AN ATYPICAL MENINGOCOCCUS ISOLATED FROM CEREBROSPINAL FLUID IN THE CALCUTTA MENINGITIS EPIDEMIC OF 1934-35

BENODE BEHARI SEN

Director, Serum Institute of India (Biological Laboratory, Union Drug Company, Ltd., Calcutta, India)

Received for publication May 2, 1936

INTRODUCTION

Between the years 1932 and 1935 there were frequent outbreaks of meningitis in an epidemic form in Calcutta and disappointing results were obtained with serum therapy in Calcutta hospitals. The same disappointing results were also reported from the United States of America. Sara Branham of the National Institute of Health, United States Public Health Service, Washington, D. C., took up the study of the meningococci involved in the outbreaks there, to find out if the strains involved might be different from those found in earlier epidemics. She typed about 375 strains of meningococci and concluded that “On the whole, the meningococci found during the recent epidemics are not essentially different from those found in 1915–18, in the sense that except for 14 strains, which seemed to be a different species, they could be placed in the same serological group” (Branham, 1932). “The remaining strains (14 strains) did not fall into any of these four serological groups represented in the Gordon-Murray classification” (Branham, 1930), and their “colonies on blood agar are less moist than those of meningococci, they produce a golden yellow pigment and do not ferment any of the carbohydrates generally used in classifying this genus, viz., dextrose, levulose, maltose and sucrose.” This is the description of a new meningococcus-like organism isolated from the cerebrospinal fluid by Sara Branham and has since been known as Neisseria flavescens. Von Lingelsheim's yellow cocci of this genus (Neis-
seria flava) and Elser and Huntoon’s chromogenic groups (Neisseria sub-flava) found earlier than this are separate classes by themselves. They ferment glucose and maltose (Berger, 1934) though they differ in the type of pigments produced.

The writer was similarly interested in this question in the year 1932 and has studied about 225 epidemic strains during the last two and a half years, all isolated from the cerebrospinal fluid only. Eighty-five strains were found to correspond with the cultural and fermentative characteristics of the meningococcus, whereas 140 strains were found to belong to a different group of Gram-negative diplococci. A preliminary report was published (Sen, 1935a) in which a group of strains was described, which failed to correspond with the meningococcus in cultural and fermentative characteristics.

This group of strains was non-chromogenic, non-hemolytic, grew readily from the primary culture in ordinary nutrient agar, requiring no enrichment fluid in the medium for their growth and did not ferment any of the sugars—glucose, maltose, lactose and sucrose, even after 60 generations, as recorded in the preliminary report.

The present report gives a fuller account of the fermentative, biochemical and cultural characteristics of this new group of strains which has been described hereafter as group 6A, since all the strains isolated with these characteristics have been found to be agglutinated by high titer serum from a particular strain, and also since the type was first isolated from bed No. 6 of a hospital on the first day of cerebrospinal fluid culture.

**EXPERIMENTAL**

*Description of group 6A organisms when first isolated from the cerebrospinal fluid*

Gram-negative, biscuit-shaped and flattened diplococci. Grow well on ordinary culture media (plain nutrient agar). Grow well at 22°C. Non-chromogenic. Moist colonies on ordinary and blood agar.

No acid formed in any of the carbohydrate media (glucose, lactose, maltose and sucrose). Do not liquefy gelatin. Agar colonies—small, circular, grayish white with erose margin. Litmus milk—unaltered. Non-hemolytic, non-motile, non-capsulated. Survive for more than
15 days at a temperature of 5°C. Aerobic, facultative. Optimum temperature, 37°C.

Virulence for laboratory animals—very high (killing guinea pigs and rabbits within 24 to 48 hours with symptoms of paralysis of the legs.) Not agglutinated by any of the four Oxford standard typed sera (then in vogue).

During our last study of cerebrospinal fever, we did not obtain *N. flavescens* (Sen, 1935b) (golden-yellow-pigment producing organism), but in the last epidemic we have obtained this chromogenic variety in a small percentage of cases.

That this group of diplococci has many peculiar characteristics is evident. After repeated subculture in ordinary agar for more than 300 generations, it is now found to give positive sugar reactions with glucose and sucrose as well as maltose. Furthermore, the group has changed other biochemical characteristics as well, as is evident from table 1.

From the recent pamphlet issued by the Standard Laboratory (Medical Research Council) along with the standard agglutinating group I and II sera, it appears that "Strains from the cerebrospinal fluid not agglutinable by either serums but otherwise typical, may be considered as group II."

We have tried agglutination tests with recently isolated as well as with 300-generations-old 6 A group of strains against freshly supplied Oxford group sera (two) with negative results. Had

<table>
<thead>
<tr>
<th>TABLE 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>6A GROUP OF GRAM-NEGATIVE DIPLOCOCCI AFTER 300 GENERATIONS (OLD)</td>
</tr>
<tr>
<td>Litmus milk</td>
</tr>
<tr>
<td>Indol reaction</td>
</tr>
<tr>
<td>Methyl red</td>
</tr>
<tr>
<td>Voges-Proskauer</td>
</tr>
<tr>
<td>Nitrate reaction</td>
</tr>
<tr>
<td>Ammonia</td>
</tr>
<tr>
<td>Methylene blue reductase</td>
</tr>
<tr>
<td>Catalase</td>
</tr>
</tbody>
</table>
they been otherwise typical, we could have classed them under group II. But they do not conform to the important typical characteristics of the meningococci.

We have studied differences in pathogenicity of freshly isolated “6 A group” meningococci and freshly-isolated typical meningococci, and standard foreign strains of meningococci. The first named is highly neurotoxic and its minimal lethal dose for the mouse is much less than that of the other two. Serum produced in horses against the 6 A group of meningococci, typical locally-isolated meningococci and standard strains, has so far given very good results in the last epidemic in the United Provinces and the Central Provinces of India. A few of our horses, when they were injected with a dose of 6 A group of meningococci more than they could bear, showed stiffness in the muscles on both sides of the spine, to the extent that they could not put their mouths into the feeding tubs easily and walked with difficulty with legs dragging and ultimate paralysis of the legs. In similar conditions with the other strains, we generally observe swelling of the joints only.

This group of strains therefore should be given a separate nomenclature as atypical meningococcus.

SUMMARY

1. A group of Gram-negative diplococci isolated from cerebrospinal fluid of epidemic meningitis cases in 1934–35 was found to differ from the typical meningococcus.

2. This group of strains was found to be capable of growing in ordinary nutrient agar from the very beginning, to have negative fermentation reactions to glucose, maltose and lactose, and to be highly virulent and negative to standard type serum, when freshly isolated and up to 300 generations.

3. After 300 generations, they were found to change their biochemical characters and to show positive fermentation reactions on glucose, maltose, and lactose, though still continuing to be negative to standard group sera.

4. Details of biochemical characters, when freshly isolated and after 300 generations have been tabulated.
I acknowledge with thanks, the assistance rendered to me by my collaborators, Dr. Sudhir Acharyya, Dr. J. Naha, Dr. Phani Dutt, Dr. K. Ganguly and Dr. Satisl Bhattacharya.

REFERENCES

Sen, B. B. 1935a Journal of Indian Medical Association, April.