STUDIES ON WHOOPING COUGH*

I. Type-Specific (S) and Dissociation (R) Forms of Hemophilus pertussis

By GERALD S. SHIBLEY, M.D., and HELENA HOELSCHER

(From the H. K. Cushing Laboratory of Experimental Medicine in the Department of Medicine of Western Reserve University, and the Medical Service of Lakeside Hospital, Cleveland)

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In the recent revival of interest regarding the etiology of whooping cough in America, two outstanding beliefs are: (*a*) that the disease is caused by *H. pertussis* (the bacillus of Bordet and Gengou), or (*b*) that the specific agent is a filtrable virus. Of the two opinions, the first named is far more generally accepted and stands practically unquestioned in Europe (1). A third hypothesis, based upon analogy with Shope's recent work on swine influenza (2), which combines the two, proposes the possibility that a filtrable agent and *H. pertussis* act together to produce the disease (3). Of the three theories the first named has seemed to us most acceptable (4), and for this reason we have undertaken and are herewith reporting detailed studies of the biological character of *H. pertussis*, with a view to clarification of some of the confusion existing in regard to its phases and dissociation forms and with the definite object of bringing these into conformity with the terminology currently employed in the description of bacteria and their variants.

H. pertussis when first isolated differs from forms found in old laboratory cultures. This difference was first described by Bordet and Sleeswyk (5) who noted that the freshly isolated organism differs serologically from old stock strains. Bordet believed that the difference was due to adaptation of the latter to agar media. Krumwiede, Mishulow, and Oldenbusch (6) described two agglutinative varieties

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or types, A and B; they failed to confirm Bordet's conclusions referred to above. From inspection of the data presented by these workers, it seems probable that their two types represent subvarieties of old stock cultures. Leslie and Gardner (7), who have recently restudied the problem, conclude that *H. pertussis* is a uniform species without fixed types when first isolated, but after subculture upon various laboratory media, it passes through a series of antigenic stages which they have called Phases I, II, III, and IV. Lawson (8) has been able to confirm their work in general but was never able to obtain their Phase II. It is not impossible that this phase exists, although we have never noted it, and that it may represent an intermediate transition form between freshly isolated strains and older stock variants.

From the findings to be reported below, it seems certain, as first suggested by Leslie and Gardner, that recently isolated strains (Phase I) correspond to the virulent or S forms and that the antigenically different stock strains (Phases III and IV) are the same as the avirulent or R variants of other bacteria. Hereafter, therefore, both for purposes of convenience and in conformity with current nomenclature, the two forms will be referred to as S and R forms. It should follow from this that the freshly isolated form of *H. pertussis* (S) represents the pathogenic phase. On this assumption transmission experiments have been commenced in an effort to produce whooping cough in apes with the S form. To eliminate the possibility of there being a virus component in the inoculum, we have maintained the organism in the S phase, by methods to be referred to below, for several months before attempting transmission experiments.[1] While so engaged, we have undertaken detailed studies of the S phase in order to establish unmistakable criteria for its identification and for its differentiation from R forms. For this purpose observations have been made upon the cultural, morphological, toxic, serological, electrophoretic, and isoelectric properties of all strains studied. It is with a consideration of these findings that the present communication is chiefly concerned.

[1] In a preliminary communication (4) we have reported what we believe to be the successful transmission of whooping cough by means of this theoretically virus-free pathogenic S form and unsuccessful transmission with the R form in chimpanzees. The S form had been subcultured more than 60 times over a period of 8 months.

Methods

Materials.—We have studied 98 strains of *H. pertussis*. Fifty-nine were freshly isolated strains and were obtained from the following sources: Cleveland strains, 12 from Dr. Toomey of the Cleveland City Hospital and 30, isolated by us, mainly from cases of whooping cough in Dr. Gerstenberger's Pediatric Clinic of the Lakeside Hospital Out-Patient Department; Boston strains, 4, from Dr. G. M. Lawson and Dr. Hans Zinsser of The Harvard Medical School; Chicago strains, 3, from Dr. L. W. Sauer of the Evanston Hospital; Albany strains, 3, from Dr. A. B. Wadsworth of the New York State Board of Health; California strains, 3, from Dr. A. P. Krueger of the University of California; and Danish strains, 3, sent us by Dr. J. J. Miller who at the time was associated with the Statens Serum Institute in Copenhagen. Thirty-nine strains were laboratory stock cultures. Nineteen of these of widely scattered origin, including an original Bordet strain, were kindly sent to us by Dr. Toomey. The remaining 20 came from: New York, 2 (Dr. Park); Albany, 3 (Dr. Wadsworth); Boston, 2 (Dr. Lawson); and 13 were derived from our own freshly isolated strains.

Cultural Methods.—For the isolation of *H. pertussis* from active cases of whooping cough, the cough plate method of Chievitz and Meyer (9) was used.[2] For cough plates and for cultivating freshly isolated strains, the Bordet-Gengou potato-glycerin-blood agar medium used was essentially that recommended by Lawson and Mueller (10) except that sterilization was done by autoclave with 15 pounds of pressure at 121°C. for 20 minutes instead of by the Arnold sterilizer. Defibrinated or citrated human blood, either placental or obtained by venepuncture, was used in 25 to 35 per cent concentration. Plates were always poured freshly with blood usually less than 5 days old and never more than 1 week old. Plates, slants, and Blake bottles were poured thick. Old stock cultures were grown on chocolate blood (5 to 10 per cent), glycerin, agar (1.5 per cent) medium.

Morphological Studies.—Colonies were examined under the Zeiss colony microscope with both oblique and indirect illumination using magnification × 20. Smears were studied with Gram stains. For capsular studies, Wright's stain diluted 2:1 with glycerin, as recommended by Lawson (8) was used.

Determination of Toxicity.—This was determined on rabbits by intradermal injection using Berkefeld filtrates of the organisms and on guinea pigs by the intraperitoneal method used by Leslie and Gardner (7).

Methods of Agglutination.—Agglutinating sera were prepared by intravenous injection of rabbits with washed organisms resuspended in formalized (0.2 per cent) normal salt solution. Titers ranged from 1:2,000 to 1:20,000.

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[2] Forty-two attempts in all were made to recover the organism. The day of the disease ranged from the 2nd to the 28th. In cases seen during the 1st week the number of positive plates was 100 per cent (6 cases), in the 2nd week 100 per cent (10 cases), in the 3rd week 59 per cent (22 cases), and in the 4th week 50 per cent (4 cases), an average of 76 per cent for all cases.

All agglutination tests were done macroscopically. Organisms were washed twice with distilled water and resuspended in formalized saline in a concentration of 2 billion per cc. before addition to serum dilutions. When cultures were granular, glycocoll-sodium phosphate-sodium acetate buffer solutions (11) at pH 7.0 were substituted for the saline (12). Readings were made after 18 hours incubation at 37°C. Incubation at 56°C. occasionally resulted in the appearance of an inhibition zone (13) and was discontinued accordingly.

Absorption of Agglutinin.—Organisms were treated as for agglutination. The absorbing dose was the growth obtained from 6 to 8 Blake bottles. Serum dilutions used ranged from 1:10 to 1:100 depending upon the agglutinative titer of the serum tested. Serum-bacteria mixtures were incubated for 3 hours at 37°C. and were placed in the ice box overnight. Reciprocal absorption as recommended by Krumwiede, Cooper, and Provost (14) was carried out in all cases.

Measurement of Potential.—Cataphoretic migration velocities were determined by means of the Northrop-Kunitz microcataphoretic apparatus (15) and are reported in terms of microns/second/volt/centimeter. Before making readings, the bacteria were washed 3 times with distilled water and were then suspended in glycocoll-sodium phosphate-sodium acetate buffer at pH 7.0, molar concentration 1:100. For determination of isoelectric points, the same buffer solution at varying pH's in molar concentration of 1:25 was used.

RESULTS

Before proceeding to a detailed consideration of our experimental findings, a brief account of the transition from the S forms of *H. pertussis* to the R dissociants is in order. When first isolated upon Bordet-Gengou medium cough plates, we have found the organism to be a small Gram-negative coccobacillus which agglutinates to full titer with sera prepared with freshly isolated, or S strains, agglutinates in very low dilution or not at all with anti-R sera and is incapable of growth upon chocolate agar. After continued subculture upon Bordet-Gengou medium, it begins first to grow scantily and later more luxuriantly upon the coagulated blood medium. When this stage is reached, the organism more than doubles its cataphoretic migration velocity and will agglutinate with R sera. While in transition, cataphoretic studies show organisms moving at both the slow rate of S and the more rapid rate of R forms. The latter probably represent the developing R variants. Eventually, as growth becomes more luxuriant on the chocolate agar, the S form drops out and the fully developed R form is established. When this is accomplished the organism is no longer ovoid but is a long, thin bacillus and no longer

agglutinates with S sera. With this brief summary, we may pass on to a detailed consideration of the recently isolated S form and to a discussion of its essential differences from R forms.

Cultural Differences between S and R Strains.—When a positive cough plate from a case of whooping cough is examined under the colony scope at the end of 24 hours, typical colonies of *H. pertussis* appear as minute, shiny, greyish white, opaque, dome-shaped or pyramidal pin-points. They are quite distinctive and are readily differentiated from the colonies of other organisms, including *H. influenzae* (16), appearing upon the plate. After 48 to 72 hours incubation, they are fully developed and take on the character of a domed, glistening half-pearl, measuring 1 to 2 mm. in diameter. The edge is quite smooth, although occasionally if the medium is dried out, there may be fine stippling. Larger colonies, due to piled up growth, may take on a moist, glossy, pearly white appearance. By 48 to 72 hours most colonies will be surrounded by a small though definite zone of hemolysis. On subculture, if growth is heavily streaked, the hemolysis is much more conspicuous. R forms on Bordet or chocolate agar media grow more rapidly and present a flatter, less compact appearance. Their color is a dull or dirty grey rather than pearly grey. They are not glossy. Although their outline is not grossly rough as is the case for example, with R pneumococci, it lacks the sharp definiteness of contour of S forms. The zone of hemolysis so typical of S strains is not present around R forms.

A characteristic difference between S and R forms is found in their behavior in saline suspension. S strains are always readily emulsified when taken off solid media and are stable. R forms at times, however, emulsify with difficulty and not infrequently are granular; on occasion, they may be so unstable in suspension as to make agglutination tests, even in buffer solution, practically impossible. This instability in suspension is frequently a characteristic of the R forms (*e.g.* R pneumococci) of other bacteria.

Morphological Differences between S and R Strains.—The recently isolated or S form when stained by the method of Gram is characteristically a small Gram-negative coccoid or oval bacillus. The organisms are quite uniform in appearance and average about 1.0 x 0.5 micra in size. Paired organisms are not infrequent. Longer bacillary

forms are rare (Fig. 1). The R form is typically a much longer bacillus, running as high as 4 or 5 micra in length but retaining about the same diameter as the S form. Occasionally ovoid bacilli may be seen (Fig. 2) in films of R strains. Attempts to show a capsule with the usual staining methods have been unsuccessful in our hands and in those of Lawson, but when Wright's stain diluted 2:1 with glycerin as suggested by Lawson is used, definite capsules as described by him (8) may be made out in young cultures of S forms (Fig. 3), while indefinitely outlined or doubtful capsules appear in R strains.

Toxicity.—At the time that Bordet and Sleeswyk noted the antigenic differences between recently isolated and stock strains of *H. pertussis*, they demonstrated that the former, upon intraperitoneal inoculation of guinea pigs, were toxic, whereas the latter were not. Leslie and Gardner (7) have repeated this work and state that their Phases I and II were toxic for guinea pigs and III and IV were not. Bordet's finding that there is no multiplication of bacteria within the animal's peritoneal cavity and his view that death is purely toxic in its mechanism received support from these authors. Lawson (8), elaborating upon the work of Teissier, Reilly, Rivalier, and Cambassedes (17) has shown that there is a toxic filtrate (endotoxin) obtainable from Phase I (S) strains which produces a skin reaction in guinea pigs and which is neutralizable both *in vivo* and *in vitro* by immune serum. He was unable to show similar toxic properties in Phase III and IV (R) cultures.

We have made no effort to study this aspect of the problem in detail. However, in seeking criteria for the identification of S strains, we have tested a few of our cultures when indicated by intradermal injection of rabbits with filtrates and by intraperitoneal injection of guinea pigs according to the technic of Leslie and Gardner and have consistently found our S strains to be toxic and our R strains to be non-toxic.

Serological Differences between S and R Strains: (a) Agglutination.—Leslie and Gardner (7) working with 16 strains isolated by them and with 4 additional fresh strains obtained from Denmark and with 12 stock cultures derived from the Lister Institute and from New York, concluded that their freshly isolated strains were a uniform antigenic species without fixed varieties or types (Phase I) and that after isolation and repeated laboratory subculturing on various media,

they passed over through a transitional phase (II) into Phases III and IV, the phases represented by their stock cultures. Lawson (8) working with Leslie's phase sera studied 18 freshly isolated strains in Boston and found that they were Phase I (Leslie and Gardner). Certain stock strains in his possession were Phases III and IV. In an effort to extend this work and particularly with a view to amplifying it in the direction of discovering whether S forms (Phase I) were a uniform type wherever derived, we have studied 59

TABLE I

Summary of Agglutination Tests of S and R Strains of H. pertussis with S and R Sera

+ indicates agglutination to full titer of the serum employed. − indicates no agglutination or slight agglutination only. Nine different S and 7 different R sera were used in the tests.

Strains of *H. pertussis*	S serum		R serum	
	No. of strains	Results	No. of strains	Results
S strains				
Cleveland	39	+	24	−
Boston	4	+	4	−
Chicago	3	+	3	−
California	3	+	1	−
Denmark	3	+	2	−
R strains				
Cleveland (Toomey)	18	−	18	+
Cleveland (from S strains)	13	−	13	+
Boston	2	−	2	+
New York City	2	−	2	+
New York State	3	−	3	+

freshly isolated strains obtained from widely distributed sources. They include strains from Boston shown by Dr. Lawson to be Phase I with English phase serum, strains from Denmark, and strains obtained in this country from Boston, Albany, Chicago, San Francisco, and Cleveland.

All of these strains, with the exception of the Albany ones, which were somewhat older and presumably had dissociated to the R form, have agglutinated to full titer with serum made with strains freshly

isolated by us or with sera made with S strains selected from each of the sources noted above. All of them failed to agglutinate or agglutinated only in low titer with sera made with stock (R) strains. These findings are summarized in Table I. In brief, all recently isolated S strains of *H. pertussis* tested by us, although obtained from widely distributed sources, are identical by the method of agglutination and are agglutinatively distinct from R forms.

We have not made detailed efforts as yet to differentiate subvarieties of R strains (Phases III and IV). As stated above, we have not noted Leslie and Gardner's Phase II (transitional S strain). Every one of the R strains including a "Phase III" sent us by Dr. Lawson has agglutinated to full titer with serum made with Strain A (Mishu-

TABLE II

Reciprocal Absorption Tests with Serum and Organisms of S Strains of H. pertussis from Cleveland (H16) and Boston (Z)

	Organism agglutinated	Titer before absorption	Agglutinative titer after absorption with	
			Strain H16	Strain Z
Serum H16	H16	1:2,560	>1:100	>1:100
	Z	1:2,560	>1:100	>1:100
Serum Z	H16	1:20,480	>1:100	>1:100
	Z	1:20,480	>1:100	>1:100

low) which was designated Phase IV by Leslie and Gardner (7), and Lawson (8). This has been true of all stock strains obtained from outside sources as well as all R forms derived from our own S strains. One strain (M1, obtained from Dr. Park) has puzzled us, as although in all other respects it has behaved as an R variant, its serum has occasionally agglutinated our S strains even though it has not been agglutinated by the anti-S sera. We are planning to study this strain and other aspects of R dissociants in the near future.

(*b*) *Agglutinin Absorption.*—In order to establish the fact that all our widely derived S strains, seemingly the same by agglutinative methods, are serologically identical, we have tested them further by absorption of agglutinin. In all cases we have used the method of reciprocal absorption insisted upon by Krumwiede (14). We have not tested all

strains but have selected single samples from each of the sources noted above. By this method in all cases tested, it has been found that absorption is reciprocally complete. Table II shows a typical experiment using S strains from Cleveland and from Boston.

As noted above, studies concerned with the classification of subvarieties of R forms have not yet been undertaken in detail. However, such work as we have done by the methods of agglutination or agglutinin absorption, have not indicated that mutually exclusive, sharply defined phases are the rule.

Cataphoretic Differentiation of S and R Strains.—Early in our studies of *H. pertussis* we noted and reported the fact (18) that recently isolated (S) strains could be sharply differentiated from stock (R) forms by means of their cataphoretic migration velocities in an electrophoretic cell. At that time we had worked with 16 S and 6 R strains. Since then we have extended our observations to include 53 S and 39 R cultures and have consistently confirmed our original findings. All S strains have shown slower migration velocities than R variants. The velocities for S strains under standard conditions have averaged 1.76 μ / sec. / v. / cm. (range 1.2 to 2.3) and for R strains the average reading has been 4.2 μ / sec. / v. / cm. (range 3.9 to 4.6). A summary of the findings appears in Table III. These results show clearly the striking difference existing between the migration rates of the two forms, the R form moving at a rate over twice that of the S form. Although the individual variations in the two categories are fairly wide, the great majority of readings have fallen fairly close to the mean, and further, these occasional variations have been noted in

TABLE III

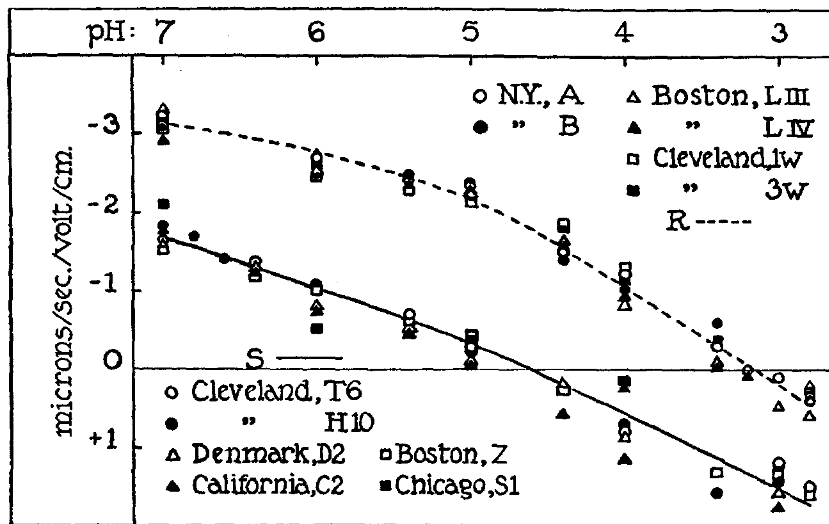
Summary of Readings Made of Cataphoretic Velocities of S and R Strains of H. pertussis

Organisms washed 3 times with distilled water and resuspended in M/100 glycocoll-sodium phosphate-sodium acetate buffer, pH 7.0.

	No. of strains	No. of determinations	Average μ/sec./v./cm.
S strains	53	201	1.78
R strains	39	162	4.20

readings made at different times upon a single strain. Some of these have been accounted for on the basis of technical difficulties.

Differences between toxigenic and non-toxigenic (S and R) diphtheria bacilli and between S and R pneumococci have already been noted (19). The findings herewith reported for *H. pertussis* would seem to parallel these and constitute a further basis for considering recently isolated (S) strains, virulent forms, and stock strains, avirulent or R dissociants.



TEXT-FIG. 1. Curves showing (*a*) the effect of varying pH upon migration velocities of S and R forms of *H. pertussis* and (*b*) their isoelectric points. The curve for the S forms is the solid line and for the R forms, the broken line. Readings for individual strains are indicated by the symbols shown on the chart.

Certain observations have been made in regard to the relationship existing between migration velocities and variation from S to R and back again. When an S strain is maintained upon Bordet-Gengou medium containing a high percentage of blood (25 to 35 per cent), it retains its typical slow rate for a long period of time, perhaps indefinitely. When the proportion of blood is reduced to 5 to 10 per cent, faster moving organisms begin to appear among the slower ones, the ratio being about 2:1. Finally, when the strain has adjusted to

chocolate agar, the slowly moving organisms are completely replaced by those of higher migration velocities and the strain is now completely R in all respects. If now the R strain is grown again on Bordet-Gengou medium with high blood percentage, provided it has not been R too long, it will revert to its slower rate and will assume all the characteristics of S strains.

One of our R strains, M1, has regularly shown a migration velocity (3.1 μ/sec./v./cm.) intermediate between those characteristic of S (1.76) and of R (4.2) forms. It is interesting to note that this is the strain referred to above which has behaved in inconsistent fashion agglutinatively and which is being investigated further in our projected detailed studies of R variants.

The Isoelectric Point of S and R Strains.—The finding of these characteristically different cataphoretic velocities for S and R strains suggested the advisability of an investigation of their respective isoelectric points. Fifteen determinations upon 12 representative S strains and 34 determinations upon 24 R variants have been made. The results show that S and R forms may be sharply distinguished by their respective isoelectric points. For recently isolated strains (S), the isoelectric point falls between pH 4.4 and 4.8, average, pH 4.61. In the case of R forms, the isoelectric point falls between pH 2.8 and 3.6, average, pH 3.19. Text-fig. 1 shows the curve for 6 representative strains in each of the categories. Differences in the isoelectric points of a virulent strain of the bacillus of rabbit septicemia and its less virulent dissociant have been reported by De Kruif (20). The finding of these differences in their isoelectric points for S and R strains of *H. pertussis*, constitutes further evidence in favor of their representing respectively the pathogenic form of the organism and the avirulent variant.

The Maintenance of S Forms.—Freshly isolated strains of *H. pertussis* persist, perhaps indefinitely, in the S form, as determined by the criteria enumerated in the foregoing sections, provided that they are kept growing upon Bordet-Gengou potato-glycerin-blood agar containing no less than 25 per cent of freshly defibrinated blood. We have 6 strains (H8, H10, H12, H15, H16, and H19) that have retained their cultural, hemolytic, morphological, serological, and cataphoretic S characteristics, under these conditions of cultivation, for periods ranging from 21 to 31 months. Strain Z used for the successful ape trans-

mission experiment referred to above, was over 8 months old and had been subcultured 60 times when used for inoculation.

If the proportion of blood is allowed to fall below 20 per cent, dissociation of S to R forms occurs. If the cultures are now seeded upon

TABLE IV

Criteria for Identification of S Form of H. pertussis and for Its Differentiation from R Forms

	S forms	R forms
Relationship to whooping cough	May be recovered in first 2 wks. of disease if suitable measures are used in practically all cases	Not present in first 4 wks. of disease
Cultural characteristics	Dome-shaped, half-pearl. Sharply circular outline	Outline circular but not sharply circumscribed. Elevation rather flat and not compact
	Glistening, mucoid or moist appearance	Not glistening in appearance
	Pearly greyish white color	Dull, dirty light grey color
	Surrounding zone of hemolysis	No hemolysis
Stability of suspension in 0.85 per cent NaCl	Emulsify smoothly to form stable suspension	Emulsify with difficulty. Frequent instability with autoagglutination
Morphological	Small Gram-negative coccobacillus, 1.0 x 0.5 μ	Long, thin bacillus, 4.5 x 0.5 μ
	Generally uniform in size	Considerable variation in size
	Capsule present	Capsule probably not present
Serological	Agglutinate to full titer with sera made with recently isolated (S) strains, wherever derived	Agglutinate to full titer with R sera
	Complete reciprocal absorption with other S strains	
	No absorption of agglutinins from R sera	No absorption of agglutinins from S sera
Toxicity	Toxic for guinea pigs upon intraperitoneal injection	Not toxic
Cataphoretic	Under standard conditions migration velocity is 1.75 μ /sec./v./cm.	Under standard conditions migration velocity is 4.2 μ /sec./v./cm.
	Isoelectric point: pH 4.6	Isoelectric point: pH 3.2

chocolate agar, they will grow luxuriantly in the R form. Strains thoroughly adapted to the last named medium will grow upon brain agar medium. R strains which have been growing for periods of

several months upon chocolate agar when placed upon Bordet-Gengou media of suitable blood concentration will return to the S form. One of our earliest strains, H1, isolated nearly 3 years ago, before we had become aware of the relationship of blood proportions to S and R dissociation, changed to the R form and remained in this phase for 16 months. Subsequent attempts, by repeated subculture upon media of high blood percentage, to restore it to the S form were successful and this strain has since remained typically S for 19 months. Attempts on our part to transform some of our very old R cultures (A, B, Mishulow; L III, L IV, Lawson) back to S by growth upon high blood Bordet-Gengou medium have so far failed, although these strains have been growing upon such media for 22 months. That old R strains will change back to S if injected intraperitoneally in guinea pigs has been shown by Toomey (21). Continued observations of the reversibility of these dissociation changes are under way in connection with our further studies of R variants.

The mechanism of these changes is still a matter for speculation. However, the results so far obtained point suggestively to the probable soundness of Bordet's original idea that the changes are a function of adaptation to conditions of growth.

SUMMARY AND DISCUSSION

The more important criteria for identification of the S form of *H. pertussis* and for its differentiation from R variants are presented in summary fashion in Table IV. The differences as indicated in detail in the foregoing sections and as shown briefly in this tabulation are so clear-cut that they call for little, if any, further comment. Of all the differential characteristics, the morphological, the serological, and the cataphoretic seem to be the most distinctive, and of these, the cataphoretic, in our hands, has been the most conclusive.

It would appear from the foregoing results that all recently isolated strains, provided that they are grown upon suitable media, fall into a single uniform serological type. This is true whether the strains are isolated from cases of whooping cough in Europe or in any part of this country. It is also apparent that when they are subcultured upon laboratory media deficient in fresh blood, dissociation occurs with the appearance of morphologically, culturally, and antigenically different variants. In keeping with the current classification of bacterial variants, we feel that the uniform recently isolated strain should be

designated the S form and the laboratory variants the R form of *H. pertussis*. Whether the R variants fall into sharply defined phases as pointed out by Leslie and Gardner, remains to be confirmed. Lawson and the writer have never noted their "Phase II" and Toomey (21) is in agreement with us that sharply defined, mutually exclusive subvarieties of R variants probably do not exist. We are undertaking further studies of this somewhat mooted point.

Dawson, as a result of detailed studies concerned with the dissociation of pneumococci (22) and of streptococci (23) has shown that these organisms have three variant forms and he proposes that the terminology currently employed for pneumococcal variants be changed to conform with the terms used in the description of corresponding variants of other bacterial species. The first form, encapsulated, at present called S, he designates *mucoid* (M); the second, at present R, he calls *smooth* (S); and the third, a new and distinct, grossly rough variant which he describes for the first time, he would call *rough* (R). Hadley (24) has found that most organisms show these three chief colony forms. From conversation with Dr. Dawson, it seemed that our S which is encapsulated and has a moist mucoid colony has the character of his M form. Whether the subvarieties of R variants (III and IV of Leslie and Gardner, A and B of Mishulow) correspond to his S and R, remains to be seen. For the time being it has seemed more practical to use the terms S and R as generally employed. As we study *H. pertussis* variants further, it is possible that these three chief colony forms, constituting the usual pattern for other bacterial species, may emerge as well defined types.

The finding that *H. pertussis* when first isolated is a single specific serological type, in S form, and that this antigenic phase may be maintained by suitable cultural management has certain definite implications. One bears upon further transmission experiments directed toward the establishing of the etiological relationship of the organism to whooping cough. Working with monkeys, Sauer (25) produced suggestive manifestations of the disease in 8 out of 76 attempts. It is very probable that his failures may be laid to the use of R forms as inoculum. Criticism directed against the conclusiveness of the occasional successful transmission experiment (Sauer (25), Rich *et al.* (26), MacDonald and MacDonald (27)) with freshly isolated *H. pertussis* put forward by those entertaining the combined *H. pertussis* and filtrable virus theory is being met in the experiments already

reported upon (4) and still being carried out in this laboratory, by subculturing the theoretically pathogenic S form long enough to preclude the presence of virus.

A second implication of importance related to the preparation of *H. pertussis* vaccines. Madsen (28), Sauer (29), and Frawley (30), the last worker using Krueger's specially prepared *H. pertussis* vaccine (31), have recently reported favorably regarding protection against whooping cough by means of suitably prepared vaccines. Sauer following the lead of Madsen insists upon the use of freshly isolated organisms; Krueger's special vaccine is made from similar strains. The ready identification of the S form of *H. pertussis* and the practicability of its maintenance brought out above puts the preparation of antigenically effective vaccines upon a sound basis. In cooperation with Dr. J. A. Doull of the Department of Hygiene and Bacteriology and Dr. H. J. Gerstenberger of the Department of Pediatrics, we have begun a carefully controlled study of the prophylactic value of *H. pertussis* vaccines made from organisms shown to be in the S form according to the criteria outlined above. By the same token, the therapeutic value of *H. pertussis* vaccine in active cases of the disease can be determined only after carefully controlled studies have been carried out with such antigenically effective preparations.

CONCLUSIONS

1. Fifty-nine recently isolated and 39 laboratory stock strains of *H. pertussis* have been studied with respect to their cultural, morphological, toxic, serological, and cataphoretic properties.
2. Recently isolated strains, whether derived from cases of whooping cough in Europe or in this country are a single specific serological type which should be designated the S form of *H. pertussis.*
3. Criteria for the identification of the S form are given.
4. The S form may be maintained in this stage by suitable cultural methods. If grown upon media deficient in blood, it will dissociate into an antigenically different form which should be designated the R form in keeping with current terminology employed in describing bacterial dissociants.

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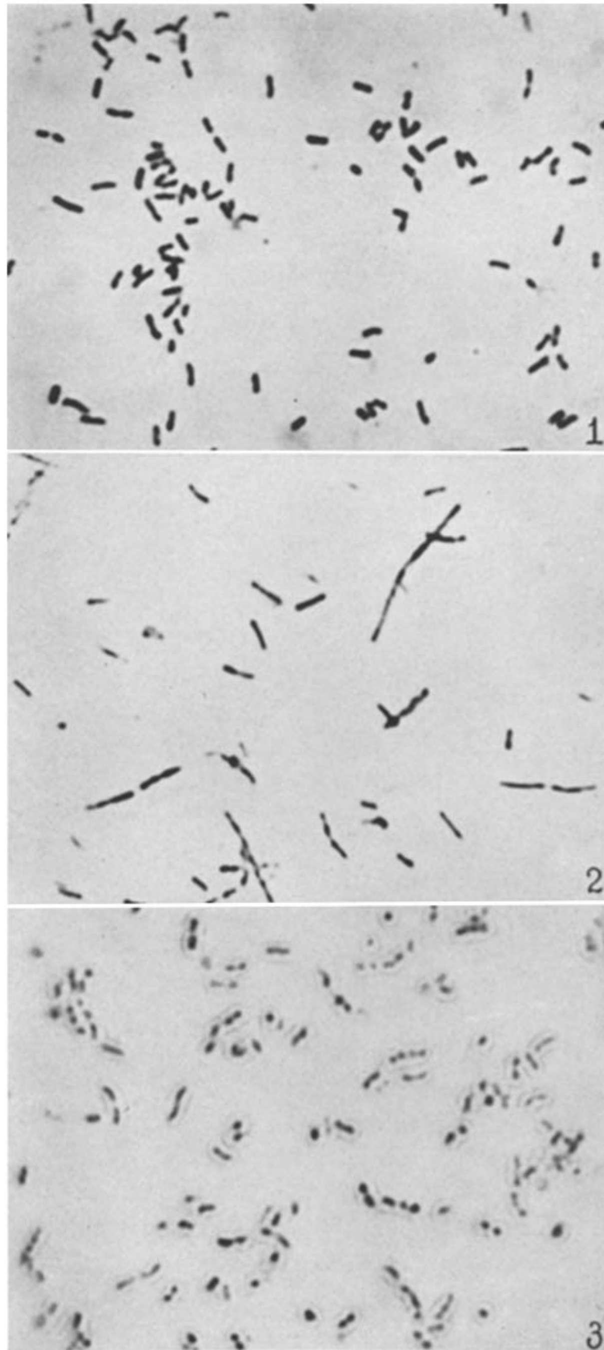
EXPLANATION OF PLATE 18

FIG. 1. S form of *H. pertussis* showing coccobacillary forms. Occasional pairing of organisms appears. × 1,250.

FIG. 2. R form of *H. pertussis* showing long bacillary morphology. × 1,250.

FIG. 3. Capsules of S forms of *H. pertussis*. Wright's stain diluted 2:1 with glycerin was used. The capsules appear bluish pink in the original smears. The color did not photograph well. × 1,250.



(Shibley and Hoelscher: Whooping cough. I)