6346

Differentiation by Cataphoretic Velocity of Fresh and Old Laboratory Strains of H. Pertussis.*

G. S. SHIBLEY. (With the technical assistance of Helena Hoelscher.)

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

In connection with studies concerned with the etiology of whooping cough we have examined the serological character of *H. pertussis*. Krumwiede, Mishulow, and Oldenbusch[1] have described 2 fixed serological varieties which they have designated as types A and B. Very recently Leslie and Gardner[2] have restudied the problem and have concluded that *H. pertussis* is a uniform species without fixed varieties or types when first isolated from the human subject, but that after subculture upon various laboratory media, it passes through a series of antigenically distinct phases which they have called Phases I, II, III, and IV. They believe the first 2 phases correspond to the smooth, and the last 2 to the rough varieties of other bacteria.

We have isolated *H. Pertussis* from early typical cases of whooping cough by the cough plate method and have studied 16 such fresh strains. In addition we have made observations on various old stock strains kindly provided us by Dr. Park in New York and Dr. Wadsworth in Albany. We have confirmed the observations of Leslie and Gardner to the extent that we have been able by agglutinative, absorptive, and toxicity studies to demonstrate the uniformity of all fresh strains obtained by us in Cleveland and to show that they pass into antigenically different phases upon continued subculture. The determination of the toxicity of strains has been made upon rabbits by the skin test method using filtrates of the organism rather than by the use of guinea pigs as carried out by Leslie and Gardner. We have not been able to divide the later phases into the 3 sharp categories described by these authors. We are still working upon this aspect of the problem.

The chief object of this communication is the reporting of the fact that freshly isolated "Phase I" strains may be rapidly and accu-

* Aided by a grant from the Rockefeller Foundation.

[1] Krumwiede, C., Mishulow, L., and Oldenbusch, C., *J. Infect. Dis.*, 1923, **32**, 22.

[2] Leslie, P. H., and Gardner, A. D., *J. Hygiene*, 1931, **31**, 423.

rately distinguished from all our old laboratory phases ("Phases II, III, and IV") by means of their cataphoretic migration velocities. Cataphoretic migration velocities were determined by means of the Northrop-Kunitz[3] micro-cataphoretic apparatus and are recorded in terms of microns, per sec., per volt, per cm. Before making readings the bacteria were washed three times in distilled water and then were suspended in glycocoll-sodium phosphate-sodium acetate buffer solution[4] at pH 7.0, 1/100 molar concentration.

All new strains have shown much slower migration velocities than the old laboratory strains and when these newly isolated strains ("Phase I") have passed over to the later phases, they have shown the much more rapid migration velocities that have characterized our old laboratory strains. This change in velocity has regularly followed the acquisition on the part of these new strains of the ability to grow upon heated blood agar. The migration velocities of all freshly isolated strains, read under standard conditions, have ranged between 1.2 and 2.1μ/sec./V/cm., average 1.6. The migration velocities of all old strains studied have varied between 3.3 and 4.6μ/sec./V/cm., average 3.9. Readings made upon our fresh strains which have passed over to the "laboratory phases" have averaged over 4.0μ/sec./V/cm.

Difference between the cataphoretic velocities of virulent and avirulent ("R" and "S") strains of various bacteria has already been noted. The differences here reported between the migration rates of freshly isolated and of old laboratory strains would seem to suggest the probability that the latter may have changed sufficiently antigenically as to be classified as rough or avirulent forms. So far all efforts to show morphological "R" and "S" colonies on many varieties of media have failed. It is interesting to note that cross "Schwartzman Phenomenon" tests have failed to indicate antigenic differences between freshly isolated and old laboratory strains.

[3] Northrop, J. H., and Kunitz, M., *J. Gen. Physiol.*, 1925, **7**, 729.

[4] Northrop, J. H., and De Kruif, P. H., *J. Gen. Physiol.*, 1921, **4**, 639.