THE NATURE OF VIRUSES

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Viruses are usually characterized by three negative properties, namely, invisibility by ordinary microscopic methods, failure to be retained by filters impervious to well-known bacteria, and inability to propagate themselves in the absence of susceptible cells. I prefer a positive characterization of the viruses, one emphasizing the intimate relation that exists between them and their host cells. The multiplication of viruses only in the presence of susceptible cells, their regeneration and production of disease in many instances in only one species of host, the marked stimulation and destruction of cells induced by their activity which on the one hand gives rise to tumors, such as Rous' sarcoma, and on the other to vesicular lesions, as fever blisters, the intracellular pathology frequently evidenced in virus diseases by inclusion bodies, and, finally, the lasting immunity that follows the majority of virus maladies, are essential phenomena that serve to stress the intimate type of parasitism encountered in working with these active agents. Such a characterization of viruses implies much, not only as concerns their biological nature which is still a moot question, but as regards their activities about which something is definitely known.

Data concerning the nature of viruses are sufficiently adequate in quantity but distinctly lacking in quality. According to reports, some of which have come from eminent investigators, most of these active agents have been seen and have been cultivated on lifeless media. If such statements are correct, viruses are autonomous living agents, and further discussion of their biological nature should deal with their place in the scale of living entities and their relation to other forms of life. Reports of work in this field are confusing, however, particularly to the uninitiated, and critical investigators are of the opinion that enough reliable data have not been acquired to establish the nature of the viruses. Inasmuch as this is a subject of fundamental biological importance, I shall review some of the recently accumulated data regarding the size, electrical charge, purification, spontaneous generation, adaptations, elementary bodies, metabolism, immunological phenomena and

cultivation of viruses that might be of assistance in the elucidation of the origin and constitution of these peculiar incitants of disease.

SIZE. The size of minute particles may be determined in several ways, namely, by direct mensuration provided the objects are capable of resolution under the microscope; by filtration and ultrafiltration if the factors that influence the passage of the particles through pores of graded diameters are known and controlled; by diffusion, and, finally, by centrifugation. All of these methods have been employed in the study of the magnitude of viruses and the results obtained will be discussed.

It is generally accepted that an object less than 0.2 μ or 200 $\mu\mu$[1] in diameter is not capable of resolution under the microscope when ordinary light is used. Furthermore, it is understood that mordants and stains usually increase the magnitude of small particles. Some of the "larger" viruses, *e.g.*, those of fowl-pox (109), smallpox, vaccinia (122, 123), and rabies, are said to be just visible after treatment with certain mordants and dyes. Consequently, one is justified in concluding that most of the viruses have a diameter of less than 200 $\mu\mu$ and in an unstained state are not mensurable by means of ordinary light. Moreover, figures regarding their size derived from stained preparations are apt to be inaccurate and misleading. The use of light of short wave lengths makes possible the mensuration of particles smaller than 0.2 μ in diameter. So far, however, this method of investigation has yielded no convincing evidence concerning the magnitude of viruses. It appears, therefore, that direct methods of mensuration only indicate that the active agents are considerably smaller than ordinary bacteria.

The sizes of at least eight viruses have been estimated by means of ultrafiltration, diffusion, or centrifugation. The results obtained for these active agents together with figures for the diameter of the hemoglobin molecule for comparison are given below.

Hemoglobin. For a number of years the molecule of hemoglobin was thought to be 30 $\mu\mu$ in diameter. Recently, however, figures (34) derived from the results of Svedberg and Nichol's (33) centrifugation experiments and Northrop and Anson's (30) diffusion experiments with hemoglobin indicate that its diameter is approximately 5.5 $\mu\mu$. Many estimations regarding the magnitude of viruses have been based on the former figure for the diameter of the hemoglobin molecule, 30 $\mu\mu$. If this figure is incorrect, many statements concerning the size of viruses are also inaccurate.

Mosaic virus. Duggar and Karrer (17) by means of ultrafiltration found the

[1] In this paper the symbol $\mu\mu$ represents 10^{-6} mm., a millimicron.

infectious particles of mosaic virus to be of the same order of magnitude as hemoglobin molecules, namely, 30 $\mu\mu$ in diameter. Vinson (34), however, says that Duggar's experiments interpreted in the light of recent work regarding the size of hemoglobin molecules indicates that the diameter of mosaic virus is about 5.5 $\mu\mu$.

Herpetic virus. Zinsser and Tang (38) by means of ultrafiltration estimated the diameter of herpetic virus to be 20–100 $\mu\mu$. Levaditi and Nicolau (27) in the same way found that the virus passed through membranes which retained toxins, hemolysins, complement, and serum globulins. Bedson (13), however, unable to confirm Levaditi and Nicolau's (27) results, obtained evidence by centrifugation that herpetic virus is probably of sufficient size to be visible.

Foot-and-mouth disease virus. Olitsky and Boëz (31), using ultrafiltration, found that the virus of foot-and-mouth disease is 20–100 $\mu\mu$ in diameter. Elford[2] by means of his special membranes estimated it to be 8–12 $\mu\mu$.

Poliomyelitic virus. By means of ultrafiltration, Krueger and Schultz (25), in 1929, found that the virus of poliomyelitis possesses a magnitude not greater than 300 $\mu\mu$. In 1931, by the same means, Clifton, Schultz, and Gebhardt (16) obtained results indicating that the diameter of the virus lies below 50 $\mu\mu$.

Fowl plague virus. By means of ultrafiltration Andriewsky (8) secured a figure of 2.5 $\mu\mu$ for the diameter of fowl plague virus, while Bechhold and Schlesinger (11) by centrifugation found it to be 120–130 $\mu\mu$.

Bacteriophage. According to d'Herelle (22) and Elford (19), both of whom used ultrafiltration, the diameter of the bacteriophage is approximately 30 $\mu\mu$. Kruger and Tamada (26) by means of purified bacteriophage preparations and ultrafiltration found it to be 5 $\mu\mu$, and Hetler and Bronfenbrenner (24) by means of a diffusion method estimated it to be 1.2–22.8 $\mu\mu$.

Rous virus. According to Zinsser and Tang (38), the Rous virus is 20–100 $\mu\mu$; according to Mendelsohn, Clifton and Lewis (29), 50 $\mu\mu$; according to Fränkel (20), 10 $\mu\mu$. All these workers obtained their figures by means of ultrafiltration.

Vaccine virus. Levaditi and Nicolau (27) reported that vaccine virus passes through membranes impervious to toxins, hemolysins, complement, and serum globulins. Bland (15), however, from the results of his centrifugation experiments not only concluded that Levaditi and Nicolau's findings are incorrect but that vaccine virus is probably large enough to be seen. Bechhold and Schlesinger (11) by means of centrifugation estimated that the active agent is 210–230 $\mu\mu$ in diameter, while Yaoi and Kasai (37) working with "purified" virus found that it diffused at the rate of fuchsin particles and is, therefore, not capable of being seen.

One cannot consider the results cited above without being amused and dismayed. Many of the workers seemed in no way concerned about the possibility that they might have been estimating not the magnitude of viruses, but the size of particles of degraded cells to which the viruses were attached. Other investigators, cognizant of the diffi-

[2] Report made before the Physiological Section of the British Association for the Advancement of Science, September 28, 1931.

culties of the problem, attempted to remove the viruses from such carriers. They were unable, however, to be assured that they had been successful and that they had obtained the correct figures for the size of the different viruses.

From the results of indirect methods of mensuration it is safe to conclude that viruses are small and that some of them may be exceedingly minute. If the figure of 210 $\mu\mu$ for the diameter of vaccine virus is accurate, there is no reason as far as size is concerned to suppose that the virus is not a living organism. On the other hand, if the figures of 1.2 $\mu\mu$, 5.5 $\mu\mu$, and 8 $\mu\mu$ for the bacteriophage, mosaic virus, and foot-and-mouth disease virus, respectively, are correct, it is obvious that these agents cannot be highly organised, because it is impossible that with such a magnitude they can consist of more than one, or at most several, molecules of protein. Unfortunately, none of the figures can be accepted without reservations. At present the exact size of no virus is known.

The numerous discordant results encountered in the literature dealing with the filterability, size, and visibility of viruses are probably due to inadequate experimentation, careless thinking, prejudice, imperfect experimental methods, and the difficult nature of the problems. One of the great needs at present is improvement in methods of microscopy, filtration, and purification of viruses in order that results obtained will approximate the true size of viruses and not the size of particles of other sorts on which the agents are adsorbed. It must be remembered, however, that the determination of the size of one virus will not establish the magnitude of another, because no more uniformity of dimensions should be expected among these agents than is found among bacteria and protozoa. Furthermore, it is not possible to derive proof of the animate or inanimate nature of viruses even from a correct estimation of their diameters, for, within limits as yet undetermined, life and death are not functions of size.

ELECTRICAL CHARGE. Most bacteria and proteins under ordinary biological conditions of hydrogen ion concentration carry a negative electrical charge.[3] When the nature of the viruses became a question of interest, attempts were made to ascertain their behavior in an electrical field in order that it might be compared with the action of proteins and bacteria under similar circumstances. Some of the results obtained with nine viruses are stated below.

[3] The pH range over which they are electronegatively charged depends on their isoelectric point.

Bacteriophage. Kligler and his co-workers (41) using a so-called "protein-free" bacteriophage found that the active agent was amphoteric in acid and decidedly alkaline solutions and chiefly negatively charged in neutral and mildly alkaline solutions. Krueger and his associates (42) stated that the bacteriophage is negatively charged between the hydrogen ion concentrations of 9.0–3.4, and positively charged at pH 3.35. Todd (48) found that the active agent carried a negative charge between the hydrogen ion concentrations of 3.36–7.6. The results of Natarajan and Hyde's (43) experiments indicate (1) that bacteriophages for typhoid bacilli and Flexner's dysentery bacilli are only electronegative between pH 4.9–9.3 and 5.4–9.3 respectively, (2) that small plaque coliphage is electronegative below pH 8.3, but with greater alkalinity moves to both poles, and (3) that large plaque coliphage is electronegative over a range of pH 5.4–6.1, while at a higher alkalinity it wanders to both poles.

Rabic virus. According to Glusman (40) and his associates fixed rabic virus is negatively charged over a range of pH 6.0–9.3.

Vaccine virus. Douglas and Smith (39) found that vaccine virus carried a negative charge between the hydrogen ion concentrations of 5.5–8.4. The experiments of Yaoi and Kasai (49) revealed that between pH 6–7 more virus collected at the positive than at the negative pole, and between pH 8–9 the active agent was demonstrable only at the anode.

Fowl-pox virus. Kligler and his co-workers (41) found fowl-pox virus in "protein-free" preparations to be positively charged on the acid side, amphoteric in neutral solutions, and negatively charged in alkaline solutions. According to Natarajan and Hyde (43), the active agent is amphoteric over a range of pH 6.4–9.3.

Foot-and-mouth disease virus. Olitsky and Boëz (44) believe that the virus of foot-and-mouth disease is positively charged, while Sichert-Modrow (47) is of the opinion that the active agent carries a negative charge over a range of pH 7.0–8.1.

Pcliomyelitic virus. According to Olitsky, Rhoads, and Long (45) poliomyelitic virus wanders to the anode.

Rous virus. Pulcher (46) found that the Rous virus was adsorbed on electropositive and not on electronegative hemoglobins and concluded that the active agent is negatively charged.

Virus of infectious myxomatosis of rabbits. According to Natarajan and Hyde (43), the virus of infectious myxomatosis of rabbits is electronegative over a range of pH 4.9–9.3.

Herpetic virus. Natarajan and Hyde (43) found herpetic virus to be electronegatively charged only between the hydrogen ion concentrations of 7.0–8.9.

From the results of the work cited above it is obvious that most workers have found that under ordinary biological conditions of hydrogen ion concentration certain viruses in an electrical field wander to the anode. Moreover, many investigators have stated that the viruses under these conditions are negatively charged and in this respect are similar to bacteria, cells, and numerous proteins. Others, however,

aware of the fact that virus preparations usually consist principally of proteins and bits of degraded cells from the host, realize that the electrical charges determined might not be those of the virus particles themselves but of their carriers, *i.e.*, material on which the virus particles are adsorbed. It is true that a few experiments have been performed with "protein-free" preparations of viruses. But an examination of the methods of purification fails to convince one that such purified viruses had been completely separated from their carriers. Therefore, at present it is impossible to state definitely what electrical charge is carried by the viruses.

PURIFICATION. Inasmuch as virus-containing emulsions consist chiefly of substances unrelated to the active agents themselves, it is natural that workers should attempt to obtain the viruses either in a pure or in a relatively pure state. Moreover, it is being realized that purified viruses are essential for the proper study of problems in this field, such as the estimation of the size of viruses, the determination of their electrical charge, and the investigation of immunological responses excited by them. In addition to the fact that purified viruses are of practical value, it is obvious that such preparations will also be of value to investigators interested in the theoretical problem of the nature of viruses. Indeed, Murphy (52, 55) has already concluded from the results of his experiments on the purification of the Rous agent that this disease-incitant is neither a virus nor a living organism. He states, "It is hardly conceivable that the active fraction which I have thus succeeded in obtaining, a substance purified by repeated precipitations, could carry with it through all these manipulations any living organism or virus. To me the enzyme-like nature of the principle seems to have been conclusively established. . . . ." However, most workers do not believe that Murphy is justified in concluding from the results of such experiments that the Rous agent is not a virus, because at least eight other viruses, *e.g.*, the incitants of infectious myxomatosis of rabbits (58), foot-and-mouth disease (64), bacteriophagy (50, 56, 57), fowl-pox (56), vaccinia (66, 68), rabies (66), poliomyelitis (65), and mosaic disease (67) have been subjected to manipulations similar to those used by Murphy and have been obtained, still active, in various states of purity.

Most methods of purification of viruses are based on the principles of precipitation by a variety of chemicals and selective adsorption and elution as used extensively in enzyme work. As yet, it is unlikely that a virus has been obtained in a state of absolute purity. Nevertheless,

the results already secured are encouraging and should excite further investigations. It may be possible in this way to attain eventually a more accurate concept of the nature of some viruses. For instance, it may be shown that in certain purified virus preparations the number of nitrogen atoms for each infectious unit or particle is insufficient to warrant the supposition that the agents are living, organized structures. Krueger and Tamada (57) have already suggested this viewpoint.

CULTIVATION. In the literature of twenty years ago it is not uncommon to encounter reports in which it was claimed that viruses had been successfully cultivated on lifeless media. These reports have not been confirmed and at present such claims are rarely made. A few, however, have been made in recent years. Frosch and Dahmen (78) stated that they were able to cultivate the virus of foot-and-mouth disease on ordinary media. But the German, English, and American Foot-and-Mouth Disease Commissions were unable to confirm their work. Olitsky (91) reported the cultivation of mosaic virus in a cell-free medium. Nevertheless, upon repeating his work he (92) has been forced to conclude that true multiplication of the virus was not obtained. Recently, Eagles and McClean (75, 76) reported that vaccine virus is capable of regeneration in a cell-free medium. A careful examination of their papers, however, leaves one in doubt as to whether some of their media were cell-free, and as to whether multiplication of the virus occurred in the nutrient materials that undoubtedly contained no cells. In my laboratory (86, 90, 93) during the last four years, Haagen, Muckenfuss, Li, and I have made numerous attempts to cultivate vaccine virus in cell-free media, many of which were similar to if not identical with those employed by Eagles and McClean. None of our efforts was successful. On the other hand, the cultivation of vaccine virus in the presence of cells surviving *in vitro* has been more consistently successful in our hands and in Maitland's (88) than it has been in Eagles and McClean's.

Although the cultivation of viruses in lifeless media has not been accomplished, it is generally conceded that these agents are capable of pullulation in the presence of susceptible cells either surviving or growing *in vitro*. The viruses of Rous' sarcoma (72), Virus III infection of rabbits (69), herpes febrilis (70), fowl-pox (77), vaccinia (79, 80, 86, 88), rabies (94), foot-and-mouth disease (83, 84, 85, 89), vesicular stomatitis (73), infectious myxomatosis of rabbits (71, 81), fowl plague (82), and probably the agents causing common colds (74) and poliomyelitis (87), have been cultivated in the presence of tissues surviving *in vitro*.

Moreover, the characteristic of species specificity possessed by many viruses is frequently reflected in their *in vitro* cultivation. For instance, fowl-pox virus (77), innocuous for mice and rats, does not regenerate in cultures of their tissues. Foot-and-mouth disease does not attack chickens and the virus (89) does not grow in cultures consisting of minced chick embryo and plasma. In addition to a species specificity, some viruses exhibit in cultivation experiments a predilection for certain kinds of cells. Fowl plague virus (82) multiplies in the presence of chick embryo skin and brain, but does not regenerate in pure cultures of fibroblasts. Foot-and-mouth disease virus (85) increases in amount when the culture medium contains minced guinea-pig embryo, but does not grow when fibroblasts or bits of heart muscle alone are present. Thus it appears that many viruses are capable of multiplication in tissue cultures and frequently retain under such conditions their species and cellular specificity. Nevertheless, it will be interesting to observe the results of further attempts to circumvent this species and cellular specificity of viruses by *in vitro* methods of cultivation.

A crucial experiment, if there be one, to decide the question of the autonomy of the viruses is their undisputed cultivation on lifeless media. It may be impossible, however, to accomplish such an experiment with all of the viruses, because some of them may be obligate parasites, as is the malarial organism. Thus in the quest for proof of the nature of viruses, we may find that many of them are invisible and incapable of regeneration in the absence of living susceptible host cells. Such a state of affairs will prevent, for a time at least, a complete definition of the nature of these peculiar incitants of disease. Nevertheless, we should obtain all the facts and make the most of them in the study of biological phenomena and in the better understanding and control of disease. For this purpose it is not absolutely essential to see and to cultivate the viruses on simple media any more than it is imperative to see and to know what electricity is in order to study the phenomena produced by it and to control its activity for our daily needs.

METABOLISM. Much of the discussion concerning the nature of viruses has centered around the question as to whether they are animate or inanimate. In this relation, one would like to know what the evidence is regarding independent metabolic activities of these active agents. Technical difficulties have hindered this type of experimentation with viruses. Nevertheless, a few investigations (95–99) have been made, the results of which were negative. One must not conclude from such negative results, however, that viruses do not possess an

independent metabolism and are, therefore, inanimate substances, because the methods used for the detection of the metabolic activities may not have been sufficiently delicate.

ADAPTATION. Certain viruses inoculated into new hosts apparently undergo changes in some of their characteristics. Smallpox virus (100, 101) passed through monkeys to rabbits and calves and then back to man is no longer smallpox virus but vaccine virus, and the disease, vaccinia, caused by it is not contagious as is smallpox. The incitant of yellow fever (106, 107) passed through a large number of mice by means of intracerebral inoculations loses much of its pathogenicity for monkeys when inoculated intravenously or intraperitoneally, but gains the power of producing a transmissible encephalitis in monkeys receiving the inoculum in the brain. Such phenomena are spoken of as adaptations of viruses to new hosts, and, inasmuch as adaptation is considered a characteristic of living rather than lifeless material, they have been cited by some investigators (103) as proof of the animate nature of the viruses. On the other hand, workers, who believe that viruses are products of cellular perversion, state that the changes observed in the characteristics of the active agents when they are inoculated into alien hosts are to be expected, inasmuch as mouse, rabbit, monkey, and human cells, because of intrinsic differences, may not always manufacture identical substances as the result of similar stimuli. Therefore, they contend that the changes and adaptations are not accomplished by the agents themselves but by their hosts and, consequently, are not admissible as proof of the living nature of the viruses.

INCLUSIONS. Within the nucleus and cytoplasm of cells injured by viruses, certain peculiar structures, inclusion bodies, are frequently observed. Although many of these bodies are of importance in diagnostic and experimental work, numerous opinions exist concerning their nature. Lipschütz believes that the inclusions in many diseases (119) consist of compact masses of virus particles, yet he is of the opinion that such structures in measles (120) are nothing more than altered central bodies. Goodpasture (113) thinks that Negri bodies in rabies are composed of degenerated mitochondria and neurofibrils, while Levaditi (118) and Manouélian (121) consider them protozoa and designate them, respectively, *Glugea lyssae* and *Encephalitozoon rabiei*. Goodpasture and his associates (124, 125) have demonstrated that the incitant of fowl-pox is intimately associated with the Bollinger bodies which are made up of a lipoid capsule within which numerous small coccoid bodies are embedded in a protein matrix. On the other hand,

Glaser (112) has presented evidence that the polyhedral bodies, the characteristic inclusions in wilt diseases of caterpillars, consist of noninfectious crystalline protein. Thus, it appears that inclusions may arise in a number of ways and that they may or may not contain virus. Consequently, generalizations regarding these peculiar structures are hazardous at present.

The small coccoid bodies found in fowl-pox by Borrel (109) and in vaccinia by Paschen (122, 123) appear to be extremely minute organisms. In fact, one is justified in asking why these bodies are not convincing evidence of the organismal nature of certain viruses. The first reason is that one cannot by morphological and tinctorial data alone determine whether autonomous life exists in such small objects. Another reason is the fact that Goodpasture, while holding the belief that the small coccoid bodies in fowl-pox (124, 125) represent the virus, stated that similar structures, seen in rabic brains (113) and considered of etiological importance by Babes (108) and Koch (114–116), are probably degenerated mitochondria. Moreover, Borrel (110) has described similar bodies in other virus diseases the etiological agents of which have been shown by ultrafiltration to be incapable of resolution by microscopic methods. Furthermore, Craciun and Oppenheimer (111), who cultivated the small bodies of vaccinia and showed that they are closely associated with the virus, made the following statement, "We have from these studies no morphological proof of an increase in the number of granules, since they cannot readily be distinguished from other granules normally seen in tissue cultures." Finally, mitochondria in some respects resemble bacteria. They may decrease or increase numerically within cells, and their size and shape may be altered by appropriate stimuli. At times, they actually divide. Nevertheless, mitochondria are not considered autonomous living agents. Consequently, so far as I am aware, there is no convincing evidence—the specific agglutination of virus elementary bodies (184) by antiviral sera will be discussed later—to invalidate the conception that cells under the stimulus of viruses may react by the formation of numerous small coccoid bodies uniform in size and intimately associated with the stimulating agents. One would not consider such bodies microorganisms or hold that they consist of virus alone. Therefore, in spite of definite proof that viruses are present in certain types of inclusions, doubt still exists regarding the organismal nature of the small coccoid bodies found within them.

Other features observed in pathological processes induced by viruses,

e.g., hyperplasia and necrosis, are fully as important as are the inclusion bodies. The excessive stimulation of cells seen in some virus diseases, *e.g.*, fowl-pox and warts, leads one by analogy to think of malignant neoplasms. Undoubtedly a number of fowl tumors are caused by agents separable from cells, and, although there is no proof that mammalian tumors arise in this way, the possibility is worthy of consideration and offers an attractive field for work. The fact, however, that some tumors are produced by filterable agents is by no means conclusive evidence that all neoplasms (217) arise through the activity of such incitants.

VIRUSES AS FILTERABLE FORMS OF BACTERIA. For a long time a few investigators have held that certain virus diseases are induced by ordinary bacteria. Now that attention is being focused on filterable forms of bacteria, workers in increasing numbers (128, 131, 132, 134, 135) are adopting the belief that viruses are merely filterable, invisible, and noncultivable elements of ordinary bacteria. It has been claimed, and evidence of a kind has been offered to substantiate the assertions, that the bacteriophage (165, 166) is a form in the life cycle of lysogenic bacteria, that the viruses of yellow fever (131, 134, 135) and hog cholera (134, 135) are invisible forms of *Leptospira icteroides* and *B. suipestifer* respectively, that the etiological agent of scarlet fever (134, 135) is a filterable form of hemolytic streptococci, and that the incitants of poliomyelitis, epidemic encephalitis, fox encephalitis, common colds, measles, and influenza represent certain stages in the life cycle of green streptococci (131). Without going into details of the available knowledge of bacterial life cycles and their invisible and noncultivable forms, one can say that proof of many of the claims regarding them is lacking. In fact, if certain reports are correct, some of the filterable forms of bacteria are much smaller than are many of the viruses. Kendall (131) recently stated that "egg white, filtered through Berkefeld W filters (after dilution with sterile physiological saline solution) is rarely sterile." Such a statement raises embarrassing questions for workers in the virus field because many viruses will not pass through W filters. Since the existence of bacterial life cycles is doubtful, it seems unwarrantable to offer the presumptive filterable forms of them as evidence upon another unsolved problem, the nature of the viruses.

EFFECT OF PHYSICAL AND CHEMICAL AGENTS ON VIRUSES. Many years ago it was discovered that bile and saponin are injurious to protozoa but with a few exceptions are innocuous for bacteria. Consequently, when the question of the nature of viruses began to attract

attention, tests were made to determine what effect bile and saponin have on these incitants of disease. Many viruses, *e.g.*, rabic virus (141, 144), were found to be inactivated and because of this fact certain workers concluded that they are protozoa. Sufficient exceptions, however, have been encountered to invalidate the test as a means either of separating bacteria from protozoa or of defining the nature of viruses. The agent causing Rous' sarcoma (140) is more resistant to ultraviolet light than are bacteria, and Murphy (220) considers this fact as evidence in favor of his hypothesis of the enzyme-like nature of the virus. On the other hand, bacteriophage (139), the living nature of which many doubt, is just as sensitive to ultraviolet light as are bacteria. Sanderson (153), using a temperature of −78°C., found no diminution in the titer of two strains of bacteriophage subjected to 20 successive freezings and thawings. Since bacteria and cells are killed by repeated freezing and thawing, he concluded that bacteriophage must be something other than a living organism. Rivers (151) showed, however, that colon bacilli, Virus III, vaccine virus, herpetic virus, bacteriophage, complement, and trypsin are all either killed or inactivated by repeated freezing (−185°C.) and thawing and that, as might be expected, some of the agents are more resistant than are others. Hence it is obvious that destruction or inactivation of an active agent by repeated freezing and thawing is not evidence that it possesses life. The observations on heat, desiccation, oxidation, and the effect of dyes have likewise yielded no convincing evidence concerning the nature of viruses. Thus it appears that a number of tests with chemical and physical agents have been devised as criteria for the presence of life or to define the nature of viruses, but no one of them has been found satisfactory.

SPONTANEOUS GENERATION OF VIRUSES. The origin as well as the nature of viruses constitutes a question of interest. The intimate relation between these active agents and their host cells has induced more than one investigator to view the host cell as the source or origin of viruses. Indeed, reports of experimental work have appeared leading to claims that normal cells have been induced to manufacture certain viruses. According to Carrel (156, 157), minced chick embryo mixed with tar, indol, or arsenic and injected into normal chickens in a small percentage of instances gives rise to tumors resembling Rous' sarcoma no. 1 and transmissible by cell-free filtrates. Fischer (163) by treating cultures of normal cells with arsenic obtained on one occasion a filterable agent capable of causing tumors. Carrel was unable to confirm Fischer's work. Murphy (52, 167), by means of a method the details

of which have not been described, reported that he was able to extract a filterable tumor-producing agent from the gonads of normal-appearing Plymouth Rock roosters. Recently, Hadley and his co-workers (166) stated that it is possible to obtain bacteriophage from normal bacterial cultures by means of enforced dissociation. Although no worker in this field has claimed to have generated living organisms from inanimate matter, it appears that a few believe that they have by certain manipulations induced cells to yield substances which possess some of the attributes of life, notably that of increasing without limit.

The observations described above are suggestive, and, if confirmed and found to warrant the interpretation given them by Carrel, Murphy, Fischer, and Hadley, will prove to be of fundamental biological importance. Unfortunately, however, all of the experiments yielding the observations referred to were conducted in laboratories where workers were actively engaged in the study of agents similar to those supposedly brought into existence. In such laboratories and with such materials it is always difficult for one to rule out the possibility of contaminating normal animals, tissues, bacteria, emulsions, and filtrates. This fact has long been appreciated by workers in vaccine virus[4] laboratories and it delayed the acceptance of the experimental transformation of smallpox virus into vaccine virus. Therefore, experiments of the nature described should never be conducted in rooms used for the study of agents similar to those for which a search is being made. The workers who believe that they have induced viruses to come into existence have not excluded the possibility of the preëxistence of latent viruses or of small amounts of virus in the supposedly normal embryos, gonads, chickens, and bacterial cultures utilized in the experiments. This possibility is emphasized by Flexner's (164) work on poliomyelitis, for he was able to demonstrate the presence of virus in the nasal washings from normal contacts. The possibility outlined is further emphasized by Andrewes and Miller's (155) experience with Virus III in rabbits, by Cole and Kuttner's (158) work with the salivary-gland virus in guinea pigs, and by the work upon virus carriers in general among animals, plants (168) and bacteria.

IMMUNITY. Most virus diseases lead to a marked and lasting immunity in recovered hosts. Not only are the hosts refractory to reinfection, but in their sera antibodies capable of neutralizing the viruses are

[4] See Nicolau, S. and L. Kopciowska. Sur la vaccine spontanée épizootique du lapin, Compt. Rend. Soc. Biol., 1931, cviii, 757.

demonstrable. What bearing have these facts upon the nature of viruses? In the first place, it is certain that viruses are highly antigenic. Furthermore, if our concept of the nature of antigens is correct, the viruses are proteins or are closely linked to proteins. Moreover, the agents are not only antigenic but they give rise to antibodies different from those excited by proteins of the host cells. This is true even of the bacteriophage (188). These facts have been adduced as evidence of the exogenous rather than the endogenous origin of the viruses. Thus the antigenic nature of viruses appears to be prejudicial to the idea that they are products of cellular activity. The notion, however, that a lifeless agent may be injurious to the cell creating it and that it may induce immunological responses independent of those excited by the cell, loses some of its fantastic qualities when one considers the well-known facts that lens protein is not species specific but organ specific and that sympathetic uveitis in the uninjured eye is caused not by microörganisms but by the reaction of the body to substances derived from injured cells of the other uveal tract.

In addition to the neutralizing antibodies, whose mode of action is not clearly understood, complement-fixing antibodies and antibodies causing flocculation in virus emulsions have been described. Schultz and his associates (191–195) contend that the latter types of antibodies are not excited by viruses and that the immunological phenomena in virus maladies are comparable to those induced by toxins. In spite of their contentions, sufficient evidence has been adduced by different workers to make it more than likely that certain virus diseases lead to the production (176, 177, 180, 199) of the antibodies mentioned. Furthermore, Ledingham (184) has recently demonstrated that Borrel bodies in fowl-pox and Paschen bodies in vaccinia are specifically agglutinated by antifowl-pox and antivaccinal sera respectively. The results of these experiments indicate to Ledingham that the elementary bodies are living organisms and represent the virus. There is no reason to doubt that specific agglutinations of the bodies occurred in the manner described by Ledingham, and one cannot deny that such a phenomenon is presumptive evidence of the organismal nature of the bodies. Yet one dare not say categorically that his experiments are unequivocal evidence that the elementary bodies represent virus alone, because it has been shown by Jones (182, 183) that collodion particles treated with a variety of proteins and then thoroughly washed are specifically agglutinated by the proper antisera. Thus, the Borrel and Paschen bodies without being organisms yet having virus adsorbed on them

might nevertheless be specifically agglutinated by appropriate antiviral sera.

Gye (181) states that Rous virus repeatedly injected into alien hosts excites two groups of antibodies, one of which acts on the virus itself, while the other operates on the "specific factor" derived from the host cell. According to him, either set of antibodies inactivates the virus. This fact is offered by him as further evidence of the dual nature of the causative agent of fowl tumors. Murphy (189) and Sittenfield (196–198) have reported the presence in Rous' sarcoma of a substance that inhibits the action of the etiological agent, and the first mentioned worker is of the opinion that the "inhibitor" differs from ordinary virus antibodies. The presence of this "inhibitor" together with other phenomena has induced Murphy (220) to believe that immunity to the Rous agent is unlike that observed in virus maladies and lends evidence to his view that the Rous agent is not a virus. Inhibiting substances, however, have been obtained from tissues infected with viruses, for example, a substance restraining the action of rabic virus has been demonstrated by Marie (186) in the brains of rabid animals. Furthermore, Andrewes' (172, 173) work appears to indicate that the immune responses excited by the filterable agents of fowl tumors may not be unique and may possess much in common with those encountered in other virus diseases.

From what has been said, it is obvious that immunological phenomena are playing an important rôle in discussions concerning the nature of viruses. As yet this method of approach has not brought us to a definite solution of the problem.

CONCEPTS OF THE NATURE OF VIRUSES. A review of the data by means of which one arrives at a concept of the nature of viruses has been presented. Now it will be interesting to see what notions certain workers have concerning some of them.

Beijerinck (202) considers the virus of mosaic disease to be a living contagious fluid; Woods (228), an oxidizing enzyme; Goldstein (212), a protozoal parasite; Vinson (67), an inanimate chemical substance. Most workers, however, believe that it is a minute living organism.

Högyes (216)[5] is of the opinion that the incitant of rabies is an enzyme or, "alternatively, that the tissues themselves might spontaneously become virulent as the result of changes in their chemical composition." At one time Remlinger[6] said, "The rabies virus, which is at once filter-

[5] See A. C. Marie (218), p. 12.

[6] See A. C. Marie (218), p. 22.

able, diffusible and capable of reproducing the disease from case to case, appears to occupy a place midway between the microbes and the diastases." Recently, however, he (221) has published an article on the evolution of the *parasite* of rabies. Levaditi (118) and others (121, 225) have presented evidence in favor of the idea that the causal agent is a protozoon. The majority of investigators hold the concept that the incitant is a living organism whose nature is not definitely known.

Numerous workers believe that the bacteriophage is an inanimate agent, while others are convinced that it is a living organism. Ideas, however, concerning the nature of the inanimate transmissible substance or the animate organism vary. For details of the different concepts one is referred to papers by Twort (226, 227), d'Herelle (103), Bordet (203), Bronfenbrenner (95), Burnet (206), and Hadley (165, 166).

The incitant of fowl-pox has been described by certain investigators as a protozoal parasite. Sanfelice (222, 223) suggested that it is a nucleoproteid poison manufactured by infected cells. Borrel (109), Goodpasture (124, 125), and Ledingham (184) hold that it is a minute coccoid organism capable of regeneration in parasitized cells.

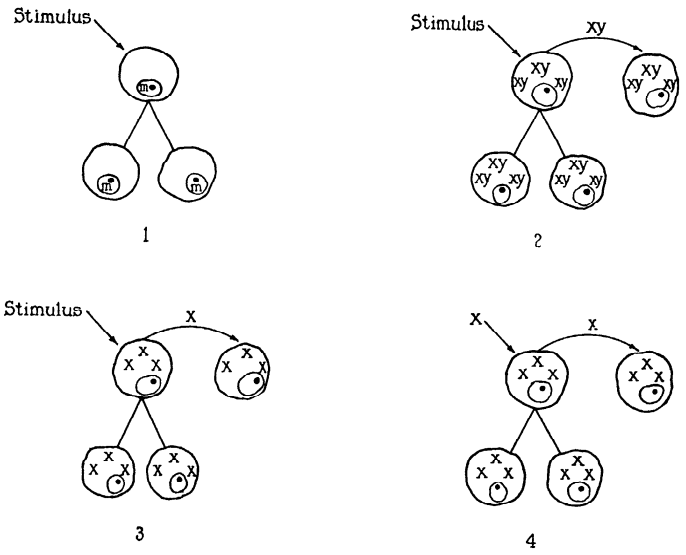
Rous and others are prepared to entertain the idea that the causal agent of Chicken Tumor No. 1 is animate. Gye (215) believes that it consists of two factors, one of which is a living exogenous organism, the other an inanimate specific factor derived from infected cells. Murphy (52, 55), at one time, spoke of the Rous agent as an enzyme-like substance. Recently, however, he (220) has compared it to filterable substances capable of transforming melitensis (204, 205) into paramelitensis organisms and of converting one type specific pneumococcus (201) into another type specific form. In regard to the matter he says (220), "Thus we have a group of agents, products of specialized cells capable of conferring the peculiar type quality to undifferentiated cells of the same species which, in turn, may produce the active factor and transmit this to their descendants." For this type of agent he proposes the name transmissible mutagens.

Sufficient ideas concerning the nature of viruses have been cited to illustrate how radically some differ from others. Many of them, particularly the ones dealing with the origin and reproduction of inanimate substances that behave in a manner similar to that of living organisms, lack precision. In a general way, however, the different concepts can be arranged in groups and it seems advisable to state and to portray diagrammatically several of the popular ones.

According to one conception, certain stimuli produce changes within

cells that are inherited by daughter cells. Once the mutations occur, cells of the new type continue to be formed though the stimuli disappear. No agents separable from the cells are demonstrable, and immunological phenomena in this type of disease differ from those observed in virus maladies. Ordinarily this idea of the causation of disease and the concepts concerning the nature of filterable viruses are not grouped together. Yet in some respects they are not dissimilar and many hold the view that malignant neoplasms arise in some such way. See figure 1.

Another notion is that appropriate stimuli induce normal cells to



Figs. 1, 2, 3 and 4

make a substance x which is closely bound to parts y of the cells. Thus an xy complex is formed. This complex, separable from the cells, yet capable of inciting its own production by them, either passes directly into daughter cells, or, having become extracellular, enters another set of normal cells. The xy complex is antigenic, and cells freed from it presumably become normal again. See figure 2.

Still another idea is that certain stimuli incite normal cells to produce a substance x which is not closely bound to parts of the cells. X, separable from cells, yet capable of impelling its formation by them, either passes directly into daughter cells, or, having become extra-

cellular, enters a new group of normal cells. X is antigenic and cells freed from it presumably become normal again. See figure 3.

Finally there is the concept most generally held that x is not a product of the perverted activity of cells but is a minute living organism. X enters cells, multiplies, produces disease, is separable from cells, and is antigenic. Cells freed from it presumably become normal again. At times, x is absorbed by particles y of host cells and evidences of an xy complex are obtained. See figure 4.

For practical purposes it makes little difference which one of the last three concepts is accepted. Theoretically, however, x of the second and third conceptions is quite different from x of the fourth. In the second and third, x, a product of cellular perversion, is an inanimate agent, while in the fourth it is an autonomous organism. No unequivocal evidence of the validity of any of the concepts has been adduced.

CONCLUSION

The confused state of our knowledge of the viruses at the present time makes it exceedingly difficult to define the nature of these active agents. The easiest way out of the dilemma, however, would be the acceptance of the presumptive evidence that viruses are minute organisms. Yet the easiest way and the one that best fits the experiences of the day may not be the right one. Furthermore, excessive skepticism and the habit of too readily accepting presumptive evidence are equally productive of sterility. Unless viruses represent a form of life unknown to us, proof of their living nature would not be a striking discovery. If, however, some of them are not animate, absolute proof of such a fact would be of fundamental biological importance. Therefore, care should be exercised that immoderate skepticism on the one hand, and the mental satisfaction secured by accepting presumptive evidence on the other, do not dull our efforts to obtain a better understanding of the viruses, some of which may be minute organisms, while others may represent forms of life unfamiliar to us, while still others may be inanimate transmissible incitants of disease. In any event, we are face to face with the "infinitely small in biology," and, if there be a sharp demarcation between life and death, then scientists, investigating the nature of viruses, are working near the line that separates infinitely small living organisms from inanimate active agents.

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