ON THE OCCURRENCE OF THE MICROCOCCUS CATARRHALIS IN NORMAL AND CATARRHAL NOSES AND ITS DIFFERENTIATION FROM OTHER GRAM-NEGATIVE COCCI.

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The *Micrococcus catarrhalis* was first isolated by Kirchner (1890) from cases of influenza-like illness. Since then several epidemics of acute catarrh and bronchitis associated with this organism have been recorded.

The first epidemic recorded in this country was that described by Dunn and Gordon (1905) as occurring in Hertfordshire; the clinical aspect of the disease was that of an acute febrile illness with variable symptoms, of which sore throat, scarlatiniform eruptions, and symptoms suggesting meningitis were the most striking features. Other epidemics have been recorded by Ghon and Pfeiffer (1902), Bezançon and de Jong (1905), and others.

In a large proportion of the cases examined Gordon obtained the *Micrococcus catarrhalis* in great numbers and often in pure culture from the nose and throat. Several writers have described the occurrence of other Gram-negative cocci in the nose.

The Meningococcus (or *Diplococcus intracellularis meningitidis* of Weichselbaum) has been found in the naso-pharynx and nasal fossae, almost exclusively in cases of meningitis or in those in close contact with meningitis patients. A few occurrences have been, however, recorded apart from obvious meningitis infection by Schiff (1898), Weichselbaum and Ghon (1905), and others.

Dunham (1906) examined cultures from the nose and throat of 16 persons suffering from epidemic meningitis, and in six found an organism resembling the meningococcus in every respect, including agglutination. Goodwin and Sholly (1906) examined the noses of

55 healthy persons not in contact with meningitis cases, and on two occasions isolated an organism which resembled the meningococcus in its cultural characteristics, but differed from it in its agglutinating properties. They also examined the noses of 45 persons in close contact with meningitis cases and found the meningococcus in five, *i.e.* 11 per cent.

v. Lingelsheim (1906) examined the naso-pharyngeal secretion of 346 persons who were in health, or ill of some complaint other than meningitis, and who were not in contact with meningitis cases: in none did he find the meningococcus. In 125 persons in more or less close contact with meningitis cases he found the meningococcus in 24 (= 19 %). On the other hand, he examined the nasal secretion in 787 cases of clinical meningitis and found the meningococcus in 182 (= 25 %). In 49 cases examined carefully in hospital in the first five days of their illness 46 (= 93·8 %) yielded the meningococcus.

Kutscher (1906) in Berlin examined the noses of 56 patients suffering from other complaints, and two of these yielded organisms which resembled the meningococcus in every respect, including agglutination; two yielded cocci like the meningococcus, but the agglutination test was not applied as the cultures died out. According to Kolle and Wassermann (1906), 114 persons were examined in Berlin with similar results.

In the identification of *Micrococcus catarrhalis* the most important organisms that require to be considered are the meningococcus, gonococcus, and certain other races of Gram-negative cocci, which occur in the nose and elsewhere, but have not yet been clearly differentiated.

The three first-mentioned organisms resemble each other very closely in several particulars.

1. In the body the cocci are often found exclusively inside the polymorphonuclear leucocytes.

2. When first removed from the body they have very feeble powers of growing on artificial media, usually requiring the addition of blood, or ascitic fluid or serum to the medium.

3. They do not usually survive long without sub-culture.

4. They are very easily killed, especially when first isolated, by drying.

5. They are very feebly, or often not at all, pathogenic for laboratory animals.

6. Morphologically there is a tendency for the cocci to vary very much in size on artificial media, especially after a few days' growth.

Individuals then occur which are two or three times the size of the ordinary forms, stain more deeply by methylene blue, and are not so readily decolourised by Gram's method.

7. They grow in pairs or tetrads, never in chains.

8. They all, when taken from the body or from a 24 hours' culture, decolourise rapidly and completely by Gram's method, except the giant forms mentioned above, which decolourise less rapidly.

The chief recognised points of difference between the several species are the following, and here I shall make use of the description by Neisser (1903) in Kolle and Wassermann's handbook unless another author is mentioned.

1. On agar with or without ascitic fluid the colonies of *M. catarrhalis* are thicker, more raised and more opaque than those of the meningococcus or gonococcus, and they appear coarsely granular under a low power of the microscope; they readily become confluent, and are of a firm consistency.

The colonies of the meningococcus, on the other hand, are soft and sticky, and appear smooth or only finely granular with low magnification. The colonies are often confluent when crowded.

Dunham (1906) considered that the mucilaginous nature of colonies of the meningococcus and some similar organisms was useful as a means of differentiating the various groups, as he found that this property caused an emulsion of these organisms to pass very slowly through filter paper.

2. Ghon and Pfeiffer (1902) say that their cultures of *M. catarrhalis* in *broth* usually formed a skin on the surface, and later a ring at the upper level of the liquid and a deposit, the broth remaining clear, but Neisser (1903) states that the broth becomes turbid and a deposit forms. Bezançon and de Jong (1905) observed a turbidity and powdery deposit.

The meningococcus causes general turbidity, and if the tube is kept very still a surface skin is said to form; whilst the gonococcus on the addition of ascitic fluid or serum to the broth grows as a coarsely granular surface skin and deposit leaving the broth clear.

3. When media containing various carbohydrates are used for cultures, the different groups do not produce acids from the same sugars. Dunn and Gordon (1905) noted the reactions in broth containing glucose, maltose, saccharose or galactose after incubation at 37° C. for seven days. He found that *M. catarrhalis* produced acid in none of these, and that the meningococcus did so in glucose,

maltose and galactose, and the gonococcus in glucose and galactose (when a few drops of ascitic fluid had been added). F. W. Andrewes (1906) adds dextrin and laevulose to those carbohydrates from which acid is formed by the meningococcus.

Dunham (1906), using litmus-glucose-serum-broth found that the meningococcus produced acid but no coagulation, and that catarrhalis-like organisms either caused no acidity or produced acid and also caused coagulation of the medium, whereas *M. catarrhalis* caused neither acidity nor coagulation.

4. Specific agglutination as a means of differentiation has proved of value as regards several kinds of Gram-negative organisms, but *M. catarrhalis* and some of the other species agglutinate spontaneously. Dunham (1906), Goodman and Sholly (1906), v. Lingelsheim (1906), and others have prepared meningococcus sera and have successfully used their agglutinating action to separate organisms otherwise indistinguishable from the meningococcus. v. Lingelsheim also prepared a *M. catarrhalis* serum which did not agglutinate the meningococcus.

Several other kinds of Gram-negative cocci have been isolated from the nose which do not conform to the characters of *M. catarrhalis*, meningococcus or gonococcus. Some of these are said to resemble catarrhalis in forming no acid in those sugars in which they were tested, others resemble catarrhalis in most respects, but produce acid in glucose, galactose, maltose and laevulose, and also in cane sugar, as pointed out by Dunn and Gordon (1905), who found as well another race which produced acid only in glucose and maltose.

v. Lingelsheim (1906) isolated five kinds of Gram-negative cocci from the naso-pharynx and differentiated them by the size and appearance of their colonies, by the production of yellow pigment, by the changes which they produced in solid media containing various carbohydrates and coloured with litmus, and by their agglutination reactions with sera prepared by injecting the meningococcus, catarrhalis or one of two other races which he had isolated.

I have taken the following as the chief differentiating characteristics of the principal Gram-negative cocci here considered:

M. catarrhalis. Growth on ordinary agar after the first isolation in translucent colonies which are whitish by reflected, and brown by transmitted light, and are seen to be coarsely granular with low magnification.

Growth fairly good on gelatin at 20° C.: inability to produce acid from any of the carbohydrates on which it has been tried.

Meningococcus. A less free growth on agar, the colonies translucent and slightly milky by reflected light; the appearance of the colonies with low magnification smooth or only finely granular; general turbidity in broth; the production of acid from maltose and usually from glucose, galactose and laevulose, but not from cane sugar. Absence of growth at 20° C. Agglutination with a serum prepared by injecting a rabbit with the meningococcus.

Gonococcus. Inability to grow on media without addition of serum or ascitic fluid, at least till several generations of artificial culture have been passed. Colonies very translucent, and not confluent. Inability to form acid in maltose or saccharose although glucose or galactose may be thus affected.

I have examined the nose in 54 instances by means of small swabs, which were introduced where possible through a speculum, and then smeared on the surface of a mixture of blood and agar, or of ascitic fluid, nutrose and agar, as recommended by Dunn and Gordon. In 19 instances I isolated *M. catarrhalis*, but from a much larger proportion of infants under one year than of older children or of adults: the last furnished proportionately the fewest strains. The proportion in catarrhal noses was no higher than in the normal noses. In only one case, a girl of 16 years of age, was *M. catarrhalis* obtained in pure culture from a nose, and this was on the fourth day of a case of acute nasal catarrh without much constitutional disturbance, when the catarrh was nearly at its height.

TABLE I. *Organisms isolated from* 53 *noses.*

	15 Normal	7 Discharging Post S. F.	26 Acutely catarrhal	5 Meningitis cases
M. catarrhalis	5	3	8	3
Percentage of cases examined in which *M. catarrhalis* was isolated	33	43	31	60
Pneumococcus	4	1	9	3
Percentage of cases examined	26	14	35	60
Hoffman's bacillus	2	4	11	—
Percentage of cases examined	13	49	42	—
Catarrhalis-like organisms	1	1	1	—

TABLE II.

Age:—	Under 1 year	1—14 years	Over 14 years	All ages
Number of noses examined ...	12	23	19	54
Number in which *M. catarrhalis* was found	7	8	4	19
Percentage with *M. catarrhalis* of those examined at each age	58	35	21	35

I examined the nose in five cases of meningitis, but not within the first five days of the illness. Many organisms appeared in the cultures, but in no instance did I find an organism resembling the meningococcus.

In order to compare the characteristics of *M. catarrhalis* with other cocci which resemble it morphologically and in their staining reactions, I have cultivated the following varieties of Gram-negative cocci.

1. Six strains of meningococcus.

2. *Strain 4M* isolated by Dr Marshall from fluid obtained by lumbar puncture in a case of meningitis supposed to be of tubercular origin.

3. *Gonococcus,* isolated by myself on blood agar from an acute case of gonorrhoea.

4. Nineteen strains of *M. catarrhalis,* which were all isolated from the nose by myself, and an additional strain, U, derived from a culture, which was very kindly furnished by Dr M. Gordon: it was obtained by him from the Hertfordshire epidemic and agreed in all particulars with my own strains.

5. *Catarrhalis-like organisms.* Of these I have isolated three strains from the nose:

Strain Y, from a catarrhal nose, which also yielded typical catarrhalis: this strain was very much like the type, but was constantly small and of unusually uniform size.

Strain Q, isolated from a normal nose, in company with the pneumococcus: it was a small coccus and its sugar reactions differed from catarrhalis.

Strain V, obtained in pure culture from the nose of a boy, where it was found in a purulent discharge which had been present since he had scarlet fever some months before. It resembled *M. catarrhalis* except in its sugar reactions.

I have grouped together the 19 strains which I consider to be *M. catarrhalis,* although they present minor differences as to vigour of growth and power of surviving on agar.

TABLE III.

		Gelatin at 20° C.	Gram.	Broth	Ascitic broth	Emulsion	Spon. agglutination	Agglutination with meningo-serum	Maltose broth	Glucose broth	Galactose broth	Saccharose broth	Laevulose broth	Lactose broth
Meningococcus	XII	0	0	Turbid	Turbid	Good	0	+	A	A	A	–	A	–
,,	XVII	0	0	,,	,,	,,	0	+	A	A	A	–	A	–
,,	XVIII	0[1]	0	,,	,,	,,	0	+	A	A	A	–	A	–
,,	XIX	0	0	,,	,,	,,	0	+	A	A	A	–	A	–
,,	XXIX	0	0	,,	,,	,,	0	+	A	–	–	–	A	–
,,	M 38	0	0	,,	...	...	...	...	A	A	A	–	A	–
4M		+	0	,,	...	Good	0	--	–	–	–	–	–	..
Catarrhalis 19 strains		+	0	Clear & granules	Turbid & ring	Bad	+	...	–	–	–	–	–	–
,, strain U		+												
Catarrhalis-like														
,,	Q	Fair	0	Clear sand	...	Bad	...	...	A	A	A	A	A	..
,,	V	Good	0	Clear granules	...	,,	+	...	A	A	A	A	A	A
,,	Y	Good	0	Clear granules	...	,,	+	...	–	–	–	–	–	–
Gonococcus[2]		0	0	...	Clear & granules	Fair	+	...	–	A	A	–	–	–

A = Acid production.

[1] XVIII did on one occasion grow on gelatin.

[2] Ascitic fluid was added to the sugar-broth for growing the gonococcus.

As regards morphology, *M. catarrhalis* is more uniform in appearance and better stained by methylene blue than meningococcus.

The growth of *M. catarrhalis* on agar at 37° C. on first isolation was rather feeble, but after a few sub-cultures it became more vigorous; the colonies on agar were firm, so that the platinum wire in making a sub-culture broke solid pieces off or detached the whole colony, whereas the meningococcus colonies were of a slimy or honey-like consistency. On gelatin at 20° C. the growth, though often feeble at first, later became whitish and more vigorous. The gelatin was not liquefied; if the tube was incubated at 37° C. the appearance of the culture resembled that in broth, and on cooling the medium completely solidified. In ordinary *broth* cultures the medium always remained clear (unless shaken) with a coarsely granular or sand-like deposit at the bottom, usually suspended in a mucus-like ball. These cultures in broth resembled those of the gonococcus in ascitic-broth, but when ascitic fluid or sugar was added to broth and *M. catarrhalis* inoculated, growth always caused turbidity.

The catarrhalis-like organisms Y, Q, and V, from the nose, all grew in broth as a granular deposit and left the medium clear: Q forming fine sand which adhered to the glass: V forming coarse granules in a glairy ball. 4M caused uniform turbidity like meningococcus.

Carbohydrate peptone broths made with 75 % peptone water, 25 % peptone beef broth, and 1 % of carbohydrate were used.

M. catarrhalis and the strains Y and 4M formed no acid in glucose, maltose, laevulose, galactose, cane sugar or lactose. Strain V formed acid in 24 hours in all these sugars, and Q in 24 hours in all of these except lactose.

Good uniform emulsions of *M. catarrhalis* and the catarrhalis-like organisms were very difficult to obtain, but the meningococcus and strain 4M were easily emulsified.

Agglutination with a specific serum as a means of differentiating *M. catarrhalis* is of little service, since all the strains cultivated by me agglutinated spontaneously, but the serum of a rabbit repeatedly inoculated intravenously with cultures of *M. catarrhalis* did not agglutinate the meningococcus or strain 4M. The gonococcus and strains Y and V all agglutinated spontaneously.

The character of spontaneous agglutination appears to be so constant as to form an important point for differentiating these groups of Gram-negative cocci, especially as the strains of meningococcus

examined by me never agglutinated spontaneously, nor can I find that other workers have observed this occurrence.

Conclusions.

1. Gram-negative cocci derived from the nose can be divided into several different races, which require very careful culture for their identification.

2. *M. catarrhalis* is present very frequently in the normal nose, especially in the young and more especially in infants.

3. Its frequency does not appear to be greater in ordinary catarrhal states than in non-catarrhal. In this respect it differs from the pneumococcus and Hoffman's bacillus.

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