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SPECIAL ARTICLES

THE INACTIVATION OF EPIDEMIC INFLUENZA VIRUS BY NASAL SECRETIONS OF HUMAN INDIVIDUALS[1]

CERTAIN difficulties have been encountered in attempting to explain susceptibility or resistance to epidemic influenza in terms of circulating antibodies to influenza virus. For example, some persons who possess little or no demonstrable antibody escape infection under the same conditions of exposure which result in the infection of other individuals with relatively high titers of neutralizing antibody. For this and other reasons, attention has been directed to the possibility that mechanisms resident in the respiratory tract itself might play a significant role in the prevention of the natural disease. An instance of local, non-immunological immunity was discovered while studying the processes of injury and repair in the respiratory mucous membrane of the ferret infected with influenza virus. It was found that, following the acute necrosis which occurs early in the disease, repair was associated with the formation of a squamous epithelium, which was refractory to further damage even by severe iontophoresis with zinc sulfate. This anatomical change was but a temporary one, although the tissues thereafter always exhibited the capacity of accelerated repair.[2]

[1] This study conducted under a grant from the International Health Division of the Rockefeller Foundation.

[2] T. Francis, Jr. and C. H. Stuart-Harris, *Jour. Exp. Med.*, 68: 789, 803, 813, 1938.

Over the last fifteen months the nasal secretions of human subjects have been studied to ascertain whether they possessed any capacity to inactivate epidemic influenza virus. Material from 31 patients in the first day or two of an acute afebrile common cold and from two hay-fever patients was collected by allowing the nasal discharge to drain directly into a bottle. The collections were ground individually with alundum and centrifuged. The supernatant fluid was removed, and 0.3 cc of it was mixed with 0.3 cc of a 1:2,000 suspension of mouse passage virus of the PR8 strain. After incubation at 37° C. for 30 minutes, 0.05 cc of the mixture, containing approximately 1,000 lethal doses of virus, was given intranasally to each of three mice. The mice were observed for ten days, all deaths recorded and the presence or absence of virus lesions in the lungs of survivors was determined at autopsy.

Secretions were obtained from fifteen normal individuals by inserting a loose pack of absorbent cotton well back into the nostrils until it became saturated. It was then removed and the clear liquid expressed. This material was tested in the same manner as the common cold secretions. Saliva from the normal individuals was also tested.

The degree of virus inactivation has been classified as complete when the mice survived without pulmonary lesions; almost complete, when the mice survived but exhibited only mild lesions; partial, when extensive

lesions were found, but the mice survived; no inactivation when the mice failed to survive the test period. On this basis the results are summarized in Table I.

TABLE I

INACTIVATION OF INFLUENZA VIRUS BY SECRETIONS OF HUMAN SUBJECTS

Secretions tested	Number of specimens	Degree of inactivation of virus			
		Complete	Almost complete	Partial	None
Common cold and hay fever	33	4	13	8	8
Normal	15	2	7	3	3
Saliva	16	0	1*	1*	14

* Negative when repeated 1 week later.

There was little difference between the results obtained with nasal secretions from patients with common colds and those from normal subjects. Approximately half caused complete or almost complete inactivation of 1,000 lethal doses of virus, while the other half exerted either slight or no inactivation. Saliva was ineffective. Secretions were obtained from six patients during the acute stage of a cold and again in a subsequent normal period. No significant differences were discernible in the results of the two tests. Of five samples of sputum from various patients, two caused partial inactivation; three had no effect.

The same phenomenon has been recently reported by Burnet, Lush and Jackson[3] who studied the action of nasal secretions of normal human subjects upon several viruses, including that of influenza. They noted no differences in the inactivating capacity of different specimens, but conducted their tests with filtered specimens. They state that the agent is destroyed at 100° C. Furthermore, they state that five hours is required for inactivation of virus to occur, and suggest that the agent is an enzyme.

Some characteristics of the inactivating agent have been outlined in the course of the present investigations. It is extremely stable at icebox temperature, remaining for at least six to eight weeks without change in potency. It is ineffective after heating at 70–75° C. for twenty minutes. In some cases, the material can exert its action after dilution, the extent of dilution varying with different samples. The highest effective dilution so far observed has been 1:8. The inactivation of virus by secretions is not the result of bacterial action, since many of the samples are either sterile or yield few colonies on blood agar plates. Furthermore, the bacteria, usually staphylococci, do not appreciably affect the test animals. The agent is not a lysozyme as measured by its action upon a susceptible micrococcus. In a series of tests with specimens exerting various degrees of inactivation upon the virus, the lysozyme content of the samples bore no parallelism to the virus-inactivating capacity but reached an approximate titer of 1:1,000 in each instance. The secretions have not been found to exert a bacteriostatic or bacteriolytic action when tested with smooth or rough pneumococcus, β. hemolytic streptococcus, *streptococcus viridans, staphylococcus aureus* or *albus, M. catarrhalis,* or meningococcus.

In the current study titrations of neutralizing antibody have been conducted in mice with serum from twenty of the patients with common colds and from all the normal subjects. While a sharp correlation between the inactivating effect of the nasal secretions and the antibody titer of the serum was not detected, it was found that the secretion from twelve of seventeen subjects with antibody titers of 1:20 or less gave either slight or no inactivation. The serum of four of the seven patients whose secretions failed to inactivate virus contained no antibodies; the other three had titers of 1:20. On the other hand, the secretions of sixteen of eighteen subjects, whose serum titers were 1:40 or more, completely or almost completely inactivated the test dose of virus. Within these broad limits a relationship is suggested. Furthermore, neutralizing antibodies in the serum are inactivated at the same temperature, 70–75° C., as the agent in the nasal secretions.

These studies are as yet incomplete. They show, nevertheless, that there exists in nasal secretions a substance capable of inactivating relatively large amounts of influenza virus. The inactivating capacity varies widely in different individuals, and in some respects resembles the so-called natural antibodies. It seems highly probable that this phenomenon is of considerable importance in relation to individual susceptibility to epidemic influenza.

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[3] F. M. Burnet, D. Lush and A. V. Jackson, *Brit. Jour. Exp. Path.*, 20: 377, 1939.

CHLOROPHYLL AS THE PROSTHETIC GROUP OF A PROTEIN IN THE GREEN LEAF[1]

IN an earlier publication,[2] it was pointed out that the differences in properties between chlorophyll dissolved in organic solvents and the green pigment as it exists in the leaf can be explained by assuming that the chlorophyll of the leaf is in combination with protein. It is now possible to present further information on the nature of this chlorophyll-protein compound.

The chloroplast material of ground-up spinach and

[1] This material was included in a paper under the same title which was presented at the photosynthesis symposium held at Columbus, Ohio, under the auspices of Section C of the American Association for the Advancement of Science on December 28, 1939.

[2] E. L. Smith, SCIENCE, 88: 170, 1938.