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SOME BIOPHYSICAL PROBLEMS OF VIRUSES*

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BIOPHYSICAL methods have done much towards obtaining purified viruses and getting from them information useful for the control of disease. The following discussion is not a review[1] of this information but rather a statement of some of the problems which must be met as further progress is made. These problems are threefold, dealing (a) with the concentration, purification and physicochemical properties of viruses, (b) with similar studies of the specific anti-substances that are an animal's response to infection, and (c) with the deeper investigation of virus-antibody interaction that purification makes possible. Their answers are bound to indicate better ways of recognizing viruses and to help in the treatment of disease with antisera and in its prevention with vaccines.

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[1] Literature references to all but current papers can be found in any one of a number of reviews (see, for example, Lennette, SCIENCE, 98: 415, 1943) and will therefore not be repeated here.

Because of the size of their particles, purified viral suspensions must have the physicochemical properties associated with colloids. The methods of colloid chemistry were developed to study particles with sizes ranging downwards from about the lower limit of microscopic vision to the larger chemical molecules. For years it was presumed that the particles in all colloidal suspensions were heterogeneous aggregates of smaller particles or molecules, the prevailing sizes being determined more by physical conditions of formation than by ultimate chemical composition. Often this is true, as with inorganic sols, many polysaccharides and the polymers that are the basis of our new plastics, textile fibers and the like: their particles vary, often widely, about some mean value. But Svedberg's demonstration that the particles of many pure proteins are molecules as alike as other molecules in size and shape brought to light an entirely different type of colloid.[2] Some proteins have molecular

weights as low as a few thousands (such as the protamines), others as great as a few millions (for instance, the erythrocruorins). The thousandfold range in weight among them is as great as that between the simplest and the most complex of all other chemical compounds. Viruses extend this range of more or less homogeneous macromolecules or particles another thousandfold before they reach their largest representatives near the lower limit of ordinary microscopic vision. For much of this region they are the only examples yet known of equi-sized particles. But this is probably more because of the attention they have drawn to themselves as producers of disease than because they alone in nature have homogeneous particles of this size. In fact, such non-pathogenic substances are now being encountered through application of the biophysical methods developed to purify viruses. Many more will probably become known as the search for them is expanded.

The biophysical problems of viruses take precedence over those of antibodies and virus-antibody mixtures because purified virus is essential to a serious study of these other questions. The principal things we wish to know now about viruses are, first, how to obtain them pure and in quantities sufficient for the study of their fundamental properties; secondly, the nature, chemical composition, the sizes, shapes and electrical characteristics of their elementary particles, their absorption spectra and the changes produced in them by various forms of radiation and by mild chemicals, and lastly their relation to other macromolecular substances in living cells.

It is obvious that the problem of getting purified virus underlies all others and that the knowledge we can accumulate will be more or less proportional to the amount of purified material available for study. Two things have contributed to make the purification of viruses especially difficult. One has been the chemical instability of most, and the other has been the minute amounts present in many infected tissues. With a few notable exceptions this instability has prevented their successful manipulation by the chemical methods developed for the purification of proteins: it is this which has brought to the fore the gentler physical methods like ultracentrifugation. Most successful purifications have been made from tissues unusually rich in virus. The yields in these cases have with a very few exceptions ranged downwards from about one part by weight per thousand of original tissue to less than a part in a million. From this it is evident that few of the known viruses can now be obtained even in the milligram amounts needed for preliminary studies with purified material. Most studies, especially those of an exploratory character, must therefore be concerned with these few without regard to whether or not they are the most desirable objects from a medical standpoint. Tobacco mosaic, for instance, will continue to command especial interest simply because it can be prepared so easily and cheaply and in such exceptionally large amounts. The quantity of purified virus needed for infection—what might be termed the absolute infectiousness—varies greatly, depending on both the virus itself and its route of invasion; for this reason the percentage of virus in an infected tissue can not be predicted before purification. When infectiousness is high only a few particles weighing perhaps not more than *ca* 10^{-14} gram are needed to produce disease; with other viruses the average infectious dose may be many millions of times greater.

Chemical methods of purification have been applied to crude virus suspensions. The most familiar of these, involving fractional salting-out in strong sulfate solutions, has often been tried. Several plant viruses, including the original preparation of tobacco mosaic, were purified in this way but not without some alteration taking place. Human[3] and mouse[4] poliomyelitis viruses have been concentrated with ammonium sulfate; but here too there is reason to believe that the treatment does not leave the virus unchanged. Selective adsorption and subsequent elution is probably less drastic in its effects on viruses than the foregoing. Large amounts of infectious material can be handled and the potentialities of the method probably have not yet been fully exploited. It was shown years ago that the virus of human poliomyelitis[5] could be partially purified by adsorption on alumina. Specific adsorption of the influenza viruses on red cells[6] has provided an especially simple way of getting large amounts of virus freed from much of the accompanying extraneous material.

A variety of physical procedures has been employed in purifying viruses. Differential ultrafiltration has been used to sort out the virus particles from a suspension of infectious tissue, but the losses are ordinarily too great and the amount of infectious material that can be handled too small to make it a practical method for preparing chemically useful amounts. Partial purifications have also been made by cataphoresis and in a number of other ways, but the methods based on high-speed centrifugation have turned out to be far more useful than others yet proposed. Not long after the initial chemical isolation of the tobacco

[2] Svedberg and Pedersen, "The Ultracentrifuge" (Oxford, 1940).

[3] Clark, Schindler and Roberts, *Jour. Bact.*, 20: 213, 1930; Clark, Rasmusson and White, *Jour. Bact.*, 42: 63, 1941.

[4] Gard and Pedersen, SCIENCE, 94: 493, 1941.

[5] Sabin, *Jour. Exp. Med.*, 56: 307, 1932; Schaeffer and Brebner, *Arch. Path.*, 15: 221, 1933.

[6] McClelland and Hare, *Canadian Publ. Health Jour.*, 32: 530, 1941; G. K. Hirst, *Jour. Exp. Med.*, 76: 195, 1942.

mosaic virus it was shown that purified virus could be obtained in large amounts and with less alteration by differential ultracentrifugation; a number of plant and animal viruses have since been purified in this fashion. Present-day ultracentrifuges hold up to 200 cc and develop fields as high as *ca* 200,000 times gravity; machines of larger capacities could be built. They will sediment particles or molecules weighing less than a million and will concentrate lighter molecules with weights down to about 20,000. They can therefore easily handle all known viruses.

Determination of the degree of purity of a refined virus preparation has proved a most difficult problem. Infectivity titrations are useful in the early stages of purification, but they are rarely accurate measures of purity. The analytical ultracentrifuge will show whether the particles in a suspension sediment with the single sharp boundary to be expected if they are alike in size and whether a partially purified preparation still contains either large colloidal or small unsedimentable material. Accurately determined rates of sedimentation also characterize a virus once it has been sufficiently purified. There are, however, several important limitations to observations with the analytical ultracentrifuge as criteria of purity. Under the most favorable conditions less than several per cents of an impurity can not be detected; under other circumstances much larger quantities of undesirable material can be missed. For asymmetric particles such as those of tobacco mosaic, rate of sedimentation is a very poor index of uniformity in particle weight, since in such cases there can be wide differences in particle weight without corresponding differences in sedimentation constant. Sensitivity is further decreased, and the efficiency of the ultracentrifugal method of fractionation can be seriously impaired in viscous tissue suspensions. Though its use for this purpose is only just beginning the electron microscope should ultimately become very helpful in controlling the purity of virus preparations.

One of the first things to be found out about any purified macromolecular substance is the size, shape and degree of uniformity of its constituent particles. Ultrafiltration gives some idea of the particle size of viruses even when applied to impure suspensions, but it can only yield approximate results because ultrafilters do not have uniform pores and filterability depends on other factors besides pore size. The development of the analytical ultracentrifuge for molecular weight determination and the large mass of data accumulated with this instrument are well known. Of the two ways to find weights from centrifugal observations only that utilizing rates of sedimentation is applicable to particles as big as viruses. Besides these rates it is necessary to know other properties both of the purified virus particles—density and shape —and of its suspensions—viscosity, rate of diffusion and degree of hydration. Diffusion can be accurately measured by one of several experimental procedures and a rough estimate of particle shape can be derived from studies of viscosity, of streaming double refraction and from other more or less indirect observations. The density of virus particles can not be measured in a straightforward manner. Perhaps the best available estimates arise from comparisons of rates of sedimentation in media of different known densities, such as strong sugar[7] or albumin[8] solutions. How closely these values approach the densities of native virus particles depends in large measure on how much the particles are altered by the abnormal osmotic environment of such solutions; in this respect determinations in albumin should be superior to those in sugar. An interesting but as yet little-used technique[9] for getting densities involves measurement of sedimentation rates in dilute suspensions made up with ordinary and heavy water. In spite of prevailing inaccuracies ultracentrifugal measurements give data about the weights of virus particles which will become increasingly accurate as time goes on. Rates of sedimentation are best determined with the optical ultracentrifuge, but they can also be estimated from biological or chemical analyses[10] of successive layers in an undisturbed tube after quantity ultracentrifugation. This is not accurate, but it can be used with crude virus suspensions and with solutions too dilute to give the optical effects required in the first procedure.

The electron microscope provides a direct and independent way of estimating the sizes and shapes of viruses. Its greatest uncertainty lies in the sure identification of the particles of virus among the numerous objects of small size that can be seen in an average preparation. This uncertainty will of course diminish as more observations are made on material of greater purity. Probably the greatest inaccuracy in electron microscopic estimates of size lies in the present lack of knowledge of the amount of shrinkage and distortion involved in making the necessary preparations. The ability to see virus particles makes many interesting questions accessible to study. For example it supplies a direct way of determining (a) how closely direct observation agrees with deductions from ultracentrifugation and other indirect measurements, (b) what if anything particle-morphology as seen in purified virus prepa-

[7] Elford and Andrewes, *Brit. Jour. Exp. Path.*, 17: 422, 1936; Smadel, Pickels and Shedlovsky, *Jour. Exp. Med.*, 68: 607, 1938.

[8] Sharp, Taylor, McLean, Beard and Beard, SCIENCE, 100: 151, 1944.

[9] Lepine, Levaditi and Guintini, *Compt. rend.*, 214: 768, 1942.

[10] Elford and Andrewes, *op. cit.;* Pickels, *Jour. Gen. Physiol.*, 26: 341, 1942–3; etc.

rations tells about viruses under actual conditions of growth and multiplication, and (c) how uniform these particles are in size and shape. In a general way ultracentrifuge and electron microscope seem to corroborate one another when observations are made on comparable material. Thus purified preparations of the tobacco mosaic virus[11] exhibit the elongated threads predicted by the ultracentrifuge and its associated methods, while the particles seen in bushy stunt virus are the roundish bodies to be expected from sedimentation data. The pictures of the papilloma virus, however, show more spherically symmetrical objects than have been predicted from other measurements.[12] It has already been stated that the electron microscope should prove helpful in controlling the purity of virus preparations. Such control will come through the study of representative fields taking account of everything present instead, as is often now the custom, of concentrating on atypical fields showing only virus particles. It is reasonable to look to the electron microscope for direct information as to how viruses multiply. For this they must be examined not in purified preparations but as nearly as possible under the conditions in which they are produced, or reproduced, in nature. During the last few years there has been much fruitless discussion as to whether viruses are "alive." This debate has been futile, not because it is inherently meaningless, as is frequently stated, but because there have been no experimental data bearing on the question. Very probably the study and photography of viruses within infected cells could give such data. Two problems are involved in the electron microscopy of sectioned material. Suggestions have been made concerning the design of special microtomes to cut thin enough sections, but they may not be needed at this time since electron microscopic pictures can apparently be made of biological material a micron thick and there are standard microtomes that can cut down to half this thickness. The other and perhaps more difficult problem concerns handling and mounting these very thin sections so that they do not suffer too much distortion during desiccation. There has also been considerable discussion of the importance of devising ways of photographing living material[13] with the electron microscope. This probably would not be as valuable as one might think if only because Brownian movement in living cells is sufficient to blot out all structures so fine that it needs the electron microscope to reveal them.

[11] Stanley and Anderson, *Jour. Biol. Chem.*, 139: 325, 1941; 146: 25, 1942.

[12] Sharp, Taylor, Beard and Beard, *Proc. Soc. Exp. Biol.*, 50: 205, 1942.

[13] von Ardenne, *Naturw.*, 29: 521, 1941; Abrams and McBain, SCIENCE, 100: 273, 1944.

The particles of a virus can not justifiably be considered as very large chemical molecules unless they are uniform in size. None of the larger viruses, such as vaccinia or influenza, has yielded the sharp centrifugal boundaries to be expected from a suspension of uniform particles. Electron microscopy too indicates that the elementary bodies of these big viruses[14] vary considerably about a mean size, though its evidence can not be convincing until more is known about how these particles change on drying. On the other hand, the boundaries from several of the smaller viruses are very sharp. This points to a great uniformity in size when the particles are nearly spherical, as they are with bushy stunt, for example.[15] Very probably such particle-uniformity is one of the essentials for the growth of well-defined crystals. Where elongated particles are involved, as with the tobacco mosaic and long-chain polymers, sharpness of sedimenting boundary is not adequate evidence of uniform particle weight. This too is borne out by electron microscopic observations that show the tobacco mosaic as consisting of particles of various lengths. These viruses give fibrous aggregates rather than well-defined crystals. Knowledge of such incompletely crystalline materials is still fragmentary, but x-ray and electron diffraction is giving more and more information about them. Some of the x-ray diffraction effects from purified tobacco mosaic virus[16] are apparently due to incomplete regularities in particle arrangement and others to repetitions within the particles themselves. Electron diffraction should prove especially valuable in the study of structures of this sort because it requires very small amounts of material and will reveal regularities in films and other thin structures. Thus it becomes feasible, especially when electron microscopy is combined with diffraction to gain a deep insight into the details of many biological structures; the important work now being done on connective tissue[17] shows how much can be learned in this fashion. These techniques may teach much about the internal structure of viruses and even about their interaction with the cells they invade.

Since there is yet no straightforward way to establish the purity of a refined virus preparation it is important to use every indirect method capable of throwing light on the subject. Electrophoresis, by providing accurate rates of migration in an electrical field, is such a method. Except for patterns demonstrating the electrophoretic homogeneity of certain

[14] Green, Anderson and Smadel, *Jour. Exp. Med.*, 75: 651, 1942; Taylor, Sharp, Beard, Beard, Dingle and Feller, *Jour. Immun.*, 47: 261, 1943; 48: 129, 361, 1944.

[15] Lauffer, *Jour. Biol. Chem.*, 143: 99, 1942.

[16] Bernal and Fankuchen, *Jour. Gen. Physiol.*, 25: 111, 147, 1941–2.

[17] Jakus, Hall and Schmitt, *Jour. Am. Chem. Soc.*, 66: 313, 1944; etc.

plant viruses,[18] of the papilloma virus[19] and of vaccinia and its antigens,[20] little work of this sort has been done with purified viruses largely because existing techniques require more material than is ordinarily available. Some dyes combine and migrate with proteins; if they react similarly with purified viruses it may be possible to work with much less virus. It will also be important to determine how much the electrical properties of a virus are influenced by other components of the tissues in which it grows. The elementary bodies of vaccinia migrate at the same rate as its soluble heat-stable antigen. The virus of African horse sickness moves[21] at the speed of albumin and it has been suggested that this may be an index of an association between the two. A complete study of the electrical mobility of a virus gives an accurate knowledge of its isoelectric point. Many determinations of isoelectric point have been made on crude suspensions of virus-diseased tissues. Such results, however, can be very misleading because the isoelectric points of tissue proteins may be different from that of virus and the predominance of these proteins may thoroughly mask what is happening to the virus.

The fundamental question of chemical composition can be approached only with considerable amounts of fully purified virus. It is therefore inevitable that the chemical work now possible will center around a few viruses, like the plentiful plant viruses, influenza, vaccinia, the equine encephalomyelitises, the rabbit papilloma and mouse poliomyelitis. A satisfactory knowledge of virus composition will probably be reached only by a process of successive approximations since much analytical work will inevitably be done on material found later to be impure. Hence for some time to come all information about composition will be provisional. Probably all viruses are nucleoproteins. The small viruses appear to be only nucleoprotein, but if vaccinia[22] is typical the larger viruses contain fat, carbohydrate and many other constituents common to microorganisms. Influenza[23] has seemed similarly complex, but this can not be considered as proved since recent work[24] has demonstrated non-viral substances of like composition in refined virus and in the fluid of undiseased embryos. Unusual amounts of copper have been found in purified vaccinia. It will be interesting to find out whether small as well as large viruses show a concentration of metal and whether it is always copper. One of the most desirable things to know would be the amino-acid content of virus protein. This analysis by classical methods is so difficult and consumes so much material that there is no hope of getting enough of any virus except the tobacco mosaic for such a study. The use of heavy isotopes and of bacteria of known amino-acid nutritional requirements is creating new analytical procedures, however, and there are several unexplored physical approaches which if developed may make possible the analysis of viruses. The presence of histidine in the rib-grass but not in other strains of tobacco mosaic[25] suggest that viruses will show significant differences in their amino acids.

Our ideas that the chemical specificity of living tissue is uniquely dependent on its proteins may be drastically modified by the recent demonstration[26] that specific nucleic acids determine the transformation of one type of pneumococcus into another. In these experiments the addition of a definite chemical substance has for the first time altered in predetermined fashion the inheritable properties of a living cell. If this is not an isolated example, then an understanding of the nucleic acids of viruses will be as valuable as a knowledge of their specific proteins. It will be important to determine if the development[27] of transmissible myxomatosis by injecting killed myxoma and living fibroma viruses together into a rabbit is due to the myxoma's nucleic acid. From a chemical standpoint nucleic acids appear simpler than proteins; if they are as numerous as the proteins their differences from one another must reside in the size of their molecules and the structural relationships between the few different nucleotides from which they are built. Nucleic acid preparations have not been crystalline and x-ray diffraction has thus far given little useful information about them. It is now evident that they are at least as easily damaged as proteins; less altered material will be needed for further studies in the ultracentrifuge and by x-ray diffraction. It remains to be seen whether the electron diffraction and microscopic techniques will help.

Viruses have been especially easy to recognize through the diseases they produce, but we are now learning that other substances of similar particle size exist. These substances frequently complicate and even render impossible the purification of viruses. In fortunate instances they are less stable or sufficiently different in size to be separable by a selective procedure such as ultracentrifugation. But cases are be-

[18] Lauffer and Ross, *Jour. Am. Chem. Soc.*, 62: 3296, 1940; McFarlane and Kekwick, *Biochem. Jour.*, 32: 1607, 1938; Melchers, *et al.*, *Biol. Zentr.*, 60: 524, 1940.

[19] Sharp, Taylor, Beard and Beard, *Jour. Biol. Chem.*, 142: 193, 1942.

[20] Shedlovsky and Smadel, *Jour. Exp. Med.*, 72: 511, 1940; Smadel, Pickels, Shedlovsky and Rivers, *ibid.*, 72: 523, 1940.

[21] Polson, *Onderstepoort J. Vet. Sci.*, 16: 51, 1941.

[22] Hoagland, *et al.*, *Jour. Exp. Med.*, 71: 737; 72: 139, 1940; 74: 69, 133, 1941; 76: 163, 1942.

[23] Taylor, Sharp, McLean, Beard, Beard, Dingle and Feller, *Jour. Immun.*, 48: 361, 1944.

[24] Knight, *Jour. Exp. Med.*, 80: 83, 1944.

[25] Knight, *Jour. Am. Chem. Soc.*, 64: 2734, 1942.

[26] Avery, MacLeod and McCarty, *Jour. Exp. Med.*, 79: 137, 1944.

[27] Berry, *Arch. Path.*, 24: 533, 1937.

ing found where viruses have much the same size and stability as components of the healthy tissues involved. Mouse lungs infected with influenza[28] provide an example. Ultrafiltration or centrifugation of normal lungs yields a suspension of particles about 100 mμ in diameter. Infectiousness is found associated with particles of this size when infected lungs are similarly treated, and it has required much work to prove that the virus itself is not a far smaller entity adsorbed to "healthy" 100 mμ particles. Allantoic fluid from infected embryos is an especially valuable starting point for the preparation of purified influenza virus, but even with this favorable material the end product is often not more than 50 per cent. virus. A similar situation has been described[29] from the study of infectious jaundice, or grasserie, of silkworms. In a recent ultracentrifugal investigation it was shown that the virus activity was sedimented at the rate of 17×10^{-13} s units; but there are macromolecules in healthy silkworms which sediment at the same rate, 16×10^{-13} s. The non-infectious macromolecules just referred to are not isolated instances of such substances. No survey has yet been made to determine how widespread they are in living tissues and we know only about those that have been encountered by accident. Many healthy plants contain macro-substances of moderate stability and complexes have been isolated which are unstable chlorophyll-protein compounds.[30] Macromolecular substances have been prepared from the tissues as well as from the allantoic fluid of healthy chicken embryos. Materials of the same order of size can be extracted from brain, spleen and from a wide variety of other tissues. It has been shown that macromolecules from lung[31] are carriers of strong thromboplastic activity. With this exception little is known of the functions of these macromolecules and whether they are primary constituents of cells or products of cell disintegration. It has been suggested that what was isolated from chicken embryos were either mitochondriae or threads of chromatin from cell nuclei. Evidently much more work will be required before there is a real understanding of these "normal" substances.

A knowledge of the mechanism of virus inactivation has become especially important with the proof that killed virus vaccines give effective protection against infection. This mechanism can only be studied with the help of pure viruses. Practical vaccine production also is increasingly concerned with purified viruses because, everything else being equal, vaccines made from them will be superior to those made with crude tissue suspensions. As yet there is little understanding of the changes that take place in a virus particle during inactivation either by chemicals or radiation. Effective killed virus vaccines are made with formaldehyde, but the reaction is in fact unknown and we are in no position to say whether the essentially empirical procedures now employed in making vaccines are the most desirable ones. A knowledge of the absorption spectrum of purified viruses provides a similar sound basis for investigations into the best way to produce vaccines through photo-inactivation. The absorption spectrum of a pure protein sometimes gives limited information about its composition. Of the few purified viruses thus far examined[32] all but the yellow fever virus have the not especially characteristic spectrum of a nucleoprotein with intense absorption around 2,600A and rather complete transmission everywhere else in the near ultraviolet and visible regions. For the yellow fever virus, however, extraordinarily intense absorption has been reported[33] from about 3,200A to the visible. The reason for this is not known, but the virus must either contain an intense chromophoric component or be associated through chemical combination or otherwise with some intensely absorbing tissue or serum protein. If this yellow fever absorption is diagnostic for virus it permits a purely physical assay of the minute amounts that are biologically significant.

Many of the problems of viral antibodies are the same as those already discussed in connection with the viruses. Compared with what is known about other antisera our knowledge of sera against viruses is very scanty. This is largely due to the relative weakness of most such sera, as shown for instance by electrophoretic analysis, and to the difficulty of making quantitative measurements of reactions between these poor sera and crude virus suspensions. Purified viral antibodies are therefore needed for fundamental biophysical work; to get them involves both the production of more potent sera and the concentration and purification of the antibodies thus produced. Sera of the highest antibody content are only obtained after often-repeated injections of large amounts of immunizing antigen. Since there is little virus by weight in most infectious tissues it is not surprising that hyperimmune sera made with them contain little viral antibody even when their neutralizing titres seem high. Their electrophoretic patterns show this clearly. It has been repeatedly demonstrated that hyperimmunization with bacteria, toxin or a purified protein enhances the small gamma-peak of a normal animal serum or introduces a new peak nearby which in-

[28] Chambers and Henle, *Jour. Bact.*, 42: 434, 1941; *Am. Jour. Path.*, 17: 442, 1941; *Jour. Exp. Med.*, 77: 3, 1943; Stanley, *Jour. Exp. Med.*, 79: 267, 1944.

[29] Glaser and Stanley, *Jour. Exp. Med.*, 77: 451, 1943.

[30] Smith and Pickels, *Jour. Gen. Physiol.*, 24: 753, 1940–1; Fishman and Moyer, *ibid.*, 25: 755, 1941–2; etc.

[31] Chargaff, Moore and Bendich, *Jour. Biol. Chem.*, 145: 593, 1942.

[32] Taylor, Sharp, Beard, Finkelstein and Beard, *Jour. Inf. Dis.*, 69: 224, 1941; etc.

[33] Pickels and Bauer, *Jour. Exp. Med.*, 71: 703, 1940.

creases with continued hyperimmunization till it predominates over all other features of the pattern. No antiviral serum has yet shown this, and few[34] that have been examined have contained enough antibody to provide a detectable electrophoretic peak, new or enhanced. It may be that hyperimmune sera will never be of value in the treatment of most viral infections, but before drawing such a conclusion at this time it is well to remember how hard it would be to prove the undisputable curative qualities of antipneumococcal or antidiphtheretic sera using preparations containing no more antibody than do present-day antiviral sera. Good hyperimmune sera against many viruses probably can not be produced in any considerable amounts until future discovery provides richer sources of virus-antigen; nevertheless, progress can now be made by working with purified viruses and by developing immunizing schedules with adjuvants that will best utilize what virus is available.

More potent antiviral sera will in themselves be purer, but it should be possible to purify them further by the kind of chemical fractionation applied to other sera. Some viral antibodies, such as those against hog cholera or the measles antibody in human blood, are concentrated by salting-out, but it is commonly supposed that others, such as some sera against encephalomyelitis, are easily destroyed by these procedures. Very little can be said about the chemical fractionation of viral antibodies until much more careful work has been carried out. In a few trials viral antibodies have not shown the pronounced resistance to peptic digestion that characterizes antitoxins and makes their purification so easy; the possibilities of using milder methods of digestion under carefully controlled conditions should be thoroughly explored.

Purified virus and antibody permit a completely new approach to problems of virus-antibody interaction. More knowledge of this interaction is needed for gauging the prophylactic and therapeutic possibilities of viral sera; it is equally essential to any serious attempt to develop and refine the various methods of virus and antibody detection based on it. There has been prolonged discussion as to whether the product of virus-antibody interaction is fundamentally different from bacterial-antibody and from other antigen-antibody combinations. The root of this debate lies in observations[35] that certain non-infectious virus-antibody mixtures become sources of disease when sufficiently diluted. This apparent recovery of free virus has been taken to prove that the virus-antibody complex dissociates readily. It is of the greatest importance to know whether this is correct because it would set an obvious limit to the sensitivity that could be attained by any diagnostic method based on the virus-antibody interaction. All that can be said at present is that, while dissociation may occur, there are other plausible explanations[36] of the dilution phenomenon, and crucial experiments have not yet been made. In any event the most direct way to increase the sensitivity of virus and antibody detection lies in finding out how to recognize minimal amounts of virus-antibody interaction. Complement-fixation is based on interactions involving virus or the associated soluble antigens, and great progress[37] has lately been made in identifying virus infections by using purer antigens and more carefully controlled conditions of test; it is not known whether the technical difficulties in converting it into a micro-method can be met. Several other ways of recognizing traces of virus-antibody suggest themselves. One of these would take advantage of our ability to see single virus particles under the electron microscope. To the degree that there is a firm union between virus and antibody it is reasonable to seek evidence for this in some alteration in the appearance of elementary virus particles or in their association together. This seems to occur when antibody is added to the tobacco mosaic virus.[38] An increase in sensitivity of virus and antibody detection may also be possible through the use of foreign particles coated with virus. As is well known, such particles, of silica or collodion, for example, may cover their surfaces with an adsorbed protein and thereby take on many of its physicochemical properties.[39] There have been several claims[40] of successful precipitation tests diagnostic for viruses performed with bacteria and with collodion particles acting as adsorbents, though careful studies[41] have failed to confirm these results. Foreign particles will behave as viruses only if their surfaces are completely virus-coated and it would seem hard to achieve this condition with crude virus or by adsorption on bacteria whose surfaces must be complex records of the varied conditions of their growth. Experiments of this type should therefore be repeated with purified virus and with collodion or other particles prepared under carefully controlled conditions. Microelectrophoretic examination of virus-coated par-

[34] Sharp, Taylor, Beard and Beard, *Proc. Soc. Exp. Biol.*, 50: 358, 1942.

[35] Sabin, *Brit. Jour. Exp. Path.*, 16: 70, 84, 158, 169, 1935; Beard, Taylor, Sharp and Beard, *Jour. Inf. Dis.*, 69: 173, 1941; etc.

[36] Morris, *Jour. Immun.*, 48: 17, 1944; Hershey, Kalmanson and Bronfenbrenner, *ibid.*, 48: 221, 1944; etc.

[37] Casals and Palacios, Jour. Exp. Med., 74: 409, 1941; *Am. Jour. Pub. Health*, 31: 1281, 1941; Havens, Watson, Green, Lavin and Smadel, *Jour. Exp. Med.*, 77: 139, 1943; Casals, SCIENCE, 97: 337, 1943.

[38] Anderson and Stanley, *Jour. Biol. Chem.*, 139: 339; 140: 3, 1941.

[39] Abramson, Moyer and Gorin, "Electrophoresis of Proteins" (New York, 1942).

[40] Goodner, SCIENCE, 94: 241, 1941; Roberts and Jones, *Proc. Soc. Exp. Biol.*, 47: 75, 1941; 49: 52, 1942; Weil, *Jour. Immun.*, 45: 187, 1942; Weil, Popken and Black, *ibid.*, 48: 355, 1944.

[41] Pearson, *Jour. Immun.*, 49: 117, 1944.

ticles[20] may provide another way of detecting traces of antibody, as well as of measuring the electrical properties of purified viruses that can not be obtained in amounts sufficient for the usual electrophoretic procedures. Some antigen-antibody reactions alter the viscosity[42] and the light-scattering of systems in which they occur; it is not yet known if these things happen when the antigen is a virus. Whether the most useful method turns out to be one of the foregoing, or some other, it seems certain that better ways of detecting minimal amounts of antibody will result from the use of purified viruses; and as antibodies are purified they will in the same fashion enhance the sensitivity of virus-detection.

OBITUARY

FREDERICK SLOCUM

AFTER an illness which had confined him to the college infirmary for about six weeks, Dr. Frederick Slocum, professor of astronomy at Wesleyan University and director of the Van Vleck Observatory, died on December 4 at the age of seventy-one years.

Professor Slocum had three leading interests, scientific research, teaching and the sea; and these three interests seemed to share his energy and his affections without dividing them. His knowledge of sailors, ships and fish enriched his teaching; and sailing and fishing gave him needed relaxation from his administrative and teaching duties.

He was born at Fairhaven, Massachusetts, on February 6, 1873, the son of Frederick and Lydia Ann Jones Slocum. His father was captain of a whaling ship, and Frederick's early years were spent in a nautical environment. During the long summers on Cuttyhunk Island he became skilful in sailing and in navigation and acquired a love of the sea which he never lost, although in later years his profession sometimes required him to live far inland.

In 1891 Mr. Slocum entered Brown University, and for eighteen years as student and teacher he was connected with that institution, receiving the degrees of A.B., A.M. and Ph.D. in 1895, 1896 and 1898, respectively. A fourth Brown degree, the honorary doctorate of science, was conferred upon him in 1938. After receiving the baccalaureate degree, he served as instructor in mathematics for five years and then as assistant professor of astronomy for nine years. During this period he was profoundly influenced by Professor Winslow Upton, by whom he was inspired to make astronomy his life work.

In 1899, while still at Brown, Dr. Slocum married Carrie E. Tripp, who was his constant companion at home and abroad until her death in 1942.

During the years spent at Brown in close association with Professor Upton, Dr. Slocum became an outstanding teacher. He remained a teacher for the rest of his life, but in 1908–09, while on leave from Brown, a year spent as volunteer assistant in the Royal Astrophysical Observatory of Potsdam marked the beginning of a fruitful research career.

After the year in Germany, Dr. Slocum returned to America to join the staff of the Yerkes Observatory of the University of Chicago. Here he worked with the recently invented spectroheliograph, studying the circulation of the solar atmosphere, and he was one of the first to call attention to the fact that matter from solar prominences seemed to move toward and into near-by sunspots. In addition to the solar work Dr. Slocum continued the stellar parallax program started by Schlesinger, introducing the use of Wallace's color filters to produce sharper photographic images.

The years at Yerkes were devoted primarily to research, but Dr. Slocum was still a teacher, and graduate students who came to Williams Bay for the observational part of their training soon learned that in him they could find a skilful guide and a friendly counselor.

In 1914 Dr. Slocum was called to Wesleyan University as professor of astronomy. There his first task was to plan the new Van Vleck Observatory and supervise its construction. In choosing for the principal instrument of the observatory a twenty-inch visual refractor, he had in mind the continuation of his stellar parallax observations and also the requirements of a fairly extensive teaching program. The observatory staff soon increased to four in number, and under Dr. Slocum's leadership a regular program of parallax and other astrometric observations was maintained until the establishment of a Naval Flight Preparatory School at Wesleyan in 1942 made it necessary for the members of the staff to devote all their time to teaching. Although beyond the usual retiring age, Professor Slocum carried his full share of the teaching load until failing health necessitated his retirement on November 1, 1944.

The continuity of Professor Slocum's work at Wesleyan was broken by an absence of three years from 1917 to 1920, when he served for one year as instructor in navigation for the United States Shipping Board and for two years as professor of nautical science at Brown University.

During his year at Potsdam and during several later trips to Europe, Professor Slocum made many friends among the astronomers of other lands, and

[42] du Noüy, "La Température critique du Sérum" (Hermann et Cie, Paris, 1936), Chap. III.