Improvement of the Nasopharyngeal Swab Method of Diagnosis in Pertussis by the Use of Penicillin*

WILLIAM L. BRADFORD, M.D., ELIZABETH DAY, AND GEORGE P. BERRY, M.D., F.A.P.H.A.

Departments of Pediatrics and Bacteriology, The University of Rochester, School of Medicine and Dentistry, Rochester, N. Y.

IN 1929 Fleming [1] demonstrated the value of penicillin in the isolation of *Hemophilus influenzae*. When penicillin was incorporated in the culture medium or when an amount of from 2 to 6 drops of it was spread over one-half a Petri plate after the infected material, such as sputum or nasal mucus, had been streaked over the surface of the medium, the growth of the penicillin-sensitive organisms was so completely inhibited that *Hemophilus influenzae* often grew in practically pure culture. Fleming stated, "In addition to its possible use in the treatment of bacterial infections penicillin is certainly useful to the bacteriologist for its power of inhibiting unwanted microbes in bacterial cultures so that penicillin-insensitive bacteria can readily be isolated."

MacLean (1937) [2] used Fleming's technique for the isolation of *Hemophilis pertussis*. He applied from 6 to 8 drops of the strong penicillin solution to one-half of the Bordet's medium in a cough plate. Of 50 such plates exposed, 47 gave positive cultures for *Hemophilus pertussis* on the penicillin-treated side of the plate as against 33 positives obtained on the untreated side. When pharyngeal swab cultures, taken after a paroxysm, were treated with penicillin, from 75 to 80 per cent yielded positive results.

Cruickshank (1944) [3] applied 4 drops (15 to 25 Oxford units) of penicillin solution to the surface of Bordet's medium (12 ml. of medium per plate). Later, this amount was increased to 8 drops. The solution was distributed over the surface of the plate with a sterile glass spreader. After the plate was incubated for from ½ to 1 hour, it was inoculated with material obtained by swabbing the posterior pharynx with a bent swab passed through the mouth. Cruickshank found that such swab cultures were equally as effective as ordinary cough plate cultures without preliminary treatment with penicillin. He indicated that more care in the taking of the post-nasal cultures would probably have given better results.

The nasopharyngeal swab method for bacteriological diagnosis of pertussis, introduced in 1940, has proved to be superior to the ordinary cough plate method in our hands.[4, 5] This superiority has been confirmed by others.[6, 7] In using this technique a small loopful of 0.85 per cent sodium chloride solution is placed upon the surface of the medium and the charged swab is passed

* This work was supported in part by grants from the John and Mary R. Markle Foundation and from The Chatterbox Fund.

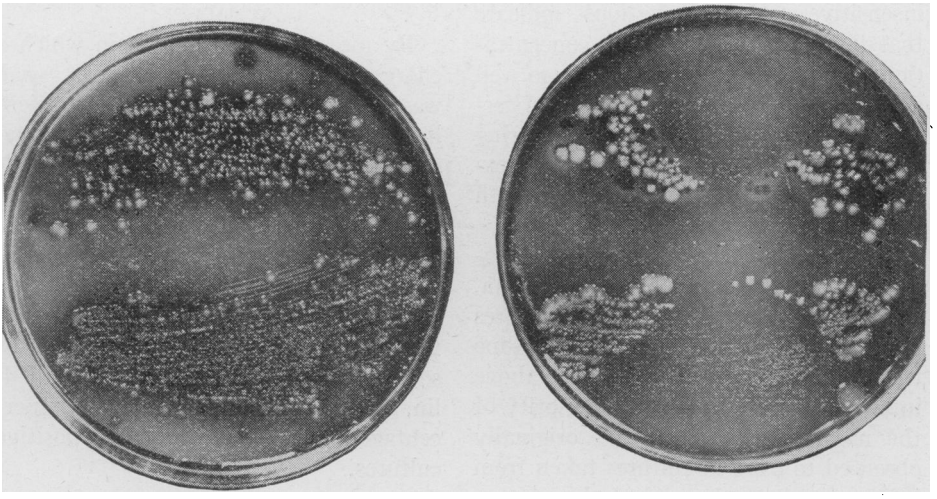

FIGURE 1—Showing the inhibiting effect of penicillin on the growth of contaminating organisms (right) to facilitate the identification of *Hemophilus pertussis* colonies on the surface of Bordet's medium. (Control plate is shown on the left.)

back and forth several times through the drop of saline. The plate is then streaked with a long, flexible loop.

In the present report are described the results of a series of cultures in which a loopful of penicillin solution containing 1,000 Oxford units per ml. was substituted for a loopful of 0.85 per cent sodium chloride solution. For comparison, cultures were taken chiefly from hospitalized patients during various stages of whooping cough, and plated simultaneously by the two methods.

RESULTS

The results shown in Table 1 clearly indicate that the use of penicillin facilitates the isolation of *Hemophilus pertussis* by inhibiting the penicillin-sensitive organisms that ordinarily appear on cultures made from the posterior pharynx.

The area of inhibition of the contaminating organisms, as usually observed, is well illustrated in Figure 1. Within this area a practically pure growth of *Hemophilus pertussis* is often observed. Moreover, the medium within this zone usually retains its original cherry-red appearance. After 3 days of incubation, the remainder of the medium is frequently discolored by the acid producing growth of contaminating organisms.

When too strong a concentration of penicillin is used, the growth of *Hemophilus pertussis* is inhibited. In spite of the fact that the organism is generally considered to be penicillin-

TABLE 1

Results of 95 Simultaneous Nasopharyngeal Swab Cultures from 52 Cases of Pertussis

Age Years	*Number Patients*	*Number of Positive Cultures*	*Number of Negative Cultures*
Penicillin			
Under 2	20	47	2
2–14	28	38	1
Over 15	4	7	0
Total	52	92 (97.6%)	3
0.85% NaCl			
Under 2	20	42	7
2–14	28	26	13
Over 15	4	7	0
Total	52	75 (76.8%)	20

Difference in Percentage of Positive cultures 20.8
Standard error of the difference 4.6
Ratio of difference in Percentages to its S.E. 4.5 to 1

insensitive, our observations indicate that it is sensitive to strong concentrations. This effect occurs *in vivo* as well as *in vitro*, for we have observed in certain experiments a significant clearing of the organism from the lungs of experimentally infected mice treated with penicillin.

Two interesting, and probably significant, findings appear in our data. First, the penicillin treated cultures gave better results than the saline treated cultures in the age groups above infancy (Table 1). (The superiority of the nasal swab method was originally observed to exist in cultures taken from infants.) Second, such cultures are more efficient after the catarrhal period of the disease (Table 2); for example, 13 of the 20 negative control cultures were obtained in the later stages of the disease when the number of organisms is usually less.

TABLE 2

Distribution of Negative Cultures According to the Stage of the Disease

	Number of Negative Cultures	
Stage of Disease	*Penicillin*	*0.85% NaCl*
Catarrhal (1st and 2nd week)	2	7
Paroxysmal (3rd and 4th week)	1	7
Decline (5th and 6th week)	0	6

SUMMARY

By means of a technique in which a charged post-nasal pharyngeal swab was moistened with a loopful of penicillin (1,000 Oxford units per ml.) and placed upon the surface of Bordet's medium, a high percentage (97.6 per cent) of positive cultures of *Hemophilus pertussis* was obtained from patients in various stages of whooping cough. Cultures taken in the same way except for the substitution of 0.85 per cent sodium chloride solution for the penicillin, yielded a significantly lower percentage (76.5 per cent) of positive cultures.

REFERENCES

1. Fleming, A. On the Antibacterial Action of Cultures of Penicillium, with Special Reference to Their Use in the Isolation of *B. Influenzae*. *Brit. J. Exper. Path.*, 10:226, 1929.

2. MacLean, I. H. A Modification of the Cough Plate Method of Diagnosis in Whooping Cough. *J. Path. & Bact.*, 45:472, 1937.

3. Cruickshank, R. Post-nasal Swab in Diagnosis of Pertussis. *Lancet*, 1:176, 1944.

4. Bradford, W. L., and Slavin, B. Nasopharyngeal Cultures in Pertussis. *Proc. Soc. Exper. Biol. & Med.*, 43:590, 1940.

5. Brooks, A. M., Bradford, W. L., and Berry, G. P. The Method of Nasopharyngeal Culture in the Diagnosis of Whooping Cough. *J.A.M.A.*, 120: 883, 1942.

6. Miller, J. J., Jr., Leach, C. W., Saito, T. M., and Humber, J. B. Comparison of the Nasopharyngeal Swab and the Cough Plate in the Diagnosis of Whooping Cough and *Hemophilus Pertussis* Carriers. *A.J.P.H.*, 33:839, 1943.

7. Bullowa, J. G. M., Buxbaum, L., and Scheinblum, I. E. Pertussis: Bacteriologic and Agglutination Studies. *J. Pediat.*, 25:299, 1944.