International Symposium

The Dynamics of Virus and Rickettsial Infections

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Introduction

An International Symposium on the Dynamics of Virus and Rickettsial Infections was held in Detroit, Michigan on October 21, 22 and 23, 1953, under the auspices of the Henry Ford Hospital. This volume constitutes the proceedings of the Symposium and contains the papers that were presented as well as discussions of them.

The idea that a Symposium on this broad subject might prove valuable had its origin with the staff of the Henry Ford Hospital. An advisory committee composed of Doctors Thomas Francis, Jr., John G. Kidd, Joseph E. Smadel, and Frederick D. Stimpert shared with a Ford Hospital committee, under the chairmanship of Dr. Frank W. Hartman and Dr. Gerald A. LoGrippo, the responsibility for the program. Thirty-three investigators in the field of virus and rickettsial infections were invited to present papers. Some traveled from distant parts of the world in order to do so: Dr. J. Ralph Audy, for example, came from Malaya, while Mr. F. C. Bawden, Dr. A. W. Downie, and Dr. F. O. MacCallum came from England, Dr. Alfred Gottschalk from Australia, and Dr. Preben von Magnus from Denmark. Other eminent workers came from twenty-one institutions located in various parts of the United States. In addition to those who gave papers, more than 400 scientists attended the Symposium, and many of these participated in the discussions.

One of the chief objectives of the Symposium was to provide an occasion for an exchange of ideas and information between workers in different areas of the virus and rickettsial fields. The subjects discussed were grouped in five major categories: Mechanisms of Virus and Rickettsial Infections, which included twelve papers; Ecology and Pathogenesis of Virus and Rickettsial Infections, with six papers; Mechanisms of Immunity in Virus and Rickettsial Infections, with six papers; Laboratory Diagnosis of Virus and Rickettsial Infections, with five papers; and Approaches to Prophylaxis and Therapy of Virus and Rickettsial Infections, with six papers. No attempt was made to draw lines between different varieties of viruses and rickettsiae. Instead, an effort was made to integrate information recently obtained in various fields concerned with this group of infectious agents. Twenty of the papers were concerned mainly with studies on viruses infecting man or animals; seven with viruses infecting bacteria; three with viruses infecting plants; and three with rickettsiae.

After the Symposium the manuscripts of the papers and transcripts of

the discussions were returned to the participants for revision. In the interest of prompt publication, editing of the revised manuscripts and transcripts has been limited to the barest essentials, and the participants have been spared the obligation of correcting proof.

Information on virus and rickettsial infections has theoretical interest for scientists in a number of disciplines, and importance for practicing physicians as well. Hence a review of current knowledge in this field should have wide usefulness. Furthermore, the amount of new information which is emerging and the concepts which are now developing in the study of these infectious agents provide additional reasons for an appraisal of the findings and ideas of a large group of prominent workers. The proceedings of this Symposium afford an opportunity for this.

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Moderator: Papers 7 to 12

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The Initiation of Cellular Infection by Viruses

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It is probably true to say that viruses represent a manifestation of life at its lowest level of size, when size is measured in units of mass. However, better than by their small size viruses as a group of living organisms are defined by their common feature of propagation in living cells only. In order to multiply the virus has to bring its genetic material into effective contact with the metabolic apparatus of the host cell. Probably those cells only are suitable host cells which can provide the chemical resources required for virus reproduction and in which the genetic units of the virus are able to gain control over the host cell's metabolic machinery diverting the enzymatic processes in the direction needed for the replication of virus genetic patterns and the eventual formation of a new generation of infective virus.

In order to get access to the intricate structure of the cytoplasma harbouring the enzymic equipment of the host cell, the virus has to make contact with the cell. It would appear that two different mechanisms exist by which a virus-host cell contact is established:

(1) One group of viruses comprising vaccinia virus, ectromelia, psittacosis and others are able to multiply in a wide range of epithelial and other animal cells. Their contact with the host cell may be visualized as a chance collision; once in contact with the surface of the cell the virus particle is taken into the cell just like other foreign bodies (charcoal, dyes, etc.). With these viruses there is no evidence for the presence at their surface of a mechanism ensuring close and selected contact with the host cell's surface.

(2) A second group of viruses including the bacterial viruses, the influenza-mumps group and the Newcastle disease virus is restricted in its propagation to cells lining a surface in contact with the environment. These viruses have evolved an elaborate effector fitting precisely a receptor at the surface of the host cell. This effector-receptor mechanism for attachment

of the virus at the surface of the host cell has been worked out over the past ten years in great detail for the influenza virus group and more recently also for the bacterial viruses; as far as can be assessed, the mechanism of virus-host cell combination with influenza and bacterial viruses is closely related. The attachment of the virus to the host cell is the initial step in viral infection and I will confine this paper mainly to the initiation of cellular infection by influenza virus; for it is on this system that since 1945 the workers at the Hall Institute in Melbourne have studied the initial phases of virus infection.

The first relevant observation was made by Hirst[1] when in 1941 he observed that influenza virus grown in the lining cells of the allantoic cavity of chick embryos agglutinates fowl, guinea pig and human erythrocytes; the virus particles act by being adsorbed to the surface of the red cells and forming bridges between the erythrocytes. Virus haemagglutination is essentially an adsorption phenomenon. Since the heat of adsorption not involving strong chemical bonds is usually small, virus haemagglutination proceeds well at 0° C. When Hirst[2] allowed virus and red cells to interact at 37° C, adsorption of the virus onto the red cells was followed by spontaneous elution of the virus into the medium. That this phenomenon of adsorption and desorption involved more than a purely physical procedure was shown by the further observation that, whereas the virus after its spontaneous elution from the cell surface is functionally intact, the red cell is irreversibly changed so as to be no longer available for virus adsorption or agglutination. Hirst clearly recognized the resemblance of the phenomena described to an enzyme-substrate interaction, the enzyme possessed by the virus forming, as it were, by its adsorption onto receptors at the red cell surface an enzyme-substrate intermediate complex, which after chemical interaction breaks down into regenerated enzyme and product, i.e. into living virus and changed red cell surface.

To obtain more direct evidence for this interpretation, an approach by biological, physical and chemical means was decided upon in our Institute.

The first major biological contribution was the preparation and purification by Burnet and co-workers[3] of a soluble enzyme from vibrio cholerae cultures. This enzyme imitates, nearly to the last detail, the activity of the enzyme supposed to be present at the virus surface. It stabilizes red cells in the same manner as does the virus, making the cells unavailable for further agglutination. The bacterial enzyme, referred to as receptor destroying enzyme (RDE), has proved of the greatest value for the progress made in further research on the subject under discussion. Burnet's finding strengthened considerably Hirst's suggestion of the enzymatic nature of the spontaneous elution of the virus from the erythrocytes.

Another observation pointing in the same direction was the change in net electrical charge of the red cells after treatment with virus or RDE. In 1948 Hanig[4] showed that the action of the PR8 strain of influenza virus on human erythrocytes resulted in a decrease in the electrophoretic mobility

of the cells as measured in phosphate buffer at pH 7.35. This investigation was extended by Ada and Stone[5] in our Institute to include various members of the influenza-mumps-Newcastle disease group of viruses. Different strains were found to reduce the electrophoretic mobility value to different levels though none of them down to the level obtained with a purified preparation of RDE. An indication of the extent of reduction is given in Figure 1.

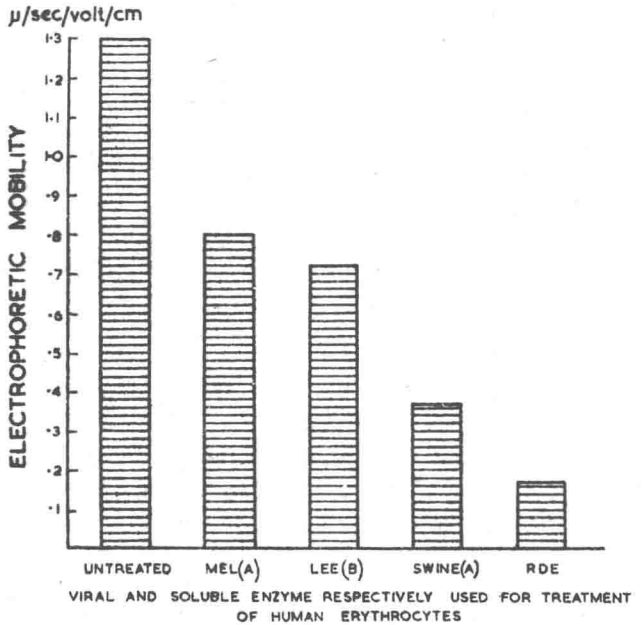

FIG. 1

Further work showed that partial or complete pretreatment of cells with any virus strain followed by treatment with RDE reduced the electrophoretic mobility of the cells to the same value as that obtained with RDE alone. The lack of any summation effect was taken as evidence that the same groups at the red cell surface which were susceptible to an attack by the various viruses were also sensitive to vibrio enzyme action. The identification of the groups responsible for the decrease in electrical charge had to await chemical analysis.

As mentioned already, red cells pretreated with RDE do not adsorb virus. The complete adsorption-elution phenomenon requires the unharmed living virus and intact receptors at the red cell surface. It is possible, however, to alter by controlled damage either of the reactants in such a way as to allow adsorption but hinder spontaneous elution. When, for instance, fowl or human red cells are treated with metaperiodate in the concentration range 0.2 to 2.0 mg. periodate per ml. of packed cells, i.e. with M/100 to

M/1000 periodate, the living virus is adsorbed to but not released from the periodated cells.[6,7] The same over-all effect is seen, when intact red cells are mixed with virus previously heated to 55° C for 30 minutes. This virus, still adsorbed to the cells and agglutinating them, has lost the capacity to change the receptors in such a way as to result in spontaneous elution of the virus.[8]

These facts, taken together, left little doubt that the red cell surface harbours receptors serving as specific substrate for the viral enzyme and the closely related or identical enzyme produced by V. cholerae. However, there could be some doubt whether it was permissible to apply the insight gained into the virus-red cell relationship to the host cells of influenza virus, since red cells do not support virus propagation. It was, therefore, very gratifying to find that practically all the relevant phenomena observed with virus and red cells were reproducible when host cells were substituted for erythrocytes. Again Hirst[9] was the first to demonstrate adsorption of the virus onto and spontaneous elution from susceptible respiratory cells of the excised ferret lung. The same sequence of events was found in the excised mouse lung and Fazekas[10] has investigated this system extensively. He found that active LEE virus is practically completely adsorbed to the lungs in 15 min. and that at 20° C 50 min. after administration spontaneous elution begins and that it comes to an end in 3 hours with about 75% recovery of the virus used. RDE is able to interfere with this sequence in two different ways: (1) When RDE is administered prior to virus installation, the respiratory surface of excised mouse lungs is rendered incapable of adsorbing the virus. (2) If

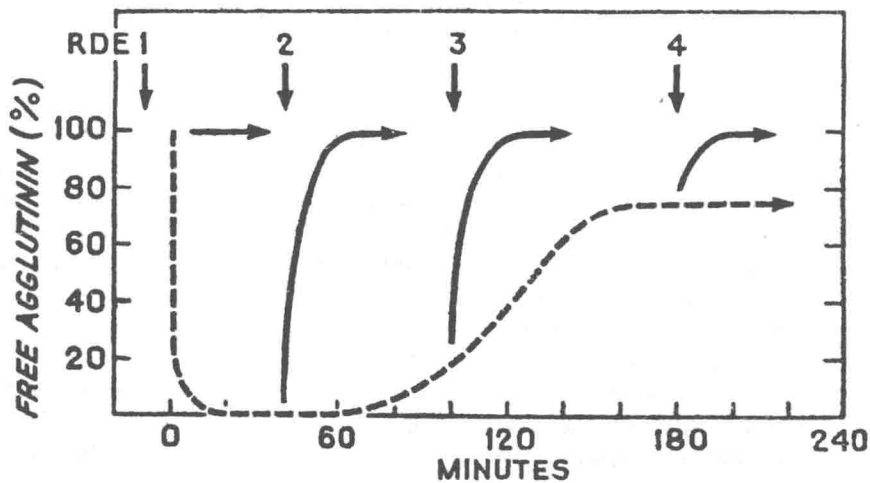

FIG. 2. Action of RDE on adsorption and elution of virus in the excised mouse lung. Dotted line represents the normal adsorption-elution curve of virus in untreated lungs. Solid lines show the result of washing out the lungs with RDE at the time indicated by vertical arrows.

administered at any time interval after virus addition, RDE liberates in 5 to 10 min. all residual virus from the lung cells. Heat inactivated virus is adsorbed to the lung cells, but without spontaneous elution following.

The allantoic cavity of the chick embryo provides another easily available system containing cells susceptible to influenza virus infection. Stone[11] has carefully studied this system with the following results:

(1) If the allantoic cavity is washed with formalin, thereby killing the embryo, the virus is adsorbed to and eluted from the lining cells of the cavity in a fashion very similar to that described for red cells and the mouse lung.

(2) When the formolized cavity is pretreated with periodate, virus is adsorbed but not eluted.

(3) Virus deposited in the allantoic cavity of living embryos is adsorbed by the susceptible cells; this adsorption is followed not by elution but rather by infection of the cells.

(4) The injection of RDE into the cavity of the living embryo prior to virus installation prevents infection for a period of 1 or 2 days, when regeneration of receptors restores suspectibility to the virus.

It would appear that the accumulated data are conclusive evidence for the presence of distinct receptors at the surface of host cells. These receptors provide the anchoring ground for the influenza virus and are, therefore, a prerequisite for virus infection. The virus at its surface carries a specific enzyme which fits the receptor and can modify it.

Obviously any further progress as to the nature of the cellular receptors and to the mode of the viral action had to come from chemical work. Three observations paved the way for the chemical approach started in Melbourne in 1948: (1) Francis[12] discovered that influenza B virus upon heating to 55° for 30 min., though not losing its capacity to agglutinate red cells, was prevented from doing so by normal serum. (2) Anderson,[13] in our Institute, showed that the inhibitory agent in serum, the Francis inhibitor, was a specific substrate for RDE and was rendered inactive by this enzyme and by the viruses of the mumps-influenza group. (3) Burnet[14] and co-workers[15] found that a great variety of mucinous material from human source (gastric mucin, ovarian cyst mucin, uterine cervical mucus, saliva, respiratory mucus) and from animals (sheep submaxillary glands, egg-white) contain a potent virus haemagglutinin inhibitor; RDE or virus treatment inactivated the inhibitor.

From the facts that the same specific enzyme irreversibly changes both the cellular receptors and the soluble mucins in such a way as to make them insensitive towards influenza virus and that the mucins are competitive inhibitors for the adsorption onto red cells of partially inactivated virus, it was concluded that the cellular receptors and the mucins contained an identical or chemically related structure; in other words we regarded the soluble inhibitory mucins as chemical analogues of the cellular receptors.

We investigated first the interaction between purified influenza virus A (Melbourne strain) and ovomucin, a protein fraction of egg-white containing the virus haemagglutinin inhibitor. It was found[16] that upon virus action a water soluble and dialyzable compound is released from the mucoprotein containing carbohydrate and a nitrogenous substance. The compound had quite unusual features. The carbohydrate was unstable against mild acid treatment at 100° for 10 min.; the nitrogenous substance or a component of it gave the Ehrlich reaction thought at that time to be specific for hexosamines; however, in contrast to the acid stability and alkali liability of the known hexosamines, glucosamine and chondrosamine, our "hexosamine" was acid-labile and alkali-stable. Though at this stage the chemical analysis of the split product was unyielding, its isolation was definite chemical proof for the enzymatic nature of the reaction studied. Treatment of ovomucin with RDE resulted in the release of a compound very similar to that obtained with the viral enzyme. No similar enzyme was detectable in the host cells of the virus or in the nutrient fluids of the chick embryo, and it could be shown[17] that the enzyme is an integral and inseparable part of the virus particle itself.

As mentioned before, ovomucin is a protein fraction and with an adequate technique it was found[18] to contain not more than 5 to 10% inhibitor. It was, therefore, of great interest and value to us that Tamm and Horsfall[19] succeeded in preparing from urine an inhibitory mucoprotein. Quite independently Tamm and Horsfall[20] and Ada and Gottschalk[21] produced by a modification of the original method an electrophoretically homogeneous substance. When highly purified influenza B virus (LEE) was allowed to act on the urine mucoprotein until its inhibitory faculty was lost, a compound[22] was separable from the reaction mixture by dialysis nearly identical with that obtained from ovomucin. It resembled in many details amino acid N–glucosides or their isomers (N–substituted isoglucosamines) which meanwhile had been synthesized in our laboratory.[23] In these synthetic compounds the carbohydrate is as unstable towards mild acid treatment as we had found it in the product released from mucoproteins by the viral enzyme. The amino acid N–glucosides or their isomers differed, however, from the natural compound in that they did not give the Ehrlich reaction (without acetylacetone). These findings strongly suggested the presence in the inhibitory mucoproteins of a nitrogenous component other than hexosamine being responsible for the Ehrlich reaction given by the split product.

Hirst[6] and Burnet and his group[24] had interpreted the modifying effect on cell receptor and inhibitory mucoproteins of low concentrations of periodate in terms of the well known oxidation of α–glycol groups in carbohydrates. The presence in the split product of a sugar[16,22] supported the view that the carbohydrate complex of the mucoprotein was subject to attack by the viral enzyme. An analysis of the carbohydrate complex of the urine mucoprotein was, therefore, undertaken hoping that such an an-