THE ISOLATION OF A NEW VIRUS ASSOCIATED WITH RESPIRATORY CLINICAL DISEASE IN HUMANS*

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The isolation of the RI-APC group of respiratory viruses through the use of tissue culture methods has greatly stimulated interest in this field.

In this paper we wish to report the isolation of a new respiratory virus (JH), which from all available data is responsible for a clinical disease in the human population.

RESULTS

Isolation of Virus.—Naso-pharyngeal washings were collected in monkey kidney maintenance media. They were shell frozen in ampules in an alcohol–dry ice mixture and stored at −50°C. When isolation attempts were made the ampule was quickly thawed and 0.1 ml. of the washing was inoculated into tubes of Hela cells as described by Hilleman and Werner.1 Our methods follow theirs exactly except the maintenance media of Eagle was substituted. The virus which will be described in this paper did not cause degeneration of the Hela cells within 240 hours, even after 3 blind passages. The twenty isolations on which most of this work has been done elicited titers of approximately $10^4$ to $10^6$ tissue culture infecting doses per 1.0 ml. of the infected monkey kidney cells. The agent multiplies in monkey kidney cells grown in 3 per cent inactivated horse serum, 5 per cent beef embryo extract, and 92 per cent 199 solution. This media contains 100 units of penicillin and streptomycin per one ml. of media. Monkey kidney cells are destroyed by the virus.

The cytopathogenic effect of this agent can usually be observed more readily in 199 medium without the above additions. However, in a few primary isolations this virus was isolated in the richer medium and not in plain 199 medium. The significance of these observations is not clear at the present time

Epidemiological and Clinical Data.—Although detailed epidemiological and clinical data will be reported elsewhere, brief mention will be made here of certain of the data.

The virus has been isolated from human respiratory cases in all seasons of the year from both adults and children. It has been isolated from two large respiratory outbreaks, as well as from individuals clinically ill with respiratory infections who came to the Johns Hopkins Hospital or to the Baltimore city hospitals for treatment. The main clinical features with which this agent is associated are malaise and coryza and mild sore throat. A low-grade fever may or may not be present. There are no physical findings to suggest lower respiratory involvement. The duration of clinical symptoms averages about three days. Bacteriological analysis of the throat and nasopharynx swabs of individuals ill with the above syndrome show normal flora. Antibody surveys on various age groups carried out so far have shown that this virus is relatively widespread, about 20 per cent of the indi-
individuals over eight years of age showing the presence of neutralizing antibodies. Complement-fixing antibodies also can be shown to be present for this agent in the sera of individuals.

Serological Characteristics of Etiological Agent.—Table 1 shows a typical neutralization test using acute-convalescent sera of individuals from whom the virus has been isolated who were clinically ill with the symptoms described above. It can be readily seen that there is a large increase in the neutralizing antibody level.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Acute Titer</th>
<th>Convalescent Titer</th>
<th>Patient</th>
<th>Acute Titer</th>
<th>Convalescent Titer</th>
</tr>
</thead>
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<td>M. W.</td>
<td>&lt;1:2</td>
<td>1:64</td>
<td>J. S.</td>
<td>&lt;1:2</td>
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<tr>
<td>D. L.</td>
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<tr>
<td>H. S.</td>
<td>&lt;1:2</td>
<td>1:128</td>
<td></td>
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</tbody>
</table>

* All convalescent sera were collected about 4 to 6 weeks after the acute sample was taken.

These tests were carried out as described above using 10 to 50 TCD₅₀. All isolations of the agent seem to be immunologically similar as determined by neutralization tests.

Table 2 shows that there was no significant increase in titer against this virus in acute-convalescent sera of the following infections: influenza, RI-APC, Q fever, Rocky Mountain spotted fever, Murine typhus, Rheumatic fever, Tuberculosis, Infectious mononucleosis, "Common cold", Mumps, Infectious hepatitis, Herpangina, Primary atypical pneumonia with rise in cold agglutinins, Nonbacterial meningitis, Measles, Rheumatoid arthritis, Coronary thrombosis.

* A four-fold rise was considered significant.
fever, Rocky Mountain spotted fever, scarlet fever, streptococcal sore throat, rheumatic fever, and poliomyelitis. All these sera showed very high increases in titer in the convalescent sera against the homologous etiological agents. Furthermore, acute-convalescent sera from various other diseases shown in Table 2 showed no increase in neutralizing titer in the convalescent sera to the JH agent. All these data indicate that the increase in neutralizing titer is specific.

No complement-fixing (CF) antigen could be prepared from monkey cells infected with the JH agent which reacted with standard RI-APC sera using methods which could readily detect RI-APC CF antigen in Hela cells infected with a RI-APC virus. The JH preparation used for these CF experiments titered out to approximately $10^6$ tissue-culture-infecting doses. Standard sera to RI-APC types 1–7 which neutralized 1,000 tissue-culture-destroying doses of these viruses did not neutralize 10 tissue-culture-destroying doses of the JH agents, all tests being carried out in monkey cells under the same conditions.

The acute-convalescent sera of the individuals shown in Table 1 did not show any hemagglutination inhibition rise to influenza A (PR8), influenza A’ (FW-1-50), influenza B (B-1-11), influenza C (strain 1233), or a strain of swine influenza. None of the sera showed rises to the RI-APC CF antigen supplied by Microbiological Associates. None of the convalescent sera showed evidence of cold or streptococcus MG agglutinins, or a rise in streptolysin O antibodies.

**Other Characteristics of the JH Agent.**—The agent can be passed in series in monkey cells without loss of titer at least 10 times, after which no further attempts were made to pass the agent. When the JH suspension, which titered about $10^{-8}$ in monkey cells, was inoculated intranasally, intracerebrally, or intraperitoneally into twenty 1-day-old mice or ten 10-day-old hamsters, no overt signs of disease were noted over the 21-day observation period. Similar negative results were obtained when ten 15–17-gm. mice or six young adult guinea pigs were inoculated intraperitoneally. The baby mice showed no lesions in their thoracic or abdominal viscera when some were sacrificed 7 or 12 days following inoculation. Intranasal inoculation of ferrets gave negative results, the animals showing normal fever curves and showing no overt signs of illness. Two blind passages of various organs and tissues under all the above conditions failed to show any evidence that the JH agent had multiplied.

Inoculation of the JH suspensions which contained about $10^2$ and $10^5$ TCD$_{50}$ into 12-day-old chick embryos by the amniotic and allantoic routes failed to reveal evidence of multiplication even after three blind passages, the fluids being harvested 72 hours after inoculation and titered in monkey cells. The amniotic and allantoic fluids did not agglutinate chicken, human "O", or guinea pig red cells at room temperature or at $4^\circ$ C.

The JH virus does not appear related to the ECHO group of viruses. The JH virus shows a very different cytopathogenic effect than the ECHO viruses in monkey kidney cells. It is not neutralized by antisera prepared to ECHO viruses 1, 2, 3, 6, 5, 7, 8, and 9. The JH virus also has very peculiar nutritional requirements which are different from those reported for any ECHO virus.

The JH virus is not neutralized by antisera prepared to Coxsackie A types 1, 2, 5, 6, 8, 9, 10 or B types 1, 2, 3, 4 or 5. The cytopathogenic effect of the JH agent in monkey kidney cells is also different from that associated with Coxsackie viruses.
Attempts To Grow JH Agent on Artificial Medium.—Infected monkey tissue culture preparations which titered out to approximately $10^{-4}$ in monkey cells were inoculated into Brewer's thioglycolate medium and sheep, human, and rabbit blood agar plates. These cultures were inoculated both aerobically and anaerobically, and none showed growth after 12 days at 30° C. The JH agent could be filtered through standard bacteriological sterile filters which completely removed all Staphylococcus aureus present in prepared suspensions.

DISCUSSION

The data presented in this paper indicate that the agent described is a new virus responsible for clinical illness in the civilian population. This statement is based on the following facts: (1) the isolation of the agent from individuals ill with a particular respiratory clinical syndrome; (2) the rise in antibodies to the agent in such people and the failure to show a rise in antibody to the agent in many other human respiratory as well as other viral, bacterial, rickettsial, and chronic diseases; (3) the failure to isolate the agent from 1,100 individuals not showing clinical signs of respiratory illness in the same seasons and from the same age group and in the same state as the ill individuals from whom the virus was isolated.5 (4) the relationship between antibody level and clinical disease in certain respiratory outbreaks. Thus, in one large respiratory outbreak where the JH virus was isolated from many sick individuals, there was a definite correlation between the individuals' blood sera neutralizing antibody titers to the JH virus one month previous to the outbreak and their developing the clinical signs of respiratory infection during the epidemic. The higher the titer to the JH virus previous to the respiratory outbreak, the less chance a person had of developing overt illness. No such relationship was found for influenza A' or RI-APC antibodies. Furthermore, 16 individuals who developed respiratory infections during this epidemic did not show the presence of the JH virus in their nasal pharyngeal washings taken one month previous to their respiratory infections. However, from 11 of these individuals the JH virus was isolated from their nasal pharyngeal washings when they were ill during the respiratory outbreak, 10 of the 11 individuals showing significant rises in neutralizing antibodies to the JH virus. Such longitudinal studies as these where the same individuals are studied for a long period of time with test samples being taken at frequent intervals, greatly help in deciding whether a virus is the cause of a disease.

The viruses responsible for the major cause of respiratory disease in the civilian population have not as yet been identified. In our extensive field-laboratory study over the last three years, less than 30 per cent of the respiratory illnesses could be identified as being caused by a known microorganism, of either bacterial or viral origin.6

The importance of the JH agent in causing illness in the civilian population as well as other problems dealing with this virus are under investigation and will be described elsewhere. The virus seems fairly widespread, as our surveys carried out so far indicate that about 20 per cent of the population over eight years of age have antibodies to this agent.

Clinically, the virus described in this paper is associated with a very mild upper respiratory infection. The major difference between this illness and the common
cold is that the JH virus is usually associated with low grade fever, as well as slight malaise, coryza, mild sore throat, and in some cases a cough. However, in view of the similarity between the mild respiratory infection associated with the JH virus and the common cold we hope that study of the JH virus may lead to some clues as to how the common cold virus may be isolated. In this connection it should be pointed out that the JH virus has a very long incubation period before producing a cytopathogenic effect in monkey kidney cells, particularly on primary isolation. Thus, a period of 25 days, as well as a blind passage at this time, has been found to be required in many of the isolations, the cytopathogenic effect showing up on the tenth to sixteenth day of the second passage.

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4 This CF antigen will react with all types of RI-APC viruses and is supplied by Microbiological Associates, Bethesda, Maryland.

REVERSIBILITY AND EQUILIBRIUM OF THE GLUTAMINASE REACTION OBSERVED CALORIMETRICALLY TO FIND THE FREE ENERGY OF ADENOSINE TRIPHOSPHATE HYDROLYSIS

By T. H. Benzinger and R. Hems

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The enzymatic hydrolysis of glutamine in the absence of adenosine phosphates is considered a practically irreversible\(^1\) reaction. Attempts have therefore been made by Levintow, Meister, and Morales\(^2\) to derive its standard free-energy change, \(\Delta F^\circ\), indirectly, from available thermal data and "third-law entropies" of asparagine and ammonium aspartate. Although the argument of analogy with asparagine has recently been shown to be invalid,\(^3\) the biological significance of thermodynamic data on glutamine hydrolysis is obvious: Elliott's glutamine synthetase reaction,\(^4\) in which glutamine synthesis is coupled with the hydrolysis of adenosine triphosphate (ATP) has a well-measurable ratio of reactants and products at equilibrium,\(^5\) and the difference between the free energies of the glutaminase and glutamine synthetase reactions is the free energy of ATP hydrolysis.

In an effort to detect with glutaminase the reverse process, glutamine synthesis, and to measure directly a possible glutaminase equilibrium, we have employed a method recently described in these PROCEEDINGS.\(^6\) Free-energy, heat, and entropy changes of equilibrium reactions may be obtained from two calorimetric measurements.