ON THE BEHAVIOR OF HEMOPHILUS INFLUENZAE IN CERTAIN DISEASES OF CHILDREN

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Dochez, Mills and Kneeland (2) have shown that on infection with the virus of "common cold," apes which had previously harbored a few *H. influenzae* in their throats may yield heavy cultures of this organism from both throat and nose. With the spread of distribution there was a change in cultural and immunological characteristics such that strains isolated during the infection fell within the category designated by Pittman (6, 7) as the smooth or fluorescent variant of *H. influenzae*. It seems that strains of this kind are responsible for most cases of influenzal meningitis, and that, as compared with the more familiar forms, they are characterized by a superior viability in the blood and tissues of experimental animals. "Fluorescent" strains may be recognized by their mucoid fluorescent growth on suitable media, by the demonstrable capsule and by the exhibition of a specific soluble substance which can be detected immunologically.[1]

It seemed desirable to supplement recent studies on the distribution of *H. influenzae* in the human upper respiratory tract (4, 13) by a consideration of the spread of this organism, both within the upper respiratory tract and elsewhere, with especial reference to the occurrence of fluorescent strains.

The nomenclature of influenzal variants is a vexed question. Since a distinct rough variant has been described (3, 10), the use of the term R for ordinary strains of influenza bacilli (6) seems likely to lead to confusion. We would suggest, therefore, the adoption of the terminology proposed by Dawson (1) for the pneumococci. In this paper, we shall designate fluorescent strains of *H. influenzae* as M (mucoid), and ordinary strains as S (smooth), reserving R for the rough variant.

METHODS AND MATERIAL

The Pediatric Service at the New Haven Hospital consists of 60 beds (including a contagious division) to which are admitted patients under the age of 13 suffering from all types of acute non-surgical diseases. Nose and throat cultures are taken on all patients on admission and are repeated irregularly.

The nose and throat swabs are streaked on separate blood agar plates, and if the illness is thought to be respiratory, the swabs are usually shaken up together in 1 cc. of broth, which is then injected into the peritoneal cavity of a mouse. If the mouse dies or is killed, subcultures are made of the peritoneal exudate and heart's blood. Aural and other purulent discharges are invariably cultured, and blood cultures are taken as indicated. Blood and spinal fluid cultures are made within 20 minutes of death on all children who have died on the service.

In addition to the wards, there is an active outpatient clinic from which many cultures come to the laboratory.

All plates are carefully examined in oblique light for the small translucent nonhemolytic colonies of influenza bacillus.

Certain gram-negative diplococci give small colonies which may be confused with those of the influenza bacillus, especially if the plates are examined at 24 hours. At 48 hours such colonies are usually larger and more opaque than the influenzal colonies. Some of these diplococcus strains have been subcultured. These did not require the accessory "V factor" of Thjötta and Avery (11). Small non-hemolytic or slightly green streptococci may form colonies readily mistaken for those of Pfeiffer's bacillus. If there are only a few colonies of *H. influenzae* on a plate, one may be unable to get a satisfactory smear, and, if mixed with other common organisms, influenza bacilli will usually be outgrown on subculture. Hence an accurate estimate of the prevalence of *H. influenzae* is hardly possible.

Certain criteria of the species *H. influenzae* are given below.

[1] By means of precipitation or agglutination tests these strains may be classified in six serological groups designated A to F.

Growth on blood agar plate	Morphology	Growth on special media	Growth requirements
Small, translucent colonies. No hemolysis.	Gram-negative rods and cocco-bacilli.	More luxuriant than on ordinary blood media.	Requires both X and V factors (11, 12).

From February to December, 1933, only strains which promised to be interesting from their predominance or from their location in the nose, or other unusual site, were tested for fluorescence. After some checking with Dr. Pittman, we came to feel considerable confidence in our recognition of fluorescent (M), borderline (±) and ordinary (S) strains. Unfortunately, a number of M strains obtained in this period were not typed. From December 23, 1933, to March 17, 1934, we attempted to study all strains systematically. All were subcultured on special media and on blood agar plates, and strains were kept on blood agar slants, transfers being made every 9 to 10 days. Dr. Pittman has kindly typed most of our M strains for us. All strains obtained in this latter period, except those lost on subculture, were subjected to the growth requirement test (11).

The technique used for this test, with slight modifications, was that communicated to us by Pittman (9) but Savita broth was used as a basic medium. The X factor was prepared by hemolyzing rabbit red cells with ether. The cells, thrice washed, were mixed with saline and ether in the proportion 10:10:4. The container was capped to prevent evaporation and kept in the icebox for 2 days. The rubber cap was then replaced by a cotton plug, and the tube placed in the incubator overnight to allow the ether to evaporate. The material was then centrifuged, and the solid residue discarded. The supernatant fluid, kept as stock in the icebox, was found to be sterile.

For the V factor about 500 cc. of unwashed brewer's yeast was mixed with 2,000 cc. of tap water. The mixture was heated slowly in an open pan with frequent stirring. When the temperature reached 80° C., the suspension was quite homogeneous. It was filtered through filter paper and through Berkefeld N candles.

To perform the test, 4 tubes were set up as follows:

A	*B*	*C*	*D*
2 cc. broth + 0.05 cc. 1/100 dilution of X factor + 0.1 cc. yeast extract (V).	2 cc. broth + 0.05 cc. 1/100 X factor.	2 cc. broth + 0.1 cc. yeast extract (V).	Same as A.

The tubes were incubated overnight to rule out contamination. A small portion of a single colony was fished with an inoculation needle into Tube A. The rack was then incubated for 24 hours. At this time *H. influenzae* would have grown well in A. A small loopful from this tube was then passed into each of the other three, and the rack was again incubated for 30 to 48 hours. Smears were made from Tube D. Tubes B and C should show no growth, if the organism was *H. influenzae*.

In our hands the "accessory factor" broth (X–V broth) has supported a more luxuriant growth than Levinthal (5, 9) or blood broth.

For reading fluorescence we have used both Levinthal agar (5, 9) and a medium prepared from the test factors (X–V agar). The latter may be made as follows: To 80 cc. of meat infusion broth add 20 cc. of yeast extract and 0.3 cc. of undiluted X factor. Mix the whole with 100 cc. melted 3 per cent agar, and pour plates. Such plates are satisfactory if used within 24 hours.

It has been our practice to streak each sector of a plate heavily with one of the strains to be tested. Plates have been read at 24 hours, each batch being controlled with a known fluorescent strain. The plate should be viewed by transmitted light against a dark background, and the opaque fluorescent appearance of a good M strain is striking. All influenza bacilli grew well on these media, but the growth of S strains is relatively meager compared to that of the M forms, and the S colonies are translucent. Certain strains are characterized by a luxuriant opaque growth with little fluorescence. These resembled certain of our stock strains, the fluorescence of which has been lost on subculture, and were classed as ± or intermediate.

If a stock mucoid strain has lost its fluorescence, one may sometimes restore it by inoculating a heavy culture into a mouse and killing the mouse

after 12 to 24 hours. It will be noted that M strains have sometimes been obtained on mouse culture only. However, the survival of *H. influenzae* in the mouse is irregular, and the death of a mouse inoculated from nose and throat swabs is seldom due to Pfeiffer's bacillus alone. The mouse is not a satisfactory medium for the routine culture of *H. influenzae*.

RESULTS

As an estimate of the frequency with which mucoid variants may be found in the upper respiratory tract, we give the figures from the latter part of this study (December, 1933, to March, 1934).

Children studied 186
Influenza bacilli found in upper respiratory tracts of 43
Mucoid variants found in upper respiratory tracts of 4
Intermediate variants found in upper respiratory tracts of 8

Thirty-four of the 67 strains studied, including all the M strains, were subjected to the growth requirement test. A number of organisms not thought to be *H. influenzae* were also tested. In all instances the results were as expected. It would seem that *H. influenzae* can usually be identified by its morphology and by the typical colony on blood agar.

Data on the entire period of study, and on some cultures taken before and after that period, are given in the tables. Material from 29 patients, who showed no M or intermediate variants in their upper air ways but had S forms in nose or ear, is summarized in Table I. Table II is based on a study of 11 children with intermediate ± strains in their upper respiratory tracts, while the 13 who had mucoid strains are reported in Table III. Table IV includes four patients who had influenza bacilli in sites other than the upper respiratory tract; these strains were all mucoid. One of these children (K.L.) had influenzal meningitis. Another (R.C.), with otitis media, appears also in Table I. This child had one positive blood culture only and made a complete recovery. A third, Eng., 6 years of age, died outside the hospital one hour after the onset of laryngitis. The strain isolated from the heart blood at autopsy was kindly given us by the Pathology Department. In the pleural fluid of the child R.F. tubercle bacilli were demonstrated by guinea-pig inoculation.

DISCUSSION

Pittman has emphasized (7) the extreme instability of freshly isolated M strains. It is possible that mucoid variants may dissociate or be overgrown during the first transplant, and that the use of media suitable both for primary isolation of *H. influenzae* and for demonstration of fluorescence might give different results from those recorded here. On the translucent media which we have used the growth of *H. influenzae* is inhibited by the presence of green streptococci or pneumococci (9). Our statement of findings must include the proviso "with the techniques used." Our conclusions must also include the qualification "in children" for bacterial behavior in adults might be quite different.

Of the cases of patients with *H. influenzae* in their noses there are three which one might hesitate to class as infections of the respiratory tract. Yet the asthma of the child F (Table II) was probably on an infectious basis, the child E.G. (Table I) had a tuberculous otitis media (proved at autopsy), and M.B. had a severe oral infection. Some of the children were seen several times, with and without respiratory infection; Pfeiffer's bacillus, often present in the nose in respiratory disease was not found there apart from such disease. The view that *H. influenzae* may spread to the nose or ear during infections of the respiratory tract is supported by the present study. The instances given include cases of scarlet fever and of Type I pneumococcus pneumonia, as well as of "common cold." Hence it is difficult to interpret the significance of this spread. In many cases we feel sure that the influenza bacillus played no great part in the disease. We cannot be sure of its rôle in otitis media. Our figures indicate that a spread of the bacillus may occur without the appearance of mucoid variants.

It is interesting that all the strains obtained from "distant sources" (Table IV) were mucoid. This finding accords well with Pittman's observation of the superior viability of M strains in the blood and tissues of experimental animals (7, 8). However, except in the case of the patient K.L. with meningitis, we have no means of

TABLE I

Isolation of S forms of H. influenzae from nose, ear, etc.†

Patient	Age	Date	Nose	Throat	Mouse	Ear	Diagnosis
C. A.....	4 years	April 17, 1933	S	S	–		Lobar pneumonia (Pn. unclassified)
M. M....	1 year	February 7, 1933	S	?	–		Bronchiolitis
R. L.....	6 weeks	January 27, 1933	S	?			Bronchitis
J. R.....	14 months	February 7, 1933	S	?			Lobar pneumonia (Pn. unclassified)
J. V.....	6 months	January 3, 1933	S	?	?		Bronchiolitis
P. de S...	7 months	March 19, 1933	S	?			Rhinopharynigitis
W. H....	5 months	September 16, 1933	S	?		–	Otitis media
E. G.....	2 years	April 5, 1933	S	S	–		Tuberculous otitis media and meningitis
H. C.....	4 weeks	March 23, 1933	S	?			Bronchiolitis
P. P.....	6 years	February 3, 1934	S	–	–		Scarlet fever
K. A.....	8 years	January 10, 1934	S	–			Strep. pharyngitis
Z. P.....	1 year	January 1, 1934	S	–			Rhinopharyngitis
B. R.....	2 years	March 9, 1934	S				Otitis media
S. D.....	8 years	November 10, 1933	–	–	–	S	Otitis media
P. A.....	2 years	March 12, 1934	S				U. R. I.
V. C.....	5 years	March 14, 1934	S				U. R. I.
F. C.....	9 months	March 4, 1933	S	?			U. R. I., tuberculous peritonitis
E. L.....	3 years	April 5, 1933	S	?		–	Scarlet fever
R. L.....	5 years	April 5, 1933	S	?			Scarlet fever
		April 25, 1933	S	?			
R. C.....	3 months	December 23, 1933	S	–		S	Otitis media
		January 4, 1934	–	–			Improved
		September 16, 1934*	–	–			Gc. vaginitis
A. K.....	2.5 years	April 21, 1933	S	?			Scarlet fever
		May 12, 1933	–	–			Well
		July 28, 1934*	–	?			Bacillary dysentery
J. M.....	5 years	January 7, 1934	S	–	S		Lobar Pn. (Pn. unclassified), bronchitis
		August 2, 1934*	–	?			Fever undiagnosed
M. B....	9 years	April 23, 1933	S	?			Dental abscess
		April 29, 1933	S	?			Cervical adenitis. Rheumatic fever
		September 28, 1934*	–	–			Congestive heart failure
D. M....	3 years	November 19, 1933	?	–			Bronchiolitis
		December 12, 1933	S	S			
		December 19, 1933	–	–			Improved
P. J.....	12 days	March 15, 1933	–	–			Bronchopneumonia (pneumococcus).
		March 16, 1933	S	–			
		March 24, 1933	–	?			
J. Mas...	6 years	January 9, 1934	S	–	–		Lobar pneumonia (Pn. I)
		April 19, 1934*	–	–			Mesenteric adenitis
A. D'A. .	6 days	November 18, 1932	–	–			Fat necrosis
		November 30, 1932	–	–			
		December 21, 1932	–	–		–	Otitis media
		May 6, 1933*	–	?		S	Otitis media
E. K.....	15 months	February 5, 1933	?	?	–		Bronchitis and bronchopneumonia
		March 7, 1933	S	?			
		March 15, 1933	S	?			
I. M.....	3 years	January 13, 1933	–	?			Cooley's anemia
		June 20, 1933*	–	–			Cooley's anemia
		September 12, 1933*	–	–			Cooley's anemia
		February 18, 1934	S	–			+ U. R. I.

† S = *H. influenzae*, S form.
? = *H. influenzae*, strain not tested.
– = No influenza bacilli recovered.
* = Readmission.
U. R. I. = Upper respiratory infection.

assaying the importance of mucoid influenza bacilli in these patients. We may note that Pittman has found that most of her meningeal strains are Type B (7, 8), and that two of our patients had Type B bacteremia without meningitis.

CONCLUSIONS

(1) A spread of *H. influenzae* to the nose may occur in a variety of infections of the respiratory tract. This organism is also frequently isolated from aural discharges.

(2) While mucoid variants are occasionally isolated from the upper respiratory passages, the spread of *H. influenzae* to the nose or ear is frequently unassociated with the appearance of such a variant. No correlation is evident between the occurrence of M forms and the type and severity of the disease.

TABLE II

Isolation of strains intermediate between S and M forms

Patient	Age	Date	Nose*	Throat	Mouse	Ear	Diagnosis
	years						
A. R.....	10	May 16, 1932	–	?			Diabetes
		February 27, 1933*	S	–	±		Diabetes and U. R. I.
B. H.....	1	December 2, 1932	?	?	?		Lobar pneumonia (Pn. XIV)
		March 3, 1933*	±	–			Otitis media
		March 9, 1933				S	
		July 9, 1933*	–	?			Lobar pneumonia (Pn. unclassified)
J. F.....	1	October 10, 1933	–	–	±		Bronchiolitis, bronchopneumonia
		December 18, 1933	S	–			
		December 25, 1933				–	
		January 2, 1934	–	?			
W. S.....	4	December 10, 1933	–	?			Lobar pneumonia (Pn. I)
		February 8, 1934	±	?			Empyema, pharynigitis
Kn.....	1	January 12, 1934	–	–	±	S	Lobar pneumonia (Pn. I), otitis media
P.....	2.5	January 25, 1934	S	–			U. R. I.
		February 10, 1934	±				
Ra.....	1	January 28, 1934	S	–	–	±	Otitis media
C. R.....	2.5	September 3, 1933	?				U. R. I.
		February 8, 1934*	±	–			Tonsillitis, adenitis
F.....	3	February 23, 1934	S	–			Asthma
		March 2, 1934*			±		Asthma
Del.....	1	March 5, 1934	±	S	–		Pharyngitis
D'Ag....	(0.4)	March 12, 1934	±	±	±		Bronchopneumonia

* ± = Intermediate form of *H. influenzae.* For other symbols see Table I.

TABLE III

Isolation of M strains of H. influenzae from upper respiratory tract

Patient	Age	Date	Nose*	Throat	Mouse	Ear	Diagnosis
	years						
R. W....	1.7	February 14, 1933	–	M			Laryngitis
E. B.....	8	February 23, 1933	M(B)	?	?		Scarlet fever
A. C.....	1.3	March 27, 1933	?	?			Scarlet fever
		April 15, 1933	M(E)	M(E)			
R. A.....	3	January 10, 1932	–	–			Lobar pneumonia (Pn. IV)
		September 19, 1932*	–	?			Bacillary dysentery
		November 23, 1932	?	?	?		U. R. I., anemia
		January 7, 1933*	–	?	–		Lobar pneumonia (Pn. IV)
		January 13, 1933	M(E)				
		January 27, 1933	–	?			
		December 25, 1933*	–	–	M(E)		Lobar pneumonia
		March 14, 1934*	–	S			Rhinopharyngitis, cervical adenitis
		March 24, 1934	S	S	S		
L. B.....	4	January 17, 1933	–	S			Thyrotoxic goiter
		September 18, 1933*	M	?	?		Thyrotoxic + pharyngitis and tonsillitis
		September 21, 1933	M	?			
		September 27, 1933	M	?			
		September 30, 1933	M	?			
A. S.....	1.5	October 12, 1933	–	–	M		Bronchopneumonia
S. R.....	4.5	February 24, 1933	–	?			Acidosis
		March 2, 1933	–	?			
		September 15, 1933*	M	?	–		Tonsillitis
E. V.....	8	October 17, 1933	M	?	–		U. R. I., epilepsy
W. K....	4.5	November 17, 1933	?	M			Pharyngitis
P. T.....	3	December 17, 1933	–	–	M(A)		Epilepsy, U. R. I.
Bi.....	2	December 28, 1933	–	S	M(E)		Lobar pneumonia (Pn. V)
R. F.....	1.4	January 23, 1934	–	–			Tuberculous effusion
		January 25, 1934	–	–			Pharyngitis
		January 29, 1934			M(E)		
		February 21, 1934			–		
Cd. R....	2	March 6, 1934		M(A)			Follicular tonsillitis
		March 12, 1934	M(A)	M(A)			Improved

* M(A) = Mucoid (fluorescent) *H. Influenzae,* Type A (Pittman).
M(B) = Mucoid (fluorescent) *H. Influenzae,* Type B (Pittman).
M(E) = Mucoid (fluorescent) *H. Influenzae,* Type E (Pittman).
For other symbols see Table I.

TABLE IV

Isolation of H. influenzae from distant sources *

Patient	Age	Upper respiratory tract	Blood	Cerebro-spinal fluid	Knee joint	Pleural (effusion)	Condition
K. L.....	3 years	?	B	B	B		Influenzal meningitis; died
Eng.....	6 years	?	B				Laryngitis; died
R. C.....	8 weeks	S	B				Otitis media; recovered
R. F.....	17 months	E 1/29				E 1/23 — 1/25 — 1/26 E 2/1 — 2/2 — 2/5	Tuberculous effusion; recovered

* S = S form
? = Strain lost
— = No influenza bacilli recovered
B = M form, Type B ("Meningeal" type)
E = M form, Type E

(3) Influenza bacilli have been isolated from sources distant from the upper respiratory tract in four cases. In each instance they have been M forms.

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