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Intranasal Inoculation of Human Individuals with the Virus of Epidemic Influenza.*

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Burnet and Lush have reported the intranasal inoculation of a series of human individuals with a strain of influenza virus propagated on the membrane of the developing egg.[1] The strain, so maintained, had lost its capacity to elicit clinical infection in ferrets and was of extremely low virulence for mice. Of the human subjects tested none showed evidence of infection. Subsequent to this exposure, the serum of none of 18 subjects examined exhibited a rise in circulating antibodies, as measured by the mouse protection test, while a minority of them showed some increase when the sera were titrated with egg passage virus on the egg membrane.

The use of the nasal route for vaccination against epidemic influenza offers several theoretical advantages as well as disadvantages. Among the former are the fact that the nasal route represents the normal route of infection and a subclinical infection so produced might result not only in antibody response to an actual, mild infection but in a protective conditioning of the respiratory membranes as well. On the other hand, the most obvious objection is the possibility of establishing infection which could be transmitted from one individual to another. It seemed likely that the essentially negative results previously mentioned were attributable to the use of an avirulent strain of virus. It was desirable, therefore, to undertake similar experiments with human subjects employing virus of known animal pathogenicity, the virulence of which could be readily titrated.

The PR8 strain of the virus of epidemic influenza had been maintained through 480 transfers in tissue culture medium for over 4 years at the time the experiments were undertaken. When tested in ferrets, the undiluted culture induced fever and nasal discharge but no pulmonary lesions. When diluted tenfold, the virus caused no elevation of temperature above the normal limit of 104°F in the ferret, nasal signs were slight, but the animal developed a high titer of neutralizing antibodies. In mice the culture of virus usually produced fatal infection when 0.05 cc of a 1:1000 dilution was given

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[1] Burnet, F. M., and Lush, D., *Brit. J. Exp. Path.*, 1938, **19**, 17.

TABLE I.

Neutralizing Titer of Serum of Human Subjects Before and After Intranasal Inoculation of the Virus of Influenza.

Neutralizing titer of serum	Test Subject										
	I.B.	H.M.	W.V.	C.S.	A.M.	D.C.	E.H.	M.L.	D.R.	M.S.	L.W.
Before inoculation	1:35	1:140	1:120	1:140	0	1:15	1:70	1:17.5	1:60	1:60	1:4
After "	1:35	1:140	1:120	1:140	1:15	1:17.5	1:100	1:15	1:60	1:50	0

intranasally and pulmonary lesions were produced by culture diluted 1:10,000.

Eleven healthy human subjects with no history of immediately recent respiratory infection were selected. Throat cultures had been made to detect any abnormal pharyngeal bacterial flora. Serum was obtained before inoculation. Fresh cultures of virus were prepared and mice were inoculated intranasally at the time of their use. The culture was diluted tenfold and with the head tilted backward, 0.25 cc of the dilution, containing approximately 500 fatal doses for mice, was allowed to run into each nostril of the human subject from a pipette. Little or none escaped into the pharynx. The head was then thrown forward. Daily leukocyte counts were done on the first 5 subjects and in all, temperatures were taken twice daily and suggestive symptoms were noted for a period of a week. Two weeks after the inoculation serum was again obtained and titrations of the content of neutralizing antibodies of both sera were done simultaneously by the method of Francis and Magill.

One subject had a rise of temperature to 99°, p.o., 30 hours after the inoculation, at which time a feeling of warmth and slight achiness was noted. One other developed an acute rhinitis without fever 8 hours after inoculation and this persisted as a common cold. A third subject complained of slight dryness in the nasopharynx on the first day after injection and on the second day labial herpes was noted. This was thought to be related to sunburn. All others remained asymptomatic and afebrile. Furthermore, titrations of the antibodies present in the sera before and after inoculation revealed that only one, the last subject mentioned above (A.M.), showed any significant elevation in antibody titer and this individual possessed no neutralizing antibodies before inoculation. Studies to be reported elsewhere regarding the inactivation of virus by nasal secretions offer an explanation for these results.

Summary. Of 11 human subjects inoculated intranasally with active cultures of the virus of epidemic influenza only one showed a rise of antibodies following the injection. None exhibited significant signs or symptoms of infection. The possible use of this procedure for preventive immunization will be further investigated.