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THE NATURE AND ORIGIN OF PLANT VIRUSES WITH AN INCURSION INTO VIRUS NOMENCLATURE

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ABSTRACT

Eversince, the chemical nature of not only the anisometric tobacco mosaic virus (TMV) but also the crystalline viruses like bushy stunt of tomato, tobacco necrosis virus, turnip yellow mosaic virus and others have been characterized, sap transmissible viruses have been shown to be good antigens and that their antisera had group specificity aiding identification. Whilst symptomatology alone was found unreliable and of insufficient means for diagnosis and classification of viruses, serology, chemical and physical structure of viruses and the modes of transmission have played an important role in virus nomenclature. However, it is premature to think of orthodox binomial and trinomial nomenclature as practised in fungal classification playing a role in virus terminology. Anisometric and crystalline viruses are comparatively simple chemical entities (as compared with animal viruses) and are essentially nucleoproteins where the RNA (ribonucleic acid) core has a shell of protein in a helical pattern serving as a protective coating. The infectivity of the nucleic acid alone (which is hardly 6% of the nucleoprotein) has been proved beyond doubt whereas, the protein (which is the bulk of the virus) does not count for infectivity but plays an important role in serological reactions and consequently is of immense value in grouping viruses. Nevertheless, size, shapes and structure of viruses are of considerable significance as a tool in virus classification of rodshaped particles. Spherical virus particles, however, have not lent themselves to this approach. A somewhat similar approach to classification has been made by using specific vector-virus relationships.

In the speculative field of origin of viruses, the free gene or plasmagene theory of Darlington seems to find little support at present. Nonetheless, a biochemical approach is still valid and as all the ribonucleic acid (RNA) in the TMV appears to be present in a single polynucleotide chain, a break in this chain has to be transmitted intact to the recipient cell to enable reduplication. That the RNA may be replicated in the cells is, therefore, not to be regarded as a shortlived messenger carrying genetic information from nuclear deoxyribonucleic acid (DNA) to protein. Stretching this argument one could conceive of a further step to the self-perpetuating RNA molecule capable of directing protein synthetic mechanisms in recipient cells to specific products.

Among the many intriguing problems tackled by the biologist the most difficult, to my mind, is the unravelling of the mystery that surrounds the entry in host cells and subsequent establishment of infection by ultramicroscopic particles called viruses. The term virus is latin in origin (meaning slimy liquid, poison), and was originally used for small pox and other infections of man and animal. Currently, the term virus disease is confined to infectious diseases for which no visible causes have yet been demonstrated, although ever since the advent of the electron microscope, the size and shape of these ultramicroscopic bodies have been studied and, indeed, serological and other chemical methods of diagnoses have been evolved in many cases. As far as plant viruses are concerned, they are obligate parasites, very much smaller than the smallest bacterium known to science with an astonishing rapidity of action when once inside the host cell of choice. Remarkable too is the specificity of some of these viruses both in regard to choice of host as well as methods by which they get transmitted. Three main methods of transmission are recognized: by grafting diseased material to healthy ones ; by rubbing infected plant sap on to leaves of healthy plants ; and by insects feeding on healthy plants having fed on diseased ones. In recent years, to this list has been added, hitherto unsuspected microscopic animals like wire worms, larvae and mites. One of the puzzling questions is how some insect vectors retain the virus in them as long as they are alive once having acquired the virus from the diseased plants and what is more, how they pass it on to their progeny. In certain cases, there is even unequivocal evidence of viruses multiplying in their vectors, although this does not seem to be a universal phenomenon.

Viruses produce many external symptoms on the host plants they invade ranging from localized brown spots or lesions to mottling of leaves, variegation in petals of flowers, sterility resulting from aborted ovary development, extreme reduction of the leaf blade and crinkling and unusual appendages along the midribs and veins called 'enations'. Internally, within cells, inclusion bodies are formed, crystals appear, derangement of normal physiologic processes such as photosynthesis and respiration occur and generally, the more virulent the virus the more drastic the internal tissue and metabolic changes. Despite all these changes that viruses bring about in their hosts, not all viruses are transmitted through the true seeds. Indeed, seeds of many leguminous plants can carry over their virus infections but many others such as the world-wide distributed tobacco mosaic virus, tobacco necrosis, severe etch of tobacco, etc., are not known to be seed transmitted. Nevertheless, seed transmission can not be ruled out in many

cases hitherto unsuspected but, by and large, it is the vegetatively propagated plants such as ornamentals, tuberous materials and grafted and layered propagules that are the source of greatest danger in the maintenance of virus infective material.

Most viruses are known to occur in nature in the form of strains with differing virulence. In fact, the more virulent a virus strain, the less the chances of its survival in nature and conversely, the less virulent, the greater the chances of survival as such plants suffer from a chronic disease with less metabolic derangement and continue to do so throughout their life. What is most interesting is that such sickly plants offer a perennial source of infective material for further propagation of disease. The discovery of plant virus strains of differing virulence started a wave of speculation as to whether the attenuated or non-virulent strain could be used to protect plants against the virulent related strains as, indeed, theoretically the surmise was right and in fact, under experimental conditions they were shown to do so. Nonetheless, this concept, akin to vaccination in the animal and human diseases caused by microbes, failed to offer a break through and evolve a much awaited control measure. Following this set-back exploratory work in this field of control of virus multiplication is now afoot after the discovery that certain chemicals show virus inhibiting properties under laboratory conditions. Substances like azaguanine, azauracil, thiouracil, etc., have been found promising. A parallel case is the discovery in animal viruses that a substance called interferon could offer some hope in this direction. Interesting in itself, interferon is not known to affect absorption or penetration of virus; in fact, it selectively interferes with the replication of the nucleic acid of virus. Interferon possibly uncouples certain essential respiratory processes of host resulting in inhibition of viruses. Such a system, however, is not known to be operative in plant viruses. Nevertheless, it should not be interpreted to mean that all work on plant virus strains has been futile and, therefore, is to be abandoned. Indeed, they have much fundamental bearing in experiments on recombination of viruses because plants inoculated with strains of one virus have been shown to yield new hybrid or recombinant strains, some showing character determinants. What is of further interest is, there is evidence to show that parent and recombinant strains all "breed true". There is, thus, possible analogy between genetic behaviour of living systems and those of plant viruses introduced into living cells. No doubt extreme views have been expressed by some workers who consider viruses to be "free genes" that escape from the chromosomes and act as independent entities. It has also been suggested that certain viruses exist in their hosts as a plasmagene, "a stable and presumably useful protein" somewhat in a state to be called "pro-virus" which is changed to a virus by grafting operations. Like many hypothetical viewpoints these are harder to prove than enunciate.

THE PURIFIED VIRUS

Apart from these problems of inactivation of viruses and virus strains, the most exciting work in recent years in the field of plant virology has been on a very high biochemical and biophysical plane. For many years, early this century, nothing was known about viruses that was incompatible with the view that they were essentially living organisms, resembling very small bacteria and having the physical property of filtrability through the finest pore size filter membranes. Whilst it was conceded they were obligate parasites, it was thought that because of their small size their food had to be prepared for them by host cells and that, apart from this dependence, they were still to be regarded as comparable to any other living organism. It was at this stage that Stanley in 1935, claimed to have isolated tobacco mosaic virus as a crystalline protein with all the properties of the virus. This assertion that the virus was crystalline created a stir in scientific circles as it was difficult to believe that a thing of the size of a molecule could possess all the properties of life. The diehard vitalist began to feel that his position was not so secure as he had believed. Indeed, it was a challenge to the older idea that attached a kind of sanctity to the word 'crystal'. What followed in subsequent years has been and still is of gripping scientific interest. Stanley's original claim that the tobacco mosaic crystal was only a protein had to be modified and it since became accepted that it was nucleo-protein where the presence of the nucleic acid was vital for infectivity. Further, it was then supposed that all plant viruses were of the tobacco mosaic type which were not true crystals but what could be regarded as asymmetrical 'liquid crystalline' substances without the three dimensional regularity of a true crystal. The ultimate particle under the electron microscope appeared as a rod shaped particle. Subsequently, however, other viruses were described which had true crystalline form, rhombic dodecahedrons, bipyramids, etc., with spherical particles and all of them were nucleo-proteins where the nucleic acid was of the ribose type. Quite recently, a number of methods have been used to break down tobacco mosaic virus into protein and nucleic acid constituents and it has resulted in a great revelation that the ribonucleic acid in the virus really carries the whole of the infective principle and the protein shell did not matter for infectivity. Highly infectious tobacco mosaic virus has now been successfully reconstituted in the laboratory with an anomalous non-infectious protein isolated from diseased plants and nucleic acid isolated from tobacco mosaic virus. It appears that virus biosynthesis occurs in two parts: (a) by reduplication of virus nucleic acid and (b) by

formation of an anomalous protein. In the host, these components appear to be produced separately and simultaneously and under proper conditions they 'polymerize' (the combination of several molecules to form a more complex molecule) to constitute the characteristic rod shaped or spherical infective virus particles.

Apart from these very fundamental discoveries on the size, shape, structure and infective properties of these virus crystals, there are other extremely interesting facts which we may consider if we are to have a more complete picture about them. The crude virus sap as well as the highly purified preparations have specific antigenic properties. In the case of pathogenic bacteria and some of the animal viruses, antisera can be prepared by immunizing animals by injecting into their blood stream graded doses of the attenuated forms of the microbes and when this immunized serum is mixed with the original organism or antigen, an observable precipitin reaction occurs. This knowledge has been used in characterizing many plant viruses, as grouping of related strains of one virus is possible by this technique.

THE ORIGINS OF VIRUSES

We shall now return to where we began and end this article by discussing some of the philosophical implications of how these virus units establish in host cells and multiply. Two questions are commonly asked: where do viruses originate, and are they living units? Whilst it is difficult to answer these with any degree of precision it may be well to consider some points of interest that govern them. Viruses are intracellular pathogens and, therefore, their individuality gets merged into that of the individual infected cell and subsequently their biological properties show themselves as the combined activities of all constituents of the infected cell. The infective nucleoproteins of purified viruses do stimulate the infected cell to new activities but it is hardly possible to compare them to, say, what a fertilized ovum does to an organism where it ultimately develops. Nevertheless, it is difficult to attribute any new independent existence to virus because, outside their normal abode—the living host cell—they can be at best regarded as substances with all potentiality of becoming dynamic and functional when returned to the cell habitat. Therefore, to treat the purified viruses as complete and self-sufficient systems or for that matter to differentiate between their biological activities as against the total activity of the infected cell would go into the realms of conjecture just at present. Despite this obvious difficulty, the recent discovery that the nucleic acid from the virus molecule is itself infective poses new questions which we shall examine further. An essential step for the purified virus particles or the infective nucleic acid component alone is probably to combine with other cell constituents. Logically, this would indicate that resistance or susceptibility in a host may depend largely on the presence of suitable components and, consequently, the varying effects produced by one virus in different hosts may result from combination with different components. This appears a simple explanation as the chemical simplicity of purified viruses may then be more an artefact than real and their behaviour outside the cell could be considered as a temporary unreal status which is soon lost when they start functioning within the cell. It should be mentioned here that purified preparations of animal viruses are chemically more complex than those of purified plant viruses but this may only indicate that the infective part of plant viruses are more readily separable from host cell constituents and no more. It is desirable to explain, if one can, variation in size and chemical complexity in terms of evolution. Attempts have been made in this direction and some workers consider that viruses represent a stage in evolution originating from pre-cellular forms of "life" and some others feel they have evolved by a process of retrograde evolution from larger organisms. The first view has little support as "saprophytic viruses" are not known whereas, the second view has considerable backing as saprophytic organisms are known to lose their ability to synthesize essential growth requirements and, therefore, have to depend on preformed supply of them. However, these two theories are unnecessarily restrictive and involve far too many assumptions. There are other theories too about the origin of viruses and since the field is so large it would be idle to discuss the many facts of these within the limitations of space imposed here.

THE VIRAL NUCLEIC ACID

It is quite obvious from all that has been said that, virus nucleic acid has a decided advantage, in terms of time, in initiating an infection over the intact virus. All results so far suggest that competition in a virus system occurs at the nucleic acid level. For this reason, the likelihood of an intact virus interfering with the establishment of an infection by their nucleic acid is slight, indeed. It has been suggested, on experimental evidence, that the ribonucleic acid (RNA) initiates an infection and completes its reproductive cycle sooner than does the intact virus leading to the conclusion that the evidence seems to be more strongly in favour of leaf RNase (the enzyme that interferes with RNA activity) indirectly enhancing the establishment of virus infection than in playing no part in the process.

All RNA viruses so far analysed have nucleic acid molecular weights of the order of 2 million (molecular weight is the weight of a molecule of a substance referred to that of an atom of oxygen as 16.00). Plant viruses are entirely of the RNA type whereas, animal and insect viruses are both DNA and RNA. Infectious RNA extracted from TMV and foot-and-

mouth disease viruses have molecular weights of the same order, i.e., all the RNA in these viruses appear to be present in a single polynucleotide chain containing some 6000 nucleotides (nucleotide: a compound of a base derived from purine, pyrimidine or pyridine, a pentose and phosphoric acid). Even with a single break in the polynucleotide chain it has to be transmitted intact to the recipient cell in order to reproduce itself and to initiate the synthesis of a virus-specific protein coat. There is presumptive evidence that RNA can carry information from one cell to another. For instance, extracts containing RNA added to bacteria have been reported to initiate the synthesis of enzymes not present in the recipient cells.

However, there is also evidence to show that the RNA may be replicated in the cells and is, therefore, not to be regarded as a short-lived messenger carrying genetic information from nuclear DNA to protein. It is but a small further step to a virus RNA molecule that is self-perpetuating and capable of redirecting the protein synthetic mechanisms of recipient cells to form specific products. These, in turn, might aggregate to coat the RNA and protect it from degradative agents (such as RNase). Transfer of RNA from cell to cell might have been 'streamlined' by natural selection so that in viruses it has become a devastatingly efficient process. The reason why a minimum RNA molecular weight of 1 to 2 million is apparently required for this process is not clear. It would seem that virus RNA has to subserve other functions during the course of self-replication. Evidence is accumulating that some plant virus mutants have normal protein subunits and fits in with this interpretation.

CLASSIFICATION AND NOMENCLATURE

(i) Systems Proposed and in Vogue:

It may be appropriate to discuss and differentiate between classification and nomenclature. Classification of viruses must obviously be based on some fundamental relationships and characteristics in which like viruses are grouped together. An ideal system of classification would be based on morphology and properties of the viruses themselves quite independently of their effects on the host plant. A system of nomenclature, on the other hand, is mainly a stopgap, pending the development of a proper classification and does not depend on fundamental properties of viruses which may be grouped in an arbitrary manner.

The first serious attempt at a classification or an orderly arrangement of viruses was by Johnson (1927). The main elegance of this system was in its simplicity of naming a virus after the first found or most important host plant and in its being adequately described. Also a number showing the chronological order of new and distinct viruses found and described on one host species has been found helpful. Thus, tobacco mosaic virus became Tobacco Virus 1 and strains of viruses came to be designated by capital letters and sub-strains by small letters. Subsequently tobacco mosaic virus became *Nicotiana Virus* 1, and various strains were called 1A, B, C, etc. The numbering and lettering systems have been adversely criticized by several workers. The chief error is to consider the system a 'classification' particularly, when the arrangement is not based on any important properties of the virus. The main criticisms of the numbering system are: (i) a number denotes nothing in respect to characteristics of a virus or the disease it causes; (ii) numbers do not permit the desired mobility and indeed a virus coming close to the first described virus as far as relationship is concerned may have to be shifted elsewhere when a new virus with somewhat common properties is discovered later. Therefore, the numerical system is at best termed a system of nomenclature and not a classification.

Holmes (1939) published a Latin binomial system for plant viruses and again enlarged it (1948) to include animal and bacterial viruses. This 'classification' is based on symptomatology which is an unreliable commodity and, what is more, it brings together groups of viruses which are unlike and attempts at a relationship that is not real. In this system, the genus *Marmor* had totally unrelated viruses differing in mode of transmission, symptomatology and other properties. In fact, *Annulaceae* contained viruses which in winter belonged to this group and to *Marmoraceae* in summer based on symptomatology.

Various workers have suggested additions and alterations to Holmes' system. As an illustration could be cited the changed name of the common tobacco mosaic virus which has been called *Tobacco virus altathermus* or *Nicotiana virus altathermus* or *Paracrystalis altathermus*. These modifications have been suggested based on the properties of viruses and, therefore, are subject to modification when more is known about the chemistry and physics of virus particles.

(ii) General considerations: Classification of organisms consists of two things, (a) the analysis of individuals into basic units, and (b) the synthesis of these units into like groups. The former constitutes no great problem with plant viruses but the latter does. Therefore, Bawden (1953, 1955, 1957, 1959) has suggested that the unit be called 'collective species' meaning a collection of clones, differing in some characteristics, notably in pathogenicity. Work with bacteriophages suggests that, when related clones multiply in a cell, they can exchange genetic characters and techniques may be developed to test whether or not such clones of plant viruses can do so, and, if they can, then it should be comparatively easy to include them in a species. The criterion that is most valuable is the sharing of common antigens and, indeed, all viruses

so far found to be related serologically also resemble each other in many intrinsic properties such as similar sizes, shapes, stabilities *in vitro* and gross chemical constitutions. Therefore, the first step in classification should be to select specific clones as types of named 'collective species' such as tobacco mosaic, potato X, potato Y, etc., and antisera prepared against each. Subsequently, by testing individual virus clones against the type antisera, the clones can be allotted to 'collective species' according to the antisera with which they react. This would eliminate synonyms and would decrease the numbers of 'recognized' viruses to manageable proportions. Another criterion that can be applied is to test whether clones can interfere with each other's multiplication in susceptible plants, which is closely tied to serological relationship. As mentioned earlier, viruses serologically unrelated to one another usually fail to depress the multiplication of each other, and plants infected simultaneously with two such viruses show more severe diseases and often produce lesions quite different from those produced by either virus by itself. By contrast, if two serologically related viruses are inoculated simultaneously to a plant, they depress each other's multiplication and the resulting symptoms are usually intermediate between those caused by either virus alone. Also, a plant systematically invaded by one virus resists invasion by a second serologically related strain, but not by one serologically unrelated. This test could help in allocating virus isolated to 'collective species'. Thus, within a 'collective species' clones might be arranged in some sequence that reflects phylogeny, but beyond this it seems impossible at present to try to use evolution as a basis for grouping. It has already been emphasized that all the plant viruses that have been purified so far have been found to be nucleoproteins with the RNA type of nucleic acid and it must be stated that their origins are 'wholly obscure'. Each 'collective species' seems to have had an equal chance of deriving from a higher plant, a micro-organism, an insect or any other kind of organism, as nucleoproteins are components of all living cells. The fact that we are not now able to group our 'collective species' by inferred phylogeny is one of the reasons that make it impracticable to use Linnaean binomial names for plant viruses, particularly as the arrangement of species into genera is an essential prerequisite in modern taxonomy and this is not now possible with our limited knowledge. The 'collective species' can, of course, be grouped on criteria other than phylogeny. The obvious thing to do is to use morphology as a criterion and, as illustrated earlier, electron microscope studies have revealed that three categories corresponding somewhat to bacilli, spirochaetes, and cocci could be made depending on whether the particles are rigid rods, flexible filaments or spheres. As all plant viruses consist only of protein and RNA; the main difference between the 'collective species' would then be the relative proportions of the two components. Elongated particulate viruses all have the same ratio of nucleic acid to protein, whereas spherical particles all have three or more times as much nucleic acid, and, therefore, this along with shape may be taxonomically significant. Recently, Brandes and Wetter (1959) have attempted a classification of elongated plant viruses on the basis of particle morphology. Shape, diameter and length of particles are morphological characteristics of elongated plant viruses. The length is the most useful feature for characterizing a distinct virus. Based upon the results of comparative measurements, a number of elongated viruses are placed in twelve groups according to their normal lengths. Viruses belonging to one group and representing distinct species are sometimes related serologically, sometimes not. Additional characteristics such as diameter, shape, thermal inactivation point and mode of transmission show correlation within each group and suggest higher systematic units into which viruses with different normal lengths can be combined. Chemistry and protein chemistry techniques are gradually becoming more helpful taxonomically and taken in juxtaposition with the facts already presented from the serological and virus-vector relationships, is bound to not only bring about a bridging of differences between clones but also help in clearly defining the limits or de-limits of a 'collective species'. Actually, Hansen (1957) considered a practical method of classification of plant viruses based on the periodical system of classifying elements using virus-vector relationships as the main criteria. Hansen arranged plant viruses on their arthropod vector-virus relationships involved in transmission. He further utilized the high correlation known to exist between their physiological effects in the main hosts and the transmission through vectors. The plant virus families were designated by symbols referring to their arthropod vector-virus relationships (for example "in", "aphi", "cica" and "xe"). Similarly, the plant virus genera, he suggested, may be designated by symbols indicating certain ways of transmission which are connected with their tissue-regional affinity.

In the generic level Maphivirus, Hansen indicated, may be taken to describe that the viruses in question are transmissible by the leaf-rubbing method and by aphid-vectors, and this combination suggests that viruses are generally non-persistent and induce mosaic mottling. Maphivirus includes three "true" genera, namely, Maphiflexus with thread-like, flexible particles, Maphicorda with rod-like, rigid particles and Maphiglobus with small spherical particles.

While considering a binomial system of nomenclature of viruses, it should be remembered that there is no reason to assume that all viruses have similar origins and it would, therefore, be inappro-

priate to adopt a uniform standardized nomenclature. It seems unlikely they are degenerate organisms. Their nearest analogues are macromolecular nucleoproteins of normal cells, and indeed, with the viral nucleic acid core, anomalous plant protein has been tagged to produce a reconstituted complete nucleoprotein virus particle. Is it logical then that this nucleoprotein receives a Latin binomial ? Article 4 of the international Rules of Botanical Nomenclature makes the essential points: (i) to aim at fixity of names and (ii) to avoid or reject the use of forms and names which may cause error or ambiguity or throw science into confusion. It is hard to understand how the first point would be better served by Latin binomials than the use of common names. Tobacco mosaic has been in use for more than fifty years without any alarming confusion whereas, Latin binomials suggested by Holmes and others such as *Marmor tabaci, Musivum tabaci,* etc., have not helped ease the situation. As pointed out by Bawden (1953) crown gall bacterium is a well understood name but has been changed in Latin binomial from *Bacterium* to *Pseudomonas,* to *Bacillus,* to *Phytomonas* and now to *Agrobacterium.* Are these changes justifiable ? Nevertheless, they fulfil point (1) of Article 4. However, the present attempts at virus nomenclature do not fulfil the obligations laid down by point (2) of Article 4, namely, that it has examples of errors and ambiguities capable of creating a situation of "science being thrown into confusion". It is obvious that binomial nomenclature almost necessarily calls for a detailed knowledge of the intrinsic properties of viruses and only then can a classification be built reflecting natural relationships and evolutionary trends. Unfortunately, despite the acquiring of increasing knowledge of properties of a few plant viruses, we are woefully ignorant of virus origins except that it has been a virgin ground for many speculative incursions not only by botanists but by biochemists and biophysicists.

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