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RECENT ADVANCES IN VIRUSES[1]

A BRIEF SURVEY OF RECENT WORK ON VIRUSES AND VIRUS DISEASES[2]

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To formulate at the present time a concise, accurate and invariant definition of a virus is impossible due to the insufficiency of our knowledge concerning the nature of these disease incitants. Because the infectious agents classified as viruses possess the capacity to multiply or reproduce, because they showed marked specificity under natural conditions for certain hosts and tissues, are able to adapt themselves to new environmental conditions and to undergo variation, it is customary to regard them as living organisms. In the past viruses were characterized, and thus differentiated from bacteria, by the possession of a size at or below the limits of resolution possible with the usual microscopic methods, by their ability to pass through mineral or collodion filters which hold back bacteria, and by their total inability to reproduce in lifeless bacteriologic media. We now know, however, that invisibility and filtrability do not constitute valid criteria—some infectious agents possessing all the attributes of a virus and classed as such are visible and approximate the smallest bacteria in size while others pass with difficulty, or not at all, through filters which permit passage of the smallest bacteria. From the biologic standpoint the outstanding difference between viruses and bacteria appears to lie in the inability of viruses to propagate unless living cells are present; yet on closer analysis even this difference approximates the relative rather than the absolute in degree. Certain pathogenic bacteria, such as *Hemophilus influenzae* and *Pasteurella tularensis,* have become so highly parasitic that their nutritional requirements are

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met only by media containing animal tissues or tissue extracts, a condition which implies that certain enzyme systems vital to free-living forms are lacking. Viruses presumably possess even fewer enzyme systems, so few that only living cells can provide them with the conditions essential to multiplication.

The biologic position of the viruses is still uncertain, although a link to the recognized bacteria may perhaps exist through the rickettsiae, which some of the larger viruses resemble in certain respects, such as size, developmental cycles, tinctorial properties, etc.

THE NATURE OF VIRUSES

The numerous early attempts to gain insight into the nature and properties of the viruses were seriously hampered by the unavoidable use of crude extracts of infected tissues. Attempts at purification by chemical or physicochemical means were not lacking, but were attended with the loss of so much activity that the preparations were unsatisfactory for study.

The success of Stanley[4] in 1935 in isolating by chemical means from the juice of tobacco plants infected with tobacco-mosaic virus a crystalline protein possessing the properties of the virus gave new impetus to this field. While purely chemical methods on the whole have been found unsuitable for purification of viruses not endowed with the unusual stability of tobacco-mosaic virus, the development of the high-speed vacuum centrifuge has provided a valuable alternate method for purifying and concentrating virus preparations. Optical, ultracentrifugation, viscosity, diffusion, x-ray and other physical, as well as chemical, studies have been carried out on such purified preparations; the significance of some of the information obtained by correlating data from examinations by these methods is presented below.

It has been found possible to concentrate and purify the less stable viruses by repeated centrifugation in a high-speed air-driven vacuum centrifuge.[5,6] The procedure depends on the use of alternate cycles of low and high speed. Material thrown down at low speed (about 10,000 g) is discarded while sediments produced at high speed (*e.g.*, 50,000 g) are resuspended and subjected to another cycle of low- and high-speed centrifugation. The cycles are repeated until a homogeneous final preparation is achieved. The degree of homogeneity of the suspension as well as the particle size of the suspended material is checked in an analytical ultracentrifuge.[5,6]

The end-product obtained by repeated alternate high- and low-speed centrifugation is a protein with which are associated all the properties of the virus. Ultracentrifugal analysis of such virus proteins indicates that they possess remarkable molecular homogeneity. Calculations of the molecular weights of the virus-proteins from their sedimentation constants show that these proteins are unusual in that they are composed of molecules much larger than those of any proteins previously described.[7] Thus, the tobacco-mosaic virus, one of the most intensively investigated, has a molecular weight of forty-three million, computed in accordance with the known asymmetry of the virus particles.[8] The viruses of rabbit papilloma[9] and of eastern equine encephalomyelitis,[10] whose particles are assumed to be spherical, have molecular weights in the neighborhood of twenty million. As pointed out by Stanley[7] the virus proteins have many of the properties of ordinary molecules, and because of their tremendous size he groups them with the macromolecules of the colloidal chemist.

The validity of the figures assigned for molecular weight or for particle shape, for example, has been checked, whenever feasible, by one or more additional methods, singly or in combinations. The plant viruses have been the most intensively studied and tobacco-mosaic virus protein is chosen here as an illustrative example; the data presented are taken from a recent review by Lauffer and Stanley.[8a] Tobacco-mosaic virus protein occurs in the liquid crystalline (mesomorphic or paracrystalline) state, and the crystals are held to be true crystals since they are double-refractive and on x-ray analysis give a typical crystalline pattern. This ability to exist in a liquid crystalline state implies that the molecules have a rodlike structure, an implication supported by the findings that the virus protein shows double refraction of flow, positive electrical birefringence and the Ganz depolarization effect. If the constants for sedimentation, specific viscosity, specific volume, diffusion and rotational diffusion for a virus protein, are known it is possible by use of the appropriate equations and various combinations of these constants to ascertain the size, shape and weight of the virus molecule. By such means the tobacco-mosaic virus protein has been calculated, as mentioned above, to have a molecular weight of forty-three million and to consist of asymmetric particles approximately 280 mμ × 15 mμ.[8b] X-ray diffraction data also indicate that the particles have a diameter of 15.2 mμ and a length greater than 120 mμ.

[4] W. M. Stanley, SCIENCE, 81: 644–645, 1935.
[5] T. Svedberg and K. O. Pedersen, "The Ultracentrifuge." Clarendon Press, Oxford, England, 478 pp., 1940.
[6] E. G. Pickels, *Chem. Reviews*, 30: 341–345, 1942.
[7] W. M. Stanley, *Jour. Phys. Chem.*, 42: 55–70, 1938.
[8] a. M. A. Lauffer and W. M. Stanley, *Chem. Reviews*, 24: 303–321, 1939. b. W. M. Stanley and C. A. Knight, *Cold Spring Harbor Symposia on Quantitative Biology*, 9: 255–262, 1941.
[9] J. W. Beard and R. W. G. Wyckoff, SCIENCE, 85: 201–202, 1937.
[10] A. R. Taylor, D. G. Sharp, Dorothy Beard and J. W. Beard, *Jour. Infect. Dis.*, 72: 31–41, 1943.

With the exception of vaccine virus, which is relatively quite stable, as well as being one of the largest known viruses, the animal viruses have enjoyed but little attention compared to the plant viruses. The large particle size of vaccine virus makes feasible its purification and concentration by comparatively simple centrifugational procedures, so that pure preparations of this virus have long been available for study. Infectivity of such preparations is attributed to the elementary bodies, which are sufficiently large to be examined under the ordinary microscope. Data derived from filtration of the virus through graded collodion membranes indicate that the virus has a particle size of 125 mμ to 175 mμ.[11] Barnard (according to Elford and Andrewes)[12] estimated the particle size, from photomicrographs taken with ultraviolet light, to be 160 mμ to 170 mμ. From the experimentally determined density and sedimentation constants and assuming an approximately spherical shape, Pickels and Smadel[13] calculated the average particle size of the elementary bodies to be 236 mμ to 252 mμ; direct measurements made by Green, Anderson and Smadel[14] from electron photomicrographs of elementary bodies are reported by Smadel and Hoagland[15] only to give values slightly lower than those obtained from ultracentrifugational analysis—no definite figures are given. However, since dehydration, an integral part of the technique of electron microscopy, is reported to decrease the diameter of elementary bodies approximately 14 per cent.,[16] a particle size somewhere around 200 mμ might be expected as compared with the particle size estimated from sedimentation experiments using hydrated elementary bodies.

In size, therefore, the elementary bodies of vaccine virus are close to the smallest bacteria. While pure plant viruses occur as liquid crystals, the elementary bodies of vaccinia morphologically resemble bacteria in structure. Electron microscope examination of the elementary bodies[14] reveals that they have a "bricklike" form within which can be seen five areas of condensation, darker than the surrounding ground substance. The nature of these condensed masses is unknown, as is true of similar masses found in many bacteria. The density of elementary bodies is 1.16,[16] greater than that of bacteria, 1.10[17] and less than that of protein, 1.33.[5] When treated with alkali, the elementary bodies swell and may burst, with resultant extrusion of the internal substance through the break in surface continuity.

None of the smaller animal viruses has been so intensively studied, from the physical standpoint, as vaccine virus, although there is now a perceptible trend to rectify this situation as evidenced by recent publications on rabbit papilloma,[18] mouse encephalomyelitis,[19] poliomyelitis,[20, 21] equine encephalomyelitis[10] and influenza A[22] viruses. The latter virus will be used to illustrate how future work may serve to alter considerably our present conception of individual viruses.

The size of the infectious unit of influenza A virus in infectious mouse lung suspension has been estimated to be 80 mμ to 120 mμ by ultrafiltration[23] and 87 mμ to 99 mμ by centrifugation methods.[24] High-speed centrifugation of such suspensions results in sedimentation of more than 99 per cent. of the infectious material.[22, 25] Electron micrographs show that the sedimented particles have a diameter of approximately 100 mμ,[22] which would be expected from the previous filtration and centrifugation studies. However, when infectious chick embryo allantoic fluid is spun in fields of centrifugal force capable of sedimenting mouse lung virus, infectivity remains about equally distributed between sediment and supernatant,[22] implying that the infectious unit in allantoic fluid actually is much smaller than in mouse lung suspensions. Since centrifugation of infectious and of normal mouse lung suspensions under identical conditions sediments particles of the same size, indistinguishable by electron microscopy or by chemical means,[22] it appears that the infectious unit is considerably smaller than the tissue particles on which it is adsorbed.[22] Bourdillon[26] has found that in infectious mouse lung suspensions only a small proportion of infectivity is associated with particles estimated from their diffusion constant to be about 6 mμ in diameter, while in allantoic fluid, which allows far less opportunity for adsorptive phenomena, up to one half of the infectivity is associated with particles of this size.

[11] W. J. Elford and C. H. Andrewes, *Brit. Jour. Exp. Path.*, 13: 36–42, 1932.
[12] W. J. Elford and C. H. Andrewes, *Brit. Jour. Exp. Path.*, 17: 422–430, 1936.
[13] E. G. Pickels and J. E. Smadel, *Jour. Exp. Med.*, 68: 583–606, 1938.
[14] R. H. Green, T. F. Anderson and J. E. Smadel, *Jour. Exp. Med.*, 75: 651–656, 1942.
[15] J. E. Smadel and C. L. Hoagland, *Bact. Rev.*, 6: 79–110, 1942.
[16] J. E. Smadel, E. G. Pickels and T. Shedlovsky, *Jour. Exp. Med.*, 68: 607–627, 1938.
[17] D. Ruffilli, *Biochem. Ztschr.*, 263: 63–74, 1933.
[18] W. R. Bryan and J. W. Beard, *Jour. Nat. Cancer Inst.*, 1: 607–673, 1941.
[19] S. Gard and K. O. Pedersen, SCIENCE, 94: 493–494, 1941.
[20] H. S. Loring and C. E. Schwerdt, *Jour. Exp. Med.*, 75: 395–406, 1942.
[21] E. Racker, SCIENCE, 96: 364–365, 1942.
[22] L. A. Chambers, W. Henle, M. A. Lauffer and T. F. Anderson, *Jour. Exp. Med.*, 77: 265–276, 1943.
[23] W. J. Elford, C. H. Andrewes and F. F. Tang, *Brit. Jour. Exp. Path.*, 17: 51–53, 1936.
[24] W. J. Elford and C. H. Andrewes, *Brit. Jour. Exp. Path.*, 17: 422–430, 1936.
[25] E. H. Lennette and F. L. Horsfall, *Jour. Exp. Med.*, 72: 233–246, 1940.
[26] J. Bourdillon, *Jour. Gen. Physiol.*, 25: 263–273, 1941.

Chambers *et al.*,[22] using infected allantoic fluid, obtained sedimentation constants which correspond to those of spherical particles 10–12.5 mμ in diameter; examination by electron microscopy of the sediments from ultracentrifugation showed a preponderance of spherical particles with a diameter of 11 mμ.

If these findings are substantiated, we must then consider influenza A virus to be one of the smallest known, and group it with the viruses of foot-and-mouth disease[27] and of poliomyelitis.[28,29] In addition, if its relatively low molecular weight, estimated at 650,000,[22] is confirmed, this would indicate that architectonically influenza A virus is closer to a virus such as tobacco-mosaic than to one like vaccinia or psittacosis, with molecular weights of $4{,}300 \times 10^{-6}$ and $8{,}500 \times 10^{-6}$ respectively.[8b]

The architectural similarity of poliomyelitis virus and of tobacco-mosaic virus perhaps is even closer, since Racker[21] has recently reported the isolation from infected mouse brains of liquid crystals which are protein in nature and which carry the infectivity of the virus.

Chemistry in the Virus

Attempts to study the composition of viruses have been, with few exceptions, restricted to elementary quantitative analyses because of the difficulty in obtaining more than minute amounts of purified material. However, important chemical differences among viruses can be shown only when the integral components, rather than the elements, of which they are built are known.

Of the twenty or so viruses which have been purified, all have been reported to contain nucleic acid. The nucleic acid present in plant virus proteins has invariably been of the ribo- or yeast-nucleic type, and hence the presence of the desoxy-ribose or thymonucleic type in the elementary bodies of vaccine virus[30] might be supposed to constitute a possible difference between viruses of plant and animal origin. Vaccine virus, however, appears to occupy, at least for the present, a unique position among the animal viruses, since the virus proteins of eastern equine encephalomyelitis[10] and of influenza A,[31] admittedly not as highly purified as elementary body suspensions, are reported to be of the yeast-nucleic type; the findings regarding chicken tumor I are contradictory.[32,33]

Tobacco-mosaic,[34] tobacco ring-spot[34] and rabbit papilloma[18] viruses appear to be composed entirely of nucleic and amino acids, since all the carbohydrate and phosphorus found can be accounted for on the basis of nucleic acid. Other plant viruses, as the latent mosaic virus,[34] may contain in addition a conjugated polysaccharide.

Lipoids have never been found associated with the plant viruses,[34] but the situation with the animal viruses is not clear—eastern equine encephalomyelitis virus appears to contain phospholipids, cholesterol and fatty acids;[10] vaccine virus contains phospholipids, cholesterol and neutral fat;[15] chicken tumor I contains a lipoid fraction of which about two thirds are probably a lecithin and the rest is unidentified,[32] while rabbit papilloma virus consists practically entirely of protein, having a fat content so small that its significance is questionable.[18]

The ability to obtain sufficient amounts of purified plant viruses has made feasible determinations of the amino acids composing the virus protein. Amino acids so far demonstrated in tobacco-mosaic virus protein include arginine, aspartic acid, cysteine, glutamic acid, leucine, lysine, phenylalanine, proline, serine, tryptophane and tyrosine;[35] alanine, histidine and glycine have not been demonstrated.[35] In addition, Stanley and Knight[8b] have been able to show that variations of tobacco-mosaic virus to produce a new strain as shown by serologic tests is also accompanied by specific changes in the amino acid content.

Antigenic Structure

The complexity in antigenic structure of a virus is reflected, within limits, by the number of distinct antibodies it is capable of producing.

The simplest viruses, antigenically, appear to be those of tobacco-mosaic[36] and of rabbit papilloma[37] since only one antibody responsible for both *in vitro* and *in vivo* immune phenomena has been detected. Most of the animal viruses studied thus far, however, give rise to at least two antibodies, one elicited by the virus proper and the other by the so-called soluble antigen, which is distinct and separable from the virus. The virus itself is presumed to give rise to the neutralizing antibodies, which when mixed with the virus *in vitro* render it noninfective. Soluble antigens, on the other hand, call forth antibodies which have no, or slight, neutralizing capacity, but react with the antigens *in vitro* to produce precipitation, agglutina-

[27] I. A. Galloway and W. J. Elford, *Brit. Jour. Exp. Path.*, 12: 407–425, 1931.
[28] Max Theiler and J. H. Bauer, *Jour. Exp. Med.*, 60: 767–772, 1934.
[29] W. J. Elford, I. A. Galloway and J. R. Perdrau, *Jour. Path. and Bact.*, 40: 135–141, 1935.
[30] C. L. Hoagland, G. I. Lavin, J. E. Smadel and T. M. Rivers, *Jour. Exp. Med.*, 72: 139–147, 1940.
[31] L. A. Chambers and W. Henle, *Jour. Exp. Med.*, 77: 251–264, 1943.
[32] A. Claude, SCIENCE, 87: 467–468, 1938.
[33] A. Claude, *Proc. Soc. Exp. Biol. and Med.*, 39: 398–403, 1938.
[34] W. M. Stanley, *Physiol. Rev.*, 19: 524–556, 1939.
[35] C. A. Knight and W. M. Stanley, *Jour. Biol. Chem.*, 141: 39–49, 1941.
[36] K. S. Chester, *Phytopathology*, 25: 702–714, 1935.
[37] J. G. Kidd, *Jour. Exp. Med.*, 68: 737–759, 1938.

tion or complement-fixation, depending upon the physical conditions present in the reacting system.

It appears likely, however, that as adequate techniques are devised additional antigens, complete or partial or both, will be discovered, since there is every reason to believe that the soluble antigen is not a single antigenic entity. Thus, the soluble antigen of infectious myxoma of rabbits has recently been found to contain two components designated A and B[38]—these are easily separable by physical methods, are heat-labile, probably protein in nature and are immunologically distinct. Vaccine virus is even more complex antigenically, since at least five antibodies appear in animals recovered from infection with this virus. Antibodies are produced against a heat-labile (L) and a heat-stable (S) antigen both of which are components of a single substance, LS,[39] shown by Smadel and Shedlovsky[40] to be an elongated protein molecule with a weight of 240,000 and an axis ratio in the neighborhood of 30:1. There is also present a nucleoprotein (NP) antigen[41] which gives rise to an antibody distinct from L and S antibodies, and like the L and S antigens, on injection into animals produces neither immunity to infection nor appreciable amounts of neutralizing antibody. The L, S and NP antigens are believed, according to recent findings, to be located on the surface of the infective unit, the elementary body.[15] Immune sera completely adsorbed with L, S and NP antigens to remove the corresponding antibodies still possess the ability to agglutinate elementary bodies and to neutralize the virus. The antigens responsible for the production of the X-agglutinins[38,41] and the neutralizing antibody are as yet little known, so that at present it is impossible to say whether they are distinct, related or identical.

DIAGNOSIS

Much has been, and is being done, to develop simple, accurate and inexpensive methods to replace wholly or in part the slow, cumbersome and costly neutralization test, which has been the basic tool in diagnosis and in epidemiologic studies.

Through refinement of the antigens employed and determination of appropriate serum-inactivation temperatures, the specificity of the complement-fixation test has been brought to the stage where it is now possible to distinguish between experimental infections induced by the viruses of rabies, eastern and western equine encephalomyelitis, lymphocytic choriomeningitis, louping ill, St. Louis encephalitis and Japanese B encephalitis.[42] Preliminary experiences with the test in the diagnosis of human infections with some of these viruses are highly encouraging.[42,43]

With a few notable exceptions, attempts to demonstrate precipitation in specific animal virus antigen-antibody systems have been almost consistently negative. Recently, however, this field has been reopened with the finding that it is possible, at least in some cases, to demonstrate specific antigen-antibody union by the addition of an "indicator system" composed of bacteria[44] or of finely dispersed particles of collodion[45] whose aggregation leads to the formation of visible floccules.

Hirst[46] has observed that chick red cells are agglutinated in the presence of influenza virus and that agglutination can be prevented by immune serum. On the basis of these observations he has devised a method for titrating the agglutination-inhibiting substance in influenza immune sera[47,48] and has been able to show that it closely parallels the neutralizing antibody titer. The full implications of Hirst's findings are still unknown, but a similar technique has already been found applicable to vaccine virus infections.[49] The simplicity of the technique and the rapidity and ease with which a large number of sera can be examined have provided a valuable method for diagnosis and especially for epidemiologic investigations of those viral diseases to which it may be found applicable.

EPIDEMIOLOGY AND EPIZOOTIOLOGY

Within the last few years our conception of the epidemiology and pathogenesis of poliomyelitis has been considerably altered. This disease was generally believed to be spread solely, or almost so, by droplet infection, the virus gaining access to the central nervous system from the nasal mucous membrane by ascent along the olfactory nerve. Recent reinvestigation, in rather minute detail, of the pathogenesis and the pathology of poliomyelitis has brought forth considerable evidence that the alimentary tract is the usual portal of entry for the virus (reviewed in[50,51,52]).

[38] J. E. Smadel, S. M. Ward and T. M. Rivers, *Jour. Exp. Med.*, 72: 129–138, 1940.

[39] a. J. Craigie and F. O. Wishart, *Jour. Exp. Med.*, 64: 819–830, 1936. b. T. Shedlovsky and J. E. Smadel, *Jour. Exp. Med.*, 75: 165–178, 1942.

[40] J. E. Smadel and T. Shedlovsky, *Annals New York Acad. Sci.*, 43: 35–46, 1942.

[41] J. E. Smadel, T. M. Rivers and C. L. Hoagland, *Arch. Path.*, 34: 275–285, 1942.

[42] J. Casals and R. Palacios, *Jour. Exp. Med.*, 74: 409–426, 1941.

[43] J. Casals, *Am. Jour. Pub. Health*, 31: 1281–1284, 1941.

[44] E. C. Roberts and L. R. Jones, *Proc. Soc. Exp. Biol. and Med.*, 49: 52–54, 1942; *ibid.*, 47: 75–76, 1941.

[45] K. Goodner, SCIENCE, 94: 241–242, 1941.

[46] G. K. Hirst, SCIENCE, 94: 22–23, 1941.

[47] G. K. Hirst, *Jour. Exp. Med.*, 75: 49–64, 1942.

[48] G. K. Hirst and E. G. Pickels, *Jour. Immunol.*, 45: 273–283, 1942.

[49] F. P. O. Nagler, *Med. Jour. Australia*, 1: 281–283, 1942.

[50] A. B. Sabin, *Jour. Am. Med. Asn.*, 117: 267–269, 1941.

[51] E. W. Schultz, *Jour. Pediat.*, 20: 110–124, 1942.

While it is possible that the virus can multiply throughout the alimentary tract, the pharynx and small intestine appear to provide the sites of choice. The virus is believed to migrate from these areas to the central nervous system via two main routes.[52] The first, which gives rise to a preponderance of bulbar symptoms, is along the cranial nerves or the parasympathetic system into the medulla; the other, which results in paralysis of the extremities, is along the visceral afferent or efferent fibers of the intestine, through the corresponding spinal or abdominal sympathetic ganglia, into the spinal cord. The total inability to demonstrate a centrifugal spread of the virus from the central nervous system is interpreted to mean that the virus multiplies in, and is eliminated from, the alimentary tract. The persistence of the virus in the stools of convalescents,[53] its presence in the stools of healthy contacts,[53] the spread of the disease by healthy carriers[54] and perhaps by flies,[55] the occurrence of cases throughout the year with a peak incidence during the summer and early fall months all point to an epidemiologic picture typical of gastrointestinal rather than respiratory routes of infection.

The comprehensive studies of Howitt[56] have demonstrated that the viruses of western equine encephalomyelitis and of St. Louis encephalitis are endemic in central California. Hammon[57] has shown that epidemics due to both viruses can occur simultaneously and that human cases due to a mixed infection probably do occur. Both Howitt and Hammon and their associates have found that the sera of certain birds and mammals in the affected areas contain neutralizing antibodies to one or both of these viruses, a finding which not only points to a possible mechanism for widespread dissemination of the virus but also implies that an arthropod vector may be involved in transmission. Further, the complete lack of epidemiologic evidence that these viruses are carried through food or water or that infections arise through direct or indirect contact, as well as the limitation of these diseases to the warm months of the year are in accord with such a view. While *Triatoma sanguisuga* Leconte has been found[58] naturally infected with the western equine virus, its distribution does not coincide with that of the virus[59a] and it is believed by Hammon and his associates not to be an efficient vector.[59a] In a search for vectors possibly associated with the Yakima Valley outbreak of encephalitis Hammon *et al.*[60] isolated five strains of the western equine virus and three of the St. Louis virus—all from a single species of mosquito, *Culex tarsalis* Coquillet. This is the first instance of isolation of the St. Louis virus from any material other than human tissues, and the first time either virus has been found in a wild-caught mosquito. Since it does not necessarily follow that an arthropod infected with virus can transmit it, proof of ability to transmit must be obtained under experimental conditions. *C. tarsalis* was quickly proved capable of transmitting the St. Louis virus experimentally[59a] and *C. pipiens, C. coronator, Aedes lateralis, A. taeniorhynchus, A. vexans* and *Theobaldia incidens* have just been added[59b] as potential vectors. The western equine virus has been transmitted under experimental conditions by *C. tarsalis* and also by *Theobaldia incidens*.[59b]

We may find eventually that other arthropods can participate in the transmission of western equine encephalitis and St. Louis encephalitis since, under laboratory conditions, many Aedes as well as *Dermacentor andersoni* can transmit the western equine virus and *Dermacentor variabilis* can transmit the St. Louis virus (reviewed in[59a]).

One of the great riddles of epidemiology is what happens to a virus during interepidemic periods, when it apparently disappears, and what influences a virus to flare up suddenly from its quiescent state. A step toward the solution of this riddle is afforded by the important discovery of Shope[61] that the virus of swine influenza is capable of existence in a masked noninfectious form from which it can be provoked into activity by appropriate stimuli. Thus, in a swine with active influenza infection, the virus is taken up by lungworms (*Metastrongylus elongatus* and *Choerostrongylus pudendo-tectus*) in the lung. Embryonated ova are passed in the feces and eventually are ingested by an earthworm, which is the intermediate host of the lungworm. In the earthworm the ova pass through developmental cycles until the third larval stage, infective for pigs, is reached. Ingestion of infected earthworms by swine leads to liberation of the larvae

[52] A. B. Sabin, *Jour. Am. Med. Asn.*, 120: 506–511, 1942.

[53] a. S. D. Kramer, A. G. Gilliam and J. G. Molner, *Pub. Health Rep.*, 54: 1914–1922, 1939. b. S. D. Kramer, B. Hoskwith and L. H. Grossman, *Jour. Exp. Med.*, 69: 49–67, 1939. c. J. D. Trask, J. R. Paul and A. J. Vignec, *Jour. Exp. Med.*, 71: 751–763, 1940. d. H. A. Howe and D. Bodian, *Jour. Infect. Dis.*, 66: 198–201, 1940.

[54] E. A. Piszczek, H. J. Shaughnessy, J. Zichis and S. O. Levinson, *Jour. Am. Med. Asn.*, 117: 1962–1965, 1941.

[55] A. B. Sabin and R. Ward, SCIENCE, 94: 590–591, 1941; *ibid.*, 95: 300–301, 1942.

[56] a. B. F. Howitt, *Proc. Sixth Pacific Sci. Cong.*, 5: 235–245, 1942; b. B. F. Howitt and W. Van Herick, *Jour. Infect. Dis.*, 71: 179–191, 1942.

[57] W. M. Hammon, *Jour. Am. Med. Asn.*, 117: 161–167, 1941.

[58] C. H. Kitselman and A. W. Grundman, *Kansas Agric. Exp. Sta. Tech. Bull.* No. 50, p. 1–15, 1940.

[59] a. W. M. Hammon, W. C. Reeves, B. Brookman and C. M. Gjullin, *Jour. Infect. Dis.*, 70: 278–283, 1942. b. W. M. Hammon, W. C. Reeves and M. Gray, *Am. Jour. Pub. Health*, 33: 201–207, 1943.

[60] W. M. Hammon, W. C. Reeves, B. Brookman and E. M. Izumi, *Jour. Infect. Dis.*, 70: 263–266, 1942.

[61] R. E. Shope, *Jour. Exp. Med.*, 74: 41–47, 1941.

which make their way to the lungs and develop into adults. The virus continues to remain in its noninfective form until it is activated by a "provoking stimulus," such as repeated injections of *H. influenzae* or of calcium chloride, when the disease supervenes. What causes fully active swine influenza virus to become noninfective when taken up by the lungworm and to resume its infectivity when it again finds itself within a susceptible swine is still unknown. Shope has shown that the virus is able to survive for at least two years in its noninfective form in the lungworm, which indicates that the virus possesses an excellent means of ensuring its survival between epizootics.

PROPHYLAXIS AND THERAPY

An effective and relatively safe method of vaccination against yellow fever has been available since 1937[62a] when a strain (17D) of virus with attenuated virulence was obtained after prolonged tissue-culture passage of the classic Asibi strain. The vaccine, prepared from chick-embryo tissue infected with the 17D strain, has been used in endemic areas of yellow fever on such a huge scale and with such good results that its protective powers are undoubted (literature in[62b]).

Vaccines have also been developed against the eastern and western equine encephalomyelitis viruses, but in this case the high virulence of the viruses has required their inactivation with formol.[63] Their protective action in animals has been shown to be of a high order, and hence they probably exert a similar effect in man—at least no cases of the disease have occurred in vaccinated laboratory workers.[63]

Encouraging results are reported, in preliminary trials, with vaccination against the tick-borne Russian spring-summer encephalitis.[64] Formol-inactivated mouse brain virus suspensions were administered to 925 individuals in an endemic area. Two cases occurred in the vaccinated group and twenty-seven in the control group of 1,185 nonvaccinated individuals, ratios of 1 in 462 and 1 in 44, respectively.

The efficacy of vaccines against influenza A is still unknown and must await further trial under controlled conditions. A recent analysis by Horsfall[65] of five independent studies of the complex influenza A vaccine[66] showed that the reduction in incidence of the disease among vaccinated persons varied from 47 per cent. to practically nil, and that on the average the number of cases in the vaccinated group was about two thirds that of the unvaccinated group. In addition any increased resistance induced by vaccination seems to be of short duration.[67] Inhalations of immune serum have been reported to exert a protective action both in prophylaxis[68] and in therapy.[69]

The results of attempts at sulfonamide therapy in virus diseases have been disappointing. This is not surprising, however, since the presence of sulfonamides in tissue culture has been found to have no appreciable effect on the multiplication of any of the viruses cultivated in such an environment.[70,71] Of the diseases usually classed with those of virus origin, only four—trachoma, inclusion blenorrhea, lymphogranuloma venereum and mouse pneumonitis—are known to respond to sulfonamide therapy.[72] Rake, Jones and Nigg,[73] however, do not believe that the success in therapy in these four diseases has any bearing on the chemotherapy of the "true" virus disease, since there is increasing evidence[73,74] that the causative agents of the lymphogranuloma venereum-psittacosis group of diseases—to which these four belong[72]—should be separated from the true viruses.

NEW VIRUSES

New viruses and diseases caused by them continue to be uncovered. In 1938 a virus responsible for the Russian spring-summer type of encephalitis was recovered from the brains of fatal human cases by Levkovitsch, Shubladze, Chumakov and Soloviev.[75,76] The virus is transmitted to man by ticks (*Ixodes persulcatus*) and is reported[76] to be related immunologically to the virus of Japanese B, or summer, encephalitis, which is mosquito transmitted. Animals immune to the Russian spring-summer encephalitis virus are resistant to inoculation of the Japanese B virus and their sera neutralize completely the Japanese virus, but in the reverse direction only partial protection or neutralization occurs, which indicates that antigenically the Russian spring-summer encephalitis virus is more complex than the Japanese virus. Casals and

[62] a. M. Theiler and H. H. Smith, *Jour. Exp. Med.*, 65: 767–786, 1937. b. J. P. Fox and A. S. Cabral, *Am. Jour. Hyg.*, 37: 93–120, 1943.

[63] D. W. Beard, H. Finkelstein and J. W. Beard, *Jour. Immunol.*, 40: 497–507, 1941.

[64] A. A. Smorodintseff, N. W. Kagan, E. N. Levkovitsch and N. L. Dankovskij, *Arch. ges. Virusforsch.*, 2: 1–25, 1941.

[65] F. L. Horsfall, Jr., *Jour. Am. Med. Asn.*, 120: 284–287, 1942.

[66] F. L. Horsfall and E. H. Lennette, SCIENCE, 91: 492–494, 1940.

[67] G. K. Hirst, E. R. Rickard, L. Whitman and F. L. Horsfall, Jr., *Jour. Exp. Med.*, 75: 495–511, 1942.

[68] R. M. Taylor, *Jour. Immunol.*, 41: 453–462, 1941.

[69] A. A. Smorodintseff, *Proc. Third Int. Cong. Microbiol.*, New York, September 2–9, 1939, p. 375 (abstract).

[70] M. Sanders, C. H. Huang and H. S. Simms, *Jour. Bact.*, 45: 81–82, 1943 (abstract).

[71] H. Koprowski and E. H. Lennette, *Am. Jour. Hyg.*

[72] G. Rake, M. F. Shaffer and P. Thygeson, *Proc. Soc. Exp. Biol. and Med.*, 49: 545–547, 1942.

[73] G. Rake, H. P. Jones and C. Nigg, *Proc. Soc. Exp. Biol. and Med.*, 49: 449–452, 1942.

[74] M. D. Eaton, W. P. Martin and M. D. Beck, *Jour. Exp. Med.*, 75: 21–33, 1942.

[75] E. N. Levkovitsch, A. K. Shubladze, M. P. Chumakov and V. D. Soloviev, *Arch. Sc. Biol.*, 52: 162–163, 1938.

[76] A. A. Smorodintseff, *Arch. ges. Virusforsch.*, 1: 468–480, 1940.

Webster[77] have reported that preliminary cross-fixation and cross-immunity tests indicate the Russian spring-summer virus to be very closely related to, if not identical with, louping-ill virus which, also transmitted by ixodid ticks, is the cause of an encephalitis in sheep in Scotland and may have been responsible for an outbreak of human encephalomyelitis (X-disease) in Australia in 1917–18.[78] If the close relationship of the Russian spring-summer encephalitis and louping-ill viruses is substantiated, one might speculate as to whether the Japanese B virus may not represent a variant of these viruses arising from prolonged association with another arthropod vector. As a matter of fact, the virus causing the Russian autumn encephalitis has been identified as that of Japanese B encephalitis, and has been recovered from naturally infected *Culex pipiens* and *C. tritaeniorhynchus*.[79]

Isolation of the West Nile virus was reported in 1940 by Smithburn, Hughes, Burke and Paul.[80] The name derives from the locality (West Nile district, Northern Province of Uganda) from which the blood specimen containing the virus was furnished, and as the specimen was taken during the course of a routine sleeping sickness survey, nothing is known of the pathologic activity of this virus in man. It has, however, some relationship to the viruses producing encephalitides in man, since it is neutralized by immune sera to both the St. Louis and Japanese B viruses, although its corresponding immune sera lack any demonstrable capacity to neutralize these viruses.[81]

Smithburn, Mahaffy and Paul[82] in 1941 reported the isolation on different occasions of a virus associated with an illness whose only features were abrupt onset, fever and severe headache and backache—the disease has been named Bwamba fever after the county in the Western Province of Uganda where it occurred.

In a number of outbreaks of undoubted influenza it proved difficult or impossible to demonstrate that the etiologic agent was the classic influenza virus, now known as influenza A virus.[83] An explanation in part was provided in 1940 when Magill[84] and Francis[85] independently and almost simultaneously announced the isolation of a second influenza virus, now designated influenza B.[83, 85] While these viruses are completely distinct immunologically, the infections they produce can not be distinguished clinically one from another. Since these viruses, alone or together, fail to account for a considerable number of cases, depending on the epidemic, it is reasonable to assume the existence of additional influenza viruses as yet unrecognized.[86]

During the last few years there has been observed with increasing frequency a respiratory disease known by several names but usually referred to as atypical pneumonia (reviewed in [87]). A number of viral agents whose association with the disease is sometimes difficult to assess and whose relationship to each other is still indeterminate, have been isolated.[88] Of these agents the best known and most completely studied is the virus isolated by Eaton, Beck and Pearson[88c] in 1940 and called meningopneumonitis virus because of its close antigenic similarity to the meningopneumonitis virus isolated in 1938 by Francis and Magill[89] from ferrets inoculated with human throat washings and believed by them to have been present in their normal ferret stock. Recently acquired evidence[88c, 90] shows that the viruses of meningopneumonitis, lymphogranuloma venereum and psittacosis are related antigenically, and in addition are characterized by the formation of elementary bodies with similar tinctorial properties and by their ability to produce meningitis, pneumonia and granulomata in the skin of suitable test animals. So similar are these viruses in their properties that it appears not improbable that they are derived from a common progenitor and that by adaptation to different tissues and animal species they have acquired certain characteristics peculiar to themselves. Eaton *et al.*[88e] have recently isolated another virus-like agent from cases of atypical pneumonia and it will be interesting to know whether the new agent will fall into the lymphogranuloma venereum-psittacosis group.

During the summer of 1941 there occurred in Oahu,

[77] J. Casals and L. T. Webster, SCIENCE, 97: 246–248, 1943.

[78] a. F. M. Burnet, *Med. Jour. Australia*, 1: 679–681, 1934. b. J. R. Perdrau, *Jour. Path. and Bact.*, 42: 59–65, 1936.

[79] A. A. Smorodintseff, A. K. Shubladse and V. D. Neustroev, *Arch. ges. Virusforsch.*, 1: 549–559, 1940.

[80] K. C. Smithburn, T. P. Hughes, A. W. Burke and J. H. Paul, *Am. Jour. Trop. Med.*, 20: 471–492, 1940.

[81] K. C. Smithburn, *Jour. Immunol.*, 44: 25–31, 1942.

[82] K. C. Smithburn, A. F. Mahaffy and J. H. Paul, *Am. Jour. Trop. Med.*, 21: 75–90, 1941.

[83] F. L. Horsfall, Jr., E. H. Lennette, E. R. Rickard, C. H. Andrewes, Wilson Smith and C. H. Stuart-Harris, *Lancet*, 2: 413, 1940.

[84] T. P. Magill, *Proc. Soc. Exp. Biol. and Med.*, 45: 162–164, 1940.

[85] T. Francis, Jr., SCIENCE, 92: 405, 1940.

[86] E. H. Lennette, E. R. Rickard, G. K. Hirst and F. L. Horsfall, Jr., *Pub. Health Rep.*, 56: 1777–1788, 1941.

[87] J. H. Dingle and M. Finland, *New England Jour. Med.*, 227: 378–385, 1942.

[88] a. J. Stokes, Jr., A. S. Kenney and D. R. Shaw, *Tr. & Stud. College Physicians, Philadelphia*, 6: 329–333, 1939. b. J. M. Weir and F. L. Horsfall, Jr., *Jour. Exp. Med.*, 72: 595–610, 1940. c. M. D. Eaton, M. D. Beck and H. E. Pearson, *Jour. Exp. Med.*, 73: 641–654, 1941. d. J. A. Baker, SCIENCE, 96: 475–476, 1942. e. M. D. Eaton, G. Meiklejohn, W. Van Herick and J. C. Talbot, SCIENCE, 96: 518–519, 1942.

[89] Thomas Francis, Jr. and T. P. Magill, *Jour. Exp. Med.*, 68: 147–163, 1938.

[90] M. D. Eaton, W. P. Martin and M. D. Beck, *Jour. Exp. Med.*, 75: 21–33, 1942.

Hawaii, a large outbreak of a highly contagious, acute type of conjunctivitis.[91] By September the epidemic, which is thought to have had its origin in Malaya,[92] had spread to the Pacific Coast of the United States, where it involved chiefly shipyard workers. In the late summer and early fall of 1942 outbreaks of the disease occurred in New York, Schenectady and Hartford.[92] From cases in the New York outbreak of the disease, now designated epidemic keratoconjunctivitis, Sanders and Alexander[93] were able on two occasions to isolate in tissue culture a virus which has proved to be pathogenic for man and for mice and rabbits. The association of their virus with the human disease appears to be established, although further work is desirable and doubtless will be forthcoming.

In conclusion, it should be emphasized that this presentation has of necessity been limited in scope and that many interesting and important contributions to our knowledge of viruses have had, perforce, to be omitted. So intensively and so sedulously have viruses and the diseases due to them been studied in recent years that any attempt, such as this, to summarize briefly the recent advances in this field, can do no more than present the high lights.

[91] Editorial, *Jour. Am. Med. Asn.*, 118: 460, 1942.

[92] J. H. Dunnington, *Symposium on Epidemic Keratoconjunctivitis* held at the College of Physicians and Surgeons, Columbia University, School of Medicine, under the direction of the Division of Preventive Medicine, Office of the Surgeon General, Department of War, and in conjunction with the Commission on Neurotropic Virus Diseases, Board for the Investigation and Control of Influenza and Other Epidemic Diseases in the Army, December 4, 1942.

[93] M. Sanders and R. C. Alexander, *Jour. Exp. Med.*, 77: 71–95, 1943.

OBITUARY

FELIX AGUILAR

THE director of the Astronomical Observatory of the University of La Plata, Dr. Félix Aguilar, died suddenly on September 28, 1943. Mr. Aguilar was not only one of the leading astronomers of the Republic of Argentina, but he was also active as president of the Comisión para la Medición de un arco de meridiano en la República Argentina, president of the Comisión Nacional de Observatorios and member of the Comisión de límites Argentino-Chilena. He served as professor of geodesy in the University of La Plata and was a member of several scientific associations such as the Sociedad Científica Argentina and the Academia Nacional de Ciencias. At the La Plata Observatory his name was connected with the creation of the Escuela Superior de Ciencias Astronómicas y Conexas and with the Instituto Geográfico Militar, where he served as head of the geodesy department. He was active in the determination of the difference in longitude between Potsdam and Belgrano (Buenos Aires), and participated in the first gravimetrical and magnetic investigations of the Instituto Geográfico. In 1936 he was placed in charge of the reorganization of the Observatorio Astronómico Nacional at Córdoba. He was the author of many scientific papers and invented a method for the use of calculating machines in the determination of latitude by a method of Gauss. He undertook one of the few astronomical leveling operations that have ever been executed. A short time before his death he arranged for two of his associates, Dr. Carlos Cesco and Dr. Jorge Sahade, to come to the United States for research in astrophysics.

OTTO STRUVE

DEATHS AND MEMORIALS

DR. ALEXANDER G. MCADIE, from 1913 to 1931 professor of meteorology at Harvard University and for eighteen years director of the Blue Hill Observatory, died on November 1 at the age of eighty years.

DR. HENRY VINECOME ARNY, who retired in 1937 as dean of the College of Pharmacy of Columbia University, died on November 3. He was seventy-five years old. Dr. Arny had served as professor of chemistry at the College of Pharmacy from 1911 to 1937 and as dean of the college from 1930 to 1937.

DR. GLENN WARREN GOLDSMITH, since 1929 professor of botany and bacteriology at the University of Texas, died on October 28. He was fifty-six years old.

DR. IRA EDWARDS, curator in geology of the Milwaukee Museum, died on October 31 at the age of fifty years.

CHARLES A. DONNEL, a former head of the Office of the U. S. Weather Bureau in Chicago, principal meteorologist and supervising forecaster for the district, died on October 29. He was sixty-two years old.

A CORRESPONDENT writes: Dr. Wilmon Newell, provost for agriculture at the University of Florida, director of the Agricultural Experiment Station and Extension Service, and plant commissioner, State Plant Board, as already reported in SCIENCE, died on October 25 at the age of sixty-five years. After serving as entomologist in Iowa, Ohio, Texas, Georgia and Louisiana, he accepted the position of plant commissioner in 1915 with the newly created State Plant Board and directed the campaign which eradicated citrus canker in Florida. In 1929 he directed a similar campaign for the eradication of the Mediterranean fruit-fly; this work was completed in 1930. He was