *Diplococcus crassus* of Jaeger and Von Lingelsheim, and the staphylococcus. Viewed in the light of present knowledge of bacterial variation and multiple infection, the problem still seems complicated but less obscure. On the evidence submitted, one may suggest the following possibilities: first, it would seem that any of a number of bacterial strains ordinarily harbored in the nasopharynx or elsewhere may become invasive and cause meningitis when the resistance of the host is reduced; second, a given strain may be present in several variant forms of type or culture phase (mucoid, smooth and rough), and third, secondary infection with another organism with its variant forms may also be present. This view of the matter reconciles many of the old ideas since it is now known that numerous bacteria may cause meningitis and any given strain may manifest itself in a number of variant forms.

One is inclined to doubt with Hadley (1927) and Sherman (1937) whether it is possible ever to classify bacteria except on
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a broad basis. Sugar fermentation, agglutination and other tests have lost much of the importance heretofore attached to them in differentiation. In our own experience many of these tests give inconstant results in the hands of different workers and even for the same person working with the same materials and same technic at different times. Certain types of bacteria indeed seem to have relatively constant characteristics, but the evidence of type and culture phase instability as demonstrated among pneumococci, Micrococcus tetragenus and staphylococci suggests that similar variation may occur among other bacterial species. Evidence of type variation among meningococci has already been published (Atkin, 1925; Branham, 1937).

SUMMARY

Two cases of meningitis associated with atypical bacteria were studied. From the first patient (case 1), a gram-negative diplococcus with characteristics different from other classified strains was isolated. The bacterium gave rise to two interchangeable colony forms. From the second case, two species of cocci were obtained; one was a type I meningococcus which had the unusual ability to grow on plain agar at room temperature and had gram-positive elements, the other was a staphylococcus which was represented by three interchangeable colony forms.

These studies suggest that under ordinary laboratory routine examination, atypical colonies may appear on culture plates. Such colonies are usually ignored or dismissed as contaminants, but they may actually be part of the pattern of variation of the bacterium concerned. Recognition of the phenomenon of bacterial variation and type transformation would remove much confusion and would probably reduce the number of separate varieties of bacteria now believed to exist.

REFERENCES


Köhlisch 1915 Bakteriologische Befunde bei einem Fall vom Meningokokkensepsis; gibt es eine Mutation bei Meningokokken? Zeit. f. Hyg., 80, 404-430.


