DEVELOPMENT OF IDEAS ON THE NATURE OF VIRUSES

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The idea that a disease can be contagious, that is, caused by direct or indirect contact with another case of the disease, is old. It appears in the Old Testament and the Decameron, to cite popular writing as evidence of a more or less general acceptance, and it was set out in a manner acceptable to scientists by Fracastorius (1546). Until bacteria had been recognized by the early microscopists no suggestions, based on evidence, were made about the nature of the infective agents. During this period the word virus was used indiscriminately to cover agents, such as snake-venoms, which cause pathological states that are not transmissible from one victim to another, and also infective agents. The restriction of the word to infective agents came with Pasteur's unequivocal demonstration of the role of bacteria in causing disease, and it was then used for any infective material, whether an organism had been recognized in it or not. As an example of this broad use Pasteur may be quoted (1889); the translation by Ruffer is used because it appeared a year earlier than the french version of this article.

> Moreover, it is not difficult to prove that rabies is a disease which cannot appear *de novo* under any physiological conditions, and that its spontaneous origin is quite impossible. We know nowadays that contagion or virulent affections are caused by small microscopic beings which are called microbes. The anthrax of cattle, malignant pustule of man, is produced by a microbe; croup is produced by a microbe . . . The microbe of rabies has not been isolated as yet, but judging by analogy, we must believe in its existence. To resume: every virus is a microbe. Although these beings are of infinite smallness, the conditions of their life and propagation are subject to the same general laws which regulate the birth and multiplication of the higher animal and vegetable beings.

On the strength of this and some other passages the suggestion that the agents causing certain diseases could be smaller than the visible bacteria is sometimes attributed to Pasteur. It is clear however that he wisely refrained from making any suggestion to explain his inability to find a rabies organism.

Ivanowski (1892) found that the sap from plants suffering from tobacco mosaic retained its infectivity after passage through a Chamberlain filter-candle but, being convinced that the disease was due to a bacterial infection, he argued that the symptoms might be due to bacteria penetrating the candle or to a toxin secreted by the bacteria. He could have disproved the latter suggestion by demonstrating that plants which showed symptoms as a result of inoculation with the filtrate had an infective sap, but he does not appear to have done so. This experiment was carried out with foot-and-mouth disease by Loeffler & Frosch (1898). They tried to make a vaccine by removing the infective particles from vesicle-lymph by passage through a filter-candle, but

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Many antigens owe their specificity to the polysaccharide moiety of the whole, among them those of the blood-groups and those responsible for type-specificity of pneumococci. A study of the former compounds is being carried out by Morgan in Britain (Morgan, 1944).[1]

[1] [*BMB* 422 & 555]

One of the factors that has held up the progress of biochemical genetics has been that only reactions involving relatively-simple compounds have been susceptible of analysis. Advances in our chemical knowledge of proteins, nucleic acids and polysaccharides cannot fail to provide chemical genetics with new methods and new sources of information.

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were surprised to find that the filtrate still produced symptoms of disease. Although well aware of the high dilution at which foot-and-mouth lymph is infective, they argued that the effect could not be due to a toxin because the lymph from animals that had been inoculated with a filtrate produced symptoms. As an alternative they suggested that the agent, although small enough to pass through the pores of the filter, multiplied in the affected animal. They also suggested that a group of other diseases might have a similar cause. In the same year, the significance of passage through a porcelain filter was also fully appreciated by Beijerinck (1898). He filtered tobacco-mosaic virus and also demonstrated the diffusion of the infective agent through agar gels. These experiments led him to suggest that the agent was not " corpuscular " but was what he called *contagium vivum fluidum.*

1. Size as a Criterion

It is not easy, at the present time, to understand what precise distinction, other than the purely quantitative one of particle-size, Beijerinck was making. Atomic theories were already 2,400 years old, and the atomic and molecular conception of matter had achieved general acceptance during the 19th century. The reality of atoms was, it is true, still doubted by Ostwald and other members of the " Energetics " school, but more perhaps as a logical exercise than as a matter of practice. Clerk Maxwell spoke confidently, in an article in the Encyclopaedia Britannica (1875), of their reality, and even made some estimates of the number of molecules in the smallest organisms and emphasized the bearing of this subject on current theories of heredity. However, as late as 1909, Perrin entitled his masterly survey of the various independent methods by which Avogadro's number could be determined " Mouvement brownien et réalité moleculaire ". When Beijerinck wrote, the nature of matter was in doubt, and even those who believed in molecules were uncertain of their size. Biologists at that date may therefore be forgiven for imagining that a fundamental qualitative difference existed between the corpuscular or particulate and the soluble or fluid states of matter; there is less excuse for the traces of the same belief that still linger in the writings of some pathologists.

Filtration through a candle was established as a practically-useful, but arbitrary, upper size-limit to the group of viruses. This was at first expressed in the phrases " filtrable microbe " or " filtrable virus ", but usage and the fact that diseases resembling the virus-diseases in other ways are sometimes not transmissible by filtrates, has led to the adoption of the simple word virus to cover this ill-defined group. There is no rigid boundary between the large viruses and the small bacteria, and a discussion on the correct category for an agent like that of pleuropneumonia might have value in law but has none in science. The lower limit of size is equally uncertain. The factors causing capsule-formation in rough strains of pneumococci are generally looked on as " substances " rather than viruses, but this distinction is not logical. Transformation is associated with the production of more of the factor, so that one has the phenomenon of multiplication of the factor in susceptible organisms. Avery, MacLeod & McCarty (1944) have evidence that transformation is due to a polymerized nucleic acid. The possibility of virus-like action with simpler substances remains open. Plant-galls can be produced by treatment with auxins; if the gall were itself rich in auxin, the state of affairs would be formally indistinguishable from an infection. An extract of the anomalous tissue would produce more of the characteristic anomaly when put on to healthy susceptible tissue. No example of this phenomenon is yet known, but it may be confidently predicted that examples will be found and that the effective auxins will be synthesizable. There will then be a smooth gradation, without any qualitative break, from synthesizable molecules at one end to microscopically-visible organisms at the other.

2. The Physico-chemical Approach

In the first quarter of this century the problem had been defined, certain infective states were classified as virus-diseases, and limits could be put to the size of the effective agents. It was not immediately obvious that the nature of a virus was a suitable theme for biochemical investigation, because our knowledge of the sizes of other biologically-important colloids was still meagre. It is unlikely that anyone giving serious thought to the matter doubted that viruses contained protein, because the unique role of proteins in biological phenomena had long been accepted; but protein-sizes had not been measured. Some tentative figures were derived from the elementary composition of proteins, especially from the iron content of haemoglobin, but it was not until the decade 1920–30 that osmotic pressure and ultracentrifugal measurements gave figures of generally-accepted validity.

The narrowing of the gap between the larger proteins and the smaller viruses stimulated many workers to investigate viruses from a chemical or physico-chemical standpoint. Two early purifications, that of vaccinia virus by Craigie (1932) and of a *Bact. coli* virus by Schlesinger (1933, 1936), were outstandingly successful. These viruses lack the geometrical or electrical simplicity that is a prerequisite for crystallization or the exhibiting of other striking physical properties, but it is probable that the preparations made in 1932 contained as high a proportion of active virus in the original state as most virus-preparations that have been described since. They were certainly more fully entitled to the designation " pure virus " than any plant-virus preparation made before 1940. Several attempts to purify tobacco-mosaic virus were made. Barton-Wright & McBain (1933) tentatively identified it with a protein-free crystalline material that they made from infective plant-sap. This suggestion was received sceptically by biochemists. A more confident identification by Stanley (1935) was at first similarly received. This scepticism was fully justified, for all the novel properties ascribed by Stanley to his crystalline globulin have proved not to be properties associated with the virus. The properties that are now accepted as those of tobacco-mosaic virus in its aggregated state were set out by Bawden, Pirie, Bernal & Fankuchen (1936) and were more fully defined by Bawden & Pirie (1937a) and by Bernal & Fankuchen (1941). Stanley has, from time to time, incorporated these properties in his description of the virus. This promotes unanimity but leads to the impression that his early description was confirmed rather than contradicted. Some parts of the description, e.g., the formation of tactoids in strong neutral solution, and fibres or " para-

crystals" rather than true crystals on precipitation with acid or on salting out, were accepted by Stanley almost immediately. Other parts were accepted more slowly, e.g. the invariable presence of nucleic acid in active preparations (Loring & Stanley, 1937; Stanley & Loring, 1938), and then only after argument (Bawden & Pirie, 1937b). The properties found with these early preparations are those of aggregates (Bawden & Pirie, 1937a; 1938c; 1946); this very important point has not yet been accepted by Stanley. It will be referred to more fully later. The sap of plants infected with various other viruses has yielded characteristic nucleoproteins which are either the viruses or are related to them. Some of these resemble tobacco-mosaic virus in forming liquid crystals, e.g. potato X (Bawden & Pirie, 1938a) and potato Y (Bawden & Pirie, 1939); others form true three-dimensional crystals, e.g. bushy stunt (Bawden & Pirie, 1938b), tobacco necrosis (Pirie, Smith, Spooner & McClement, 1938; Bawden & Pirie, 1942) and turnip yellow mosaic (Markham & Smith, 1946).

3. Microbes or Molecules?

The preparation of so many plant-viruses in forms with characteristic chemical and physical properties has fostered two unfortunate illusions: that there is a qualitative distinction between plant- and animal-viruses and that plant-viruses have been shown to be substances or molecules rather than organisms. More than 200 plant-viruses or virus-strains are known, and only about 20 have been subjected to serious chemical or physical study. These have been selected for detailed study largely because they give a highly infective sap, which retains its infectivity in spite of the various changes that go on in ageing leaf-extracts. These are more or less chemical criteria, and any uniformity which appears to run through the group of plant-viruses is at least as likely to be due to this initial selection as it is to be due to a genuine uniformity in the viruses. Animal-viruses have, from a chemical point of view, been chosen at random; that is to say they have been chosen for their social or economic importance. Those so far investigated, notably by Beard and his associates, have tended to be larger and more complex chemically than the plant-viruses, but it would be premature to make any generalizations until many more have been investigated.

The distinction between molecules and organisms, in any region where this distinction could be uncertain, is purely verbal. In so far as it can be defined, it is between materials that are handled by the methods characteristic of a biochemical laboratory and materials handled by methods characteristic of a biological laboratory. But many well-known materials are habitually handled in both ways. We all agree that *Bact. coli* is an organism, but when it is being disintegrated to make an enzyme-preparation or hydrolyzed as a prelude to amino-acid analysis, it is being treated as a substance and, but for the preliminary agreement, might be looked on as a molecule. The position with viruses is similar. No one has demonstrated that some of them are substances or macromolecules. Indeed, such a thing is not susceptible of demonstration; it is not a statement about the viruses but about the mental approach and technique of the research-worker. It has, however, been amply demonstrated that, as Beijerinck suspected, valuable information about their properties can be won by the methods of biochemistry.

Valuable as the contributions of biochemistry to the study of viruses have been, they are not without danger. The application of some chemical methods to the study of a group of substances does not guarantee the amenability of these substances to all other chemical criteria. Uniformity is the most important of these. In the study of the simpler molecules, on which the main structure of chemistry rests, any difference in composition between two molecules is quantitatively significant because the weight of an atom is an appreciable fraction of that of the molecule. Even the smaller proteins are so large that this is no longer true, and the replacement of one amino-acid residue by a different but similar one would not be detected by any of the elementary analyses or physical measurements so far made. *A fortiori* such a replacement would not be detectable in a virus. In the absence of any possibility of demonstrating that all the particles in a virus-preparation are identical, there seems to be little point in asserting that they are molecules. The only effect of this assertion is to rob a useful word of the meaning that it has in the more rigid discipline of chemistry. As soon as a particle has become so big that the introduction of, for example, a $—CH_2—$ group has no longer an observable effect on the particle-size or analytical composition, the word molecule has become misleading (cf. Pirie, 1940; 1946). Clerk Maxwell (1875) distinguished clearly the use of the word by chemists, to mean identical primary particles of a substance, from the use by physicists, to mean a particle in a fluid that did not break up during the observation. This distinction is in danger of being disregarded, and the demonstration of physical molecularity is loosely taken to imply chemical molecularity as well.

The argument in the preceding paragraph is designed to show that, even if successive preparations of the same virus from different sources cannot be shown either to differ from one another or to be inhomogeneous, there is still no basis for the claim that all the particles are identical in the sense that those of, for example, sucrose are. Such an argument is not, strictly speaking, necessary, for even those viruses for which the molecular claim has been made most persistently are demonstrably variable and inhomogeneous. The particle-weight and analytical composition attributed by Stanley and his colleagues to tobacco mosaic have ranged over a wide field. Even in the same paper different values may be given; thus Gaw & Stanley (1947) quote phosphorus-values, one of which is 25% higher than the other, as evidence that a strain of tobacco-mosaic virus is the same when grown on tobacco as when grown on phlox. It is unlikely that such evidence would be acceptable in other fields of chemical research.

It is clear, both from first principles and from a consideration of the type of evidence that is actually published, that the idea of viruses as molecules in the strict chemical sense is misleading. Beard (1945; Hook *et al.* 1946) has ably presented the case against regarding some of the animal and bacterial viruses as molecules. He tends to contrast them, because of their variability in composition and particle-size, with the plant-viruses and other materials often classified as macromolecular. An experience of a fairly wide range of these substances, including plant-viruses, proteins, bacterial antigens, agar and hyaluronic acid, suggests that this distinction is unreal. The variability of these substanees may not be so great as that of the

viruses which Beard has studied, but the difference is quantitative only.

4. Nature of the Fundamental Virus-Particles

Variation between individual virus-particles or between successive preparations of the same virus can arise in many ways. There is no *a priori* reason why the property of being virus, that is to say of transmitting a certain set of symptoms or behaving in some other defined way, should be associated with a unique chemical structure. The virus-building mechanism may make a range of products, all active and similar, but not identical. It is equally probable that initially-identical particles should become non-uniform through association, dissociation, or the adsorbtion of components from the environment. Bawden & Pirie (1945) have some evidence that the last two processes operate to control the properties of tobacco-mosaic virus. In their view, the free virus aggregates in solution, but this aggregation can be prevented by other adsorbed materials. They suggest tentatively (1937a, 1945) that the primary particle is more or less spherical and smaller than the rods which are the predominant constituent of old or roughly-handled virus-preparations. Stanley stoutly defends the position that the rods, which Bawden and Pirie had described, are the fundamental particles, and that all others are derived from them. He has (Oster & Stanley, 1946; Sigurgeirsson & Stanley, 1947) published electron-micrograms purporting to show the original state of the virus in the cell. His technique, however, involves exposing the virus to that unphysiological fluid "leaf sap" which, as Bawden & Pirie (1945) have demonstrated, is a powerful aggregating agent. Furthermore, there are serious limitations to the use of electron-micrograms in getting evidence of this type (cf. Pirie, 1945; Crook & Sheffield, 1946). Stanley's position has the obvious merit of simplicity, but this simplicity can be achieved only by disregarding much of the available evidence.

5. Combination of Virus with Tissue-Constituents

The presence of material, attached to the particles in a virus-preparation but not essential for the activity of the preparation, has been recognized with many other viruses. Curnen & Horsfall (1947, and earlier papers) have studied the complex that the pneumonia virus of mice makes with a constituent of lung and other tissues. Free virus can be made, and this will then combine with the tissue-constituent in vitro. It therefore seems probable that the complex is an artefact made during the extraction of lung by the original methods as a result of bringing together components not normally in contact in the tissue. Influenza-virus preparations contain material reacting with antisera against chick-embryo or mouse, depending on which host has been used for cultivating the virus (Knight, 1946). Virus free from these components has not yet been made, and it is therefore not possible to say whether, here also, the complex is an artefact, or whether some components of the host are essential for the stability of the virus-particle. Cohen (1946) has described a phenomenon in the T2 virus of *Bact. coli* which may have a similar origin. When grown on a synthetic medium, the virus is associated with extra desoxyribose nucleic acid and this can be removed from it, without loss of virus-activity, by digestion with desoxyribonuclease. Here, therefore, an apparently homogeneous virus-preparation contains material inessential for virus-activity. There is no evidence showing whether it has any relationship with normal host-cell components or not. The tumour-viruses give many examples of similar phenomena. Carr, for example, has good evidence (1944) that the antibodies, built up in an animal carrying a Rous No. 1 sarcoma, progressively reduce the filterability of the tumour-virus, presumably by precipitating it in the interstices of the tissue. The only apparent difference between this and the other examples of complex-formation quoted is that the complex never appears in suspension. There is no evidence for anything analogous to antibody-formation in plants; it is therefore unlikely that precipitation of this type explains Bawden & Pirie's (1946) observation that the greater part of the virus in plants with tobacco-mosaic is not in solution but firmly attached to the structure of the leaf. If this phenomenon is at all general, it greatly widens the field of complex-formation.

6. Conclusions

In recapitulation it may be said that our ideas about the nature of viruses are now based on much information, but that this has increased rather than diminished the complexity of the subject. It is a matter of observation that there is no clear demarcation between viruses and bacteria, and there is probably no demarcation between viruses and physiologically-active but synthesizable molecules. All the viruses purified so far have contained nucleoprotein, but this generalization may lack significance because the viruses that have been studied are a group selected to some extent on a chemical basis. Furthermore, the purified virus is the minimum unit capable of starting the infective process in a susceptible cell. It may well be that an essential preliminary to the process of multiplication is the accretion to the virus of various other cell-constituents. This view has for long seemed probable *a priori* (cf. Dale, 1935); the frequency with which viruses, if isolated by gentle procedures, are associated with host-cell constituents is confirmatory evidence for it. It becomes a matter of arbitrary definition at what point, in stripping away host-components from a virus, one is purifying it, and at what point one is modifying it. These considerations need emphasis because of the frequency with which the supposed facts of virus-research are used in discussions on other branches of biology. There are clearly resemblances between viruses and genes (Muller, 1922) or other tissue-components (Claude, 1941) but caution is necessary in arguing from these resemblances. In commenting on the position it would be difficult to improve on Beard's (1945) felicitous phrase: "Viruses are said to be living molecules and autocatalytic enzymes and are likened to genes and mitochondria—in short, a fabric of concepts has been woven of a plethora of woof with a paucity of warp."

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