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Study of the Virus of the Common Cold and Its Cultivation in Tissue Medium.*

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In previous papers¹ we reported the evidence which supports our belief that the common cold in man is caused by a filterable virus. This view is strengthened by recent work published by Long and Doull.² In addition to the effort to decide the question of etiology one of the objects of this investigation has been to explore the possibility of developing a specific prophylaxis for this disease. The first steps in the successful carrying out of such a procedure must naturally be directed toward an attempt to preserve the virus in an active form outside the animal body and an effort to cause this agent to reproduce itself *in vitro*.

In order to test survival of the virus after removal from the human body, naso-pharyngeal washings were obtained in the manner previously described, from individuals within the first 24 hours of a typical attack of common cold. The material was quickly passed through a Seitz filter and the filtrate was subsequently tested for its capacity to induce the symptoms of the common cold in chimpanzees and in human volunteers. The filtrates with cysteine hydrochloride added were preserved anaerobically under vaseline seal both at room and at ice-box temperature. In a few instances the filtrate was concentrated to approximately one-seventh its original volume by vacuum distillation. Of 12 inoculations of filtrates preserved in this manner 9 have produced in the inoculated subject typical symptoms of the common cold. The duration of time of preservation of active filtrates has ranged from 4 to 13 days. Tests of survival beyond the thirteenth day have not been made. Positive results have been obtained with filtrates kept at room temperature and with those kept at ice-box temperature, and with both the unconcentrated filtrate and with that concentrated by vacuum distillation. From these experiments we conclude that the virus of the common cold

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¹ Dochez, A. R., Shibley, G. S., and Mills, K. C., *Proc. Soc. Exp. Biol. and Med.*, 1929, **26**, 562; **27**, 59; *J. Exp. Med.*, 1930, **52**, 701; Shibley, G. S., Mills, K. C., and Dochez, A. R., *J. Am. Med. Assn.*, 1930, **95**, 1553.

² Long, P. H., and Doull, J. A., *Proc. Soc. Exp. Biol. and Med.*, 1930, **28**, 53.

survives under suitable conditions for at least 13 days after removal from the human body.

In view of the capacity of this virus to survive in what might be considered an adverse environment an effort was next made to cultivate the agent in living tissue medium. Foster³ has previously reported the cultivation of an organism from patients suffering from the common cold. The method of culture chosen by us has been that developed by Maitland and Maitland⁴ and by Li and Rivers.⁵ Ten day chick embryos are hashed and suspended either in Tyrode's solution or in buffered bouillon prepared from a special peptone† and to which 0.1% gelatin has been added. All solutions, both those used for naso-pharyngeal washing and for cultivation, contain 1 to 2000 cysteine hydrochloride. At all times an effort has been made to protect the agent against the deleterious action of peroxides. The cultures were incubated under vaseline seal at 37°C. for from 4 to 9 days. The material used for cultivation was obtained in the following manner: naso-pharyngeal washings were made from a patient with a typical cold of more than moderate severity. The washings were passed through a Seitz filter and preserved at ice-box temperature under vaseline seal in the special peptone broth described. On the ninth day of preservation a volunteer was inoculated intranasally and within 24 hours developed typical symptoms of the common cold. As soon as the signs of the disease were well-marked, naso-pharyngeal washings were prepared in the usual manner and preserved for 5 days at ice-box temperature. After this interval a second volunteer was inoculated who also developed symptoms of a cold within the usual incubation period. Naso-pharyngeal washings were again prepared, filtered through a Seitz filter and concentrated by vacuum distillation. After 10 days' preservation at ice-box temperature a third volunteer was inoculated. This individual developed a cold of unusual severity, possibly indicating an accession of virulence by the virus. Naso-pharyngeal washings were prepared from this individual after the usual interval, filtered through a Seitz filter, concentrated 7 times by vacuum distillation and preserved for 5 days at ice-box temperature. After the lapse of this interval 0.25 cc. of the concentrated material was inoculated

³ Foster, G. B., Jr., *J. Am. Med. Assn.*, 1916, **66**, 1180; *J. Infect. Dis.*, 1917, **21**, 451.

⁴ Maitland, H. B., and Maitland, M. C., *Lancet*, 1928, **2**, 596.

⁵ Li, C. P., and Rivers, T. M., *J. Exp. Med.*, 1930, **52**, 465.

† Especially prepared peptone obtained through the kindness of Dr. René Dubos of the Hospital of the Rockefeller Institute.

into tissue medium of the nature described. The culture was incubated for 5 days at 37°C. At the end of this time a human volunteer was inoculated and within the usual incubation period developed a cold with symptoms of rather a mild character. From this time on the material was carried in tissue medium and transferred at intervals of from 3 to 9 days. We believe the preferable interval between transfers to be 4 days. From time to time cultures were chosen for the inoculation of experimental subjects. The third culture representing a dilution of the original material of approximately 1-2000 was inoculated into a chimpanzee. This animal developed symptoms of a cold after an incubation period of 4½ days. An uninoculated companion animal kept in the same cage developed symptoms of a cold at the same time. The sixth culture, representing a dilution of the original material of approximately 1-2,000,000, was inoculated into 3 human volunteers. Of these, 2 developed after 24 hours typical colds of moderate severity. The tenth culture, representing a dilution of the original material of approximately 1-20,000,000,000 was inoculated into 3 human volunteers. Of these one developed such mild symptoms of respiratory infection as to be considered doubtful. The twelfth culture representing a dilution of approximately 1-2 trillion was inoculated into 3 human volunteers, and of these one developed a cold with mild symptoms. The fifteenth culture, representing a dilution of approximately 1-2 quadrillion, was inoculated into 3 human volunteers. Of these 2 developed colds of unusually severe symptoms, complete nasal obstruction, frontal headache, malaise, loss of appetite, nasal discharge, and pronounced cough. Naso-pharyngeal washings were prepared from these 2 individuals, filtered through a Seitz filter and concentrated by vacuum distillation. Two human volunteers were inoculated after a short interval with these concentrated filtrates and within 24 hours both developed colds, one with mild symptoms and the other with the typical features of a head cold.

The evidence presented by these experiments indicates that the virus of the common cold survives for a considerable length of time after removal from the human naso-pharynx and that the agent reproduces itself *in vitro* when cultivated in suitable tissue medium. The total duration of life of the culture virus from the time of removal from the human subject has been 74 days. The final dilution of the original material is so great as to rule out conclusively the presence of any significant amount of the original virus. Controls of the constituents of the medium and of the tissue medium have been negative. In one instance when a volunteer received active cul-

ture heated for one-half hour at 80°C. there appeared symptoms of upper respiratory irritation which lasted for less than 24 hours. We realize the difficulty of a final judgment concerning the successful cultivation of an invisible agent and simply present the facts as we have observed them.

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Relationship of Sera and Spinal Fluids to Agglutination and Flocculation by Dyes.

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It has been known for a long time¹ that human red blood cells and bacteria could be agglutinated by solution of basic dyes. In addition M. Gutman² showed that whole serum could be flocculated by the same dyes. In our experiments we found that these phenomena were much more complex.

The human red blood cells used in the experiments were washed 3 times in normal saline (0.85%) in order to free them from all traces of serum. A 2% suspension of red blood cells was used. Whenever human serum was used in conjunction with the red blood cells the same types were employed in order to avoid agglutination of the cells by the serum. The standard dye used was a gentian violet solution 1:2500 in normal saline. The ingredients were mixed in equal amounts in narrow glass tubes by thorough shaking. Agglutination and flocculation could easily be observed macroscopically. For microscopic examination drops of the same ingredients were placed on glass slides and mixed with glass rods.

Results. 1. *Flocculation of human sera:* The flocculation of whole human serum by basic dyes noted by Gutman takes place only if relatively strong solutions are added. (*e. g.*, ½-1% aqueous solutions of safranin or gentian violet). But by using weaker solutions different results were obtained. Whole human serum, or serum diluted with normal saline (0.85%) up to 1:6 is not flocculated by our standard solution of gentian violet. Flocculation here first occurs with a serum diluted 1:8 and increases in strength with higher dilutions of sera. In dilutions of serum of 1:64 floccula-

¹ Brossa, G. A., *Z. f. Immunitätsforsch.*, 1923, **37**, 221.

² Gutman, M., *Centralbl. f. Bakt.*, I Abt., Orig., 1928, **56**, 68.