International Symposium on the Dynamics of Virus Infections

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AN International Symposium on the Dynamics of Virus Infections, under the auspices of the Henry Ford Hospital, was held Oct. 21-23, 1953, in Detroit, Michigan. It was the first of an annual series on medical subjects planned by the staff of the Henry Ford Hospital.

More than 400 physicians and investigators attended the three-day conference which took place in the remarkably well-appointed new auditorium of the Henry Ford Hospital. Thirty-three investigators were invited to present papers. Six scientists who gave papers came from countries other than the United States.

Workers in the three major categories of the virus field—animal, bacterial, and plant—presented the results of their recent studies bearing on the following general subjects: (1) Mechanisms of virus and rickettsial infections, (2) Ecology and pathogenesis, (3) Mechanisms of immunity, (4) Methods of virus and rickettsial diagnosis, and (5) Chemical approaches to prophylaxis and therapy of virus and rickettsial diseases. No attempt is made here to summarize all the findings reported or all the concepts put forward. The communications as well as the discussions which followed them are to be published in full by Blakiston Company, Inc.

It was obvious during the symposium that much attention is being given to the mechanism of virus reproduction and that this problem is one of the foremost in the animal, bacterial, and plant virus fields. The approaches are diverse and include studies on surface interactions, cell penetration, metabolic transformations, continuity of virus characters, composition of viruses, and by-products of reproduction.

A surface reactant for the influenza-Newcastle-mumps group, consisting of a carbohydrate complex containing four sugars and carboxy-pyrrole, has been identified, and is thought to represent the small prosthetic group of large mucoproteins which react with these viruses. That the same substance is present at the surface of infectable host cells remains to be demonstrated directly.

The attachment of phages (T series) to *E. coli* has been found to be dependent upon electrolytes and appears to be attributable to linkages between bacterial surface carboxyl groups and phage amino groups. This interaction is reversible. Electrostatic triggering then is thought to modify both the cell surface and the phage particle so that penetration of phage DNA can occur. This step is irreversible. Two distinct processes appear to be initiated by phage; the reproductive process resulting in the production of new phage, and the lytic process leading to disorganization of the host cell. Under conditions that lead to so-called abortive infection—infection not resulting in infective progeny—these processes can be separated.

A new pyrimidine, 5-hydroxy methyl cytosine, is present in the virus nucleic acid of the T even phages. This is not a metabolic intermediate normally present in the host cell. It is thought that the infecting phage particle may provide the necessary enzyme or coenzyme, or possibly evoke a bacterial enzyme not normally used. In addition, in thymineless *E. coli* mutants, infection with T2 phage results in the synthesis of both thymine and 5-hydroxy methyl cytosine. Thus, in this instance, the only pyrimidines synthesized are those that the uninfected host cell cannot make.

Phage DNA is thought to start off infection with T2, and only a few molecules per bacterium are needed. The bacterial DNA appears to be efficiently used in the production of new phage DNA. The precursors of infective phage particles, so-called vegetative or noninfective particles, multiply, interact, and presumably contain the same nucleic acids as the mature particles. Phage protein appears to have no function during replication and is thought to be synthesized later than phage nucleic acid. DNA is considered to be the sole agent of genetic continuity in phage.

The formation of both the head and the tail antigens of phage precedes the production of mature particles. Both antigens arise during the multiplication of vegetative phage. Maturation—the completion of mature, infective particles—appears to be a stepwise process; the synthesis of the specific phage materials, that is, DNA and proteins, precedes the less specific assembly of the mature particle.

The reproduction of tobacco mosaic virus is preceded by an increase in the insoluble nucleoprotein of the host tissue. It is thought that this is related to the formation of cell particulates which are the site of synthesis of virus protein. Such protein then is associated with accumulated virus RNA to yield mature virus particles. TMV reproduction is accompanied by the synthesis of three new low molecular weight (*ca* 30,000) proteins not associated with nucleic acids. The new proteins appear later than the virus, when the excess nucleic acid has disappeared; they are serologically related to TMV and can be polymerized. With plant viruses, in sharp contrast to bacterial viruses, genetic information apparently cannot be transmitted by DNA, for none is present. Whether RNA carries out such a function for plant viruses remains to be shown.

All stages of plant virus infection seem to be affected by the metabolism of the host cells. Both susceptibility and the amount of virus formed are increased by nutrients that increase plant growth. Susceptibility and virus multiplication can be affected separately by some chemical substances; however, thiouracil decreases both and is most effective when plant metabolism is high and would normally support most virus reproduction. Virus disease in plants is thought to result from aberrations in host protein metabolism.

The metabolic transformations associated with the reproduction of animal viruses remain largely unexplored. In addition, there is as yet no definite evidence on the nature of the material that transmits genetic information for these agents. The difficulty of demonstrating a possible transfer of genetic material between animal viruses remains largely unresolved. However, with influenza viruses the results of double infection experiments indicate that phenotypic mixing may occur and that a state analogous to heterozygosis can develop. It appears that single particles can carry the potentiality of giving rise to particles corresponding to both parental types. With influenza viruses, earlier indications of genetic recombination seem now to be best explained on the basis of phenotypic mixing. Similar transient alterations in the phenotype also have been demonstrated with certain T phages.

Animal virus reproduction appears to be associated with the production of new proteins separable from the virus particle, for example, soluble complement fixing antigens. In addition, there is evidence that noninfective, or "incomplete," influenza virus particles may appear in high yield under unusual experimental conditions with very large inocula. Except that they lack the highly unstable property of infectivity, such particles appear not to be markedly different from fully active particles. It has yet to be shown decisively that immature particles, comparable to those obtained with phage, are necessary precursors in the reproduction of animal viruses. Relative to cellular structures, mitochondria are thought to represent the intracellular sites of multiplication of herpes virus, possibly also of poliomyelitis virus, and may play a similar role with rabies virus.

Virus infection and bacterial transformation have as common features an invasive and replicative series of events. Each of the individual DNA transformers can be considered as an independent agent capable of invading, producing a modified bacterial cell, and being reproduced like a virus. In addition, DNA-induced transformations are closely analogous to genetic mutations, with the difference that they are directed. The stepwise transformation to drug resistance achieved with DNA appears to correspond exactly to the series of spontaneous mutations undergone by the donor strain during the acquisition of resistance.

The importance of reservoir hosts in the ecology of certain animal virus diseases—bovine pseudorabies, salmon poisoning, and swine influenza—appears to be well established. Such hosts determine the endemicity of these infections. In virus diseases of man, there is only incomplete knowledge of the whole ecological cycle in most instances. The sites at which viruses leave an infected host, as well as the means by which the agents are transmitted to and enter new hosts, are widely diverse. The role of the immune host in the transmission chain seems to be variable. As an example, it appears that measles and chicken pox do not have healthy carriers, but it is thought that infectious hepatitis and poliomyelitis may.

Population fluctuations of pathogenic agents, reservoirs, vectors, and hosts are affected by deforestation and human settlement. These fluctuations encourage evolutionary adaptation, especially evident in the genus *Rattus* and among *Trombiculid* mites in Southeast Asia, and bear on the ecology of scrub typhus. In the plant virus field, it has been demonstrated that the leaf-hopper is not only an efficient transmitter of aster yellows virus but it also is a superb reservoir for this agent. In the main, the vector remains infective throughout its life.

In poliomyelitis, there is uncertainty as to the common route by which the virus migrates from the alimentary tract to the central nervous system. There appears to be no doubt that the agent can reach lymphatic organs, enter the blood stream, and travel along nerve fibers. It is clear that the virus can multiply in the alimentary tract, the CNS, and possibly also in lymphatic tissue. The function of immune barriers in poliomyelitis seems to depend on the hypothesis favored for transmission of the virus from the periphery to the CNS.

Inapparent natural infections are thought to lead to as effective immunity to virus diseases in man and animals as recovery from manifest infections. Infective virus vaccines, prepared from variant strains, appear to mimic inapparent infections and may induce substantial immunity. Widespread application of such vaccines, excluding those against smallpox and yellow fever, has been prevented by the difficulty of securing and maintaining appropriately avirulent variants, as well as the potential hazard of causing frank disease. To induce immunity, it appears that the variant virus in an infective vaccine must multiply in the tissues of the vaccinated host. That such agents do not cause obvious disease is correlated with the finding that the rate of increase in virus concentration as well as the final yield of virus are lower than with virulent strains.

Inactivated virus vaccines, although incapable of causing infectious disease if actually free of infectious agents, are not without disadvantages. A relatively large amount of antigenic material is needed to give a satisfactory immune response and, in the absence of an adjuvant, this may be of short duration. Repeated injections may lead, if considerable foreign tissue material is present, to hyperreactive states.

The problem of early diagnosis of virus diseases of man has been solved in relatively few instances. Smallpox remains the best example and a specific diagnosis can be made rapidly through detection of the virus

antigen by *in vitro* procedures. For other viruses and rickettsiae, there are not yet available simple and reliable technics for the detection of specific antigens early in the disease process. Recovery and identification of the infectious agent and the demonstration of a specific antibody response after the early stages of the disease constitute the mainstays of laboratory diagnosis. Both have the disadvantages of requiring considerable effort and time and therefore tend to yield information only of retrospective value. Recent advances in tissue culture methods have simplified the diagnostic problem in some virus diseases, particularly in poliomyelitis. In this disease, tissue culture has largely replaced the experimental animal as a means for recovery and identification of the virus and measurement of specific antibodies. In addition, the newer tissue culture technics have made feasible the propagation of varicella and herpes zoster viruses, possibly also common cold virus, and have resulted in the recovery of a number of seemingly new infectious agents.

Although effective chemotherapeutic agents are now available for all rickettsial diseases and for those diseases caused by the psittacosis-lymphogranuloma venereum group of agents, there is still no good evidence that substances, useful in man, have been found for other virus infections. However, vigorous efforts in this direction continue and some substances capable of inhibiting the reproduction of certain animal, bacterial, or plant viruses under experimental conditions are now known. The effects of proflavine on phage multiplication appear to be best understood. The compound is thought to prevent the final assembly of the mature virus particle but does not affect the earlier production of either phage proteins or nucleic acid. However, despite its effect upon the bacterial virus, proflavine fails to provide any benefit to the infected bacterial host which proceeds to die at the expected time. Inhibitors of plant virus multiplication have received relatively little detailed study, partly because of technical difficulties, and it is doubtful that the effects of any can yet be described in mechanistic terms. In the animal virus field a variety of substances of widely dissimilar nature have been investigated intensively but only in a few instances, for example, with mumps and influenza viruses, have kinetic studies on inhibitor activity been undertaken. Chief interest appears to be in compounds that may act as inhibitors of required biosynthetic processes. Various so-called antimetabolites such as analogues of amino acids and derivatives of benzimidazole, cause effects in some virus infections that may prove to be of importance.

The Radioactivity of the Human Being

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THE amount of radioactive substances deposited naturally in the normal human being has become one of the key figures in recent discussions on "tolerance" dose, "permissible" dose and "damaging" dose in repeated, as well as in single, total body exposures to ionizing radiations. Its magnitude, originally reported by Krebs (*1–3*) as close to the accepted permissible body content of 1×10^{-7} gram radium element permanently fixed in the body, became uncertain, when Hursh and Gates (*4*), in 1950, found values 100 to 1000 times smaller than the accepted permissible content. While the reasons for this discrepancy were under discussion, Sievert (*5*), in 1951, using a special gamma-ray sensitive device for measurements on the intact living body as a whole, reported an average radioactivity of the human being close to the values given by Krebs and, thus, close to the permissible content. In connection with present considerations on the extension to man of the findings from experimental whole body irradiation studies (*6*), a critical review of the present situation seems adequate in order to come to an understanding of the reported values.

The data on total body radioactivity of the human being so far reported are presented in Fig. 1. They were obtained by Krebs in the study of 17 cases in the age range 50 to 91 yr, by Hursh and Gates in 25 cases in the age range 33 to 85 yr, and by Sievert in 12 cases in the age range 15 to 58 yr. Krebs measured his samples with an especially developed alpha-particle counter and with the emanation method. His values are given in amounts of radioactive substance equivalent to radium and/or radium element. Hursh and Gates used exclusively the emanation method and reported their findings in radium element. Sievert measured the radioactivity of the living individual with

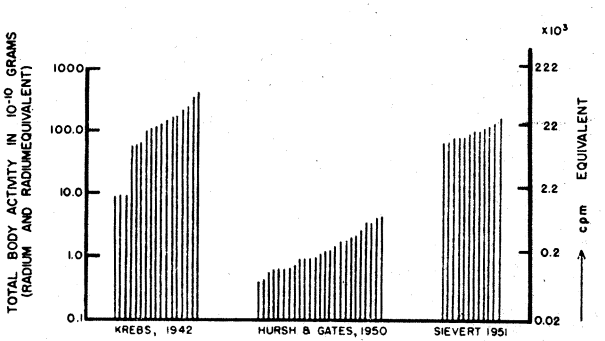

FIG. 1. Radioactivity of the normal human being.