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Variation of H. Influenzae During Acute Respiratory Infection in the Chimpanzee.

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In a previous communication[1] the striking resemblance of the bacterial flora of the upper respiratory tract of the chimpanzee to that of human beings has been described. To what extent these animals possess a similar flora in their native environment, or to what extent the organisms present represent new acquisitions after landing in the United States is not known.

The chimpanzee is highly susceptible to the common cold, whether naturally communicated by contact with infected human beings or artificially induced by bacterium-free filtrates of naso-pharyngeal washings from subjects suffering from acute colds. One of the striking phenomena of these chimpanzee colds is the great increase both in numbers and in area of distribution during the early stages of the cold, whether spontaneous or experimental, of the common pathogens of the upper respiratory tract such as *H. influenzae, pneumococcus* and *S. hemolyticus.*

In particular, we have noticed from the beginning of our study of the common cold in apes, during periods of infection, a change in the number and character of the colonies of *H. influenzae* on rabbit's blood agar plates. During the healthy period cultures from the naso-pharynx and nose give few colonies of *H. influenzae* from the naso-pharynx and usually none from the nose. The colonies themselves are small and opaque. During the period of infection numerous colonies of *H. influenzae* are obtained both from the naso-pharynx and the nose. Furthermore, the colonies themselves are of an obviously different character, very large, translucent and moist.

A more careful study of this phenomenon has revealed facts of great interest and importance. In April, 1932, an outbreak of spontaneous colds occurred among our chimpanzees. Eight animals became infected and from all of them during the first days of the cold *H. influenzae* was isolated. Four of the strains were carefully studied bacteriologically and serologically by Dr. Margaret Pittman

[1] Dochez, A. R., Shibley, G. S., and Mills, K. C., *J. Exp. Med.*, 1930, **52**, 701.

of the Hospital of the Rockefeller Institute. By the methods[2] which she has devised for the classification of *H. influenzae* all 4 strains were demonstrated by the use of the Levinthal medium to be the S. form of *H. influenzae*. Serological study further revealed that 3 of the strains belonged to immunologic Group A and one to Group B. From 5 to 8 weeks later the same 4 animals were examined for the presence of *H. influenzae*. From one no *H. influenzae* was recovered. From 3, *H. influenzae* was obtained and the 3 strains isolated were proven bacteriologically to be the R. form of the organism. No S. forms were present on the plates made at this time. One animal that developed bronchopneumonia as a sequel of its cold carried the S. form for 5 weeks.

In June, 1932, another spontaneous outbreak of colds occurred among the animals. From 7 of the 8 animals having colds at this time *H. influenzae* was isolated. All of the 7 strains obtained represented the S. form of *H. influenzae*. Serological examination of the strains isolated showed 4 of the animals to harbor the Group A variety, 2 Group B, and one both Group A and B. Twelve colonies picked from a single plate were all of the S. form. Practically all of the colonies obtained at this time by frequent plating of the upper respiratory flora were examples of the S. form of *H. influenzae*. Only very rarely was the R. form of colony visible on the plate. The serologic variety of *H. influenzae* obtained from the 4 animals studied during the June outbreak was in each instance the same as that obtained in the previous attack of cold studied. Of 2 animals kept in the same cage one harbored *H. influenzae* Group A, and the other *H. influenzae* Group B.

To throw further light on this phenomenon, 2 chimpanzees were inoculated with fresh bacteria-free filtrates of the naso-pharyngeal washings from human beings with acute colds. Chimpanzee No. 1 was carefully studied for the presence and type of *H. influenzae* during a foreperiod of several days. *H. influenzae* was obtained by plate culture and numerous colonies examined were found to be of the R. form. The animal was then inoculated intranasally with the fresh filtrate from a human cold. Cultures from the naso-pharynx within 48 hours from the time of inoculation revealed the presence of numerous S. forms of *H. influenzae*. By the third day the S. form had entirely disappeared and only R. forms were obtained. This animal developed no clinical symptoms of the common cold.

Chimpanzee No. 2 was studied during a similar foreperiod under

[2] Pittman, Margaret, *J. Exp. Med.*, 1931, **53**, 471.

careful quarantine and again only R. forms of *H. influenzae* were isolated from the naso-pharynx. The animal was then inoculated with filtrate from a common cold and the naso-pharyngeal flora studied bacteriologically. Again, within 48 hours large numbers of colonies of *H. influenzae* appeared on the plates and all colonies examined were of the S. form. In this instance the S. form of *H. influenzae* was obtainable on cultures during a period of 6 days. The animal showed symptoms of a mild cold. Serological study of the type of *H. influenzae* present following experimental inoculation showed this type to be the same as that observed in each animal during the previous periods of natural infection, in each instance Group A.

These observations indicate that under the influence of infection of chimpanzees with the virus of the common cold, a transformation takes place from the serologically non-type specific R. form of *H. influenzae* to the serologically specific S. form. During health periods there is reversion to the R. form. The presence of such small numbers of S. forms as to be undemonstrable during healthy periods and an increase to the point of demonstrability during periods of infection cannot be ruled out, but seems improbable. Introduction of S. forms from the outside cannot be an explanation of the phenomena observed because of the presence of more than one serological type of *H. influenzae* in the group of animals during the epidemic period, and because of the recurrence in each animal of the identical serologic type of organism throughout a series of infections. Furthermore, in one instance of the controlled experimental study of the phenomenon such a careful quarantine was maintained that introduction of organisms from the outside environment was rendered extremely unlikely. These observations confirm our previous studies which indicate that one of the most important effects of the virus of the common cold is to incite activity on the part of potentially pathogenic micro-organisms present in the naso-pharynx at the time of infection. Whether this action is directly operative upon the micro-organism influenced or is an indirect effect of tissue reaction cannot be stated.