An unusual *Neisseria* isolated from a case of meningitis

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SYNOPSIS A *Neisseria* was isolated from blood culture and cerebrospinal fluid of a child with clinical meningitis. On first isolation this organism failed to produce acid in the usual carbohydrates, and was heavily capsulated. After mouse passage it developed many of the characters of *N. meningitidis* group C, but was still able to grow at room temperature. Its nature is discussed.

There are scattered but persistent reports in the literature of meningitis due to *Neisseria* spp. other than *Neisseria meningitidis*. For example, Branham (1930) reported an outbreak due to an organism which she called *N. flavescens*; Newing and Christie (1947) reported isolation of *N. catarrhalis* from the cerebrospinal fluid of a case of meningitis; Christie and Cook (1947) reported a fatal case due to a capsulated *Neisseria* and reviewed the literature before that date, whilst Courtois, Thys, and Verselder (1954) reported two cases due to an organism which they called *N. capsulata*.

CASE REPORT

A 4-year-old coloured child who had come to Britain three years previously was admitted with a history that he was well until 2.30 pm on the day of admission, when he started to cry and complained of pain in the left arm and leg. At 6.30 pm he vomited profusely and started to twitch and jerk. He had been anorexic the whole day and was very feverish. There was no history of recent illness in other members of the family.

On examination, axillary temperature was 101.6°F and pulse rate 160 per min. He was sleepy but could talk and answer questions. There was a pinkish rash on the body, particularly the chest. The tonsillar lymph nodes were enlarged, and the tonsils themselves enlarged and infected. There was marked neck stiffness and a positive Kernig's sign. The pupils were equal. The fundi were not seen. The right plantar reflex was extensor, the left flexor.

Lumbar puncture showed a cerebrospinal fluid pressure of 130 mm. Protein was 30 mg per 100 ml, sugar 77 mg per 100 ml, total WBCs 106 per cu mm (differential not reported), total RBC 45 per cu mm.

Crystalline penicillin, 10,000 units, was given intrathecally, and this was followed by 125 mg penicillin V, six hourly for six days.

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Culture of cerebrospinal fluid on blood agar at 37°C in CO₂ (candle jar) showed a scanty pure growth of a *Neisseria* colonially resembling *N. meningitidis*, and a blood culture taken at the same time yielded an organism which was apparently the same. Both organisms were sensitive to penicillin, ampicillin, chloramphenicol, streptomycin, and erythromycin.

The day after admission his temperature had fallen to normal, although there was still marked neck stiffness, and a second lumbar puncture showed a cerebrospinal fluid pressure of 250 mm. Protein was now 100 mg per 100 ml and there were 6 polymorphs, 10 lymphocytes, and 4 RBCs per cu mm. Clinical improvement was maintained, and he was discharged 10 days after admission.

STAGE 1 BACTERIOLOGICAL INVESTIGATIONS The organisms isolated from cerebrospinal fluid and blood both had the following characteristics when first examined. (We shall refer to these cultures as phase 1 for convenience in description.) They were oval or spherical Gram-negative diplococci of *Neisseria* type and were heavily capsulated. After 24 hours at 37°C on horse blood agar the colonies resembled those of classical *N. meningitidis*. After 12 hours' further incubation at 37°C, the colonies became rough and the organisms autolysed. Growth did not appear to be influenced by CO₂. Subculture on a nutrient (Hartley's broth solidified by adding agar) agar slope at room temperature showed some growth after 24 hours. There was no change in glucose, saccharose, or maltose (serum agar slopes at 37°C). The isolates were oxidase and catalase positive. A saline suspension killed by heating at 60°C for one hour was not agglutinated by polyvalent meningococcus antiserum.

STAGE 2 BACTERIOLOGICAL INVESTIGATIONS Of heavy suspensions in saline from overnight cultures on blood agar at 37°C, 0.5 ml aliquots were inoculated intraperitoneally into two white mice, one receiving the culture from cerebrospinal fluid, the other from blood. The mice were sacrificed 18 hours later and the organisms

were recovered from heart blood and peritoneal cavities of both mice.

These cultures, on blood agar at 37°C, are referred to as phase 2, and were retested with the following results. Morphology was as phase 1, but capsules were less evident. Cultures on horse blood agar at 37°C were similar to those in phase 1, and growth still occurred on nutrient agar at room temperature. However, the organisms now produced acid from glucose and maltose on serum agar slopes at 37°C. They were saccharose negative, but oxidase and catalase positive. Live and heat-killed suspension (one hour at 60°C) were now both agglutinated on slides by polyvalent meningococcus antiserum.

FURTHER SEROLOGICAL INVESTIGATIONS. Phase 2 organisms were tested by slide agglutination against groups A, B, C, and D meningococcus antiserum. Rapid agglutination occurred with anti-C. There was no agglutination with the other three sera. This agglutination occurred with live organisms and also with saline suspensions, killed by heating at 60°C for one hour. (We conclude that the inagglutinability of phase 1 organisms was not due to heating. Live phase 1 organisms were no longer available owing to autolysis of the cultures.)

Group C meningococcus antiserum (Burroughs Wellcome & Co. K. 6657) was absorbed for four hours at 37°C with equal parts of a heavy suspension of phase 1 organisms heated at 60°C for one hour. The supernatant was then tested on slides against phase 2 organisms and against *N. meningitidis* group C (Wellcome Research Laboratories C.N. 5535). No agglutination occurred. In the same way, phase 2 organisms absorbed agglutinins for the standard group C meningococcus. The group C meningococcus antiserum was then absorbed in the same way with *N. pharyngis* (N.C.T.C. 4590). The slide agglutination of our phase 2 and of *N. meningitidis* group C still occurred.

Since attempts to reproduce these results in tubes were only partly successful, another method was sought.

Seven wells were cut in a sheet of 1% Oxoid purified agar, with barbiturate buffer of ionic strength 0.025 and pH 8.6. Into the centre well was placed group C meningococcus antiserum, and into the peripheral wells, in the form of a hexagon, suspensions in 0.25% phenol saline heated at 60°C for one hour, of our phase 2 *Neisseria*, both from cerebrospinal fluid and from blood culture, of *N. meningitidis* (Wellcome Research Laboratories C.N. 5535), and of *N. pharyngis* (N.C.T.C. 4590). No precipitation lines were seen after diffusion for seven days at refrigerator temperature, but staining with Chromassie blue (Gurr), showed lines suggesting identity between our phase 2 *Neisseria* and *N. meningitidis* group C.

Attempts to reproduce this experiment met with only partial success. We therefore modified the medium by adding 0.003% ethylenediamine tetraacetate (Reising and Kellogg, 1965) and carried out diffusion at room temperature for four days. Precipitation lines were now visible without the need for staining, a reaction of identity appearing between our phase 2 *Neisseria* (from cerebrospinal fluid and blood) and the standard

FIG. 1. Agar gel diffusion in duplicate, stained with amido-black (natural size). The central well contains *Meningococcus* group C antiserum. The peripheral wells, starting at the pointer (top left) and reading clockwise, contain suspensions of *Neisseria* from cerebrospinal fluid, *Neisseria* from blood culture, *Meningococcus* group C, *Neisseria* from cerebrospinal fluid, *Neisseria pharyngis*, *Meningococcus* group C.

group C meningococcus. A reaction of partial identity, with a pronounced spur, occurred where our phase 2 *Neisseria* and the group C meningococcus were placed one on either side of *N. pharyngis* (Fig. 1). Similar results were obtained after four days' diffusion in the refrigerator, but all lines were fainter. Attempts to reproduce these results with the phenol extraction method described by Reising and Kellogg (1965) have so far failed.

DISCUSSION

We suggest that this isolate is an atypical strain of *N. meningitidis* group C for the reason that (1) it was isolated from blood and cerebrospinal fluid of an early case of meningitis which responded rapidly to penicillin. (2) After mouse passage it fermented glucose and maltose, but not saccharose, and was agglutinated on slides by group C meningococcus antiserum, from which it absorbed agglutinins for a strain of group C meningococcus. Cultures before passage were also able to absorb these agglutinins. (3) After mouse passage, on immunodiffusion against group C meningococcus antiserum, a reaction of identity with a standard group C meningococcus was seen.

Against our suggestion are (1) failure in this case to exclude viral aetiology; (2) ability of the organisms to grow at room temperature (growth on nutrient agar has been reported, *eg*, Cruickshank, 1965); (3) failure to confirm results of slide agglutination satisfactorily in tubes.

What relationship this isolate bears to *N. capsulata* of Courtois *et al.* (1954) must be a matter for speculation, but as first isolated it resembles it in being grown from a case of meningitis, in being strongly capsulated and inagglutinable by meningococcus antiserum, in growing at temperatures below 37°C (*N. capsulata* grew at 28°C) in failing to produce acid from glucose, saccharose, or maltose

and in undergoing such rapid autolysis that some of our cultures and those of the Belgian authors were lost.

Inagglutinable strains of *N. meningitidis* have been reported as not infrequent during inter-epidemic periods (Wilson and Miles, 1964).

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