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PLANT VIRUSES

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PLANT VIRUSES

by

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WITH 8 PLATES
AND 3 TEXT ILLUSTRATIONS

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PREFACE TO SECOND EDITION

IN the decade or so that has elapsed since this little book was first published, the study of viruses, and particularly of plant viruses, has made such rapid strides that the original text had become completely out of date. The isolation and crystallization of the viruses themselves, the studies on the virus proteins, the measurement of virus particle size and the photography of virus particles by means of the electron microscope are all advances which have taken place in the last ten years.

The text has, therefore, been entirely re-written and is now based upon a short course of lectures given annually in the Botany School in the University of Cambridge. It is intended for students without previous knowledge of viruses who wish to know the broad outlines of the work in this particular field.

Grateful acknowledgements are due to Mr. G. Crowe for the electron micrographs in Plate 7 and to Dr. Roy Markham for preparing Fig. 2 and for taking many of the photographs. Dr. Williams and Dr. Wyckoff kindly supplied prints for Plates 5 and 6, and Dr. Darlington and the editors of *Nature* allowed reproduction of Fig. 3.

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CHAPTER I

INTRODUCTORY

Historical : Economic importance

HISTORICAL

VIRUS diseases of plants, although not of course recognized as such, were known long before the discovery of bacteria. The first record in the literature of which we have knowledge is a description published in 1576 by Charles l'Ecluse or Carolus Clusius [31] of a variegation in the colour of tulips, which is now called ' breaking ' and is recognized to be due to an aphis-transmitted virus of the mosaic type. ' Broken ' tulips are figured in *Theatrum Florae*, published in 1662 ; these illustrations have been identified as the work of the painter Daniel Rabel.[61] A somewhat later account published in *Traité des Tulips* about 1670 contains the first suggestion that the variegation in the flower colour might be due to a disease. In 1715 an account of an infectious chlorosis of *Jasminum* was published in the *Art of Gardening*.

About fifty years later the so-called ' curl ' disease of potatoes came into prominence, and about the cause of this there raged for many years a great controversy. The favourite explanation was that of ' degeneration ', a kind of senile decay caused by long-continued vegetative propagation. It was pointed out, however, that in certain secluded districts, high up on mountains or in wind-blown areas near the sea, it was possible to grow the same variety of potato for many years, saving the ' seed ' each year from the current year's crop, without any sign of degeneration. It was the discovery that potato leaf-roll was an infectious virus disease which

finally settled this controversy and showed that the degeneration of the potato crop was due solely to a gradual infiltration of viruses into the stocks.

About 1868 the variegated plant *Abutilon*, probably *A. striatum* var. *Thompsonii*, appeared in Europe and became popular as an ornamental plant. By grafting scions of variegated plants to green shoots of normal plants it was discovered that this variegation was infectious. Now the variegation in *Abutilon* is known to be due to a virus infection.

In 1886 Mayer described a disease of the tobacco plant which he called *Mosaikkrankheit* and this term, or its English equivalent, is now widely used for describing the mottling type of virus disease. Mayer showed that this mosaic disease of tobacco could be communicated to a healthy tobacco plant by inoculation with the sap of the infected plant. Two years later Erwin F. Smith proved that the disease known as 'peach yellows' was also communicable and could be transmitted by budding.

It was not, however, till 1892 that the first scientific proof of the existence of a virus was given. Iwanowsky [45] working with the mosaic disease of tobacco, described by Mayer, proved that sap from such a diseased plant was capable of inducing the mosaic disease in healthy tobacco plants *after* it had been passed through a bacteria-proof filter candle and was bacteriologically sterile. Curiously enough, Iwanowsky himself did not seem to grasp the true significance of this and his discovery passed almost unnoticed until the work was repeated seven years later by Beijerinck,[11] who then propounded his theory of a *contagium vivum fluidum*.

The discovery of the relationship between viruses and insects was not made in a day, and a period of years elapsed between the time when insects were first suspected and the actual demonstration of this method of transmission. The first to prove experimentally the

relationship between an insect and a plant virus seems to have been a Japanese farmer, Hashimoto, who worked in 1894 with the dwarf disease of rice and the leaf-hopper, *Nephotettix apicalis* var. *cincticeps*.

About 1907 three workers in America, Ball, Adams, and Shaw, suggested that there was some connexion between curly-top of sugar beet and the leaf-hopper *Eutettix tenella*. In 1915 Smith and Boncquet [87] confirmed this and showed that a single insect from an infected plant placed on a healthy plant for five minutes would produce the disease.

In his historical review of plant viruses and virus diseases, Cook [27] divides the history of virus study into three arbitrary periods. The initial period begins with the first records of a virus disease, that of tulip ' breaking ' or mosaic by Carolus Clusius in 1576, and may be said to end in 1868 with a description of the variegation of *Abutilon striatum*. During this period there was no research as that is understood at the present time, but there were some discoveries of importance. These were (1) that the ' breaking ' of tulips (see Plate 1) was transmitted by bulbs from plants showing these characteristics; (2) that peach yellows and the mottlings of *Abutilon striatum* var. *Thompsonii* were transmissible by budding, and (3) that when a mottled branch of *A. striatum* var. *Thompsonii* was grafted into a fresh plant, the mottling appeared in the new green leaves.

The second period may be said to begin with the work of Mayer, who in 1888 made a study of the mosaic disease of tobacco and showed it to be transmissible. This period also includes the work of Iwanowsky and Beijerinck already referred to. Cook puts the beginning of the third period at about 1906 when the study of plant viruses was commencing, but this did not become intensive until at least two decades had passed.

To Cook's three periods the writer would add a fourth, which may be said to date from 1935 with Stanley's isolation of the tobacco mosaic virus.[89] That discovery

enabled workers to visualize a virus as a definite entity rather than a mysterious agent whose existence was only deduced from the disease it produced. It is during the last decade that the really serious study of the virus as distinct from the virus disease has been carried out. The physicist with his exact methods, the biochemist and the serologist have all joined in, and what was once the domain of the biologist alone is now shared by workers in all these different fields. By means of the electron microscope and the new technique of shadow micrography,[101] by X-ray diffraction studies and with the aid of the ultra-centrifuge much information on the size and shape of the virus particles has been obtained. From the biochemical standpoint, too, great progress has been made whereby a number of plant viruses have been isolated and crystallized and studies on the chemical composition carried out.

We are still ignorant, however, of the method of reproduction of the viruses, and for information on that vital and fundamental process we must presumably return to the plant itself, or at all events to the living cell, aided no doubt by the electron microscope.

ECONOMIC IMPORTANCE

In 1938 the late Sir Patrick Laidlaw [59] said, ' these [virus] diseases are of great importance and the sum total of the disharmony they produce rivals that caused by the visible bacteria '. Laidlaw was referring to virus diseases as a whole and not only to those attacking plants. If we consider plant viruses alone, the damage they do probably equals if it does not exceed that due to all other disease agents. That this damage is increasing no one will deny, but whether it is due to the appearance of new viruses or to more extensive distribution of existing viruses is not at present clear.

So far as Great Britain is concerned, the most important losses from viruses are those suffered by the

following crops : potatoes, sugar-beet, cruciferous crops generally but especially brassicae, strawberries and raspberries. Much damage is also done by viruses to the tomato crop and to flowering plants of all kinds, particularly dahlias.

The extensive trade in seed potatoes, due entirely to virus infection, involves the dispatch to England of some 400,000 tons annually from Scotland and Ireland, and indicates the importance of virus diseases to the potato-grower in England. The sugar-beet industry, especially in East Anglia, suffers severe losses from virus diseases, particularly the so-called virus yellows, which may reduce the crop from 15 tons per acre to 3, whilst a crop heavily infected in July may lose 50 per cent of its sugar.

The viruses attacking cruciferous crops, particularly swedes, turnips, and the brassicae, are becoming of first-class importance and will need serious attention before long. There are several viruses concerned, some of which are aphis-transmitted ; they cause mottling, distorting, and stunting of the plants and make cauliflowers unsaleable by preventing formation of the curd.

One of these turnip viruses has recently been shown to be transmitted by a flea beetle (*Phyllotreta cruciferae*), the first virus in this country known to have a biting insect as vector and the first record of the insect transmission of a crystalline virus.

The crinkle and yellow-edge diseases of strawberries are familiar enough to all growers, as is also the mosaic disease of raspberries, and now the latter crop is menaced by the appearance of a new virus disease—or possibly the reappearance of an old one—known as leaf-curl.

CHAPTER II

VIRUS DISEASES

Symptomatology : Local lesions : Movement of virus in the plant : Metabolism and growth of virus-diseased plants : Strains and immunity

SYMPTOMATOLOGY

EXTERNAL SYMPTOMS. The symptoms produced by viruses in plants are very varied, and indeed seem to cover every kind of reaction which a plant could conceivably make to a disease stimulus. Thus, virus-affected plants are known to show mottlings, concentric rings, change in flower colour, stripes, streaks, and necrosis generally, leaf rolling, leaf distortions, suppression of leaf blade, outgrowths and tumours, splitting of stems and petioles, enlargement and distorting of veins, &c. It is possible to make a rough classification of these varied types of symptoms, but it must be emphasized that this is only an arbitrary arrangement of *diseases* and in no sense a classification of *viruses*. It is essential for the reader to realize that the terms *virus* and *virus disease* are in no way synonymous since a single virus is capable of causing half a dozen distinct diseases according to the type of host plant infected.

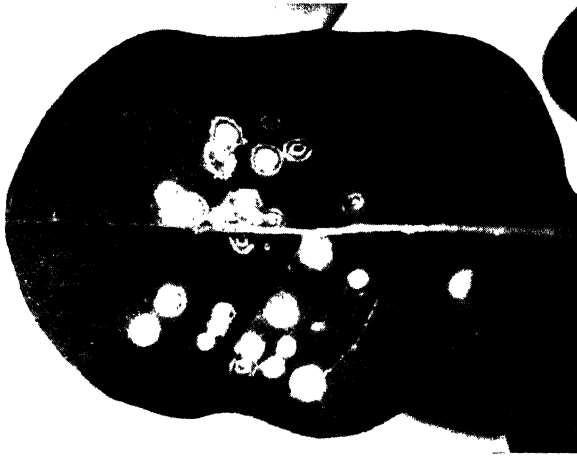
We can, then, group the virus diseases of plants as follows, according to the symptom picture alone :

(1) *Mosaic diseases* where the main symptom is a mottling of the leaf ; the mottling may consist of light or dark green, yellow, and even white. Many mosaic viruses cause a ' break ' or change in the colour of the flower, as in wallflowers and tulips. Included in this category are the *ringspot* diseases in which numbers of necrotic or chlorotic rings, sometimes concentric, some-

Self-coloured tulips affected with mosaic, showing the change or ' break ' in the colour

PLATE 2

(*a*) ' Clearing of the veins ', a common initial symptom in the development of a virus disease



(*b*) The ' ringspot ' type of virus disease

times single, and usually with a central spot, develop on the leaves of affected plant (Plate 2*b*).

(2) *Distorting Diseases.* In this type there is no mottling, and as a rule not much necrosis of the cells. The distortion takes various forms, suppression of the leaf-blade and the formation of filiform leaves are common on tomato plants affected with cucumber mosaic virus and some strains of tobacco mosaic virus. The names of the following virus diseases are self explanatory: potato leaf-roll, tobacco vein-distorting disease, tomato big bud, cranberry false-blossom; most diseases of this type, contrary to the mosaic type, are not transmissible by mechanical means but have a specific insect vector.

(3) *Necrotic Diseases.* With this type also there is little or no mottling and the following may be quoted as typical; tobacco necrosis, tomato black ring, tomato streak. In these diseases the cells are killed by necrosis, which may be confined to the leaves, as with tobacco necrosis, or may be systemic and frequently lethal, as with the other two.

(4) *Outgrowths and Tumours.* The commonest type of outgrowth is that known as an *enation*, and consists of a secondary leaf growing out from the underside of another leaf. These enations vary greatly in size from a few millimetres to two inches or more in diameter (Plate 3). They are caused by quite a number of viruses, but only on certain hosts; the following viruses have been observed to produce enations. The tobacco rosette virus complex on tobacco and related species of *Nicotiana*, tomato black ring virus on frame cucumber plants, various strains of tobacco mosaic virus, some on tobacco and some on tomato, and tobacco leaf-curl virus on tobacco.

Several animal viruses are known to give rise to tumours, such as those of the rabbit papilloma and the fowl sarcoma. Comparable virus cancers in plants are rare, but one has recently been described from

America affecting leguminous and other plants and has been named the ' wound-tumour virus '. Quite large swellings with apparent potentialities for unlimited growth develop on the roots and stems of affected plants of white sweet clover, *Melilotus alba.*

(5) *Yellows Diseases.* One or two viruses give rise to a uniform yellowing of the leaves of the host plant. This is a different effect from the mosaic mottling, where there is a combination of colour shades. There is no mottling in the yellows disease and the condition is not common. Aster yellows and sugar-beet yellows are examples of this type of virus disease.

INTERNAL SYMPTOMS. Apart from histo-pathological conditions specific to certain viruses which will be briefly described a little later, there is one internal pathological effect which is characteristic of several viruses. X-bodies, as they are sometimes called, or intra-cellular inclusions, are amoeboid in shape and are usually in close association with the cell nucleus. They have one or more vacuoles and bear a superficial resemblance to certain protozoa, with which at one time they were actually identified. In reality, these inclusions are aggregations of diseased cytoplasm, and are thus in no way analogous with certain intracellular inclusions found in animal virus diseases which in some cases appear to be the actual virus. These X-bodies are nearly always intracellular, though intranuclear inclusions have been described in one virus disease.[50]

In Iwanowsky's original paper on tobacco mosaic [45] he described a second type of intracellular inclusion to which the name ' striate material ' was given. These inclusions are of a crystalline character, and there is now a certain amount of evidence suggesting that they are actually crystalline aggregates of the tobacco mosaic virus itself.[73] If this is true, however, it is a crystalline form of tobacco mosaic virus which has not yet been achieved by artificial means. Necrosis of the phloem

is an internal symptom which is found in several virus diseases, such as potato leaf-roll and the phloem necrosis disease of tea. In the tobacco rosette disease certain characteristic changes take place in the vascular bundles where abnormal tissue is laid down which gives rise to necrosis, followed by a splitting of the epidermis.[81]

Another internal change characteristic of several virus diseases is the abnormal accumulation of starch. This is particularly true of potato leaf-roll, where the chloroplasts may become so charged with starch that they burst, curly-top of sugar-beet, and aster yellows.

In mosaic diseases it has been shown that the chlorotic areas are thinner than the green areas, that there is an inhibition of the cell structure, that the mesophyll is compact, and the palisade tissue reduced.[27] In sugar-beet seedlings infected with curly-top there appear to be two phases in the changes which take place in the cells; first an increase in nuclear and chromatic material, and, secondly, breakdown of the nucleus with the possible emission of chromatin into the cytoplasm.[2]

SYMPTOMLESS CARRIERS. The symptomless carrier of a virus is a common phenomenon among plants, and is of considerable interest and importance. These carriers can be of two kinds; in one there is an initial reaction to the virus which may be mild and fleeting or quite severe. The symptoms then disappear and the plant appears normal, though still infected with the virus. This is the usual reaction of the tobacco plant to viruses of the ringspot type, and the question is further discussed later in this chapter under the heading of *Strains and Immunity*. In the second type of carrier there is no initial reaction to infection, and the plant appears normal in every way. Virus carriers of this type occur commonly in potatoes, strawberries, raspberries, hops, and dahlias.

Mention may be made here of a recently discovered virus, known as *tomato black ring* from the outstanding initial symptoms on that plant.[82] Plants infected with

this virus seem to fall into both categories of carriers; some, such as tomato, tobacco, cucumber, &c., show a marked initial reaction to infection but later grow out of all disease symptoms. A surprisingly large number of other plants receive the virus without any visible reaction, but retain it nevertheless for long periods and are true symptomless carriers; among these may be mentioned the deadly nightshade, *Atropa belladonna*; the wallflower, *Cheiranthus cheiri*; the snapdragon, *Antirrhinum majus*, and the common teasel, *Dipsacus sylvestris*.

Among the most interesting plant carriers of viruses is the potato variety King Edward. It has been shown by Salaman and Le Pelley [75] that all plants of this potato variety are infected with a virus to which they have given the name *paracrinkle*. The interesting points about this virus are that it is not mechanically transmissible, has no known insect vector, and has never been known to spread naturally in the field to other potatoes or any other plant. One must, therefore, suppose either that the original seedling became infected or else that the virus is a normal protein constituent of the King Edward potato plant. It is not, however, apparently transmitted through the seed.[25] As an example of virus carrying by a particular plant variety may be cited the dahlia, Bishop of Llandaff, which is a symptomless carrier of cucumber mosaic virus.

LOCAL LESIONS

Certain host plants react to infection with certain viruses in such a way that the virus is localized in the inoculated leaf (Plate 4). This localization may be permanent or it may be only temporary, followed by systemic spread of the virus throughout the host plant. The usual reaction on the part of the plant in this type of infection is the development of numerous necrotic

spots or rings, termed *local lesions*, on the leaf inoculated. In those cases where there is no systemic spread of the virus, as with tobacco mosaic virus on *Nicotiana glutinosa* and *Phaseolus vulgaris*, the use of local lesions allows the recognition of large numbers of successful transmissions on single plants. This method, which has been compared with Koch's plate method with bacterial cultures, makes possible the quantitative study of plant viruses and allows for comparative estimates of virus concentrations. When highly diluted samples of virus are used and small numbers of lesions develop on the leaves of the host plant, it seems possible that each lesion has resulted from a single virus particle.[40] At higher concentrations of the virus there is no direct and simple relationship between the concentration and the numbers of lesions produced, but within certain limits it is possible to tell which of two samples of virus is the more concentrated, and to gain some idea of their relative virus content. Important points to be observed in using the local lesion method for quantitative work are the adoption of a standard method of inoculation and the comparison of virus samples by inoculation on opposite halves of the same leaves or on single leaves arranged in such a way as to eliminate the extreme effects of variation in susceptibility.[76, 102] The kind and degree of this variation were shown by the statistical analysis of experiments in which plants of *Nicotiana glutinosa* were inoculated with tobacco mosaic virus and the numbers of lesions produced were counted. The data were submitted to reduction by the analysis of variance.[102] Plants differed greatly in their reaction to inoculation and a gradient of susceptibility was established between the different leaf positions. The nature of the gradient varied with different sets of plants. It was shown that the right and left halves of a leaf responded equally to the inoculation procedure used in the experiments.

It will be understood that the local lesion method of

study, by its nature, is applicable only to those viruses which produce lesions at the site of inoculation. The number of entry points for a virus and so the number of local lesions can be greatly increased by the addition to the inoculum of a fine abrasive such as fine carborundum powder or celite.

MOVEMENT OF VIRUS IN THE PLANT

The study of the movement, or translocation, of viruses in plants can be approached from several viewpoints. There is, firstly, the *type* of tissue in which the virus moves, secondly, *rate* and *direction* of movement, and thirdly, the *mechanism* involved in the movement.

As regards the type of tissue involved, this depends a good deal upon the kind of virus concerned. These tissue relationships seem to be of three kinds.[16]

(1) A relation in which virus is more or less restricted to parenchyma.

(2) A relation in which virus is more or less restricted to the phloem.

(3) A relation in which virus occurs extensively in both phloem and parenchyma.

(4) To these may be added a fourth type where the virus is apparently confined to the xylem. Pierce's disease of grapes (= alfalfa dwarf disease ?) is transmitted only by leaf hoppers, which feed in the xylem. If the insects were prevented mechanically from reaching the xylem when feeding, infection did not occur.[44]

Viruses confined to the parenchyma would obviously be greatly handicapped in their movement through the plant, and it is probably only in local lesions formed by some viruses on certain plants that this relationship holds good, though the virus of phony disease of peach may be confined to the parenchyma. Of viruses confined to the phloem, those of curly-top of sugar-beet

and raspberry leaf-curl have been most studied. Bennett [12] has shown that these viruses may be confined to certain parts of an infected plant by destroying the phloem connexions between the inoculated portion and other parts of the plant at the time of inoculation.

Another virus which may possibly be confined to the phloem is that known as the tobacco vein-distorting virus.[81] Such viruses are rarely transmissible by sap-inoculation, but rely upon an insect vector to inject them directly into the phloem.

Those viruses which occur in both parenchyma and phloem are of the mosaic type, and the best known example of these is the tobacco mosaic virus. The breaking of a leaf hair with a virus-contaminated instrument is sufficient to allow virus to enter an epidermal cell. The movement at first is slow, the virus passing from cell to cell until it reaches a vein, after which movement becomes more rapid.

The rate of movement depends to some extent upon the virus and also upon the kind of plant infected. Thus the virus of curly-top moves at a much greater speed in sugar-beet than in tobacco. The measured rates of virus movement following introduction into the plant vary from one-tenth of a centimetre per hour for the virus of tomato mosaic in tomato, to 152·4 centimetres per hour for the virus of curly-top in sugar-beet.[16]

As regards direction of movement, it has been shown by Kunkel [55] that the virus of tobacco mosaic in tomato can move in two directions. His data show that, on reaching the stem, virus frequently travelled both upward and downward, but also frequently travelled downward only and occasionally upward only. This brings us to the question of the mechanism of virus movement in the plant. It seems clear that two kinds of movement must be visualized. There is first the slow cell-to-cell movement via the connecting protoplasmic bridges or plasmodesms; such a movement

presumably takes place following the infection, for example, of a trichome with tobacco mosaic virus. Secondly, there is the more rapid movement via the phloem. In the first movement the virus is presumably carried round the cell by diffusion and protoplasmic stream, passing via the plasmodesms from cell to cell. Viruses cannot pass through the cell-wall by diffusion. In the more rapid movement in the phloem these forces presumably play no part, but viruses have been shown to move rapidly in directions of food utilization and storage and slowly in opposite directions. Bennett [16] considers that in the light of present knowledge it seems probable that the mechanism responsible for virus transport in the phloem is able to effect movements essentially similar to those that would be expected to result if a pressure-flow mechanism such as that proposed by Münch [67] were operating in the transport of elaborated food materials.

METABOLISM AND GROWTH OF VIRUS-DISEASED PLANTS

On the whole the amount of investigation into the physiology of virus-diseased plants has been small and, probably because of the effect of mosaic diseases on the chlorophyll, the greater part of these investigations has been directed to the study of respiration.

In the case of potato leaf-roll, the respiration rates are higher in the diseased than in the healthy plant. According to Whitehead,[99] except for a short period covering the end of dormancy of the tuber to the first unfolding of the leaves, the leaf-roll-infected potato plant respires at a much higher rate than does the healthy one. He concluded that the virus affects the respiration rate not directly, but only by interfering with the translocation of the respirable substrate.

As regards tobacco mosaic, Caldwell [24] inoculated tomato plants with the aucuba or yellow mosaic, both

as seedlings and at the five-leaf stage, and measured the respiration of the diseased tops. He found that tops of plants inoculated at the five-leaf stage evolved more carbon dioxide than the healthy controls, but those inoculated as seedlings showed a lower output at the beginning, followed by a higher output than the healthy tops. On the other hand, Lemmon,[60] using discs of healthy and mosaic-infected tobacco leaves, found that the respiration rate of healthy leaves was always higher than that of the diseased, while Kempner [52] was unable to find any change in the respiration of mosaic-infected tobacco leaves. Glasstone [36] carried out experiments with entire plants in nutrient solution to avoid effects due to the passage of air from other organs or to cutting, handling, drying out, &c. An apparatus was designed for comparing the respiration of entire healthy and mosaic-diseased tobacco plants from the time of inoculation until the appearance of the mottling disease. Glasstone found that the respiration ratio of the diseased plants and healthy plants remained at the same level until the disease became systemic. When rapid movement and increase of the virus as indicated by ' clearing of the veins ' were in progress (Plate 2*a*), the respiration rate of the diseased plants rose rapidly, followed by a decrease until in the older plants it became approximately equal to that of the healthy plants by the time that the mosaic mottling had developed. The percentage increase in respiration rate was approximately 50 per cent higher than the rate of the corresponding healthy plants.

While it cannot be said that viruses stimulate the rate of growth in plants, there is no doubt that some viruses cause excessive growth in certain plant organs. For example, Kunkel [57] has shown the stimulating effect on the flower trusses of tomato of the virus of cranberry false-blossom. The sepals of the diseased flowers are much larger than the sepals of normal flowers and fuse to form a sac-shaped structure, and the diseased truss

itself is about four times as long as the healthy truss. Some strains of cranberry false-blossom virus cause a severe check to longitudinal growth but stimulate transverse growth, other strains stimulate longitudinal growth and check transverse growth. Gigantism in flowers of *Calendula* and *Nicotiana glutinosa* is frequently caused by the false-blossom virus of the ordinary type. Outgrowths from the leaves, or *enations*, are common in some virus diseases ; the virus of tomato blackring, for example, produces outgrowths on the leaves of frame cucumber plants which may be an inch or more in depth. The wound-tumour virus produces galls on the stems and roots of clover and the Fiji disease virus causes well-marked galls in the phloem tissues of sugar-cane.

Another effect of some viruses is to repress dormancy and maturity. For example, aster plants affected with aster yellows virus continue to grow after all the normal plants have died and only cease growing when they are killed by the frost. Potato plants affected with the witches' broom virus do not mature and die but continue to grow for an indefinite period if protected from low temperatures. Similarly, peach trees affected by the yellows disease do not stop growing as cold weather comes on but continue vegetative growth until the tender tips of branches are frozen and killed.[57]

STRAINS AND IMMUNITY

There seems little doubt that plant viruses share with animal viruses that characteristic of ' living ' things—the power to mutate. In consequence, many viruses occur in similar and related forms, call them strains, variations or bio-types. The virus which seems to mutate most frequently and of which there are many variants is the tobacco mosaic virus, and an interesting strain has recently been described affecting *Plantago spp.* in the U.S.A.[43] The most commonly occurring

mutation is of the ' yellow-mosaic ' type ; bright yellow spots regularly appear in the leaves of tobacco or tomato plants affected with tobacco mosaic. If such a yellow spot is cut out carefully, so as to avoid bringing any green tissue with it, a virus differing sharply in its symptoms from the type virus can be isolated. A simple method of sub-culturing from such a yellow spot is to prick through it with a sterile needle into the leaf of a healthy tobacco plant held just below. Jensen [46] made some twenty-six isolations of yellow mosaics from naturally occurring yellow spots, and many of them differed markedly in their symptomatology and infectivity from the type virus. Jensen brought forward evidence to show that such apparent mutations did arise spontaneously by the following experiment to purify his virus. He inoculated a plant of *Nicotiana glutinosa* with a dilute solution of tobacco mosaic virus, and when the local lesions were produced, cut out one and made ten serial transfers through *N. glutinosa*. The eleventh transfer was to tobacco which developed the usual systemic disease and in which in due course appeared more yellow spots. Since there is good evidence that a single lesion is caused by a single virus particle, and since many of the ' yellow ' variants are very uninfectious, it seems unlikely that the yellow type viruses could have been carried through ten serial transfers of local lesions and so must have arisen afresh in the tobacco plant inoculated at the eleventh transfer. Studies have also been made on the derivatives from an unusual strain of tobacco mosaic virus and there was some evidence of the possible repeated occurrence of the same mutation.[68] A very similar state of affairs exists in the case of cucumber mosaic virus, and yellow variant strains can be isolated from this virus also. Naturally occurring variants exist with other viruses, notably in sugar-beet curly-top, aster yellows and potato yellow dwarf. Two closely similar viruses occur both with curly-top and potato yellow dwarf, and it is

interesting to find that in each case the respective strains have their specific insect vector. With aster yellows virus also there are two strains, one in California and one in New York, which have a slightly different host range.

It is possible, to a certain extent, to produce or speed up mutations in plant viruses by artificial means, especially by heat, and attenuated strains of tobacco mosaic virus have been produced by this means. Kunkel [54] has shown that if viruliferous individuals of the vector of aster yellows, *Cicadula sexnotata*, are exposed to heat they frequently transmit a mild strain of the virus. He favours the explanation, however, that there has been some kind of selective action exerted by the heat on strains already present in the insect rather than an actual production of a new strain. The claim has been made that new virus strains can be produced by irradiation with X-rays and that strains of the yellow or Aucuba mosaic arise when the type strain of tobacco mosaic virus is irradiated. In experiments at Cambridge a new strain of tobacco mosaic virus was isolated from irradiated samples of the type virus. This new strain was peculiar in that it produced intense necrosis without mottling. It cannot be stated definitely, however, that this strain was a mutation induced by the irradiation alone.

Plants may show a natural immunity to a particular virus, but the usual type of induced immunity is of the non-sterile kind and occurs only between strains and like viruses. There is little evidence yet that plants possess anything comparable to the antibodies of animals. Since acquired immunity exists only between related viruses, a convenient method is at hand for distinguishing virus relationships. This is particularly useful in the differentiation of some of the mosaic viruses such as those of tobacco mosaic, cucumber mosaic, and also potato virus X. The mechanism of this type of acquired immunity is not clear, though it is

necessary for the cells of the plant to be completely invaded by the first virus if the second virus is not to enter.[69]

An acquired immunity of an apparently different kind has been described recently by Wallace [97] working with the curly-top virus. In order to make this phenomenon clear to the reader who is not a plant virus specialist, it is necessary to make a short digression. With certain virus infections, especially of the ringspot type, plants recover completely from the symptoms while retaining the virus systemically within them. This is true also of tobacco plants infected with curly-top, but not of tomatoes, which apparently do not recover from curly-top symptoms. Wallace, then, found that tomato plants infected with the curly-top virus either by means of the leaf hopper or by grafting from other similarly infected tomato plants invariably became severely diseased. On the other hand, tomato plants infected by grafting with scions of Turkish tobacco which had recovered from the symptoms of severe curly-top, developed only a mild disease. This is interpreted by Wallace as a type of passive immunization, some protective substances apparently being transmitted to the tomatoes by the grafts from recovered tobacco plants. Furthermore, if back transmissions are made by means of the leaf hopper from those tomato plants with the mild disease to healthy tomato and tobacco plants, the typically severe disease develops in them, showing that it is not a case of change in virulence of the virus itself. This work has recently been criticized by Price,[70] who suggests an alternative explanation. He found that severity of the curly-top disease in tomatoes when transmitted by leaf-hoppers depended upon the portion of the plant upon which the insect fed. From which he concludes that the mild symptoms induced in some plants by grafting with scions of recovered plants were due to the point of inoculation and not necessarily nor

probably to anything in the nature of antibodies that might have been carried by the scion. Price explains the difference in symptoms largely on the basis of the size of the virus dose and the point of entry of the virus. Thus when the insect vector feeds on tissue near the growing-point a comparatively large dose of virus is injected into the immature tissue which is most liable to become badly malformed. Similarly, the variation in symptoms produced by grafting with scions from recovered plants can be explained on the basis of variations in quantity of virus that moved from the point of union to the growing-points of the plant. This would explain the difference in severity of symptoms in various axillary shoots on the same grafted plant.

CHAPTER III

MODES OF TRANSMISSION OF VIRUSES

Natural and artificial

THE most important natural method of plant virus transmission is by the agency of insect vectors. Since the relationship between viruses and insects is an interesting and important subject in itself it is dealt with separately in the next chapter. At this juncture it may be sufficient to note that the majority of insect vectors belong to the Order Homiptera and are of the sap-sucking and not of the biting type. It has just recently been shown, however, that the turnip yellow mosaic virus is spread by a flea beetle which is a leaf-eating insect.[85]

Apart from the agency of insects the natural modes of transmission are few and may be classed as follows. (1) By contact, (2) By seed, (3) Through the soil, and (4) By all methods of vegetative propagation.

TRANSMISSION BY CONTACT

Except under unusual circumstances, only the more infectious viruses, i.e. those occurring in high concentration in their host plants, are spread by contact between diseased and healthy plants. Examples of this method of spread are given by tobacco mosaic virus, potato virus X, and possibly by turnip yellow mosaic virus. Since tobacco mosaic virus is so infectious, it spreads not only by contact of diseased and healthy plants, but is also carried mechanically on contaminated implements. This is the chief method of spread of the virus on tomatoes under glass in this country, particularly during the process of tying up and removing

side-shoots. It should also be remembered that most commercial brands of tobacco contain the virus in a viable state and that cigarettes are frequently a source of infection. For a virus to spread from a diseased to a healthy plant by contact, it is necessary for a wound, however slight, to be made to allow for entrance of the virus.

Potato virus X can spread in a very effective manner by contact of diseased and healthy haulms in the rows, and Roberts (*Nature*, **158**, 663, 1946) has demonstrated that there is underground spread of the virus as well. It has long been a moot point whether virus X could also be spread during the process of cutting the seed pieces, but it has recently been shown that this does not occur unless the contaminated knife passes through an eye that has already sprouted.

TRANSMISSION BY SEED

Transmission of viruses by seed is comparatively rare, but there are a few authentic cases. The exact reason for this rarity of transmission is not known, though it may be due to the anatomical isolation of the embryo in the seed and the lack of connecting plasmodesms. The mosaic of bean (*Phaseolus*) is the best known case of seed transmission, although seed from mosaic beans do not always give rise to diseased plants, the percentage varying from 13 to 50.

It has long been a matter of controversy as to whether the virus of tomato mosaic, which is the same virus as that causing tobacco mosaic, is transmitted through the seed. If such transmission does occur it seems to be rare. Infection may sometimes arise through the cotyledons becoming contaminated on rupturing the seed-coat and some recent work supports this possibility. It was found that a proportion of tomato seedlings arising from seed freshly extracted from a mosaic fruit developed the disease if planted direct from the fruit into the soil, but no disease developed

Outgrowths or 'enations' are produced on the undersides of leaves by some viruses

PLATE 4

Local lesions, a type of symptom which allows of the quantitative study of viruses

in seedlings arising from seed which had been dried for several months.

In the curly-top disease of sugar-beets the virus appears to be confined to the phloem elements and to be unable to flourish in the parenchymatous tissue. This fact may account for its non-transmission by the seed since there is no vascular connection between the mother plant and the young sporophyte. There are other records in the literature of seed transmission of viruses, some of which need confirmation, but there is no doubt that the virus of lettuce mosaic is so transmitted. In certain cases there appears to be seed-transmission of a virus from one host but not from another. A case in point is that of tobacco ringspot virus which is said to be transmitted through the seed of *Petunia violacea* but not through the seed of tobacco. Similarly with the infectious variegation of *Abutilon* spp., no transmission was obtained through the seed of *Abutilon regnelli*, but a few infected seedlings of *Abutilon striatum* var. *Thompsoni* were obtained.

TRANSMISSION THROUGH THE SOIL

Soil transmission of viruses is rare, but there are two authentic cases. The first of these is tobacco necrosis virus which occurs in the roots of apparently normal tobacco and other plants. The virus, which is highly resistant, is washed down into the soil where it comes into contact with the roots. Here again a wound of some sort is necessary to allow entry of the virus, and this condition is fulfilled by the breaking of the root-hairs during the movement of the roots through the soil. If, however, a susceptible plant is grown in a culture solution containing virus, infection does not result since the root-hairs are not broken and no entry point is afforded to the virus.[79]

The other instance of soil transmission occurs with the mosaic of winter wheat,[63] but the exact mechanism

of spread is still unknown. Some workers think that a subterranean insect or nematode worm is the vector, but this seems unlikely in view of the fact that the disease will develop in seedlings grown in virus-infested soil which has been retained in an air-dried condition for three years.[48, 49] It is possible that the mechanism of infection is similar to that which occurs with tobacco necrosis virus and that the wheat mosaic virus retains its infectivity in the soil for long periods and enters through the ruptured root-hairs during growth of the roots. Occasional cases of transmission of tobacco mosaic virus by contamination of healthy plants with virus material left in the soil may occur, but this is exceptional and in any case it is difficult to infect a tobacco plant with the mosaic virus by the roots. The virus may enter the root, but apparently has difficulty in spreading from there to the rest of the plant.

TRANSMISSION BY VEGETATIVE PROPAGATION

Since the majority of plant viruses are systemic in their hosts, all organs of the plant with the usual exception of the seed being invaded, the virus persists from year to year in the organs of vegetative reproduction such as tubers, rhizomes, and bulbs.

There are many examples of such propagation of virus diseases. The classic case of course occurs in the potato plant, the tubers of which pass on the viruses with which the plant gets infected year by year until a state of complete ' degeneration ' has set in. It is this propagation of viruses by the tuber which necessitates replacement by Scotch ' seed ' after one or two years' growth in England. All tuberous, bulbous, or rhizomatous plants behave in a similar manner. Dahlias infected with spotted wilt or mosaic, irises and daffodils infected with mosaic or stripe, reproduce the diseases indefinitely. Propagation by cuttings or suckers from infected plants also results in the production of diseased

plants. This occurs with mosaic and leaf-curl of raspberry, crinkle and yellow-edge of strawberries, bunchy-top of bananas, and so on.

TRANSMISSION BY ARTIFICIAL MEANS

A plant virus may enter its host through an extremely trivial wound, the breaking of a trichome is sufficient to allow entry of the more infectious viruses, but it appears to be an accepted fact that a wound of some sort is essential for infection to occur.

BY INOCULATION

In this context the word inoculation is used to describe the introduction of virus sap into the tissues of a healthy plant. Not all plant viruses are sap-transmissible, but most of the mosaic-type viruses can be spread by this means. Viruses do not easily enter wounds made prior to inoculation but require wounds made in their presence. That the entry of virus into such wounds is practically instantaneous is shown by the fact that washing the surface of a leaf immediately after inoculation in no way affects the subsequent development of the disease.

Various implements can be used for inoculating plants; the pestle, with which the infected leaves used as a source of inoculum have been crushed, serves the purpose if used lightly. Small pieces of muslin, cotton-wool or the cut edges of filter-paper dipped in virus sap and rubbed gently over the surface of healthy leaves are quite effective. Spatulae with a ground glass surface or small pieces of rubber sponge may also be used. Probably, however, the tip of the fore-finger dipped into the inoculum and rubbed lightly over the healthy leaf is the most effective of all.

It is a good plan to support the leaf to be inoculated on a piece of waxed paper or a wooden label to avoid

possible contamination by the fingers. A gentle rubbing achieves the best results and the addition of an abrasive like fine carborundum powder or celite greatly increases the likelihood of infection. In most experimental transmissions with viruses it is important to use young and vigorous plants.

BY GRAFTING

In order to make a successful graft it is essential that actual organic union between stock and scion be effected. All plant viruses which are systemic in their hosts can be transmitted by grafting and there are several methods, varying according to the type of plant used.

For plants with soft sappy stems like potatoes and tomatoes, the best method is the ordinary cleft graft in which the stem of the scion, cut to a wedge shape, is inserted in a cleft in the stock. The graft is then bound with fine rubber tape and the loose end secured with a drop of ordinary rubber solution. Rubber tape is more suitable than bast because it does not constrict the stem of the plant but gradually perishes and falls away by the time organic union is completed.

For other types of plants such as strawberries or lilies, inarching may be employed. This method consists essentially in removing by means of a razor blade a small slice from the side of each runner or stem, as the case may be, and then binding them together as before, leaving the roots of each plant undisturbed.

There are two types of tuber grafting which may be practised with potatoes, tulips and similar plants. 'Core-grafting' consists in the removal, by means of a cork-borer, of a core from the infected tuber and its insertion in a hole made in the healthy tuber with a cork-borer one size smaller. Alternatively the cut surfaces of the two halves of diseased and healthy tubers respectively may be placed in contact and bound

together with raffia. In the case of potato tubers the diseased half should be disbudded.

It is sometimes useful to study a virus in a new host plant which the virus would not ordinarily infect. For example, viruses like those of cranberry false-blossom and peach rosette are more conveniently studied in the tomato and tobacco plants. But it is not possible to graft cranberry or peach to tomatoes and the insect vectors will not feed on such unfamiliar host plants. This difficulty can be got over by the use of the parasitic plant, dodder, *Cuscuta spp.*, which, by parasitizing two plants simultaneously, acts as a kind of graft. There are of course limits to this procedure set by the dodder itself which will not parasitize every kind of plant nor transmit all viruses.[15]

CHAPTER IV

THE VIRUSES IN THE INSECT VECTORS

Types of insect vectors: Specificity of insect vectors: Relationship of viruses with insects

THE relationship of plant viruses with their insect vectors is one of the most interesting chapters in virus research, and much intensive study has been carried out upon it. In spite, however, of the impressive amount of facts gained thereby we have little real knowledge of the true relationship between insects and viruses. For example, we do not know the answers to the following questions, although in some cases we may make some fairly justifiable assumptions. Why are some viruses insect-borne and others are not? Why are the very infectious viruses, i.e. those which occur in high concentration in their host plants, not usually insect-transmitted? Or if they are, is it by a mechanical process only? Do plant viruses multiply in their insect vectors? And what is the true explanation of the so-called 'incubation period' (better described as a delay in the development of infective power) of a virus in an insect vector?

TYPES OF INSECT VECTORS

The power to transmit viruses is a very specialized one and the majority of insect vectors are to be found in a subdivision (Homoptera) of the plant-sucking insects (Hemiptera). Both by their food and their method of obtaining it, the Hemiptera are the most likely insects to act as vectors of disease agents. They obtain their food, the sap, by means of a long delicate sucking beak, an ideal injection apparatus, which is thrust into the plant tissue (see Fig. 1). This beak

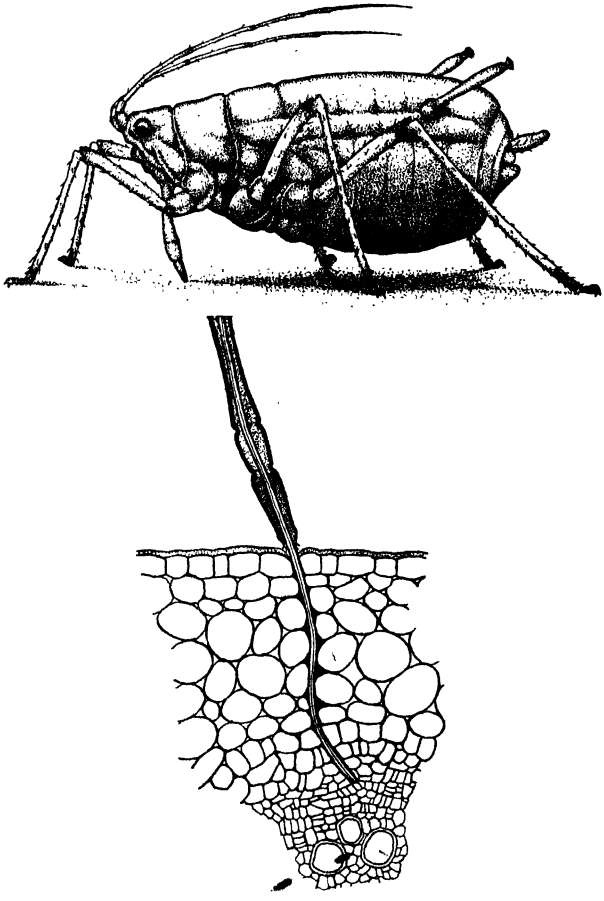


FIG. 1.—Drawing of the aphis, *Myzus persicae* Sulz., in the act of feeding. Note the intercellular path, followed by the stylets through the plant tissue to reach the phloem objective. The ' stylet sheath ', mainly formed from the insect's secretions, is shown in black surrounding the stylets.

contains two parallel channels down one of which flows the saliva which mixes with the sap in the plant, while up the other flows a mixture of sap and saliva drawn upwards by the muscular pharyngeal pump situated in the head. The saliva contains digestive enzymes which dissolve the starch in the plant cells. In sucking up the sap of a virus-diseased plant, the insect naturally draws the virus up also, and this finds its way back to the saliva, with which it is discharged into other possibly healthy and susceptible plants which thereby become infected. Furthermore, most of these insects tap the phloem in search of their food, and in so doing inject the virus directly into an area most suitable both for its multiplication and its rapid distribution about the plant.

SPECIFICITY OF INSECT VECTORS

One of the outstanding characteristics of insect-virus relationships is the specificity of insect vectors, and because of this some interesting facts have come to light. For example, it was through the selectivity in transmission shown by an aphis that it was discovered that a certain common potato virus disease was caused by two viruses and not by one.[77] When parallel transmissions were made from the same diseased potato plant to separate 'indicator' hosts, such as tobacco, by sap-inoculation on the one hand and by aphis on the other, two separate and different diseases were produced in the respective tobacco plants. Further investigation revealed the fact that there were two viruses present in the potato plant, both of which were sap-transmissible but only one of which was aphis-borne.

It sometimes happens, also, that two aphis-transmitted viruses occur together in certain brassica plants; both these viruses are spread by the common cabbage aphis (*Brevicoryne brassicae*). If, however, an aphis of an entirely different species, and one that does not normally

feed on brassicae, is colonized on a plant infected with these two viruses, it selects out one of the viruses but is unable to pick up the other one.[58] Another interesting phenomenon occurs in the aphis-transmission of the tobacco rosette disease. This also is a complex virus disease, but the two component viruses behave quite differently in their insect relationships from those forming the potato disease complex just mentioned. In the rosette disease of tobacco, of the two viruses concerned only one is mechanically transmissible, but both are aphis-borne so long as they are together in the plant. When, however, they are separated, one of the viruses can no longer be picked up by the aphis. At the moment there seems to be no satisfactory explanation of this phenomenon.[83]

It is now a well-established fact that viruses mutate and occur in closely similar forms or variants. In certain cases it appears that a related strain of a virus may have its own specific insect vector which is a different one from that transmitting the type strain of virus. The specificity seems to be absolute and neither insect can transmit the other virus. This phenomenon occurs in the disease known as potato yellow dwarf which is found in two variants, called the New York and New Jersey strains. The leaf-hopper which transmits the New York strain is *Aceratagallia sanguinolenta* Prov., but this insect cannot spread the New Jersey strain of virus which is transmitted only by another leaf-hopper, *Agallia constricta* Van Duzee.[22, 23] A similar state of affairs seems to exist in the curly-top disease of sugar-beet in which the North American virus has a specific leaf-hopper vector, *Eutettix tenellus* Bak., whereas the Argentine strain of the virus is transmitted only by another species of leaf-hopper, *Agalliana ensigera* Oman.[17]

RELATIONSHIP OF VIRUSES WITH INSECTS

There are several distinct types of transmission between insect vectors and particular groups of viruses which may be broadly divided up as follows: (1) Those viruses which are rapidly lost by the insect if it has not access to a fresh source of virus; that is to say, in a series of successive 24-hour transfers from plant to plant only the first plant in the series is infected. This type of virus is usually spoken of as *non-persistent*. (2) Those viruses which are retained for long periods by the insect, frequently for the rest of its life, without again having access to a fresh source of virus. This type of virus is known as *persistent* and in a series of successive transfers the first plant is usually not infected while the others are.[98] The first one or two plants in the series remain uninfected because of the delay in the development of infective power or ' incubation period ' of the virus in the insect, referred to at the beginning of this chapter. (3) The third type of transmission may be termed mechanical and refers to the purely passive transfer of virus by contamination of the jaws of biting insects.

It is possible that the difference between the persistent and non-persistent viruses is a quantitative rather than a qualitative one, and this may be one reason why non-persistent viruses, in contrast to persistent viruses, are usually sap-transmissible. If we suppose that the non-persistent viruses are all present in high concentration in their source plants they would be easily transmissible by sap-inoculation, whereas a virus present in the sap in concentration too low to be sap-transmitted might nevertheless be insect-transmitted since this is a more efficient method of transmission than by mechanical means. As a rule the so-called persistent viruses are not sap-transmissible, but there are exceptions such as the viruses of potato yellow dwarf and tobacco mottle.[84]

One of the questions posed at the beginning of this chapter dealt with the possibility that a plant virus may multiply in the body of an insect vector. This question has more than an academic interest because if a plant virus multiplies within the body of an insect it becomes in part an animal virus also and so suggests affinities between the two types of viruses. The most recent evidence supporting this hypothesis is the work of Black,[21] who colonized a number of leaf-hoppers of uniform size and age upon a source of aster yellows virus for a given period. The insects were then removed and colonized upon a rye plant which is immune to the virus; thus all the insects received the same dose of virus. At intervals of forty-eight hours or so, about fifty leaf-hoppers were removed, ground up in various dilutions, and inoculated to virus-free leaf-hoppers which were then caged on healthy aster seedlings. Black found that the insects which had been longest on the rye plant would withstand the highest dilution while still producing infection when inoculated to virus-free leaf-hoppers. He interprets this as indicating virus multiplication since all insects presumably received the same initial dose of virus.

Kunkel has also carried out some interesting experiments with leaf-hoppers and the aster yellows virus which he considers support the hypothesis of virus multiplication in the insect. He found that if the insects, infected with the aster yellows virus, were heated for a long period, they lost the power to infect unless recolonized on a source of virus. If, however, the insects were heated for a shorter period, they too lost the power to infect but gradually regained it after an interval. This is interpreted by Kunkel to mean that in the first case the virus was completely destroyed by the prolonged heating and in the second case it was reduced below the threshold of infection but regained it after a lapse of time by multiplication in the insect's body.[54]

There are, however, alternative interpretations to both these results and certain other findings militate against the theory of virus multiplication. For example, it has been shown that the length of time a leaf-hopper retains the virus of sugar-beet curly-top, or for that matter aster yellows virus also, is correlated with the time of feeding on the source of virus. This suggests a storage, rather than a multiplication, of virus.

The fate of the virus, once swallowed, in the body of the insect is an interesting subject and some light has been thrown on it by the work of Storey on the streak disease of maize.[91] He has shown that there exist two distinct races of the insect vector of that virus, the leaf-hopper *Cicadulina mbila*. There is no visible difference between these two races, which are both of the same species. The difference lies in the fact that one race can transmit the streak virus (active), while the other race is unable to do so (inactive). Now if the wall of the alimentary canal be punctured by a fine needle either just before or just after the insect has fed on a streak-diseased plant, the inactive insect becomes an active one; in other words, it is now able to transmit the virus. This seems to suggest that for some reason the virus is unable to diffuse through the walls of the gut in the inactive insect and to reach the salivary glands. That the inactive insect does actually imbibe the virus is shown by the recovery of the infective agent from the faeces. On the face of it, therefore, it would appear that permeability or otherwise of the gut-wall may play a part in determining the ability of an insect to transmit a plant virus, although there are other factors as well. In many Homopterous insects—the chief vectors of plant viruses—there is a special modification in the digestive system to deal with the excess, due to their mode of feeding, of water and sugars imbibed, and this modification may also have a bearing on virus transmission. Instead of the superfluous fluids being taken into the blood and then

eliminated by the Malpighian tubules, they are absorbed, or perhaps filtered, directly into the hindgut and so discharged. This is achieved by means of a dilated loop of the foregut, the ' filter chamber ', which has very delicate walls and is invaginated into the rectum. Here again the permeability of this filter chamber may affect the insect's power to transmit a virus.

The question of the inheritance of a plant virus by the progeny of an infected insect is an interesting one, but there is only one apparently authentic case of such inheritance in the literature. Fukushi,[35] working with the dwarf disease of rice, showed that the virus was passed from parent to offspring to the third generation without recourse to a fresh source of virus. For the virus to be inherited by the progeny it was necessary for the female parent to be infective ; if only the male parent was infected the offspring were all virus-free. This instance of virus inheritance is often quoted as evidence of multiplication of a plant virus in an insect vector, but there seems no good reason to suppose that the facts could not also be explained on the basis of a storage rather than a multiplication of virus. Unfortunately there appear to be no data correlating length of time of feeding on a source of virus with inheritance.

There is good evidence to show that some animal viruses are transmitted from the parent vector to the offspring, though these are not usually insects but other arthropods. For example, it has been recently shown that the virus of St. Louis Encephalitis is transmitted through the eggs of the chicken mite, *Dermanyssus gallinae*.[86]

The question of the insect's saliva is an important one in relation to virus transmission, and there seems no doubt that the saliva is the actual vehicle of virus transfer. This can be demonstrated by feeding leafhoppers which are infected with the curly-top virus on drops of sugar-water. If known virus-free leafhoppers are then fed on the same drops and subsequently

colonized on sugar-beet seedlings, they will infect a proportion of the seedlings with curly-top, having picked up the virus left in the sugar-drops with the salivary secretions of the first lot of insects.[80]

On contact with the air the saliva of Hemipterous insects sets into a gel, and because of that property a so-called 'stylet sheath' surrounds the path of penetration by the stylets into plant tissue (see Fig. 1). It has been suggested by Sukhov[92] that this stylet sheath acts as a filter which prevents certain viruses such as that of tobacco mosaic from gaining access to the plant. This seems to be altogether too facile an explanation, as if it was true there is no reason why any virus should be aphis-transmitted unless it be assumed that the tobacco mosaic virus was unable to pass out of the stylet track because of its rod-like particles.

The salivary secretions of some Hemipterous insects, notably the Capsidae, are injurious to plant cells, and this may be one reason why capsid bugs do not transmit plant viruses. The toxic saliva kills the cells and thus cuts off the multiplication and spread of any virus which might be in the saliva. The first requirement for multiplication of a virus, a living cell, is thereby not fulfilled.

It has been stated that in the case of the persistent viruses there is frequently a so-called 'incubation period' of the virus in the insect, better called a delay in the development of infective power. Working with the virus of aster yellows, Kunkel showed that this delay may be as long as nine days, which is actually longer than the nymphal life of the insect vector, and in such cases the nymphs are unable to transmit the virus. If, however, the nymphal stages are exposed to low temperatures, the larval development is retarded but not the development of infective power. Under such conditions the nymph is able to transmit the virus.

The transmission of tomato spotted wilt virus by the

thrips presents an interesting anomaly. This virus can be transmitted by the larval thrips but not by the adult thrips *unless* it has fed as a larva on a source of virus. In other words, the adult thrip cannot pick up the virus *de novo*. The reasons for this curious behaviour are quite obscure for the time being, and although various suggestions have been put forward to account for it, none is very convincing.

If virus-carrying insects such as aphides or leafhoppers are ground up and the resulting material is inoculated to susceptible plants, no infection follows. This is due to the presence in the insect of an inhibitor, probably protein in nature, which prevents infection of the plant. The virus and the inhibitor can be separated by high dilution or by spinning on the high-speed centrifuge when the virus is thrown down and the inhibitor, being much smaller than the virus, remains in solution.[20]

It is clear, then, from this short account that the relationship between viruses and their insect vectors is a complicated one and that there are many aspects of this relationship which need clarification.

CHAPTER V

THE VIRUSES THEMSELVES

Isolation : Size and shape : Chemical and physical properties

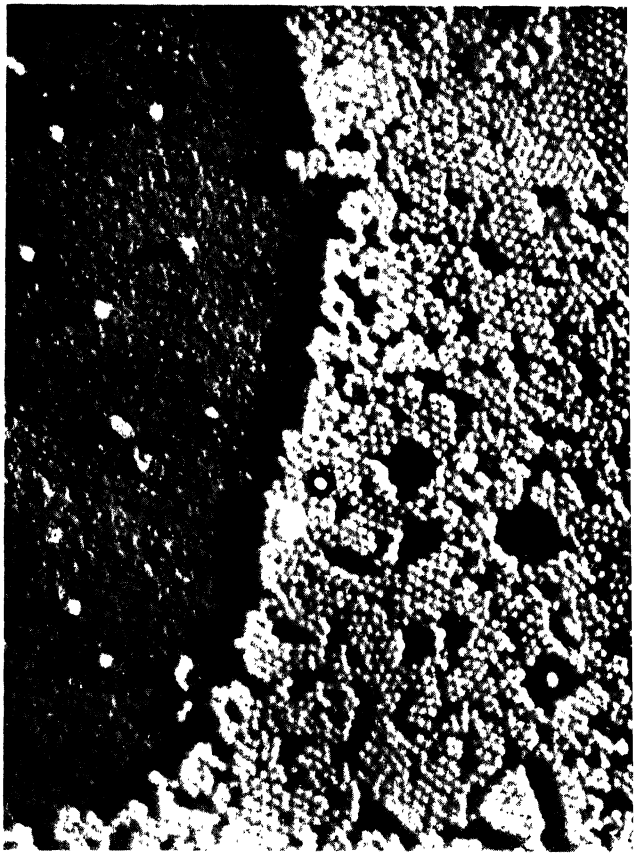
ALLARD in 1916 [1] seems to have been the first to visualize a plant virus as a separate entity and, in an attempt to isolate the virus of tobacco mosaic, he adsorbed an active fraction from the juice of diseased plants by means of talc and aluminium hydroxide. Some years later McKinney [62] attempted to purify the same virus by centrifugation. He spun crude extracted sap at a high speed for a short time to remove extraneous plant materials ; he then heated the supernatant to 65° C. and centrifuged again for five to ten minutes.

The first serious attempt to precipitate and isolate the tobacco mosaic virus by chemical means was made by Vinson,[95] who used acetone, ammonium sulphate and safranin. Later Vinson and Petre [96] showed that the safranin-virus precipitate was inactive but that activity was restored when the safranin was removed with amyl alcohol. From these results they considered that the behaviour of the virus was in many ways analogous to that of a chemical substance.

In 1933 Barton-Wright and McBain [3] tried to precipitate the virus, using ammonium sulphate. They obtained some crystals which, however, were not virus crystals. Then in 1935 Stanley,[89] working at the Rockefeller Institute, Princeton, U.S.A., gave the first description of the crystallization of the virus. He was also the first to isolate the virus as a tangible entity and show it to be a protein, which none of the earlier workers had succeeded in doing. In 1936 Best,[18] working independently in Australia, precipitated the

Tobacco mosaic virus : photographed on the electron microscope by the shadow technique. × 46,000

PLATE 6



Tomato bushy stunt virus: photographed on the electron microscope by the shadow technique. × 120,000

tobacco mosaic virus at its isoelectric point and showed that the precipitate gave positive tests for protein. In the same year Bawden, Pirie, Bernal and Fankuchen [9] showed that the tobacco mosaic virus protein could exist in a mesomorphic or liquid cyrstalline condition and was probably rod-shaped. Bawden and Pirie showed that the virus was a nucleo-protein. All these earlier experiments were made with the virus of tobacco mosaic, which by its stability and high concentration in its host plant lends itself to this type of chemical work.

In 1938 Bawden and Pirie [7] isolated the virus of tomato bushy stunt in a pure state and showed that it formed true three-dimensional crystals, dodecahedra as compared with the paracrystals or liquid crystals of tobacco mosaic virus. The next virus to be crystallized was that of tobacco necrosis, which formed thin plate-like laminae. After that came first the virus of southern bean mosaic which crystallizes in the alternative forms of rhombic prisms and bipyramids, and then that of turnip yellow mosaic which crystallizes in small octahedra.[65]

METHODS OF PURIFICATION OF VIRUSES

There are two main methods of purification of plant viruses, by chemical precipitation methods and by spinning out the virus on the high-speed centrifuge.

To extract the virus the diseased plants are usually frozen, thawed and minced; the wet pulp is pressed by hand through muslin and the sap collected. The pulp residue is then put in a hand or hydraulic press and the remainder of the sap collected. In the case of tobacco mosaic virus it has recently been shown that much more virus can be obtained after the above processes if the remaining fibres are digested with enzymes from snail-gut. Except for potato virus X and alfalfa mosaic virus, most plant viruses are resistant to the action of trypsin, and this enzyme can be used in removing normal plant proteins.

The two precipitating agents most commonly used are alcohol and ammonium sulphate. The alcohol can be used for precipitating either the virus or the extraneous plant proteins according to the particular virus being used or the strength of the alcohol. Some viruses, that of tobacco mosaic, for example, are insoluble at their isoelectric points and so can be thrown out of solution by suitable adjustment of the pH.

Not all viruses are suitable for the chemical methods of purification, and some which are rather unstable are better isolated by means of the high-speed centrifuge, which avoids the deleterious effect of ammonium sulphate and changes in hydrogen-ion concentration. The crude virus sap is first clarified, either by heat to coagulate the plant proteins, by addition of basic phosphate or alcohol, or by low-speed centrifugation, and then spun on the high-speed centrifuge. In the case of tobacco mosaic virus centrifugation for about two hours at 60,000 times gravity results in a solid pellet at the bottom of the tubes. The pellets are dissolved in water and the insoluble matter centrifuged off at low speeds. Two or three repetitions of this treatment result in the production of virus proteins similar in every respect to the virus proteins produced by chemical methods. The ordinary laboratory type of Sharples centrifuge is quite suitable for spinning down most plant viruses.

It may perhaps make things clearer to the reader if the purification of two plant viruses is described in some detail, first the isolation and crystallization of turnip yellow mosaic by chemical methods, and secondly the isolation and crystallization of tobacco necrosis virus by means of the Sharples centrifuge. The crystalline preparations of the two viruses obtained by these methods are illustrated in Plate 8. In the preparation of the turnip yellow mosaic virus, infected plants of Chinese cabbage were used.[65] The plants were frozen overnight, allowed to thaw, and then

passed through a hand mincer and the sap collected. The crude sap was centrifuged for about twenty minutes at 3,000 r.p.m. to remove as much of the extraneous plant material as possible. To this clarified sap was next added 0·30 volume of 87 per cent alcohol to bring it to 23 per cent concentration; it was allowed to stand a few minutes until precipitation was complete. It was then centrifuged for thirty minutes and the heavy precipitate discarded. To the supernatant fluid was added half its volume of saturated ammonium sulphate; this was allowed to stand in the icebox overnight. In the morning the precipitate was found to consist of many crystals of the type illustrated in Plate 8*a*. The precipitation should be repeated several times.

In the preparation of tobacco necrosis crystals on the centrifuge, the following procedure was adopted; 730 ml. of crude sap were extracted from infected tobacco plants and made up to a litre with absolute alcohol. This was centrifuged on the low-speed centrifuge and the precipitate discarded. The supernatant fluid was then spun on the Sharples centrifuge in aliquots of 250 ml. for five hours. The precipitate was resuspended in 40 ml. of distilled water, centrifuged at low speed and the insoluble matter discarded. On the addition of ammonium sulphate to one-third saturation large numbers of needle-like crystals were formed and are illustrated in Plate 8*b*. The actual shape of the crystal is a thin plate, but this easily fractures into the needle-like crystals shown in the plate.

LIST OF PLANT VIRUSES WHICH HAVE BEEN OBTAINED IN CRYSTALLINE AND PARACRYSTALLINE FORM.

Tobacco mosaic virus and its various strains Cucumber viruses 3 and 4 Potato virus X	All these viruses are rod-shaped and do not form three-dimensional crystals but paracrystals or liquid crystals

Tomato bushy stunt virus	Rhombic dodecahedra
Tobacco necrosis viruses	Thin lozenge-shaped plates, hexagonal prisms, dodecahedra bipyramids
Southern bean mosaic virus	Rhombic prisms and rhombic bipyramids
Turnip yellow mosaic virus	Octahedra

SIZE AND SHAPE OF VIRUS PARTICLES

By the application of the exact methods of the physicist to the study of viruses much information has been obtained on the size and shape of virus particles. These methods have recently been reviewed at length [66] and may be listed as follows: (1) Ultra-violet light and electron microscopy, (2) X-ray diffraction, (3) Sedimentation and diffusion, (4) Filtration, (5) Radiation inactivation. It had long been suspected, however, from the optical properties displayed by clarified sap from tobacco mosaic infected plants that this virus was not a sphere but a rod. Because of this shape the earlier attempts to measure the size of this and other rod-shaped viruses such as potato virus X by ultrafiltration were invalidated. There is in fact still a good deal of doubt as to the exact particle size of the tobacco mosaic virus. We can say with fair certainty that the diameter of the rod is 15·2 mμ, but we do not know what is the exact length of the individual particle nor is it certain that the virus as it exists in the plant is a rod at all. This possibility is supported by the fact that true three-dimensional crystals which may be virus crystals occur in the cells of tobacco plants affected with tobacco mosaic, but the virus when extracted from the plant forms only liquid, and never three-dimensional, crystals. Liquid crystals are composed of rather asymmetrical particles and three-dimensional crystals of more symmetrical particles. The most direct method

of finding the size of a virus is by ultra-violet light or the electron microscope. However, since the wavelength of ultra-violet light which can be used with a quartz objective is about 2,000 Å, only the larger viruses of 100 mμ upwards can be measured by this means. This does not allow of the plant viruses to be photographed. The wavelengths of electrons of about 60 kV. energy being 0·05 Å, the theoretical limit of resolution of the electron microscope is well beyond that necessary for the accurate measurement of even the smallest viruses. However, with present instruments the resolution is of the order of 5 mμ, which is smaller than any plant virus at present known. Photographs of plant viruses taken with the electron microscope are shown in Plates 5 and 6. These were obtained by means of the new shadow technique of Williams and Wyckoff. Put very briefly, this is done by volatilizing a metal-gold in a high vacuum and the beam of gold thus produced is directed obliquely on to the virus particles. The gold having a great density is more opaque to electrons than are the virus particles; a shadow is thus thrown by the particle, giving the three-dimensional effect shown in the photographs.

The great merit of the use of the electron microscope is that the measurement of size is direct and is not subject to error due to the necessity of interpreting the measurements of theoretical formulae of the validity of which it is difficult to be certain, but on the other hand it is not easy to measure the exact magnification and the particles have to be exposed dry, in a high vacuum, to bombardment by electrons. The second method on our list, by X-ray diffraction, is only applicable to viruses obtainable in a crystalline or semi-crystalline form and has so far been successfully applied only to tomato bushy stunt virus and to the tobacco mosaic group of viruses. In a bushy stunt virus crystal or a tobacco mosaic virus gel, the virus particles are arranged in a regular array or pattern. This array

serves as a diffraction grating to a beam of X-rays, and measurement of the angles at which the X-rays of known wave-length are diffracted enables the plan of the pattern and the repeat interval to be established. Thus in the case of wet crystals of bushy stunt virus, it has been found that the pattern is a body-centred cubic lattice of edge 394 Å. In other words, if one imagines the crystal to be built up out of cubes of this size, then at the centre of each cube, and at every corner, there is either one virus particle or an identical group of virus particles. There will thus be two particles, or groups, per cube of edge 394 Å, and knowing the density of the crystal the molecular weight of the particle or group can be calculated. In the absence of information to the contrary it has been assumed that the element in the lattice is a single particle and not a group.

In measuring the size of virus particles by means of sedimentation and diffusion, the experiments consist of determining S_{20} and D_{20}, the sedimentation and diffusion constant respectively, the former being the rate of sedimentation in an ultra-centrifuge in centimetres per second under a centrifugal acceleration of 1 dyne/g., and the latter being the rate of transference of virus across unit area under a unit concentration gradient. For a spherical, non-hydrated virus, the partial specific volume of which is known, measurement of either S_{20} or D_{20} alone gives the size of the virus.

The method of determining the size of a virus by filtration through collodion membranes of graded porosity depends upon (*a*) determining the pore size of each membrane used in terms of a conventional measure known as the ' average pore diameter ' (A.P.D.), (*b*) relating for the type of membrane used, the A.P.D. to the size of the particle just stopped by the membrane. This is the method used by Elford.[32] The actual size of the particle is calculated by the ratio size of particle/A.P.D. and a revised estimate of this relation-

ship is given in the review article previously mentioned[66] as 0·55–0·95 of the A.P.D. of the retaining membrane.

The recent method of radiation inactivation is based on the fact that, when a virus is inactivated by ionizing radiation, i.e. X-rays or a radio-active radiation, but not ultra-violet light, it is possible to calculate from the amount of inactivation produced by known doses of radiation what may be called the radio-sensitive volume of the virus, i.e. the volume of that part of the virus within which energy must be absorbed from the radiation for inactivation to occur. Absorption is a highly localized phenomenon, and is sufficiently energetic for it to be tolerably certain that when energy absorption, i.e. ionization, occurs in a particular atom, the molecule or radicle of which that atom is a part suffers chemical change. The radiation method thus measures the total volume of all those molecules or radicles which are so essential to the virus that infectivity is no longer retained when any one of them suffers chemical change. In a large virus such as vaccinia a great deal of the material in the virus is not essential in this strict sense, and the radio-sensitive volume is only a very small fraction of the volume of the virus. On the other hand, a virus which crystallizes is presumably a single molecular species, and we can reasonably expect that chemical change almost anywhere in the molecule will cause loss of infectivity or change in the symptoms. In this event the radio-sensitive volume will be identical with the volume of one molecule of the virus, i.e. the least quantity of the nucleo-protein having the characteristic properties of the virus. It is on this basis that the method has been proposed as a means of determining the sizes of the crystallizable plant viruses. In Fig. 2 is given a diagrammatic representation of the particle sizes of a few representative plant and animal viruses which have been obtained by some of the methods briefly outlined above.

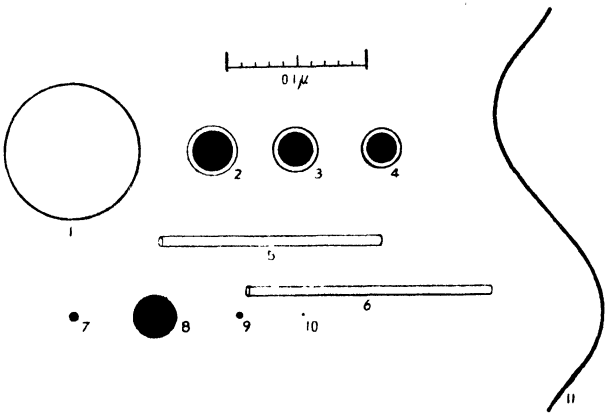


FIG. 2.—Chart showing the comparative sizes of some representative viruses and protein molecules.

(1) Influenza virus ; (2) Tomato bushy stunt virus ; (3) Southern bean mosaic virus ; (4) Turnip yellow mosaic virus ; (5) Tobacco mosaic virus ; (6) Potato virus X ; (7) Haemoglobin ; (8) *Helix haemocyanin* ; (9) Ovalbumen ; (10) Cytochrome C ; (11) Wavelength of green light.

Note.—The outer circle in 2, 3, and 4 represents the size of the hydrated molecule.

PHYSICAL AND CHEMICAL PROPERTIES

It was first demonstrated by Takahashi and Rawlins[93] that the virus of tobacco mosaic when extracted from the plant was a rod and not a sphere. They noticed that when sap from a mosaic-infected tobacco plant was viewed by polarized light between crossed nicol-prisms it showed the phenomenon of ' anisotropy of flow '. Minute rods, discs, or leaf-shaped particles which are contained in a flowing liquid tend to become orientated with their long axes parallel to the direction of flow rather like logs in a stream. Under these conditions a liquid containing rods having a different refractive index

from that of the liquid is doubly refractive when the direction of transmission of the incident light is perpendicular to the direction of flow. This is what is meant by ' anisotropy of flow '. It has been shown by Bawden and Pirie [5] that when concentrated solutions of tobacco mosaic virus are allowed to stand undisturbed, they separate into two liquid layers. The upper is the more opalescent, though it is more dilute than the lower. If these two solutions are viewed by polarized light, it will be seen that the lower layer is birefringent spontaneously, i.e. is liquid crystalline, but the upper is not birefringent while stationary but becomes so if gently agitated.

Potato virus X and cucumber viruses 3 and 4 also have rod-shaped particles and behave in a similar manner to the tobacco mosaic virus.

A number of plant viruses have been shown to be spherical; tomato bushy stunt, tobacco necrosis, southern bean mosaic and turnip yellow mosaic viruses are all spheres. Very complete data are available on the bushy stunt virus and it would appear to have as much claim to homogeneity as any other protein. It has a molecular weight of 10·6 million and is of interest as the first protein in which all reliable data point to the conclusion that it is considerably solvated, the amount of water found being about 0·7 g/g.[64]

All the plant viruses so far isolated seem to be nucleo-proteins with the possible exception of southern bean mosaic virus. The nucleic acid seems to be of the same type, in each case being related rather to the nucleic acid of yeast than to the thymus nucleic acid containing a pentose and not a desoxy-pentose. On the other hand, the nucleic acid of an animal virus like vaccinia appears to be of the thymus variety.

Studies on amino-acid content have so far been carried out only on tobacco mosaic virus. According to Ross [74] this virus contains 3·9 per cent tyrosine, 4·5 per cent tryptophane, 4·7 per cent proline, 9·0 per

cent arginine, 6·7 per cent phenylalanine, 6·4 per cent serine, and about 5·3 per cent threonine. Glycine and histidine appear to be absent. To these may be added glutamic acid, 6·3 per cent, aspartic acid, 2·4 per cent, leucine, 6·1 per cent, valine, 3·9 per cent, and alanine, 2·4 per cent. These bring the total quantity of the known constituents of the virus to about 68 per cent. In Table I are given the elementary analyses on seven viruses which have been purified. It will be seen that significant differences occur only in the figures for phosphorus and carbohydrate.

TABLE I

Elementary Analyses of Purified Virus Preparations

Virus	Carbon %	Hydrogen %	Nitrogen %	Phosphorus %	Carbohydrate %
Tobacco mosaic	50	7·3	16·5	0·5	2·5
Potato virus X	49	7·4	16·4	0·45	2·7
Potato virus Y	50	—	16·0	0·4	3·0
Tomato bushy stunt	49	7·7	16·1	1·4	5·5
Tobacco necrosis	45	6·5	16·3	1·65	6·5
Tobacco ring-spot	51	7·6	14·6	4·1	18·0

(*After Bawden.*)

CHAPTER VI

SEROLOGY OF PLANT VIRUSES: CLASSIFICATION

SEROLOGY OF PLANT VIRUSES

IN describing very briefly the serology of plant viruses, it may be helpful to explain certain of the terms used. An *antigen* is any substance which, when introduced into the animal tissues, stimulates the production of an antibody, and when mixed with that antibody reacts with it in some observable way. An *antibody* is defined as any substance which makes its appearance in the blood, serum, or body fluids of an animal in response to the stimulus provided by the introduction of an antigen into the tissues.

Since it was first shown by Purdy (Beale) in 1928 [71] that the juices of mosaic-diseased tobacco plants contain an antigen specific for virus-containing extracts and not present in the sap of healthy plants much research has been carried out on the antigenicity of plant viruses.

Three types of reaction have been considered,

(1) *Neutralization* of the properties of the virus.

(2) *Complement fixation test.* When antigens are mixed with their specific antibodies the mixture has the property of removing the power of normal serum to haemolyse sensitized red corpuscles. It is a kind of delicate colour indicator test. *Complement* is a heat-labile substance present in normal blood serum.

(3) *Precipitin reaction.* A precipitate is formed when the virus is added to its specific antiserum in saline at different dilutions and warmed in a

water-bath. In precipitation the antibody is referred to as *precipitin*.

To obtain the antiserum the rabbit is the animal generally used, though the domestic fowl has been employed on occasions. The injections are either intraperitoneal or intraveinal and the quantity of virus (antigen) used at each injection is about 5 c.c.

A number of injections are made at three- to four-day intervals and about ten days after the last injection the animal is bled, the blood is allowed to clot and the immune serum is collected. The number of injections, however, depends on the stability and concentration of the virus being used.

Since the pioneer work of Purdy-Beale on the serology of tobacco mosaic virus, antisera have been prepared for a very large number of plant viruses and the technique has proved a useful one in various ways.

In carrying out experimental work of this nature it must be remembered that the proteins present in normal plant sap are themselves antigenic. Therefore any antigenic property shown by a virus-diseased plant might be due to (1) alteration by the virus of the normal healthy antigenic constituents of the plant, (2) linkage of the virus to the normal healthy constituents of the plant, (3) the virus itself.

Now it has been shown by means of precipitin tests that antisera prepared for virus suspensions which have been freed from the normal plant proteins will still react with crude virus-containing plant juice but not with the crude juice of healthy plants. Furthermore, purified preparations of tobacco mosaic virus from tomato plants will react with antisera for crude virus-containing juice from tobacco plants and with the antisera for purified preparations but not with the juice from healthy tobacco plants. These facts show that the reactions secured are due to the virus and not to the plant proteins.[19, 51]

It has been shown that plants of tobacco, *Datura* and potato, when infected with potato virus X, contain a common antigen which can be obtained in a relatively pure condition by carbon dioxide precipitation from infected plant saps. This antigen flocculates and fixes complement with the sera of rabbits immunized with either crude sap from infected tobacco plants or with the purified virus suspension, but not with the sera of rabbits immunized with *healthy* tobacco sap or with normal rabbit serum. The anti-virus sera at a dilution of 1 : 10 neutralize the infectivity of purified virus suspensions whilst anti-healthy and normal rabbit serum do not. The virus antigen is specific to virus X and the closely related potato virus D. It was not found in the sap of tobacco plants infected with the viruses of tobacco mosaic, tobacco ringspot or potato virus Y. No differences were detected between any of the different strains of the X-virus used. Sera prepared against one strain reacted equally well with purified suspensions of any other strain. That the antigen is closely associated with the virus was shown by filtration experiments. Filtrates through collodion membranes which were infective flocculated serum, those which were not infective did not do so.[88]

There is further evidence that these precipitin tests are specific and this phenomenon may be used in the classification and differentiation of plant viruses. Thus, extracts of a number of different Solanaceous plants affected with tobacco mosaic, attenuated tobacco mosaic, and yellow (aucuba) mosaic all yielded extracts giving a positive precipitin reaction with antiserum to tobacco mosaic virus. On the other hand, extracts of plants affected with mosaic diseases other than tobacco mosaic reacted negatively with antiserum to tobacco mosaic virus.[72]

Since it is possible to distinguish serologically between viruses a method is available for finding out relationships between viruses which on their host relationships are

entirely distinct and alternatively for demonstrating differences between viruses which on their host relationships are apparently identical. In the first case Bawden and Pirie have shown [6] that the viruses known as Cucumber Viruses 3 and 4 have certain antigens in common with tobacco mosaic virus. This relationship could not have been determined by means of the cross-immunity test on host plants (see Chapter II), because these cucumber mosaic viruses and the tobacco mosaic virus have no common host plant. In the second case, it has been shown [8] that the virus of tobacco necrosis is in reality a number of viruses biologically similar but serologically unrelated. This could not have been demonstrated by the symptomatology because the lesions produced on the plant are all identical.

Another application of the precipitin reaction is to detect a latent virus in a carrier plant, and this is particularly useful in the production of virus-free seed potatoes when all virus-infected plants must be eliminated. A latent form of potato virus X is common in potato plants and its presence cannot be detected from the appearance of the plant alone. The ordinary method of testing for the presence of potato virus X is to inoculate the sap of the suspected potato plant into a more susceptible indicator host such as *Datura Stramonium*. This takes at least a week or ten days, but the precipitin test can be carried out with a few precautions on the spot actually in the field and takes a few moments only.

It is possible to inactivate a virus under certain conditions without affecting its immunological properties; thus it was found that some viruses which had been rendered non-infective by treatment with hydrogen peroxide, formaldehyde, nitrous acid, X-rays or ultra-violet light still reacted to antisera. It has been shown[10] that if partially purified preparations of potato virus X are treated with nitrous acid, the virus is inactivated. Nevertheless, it remains fully antigenic and is capable

of stimulating the formation of antibodies *in vivo* as well as reacting with them *in vitro*.

Bawden [4] has pointed out that the particle-shape of the virus has an effect on the serological reactions and the character of the specific precipitate is almost certainly determined by the shape of the virus particle. Thus viruses which are known to have rod-shaped particles are agglutinated rapidly and form large clumps which resemble the type of precipitate given by flagellate bacteria, whereas viruses which are spherical, or nearly so, such as those of tomato bushy stunt and tobacco necrosis, are agglutinated more slowly and form smaller denser clumps characteristic of the type of precipitate given by the bodies of bacteria.

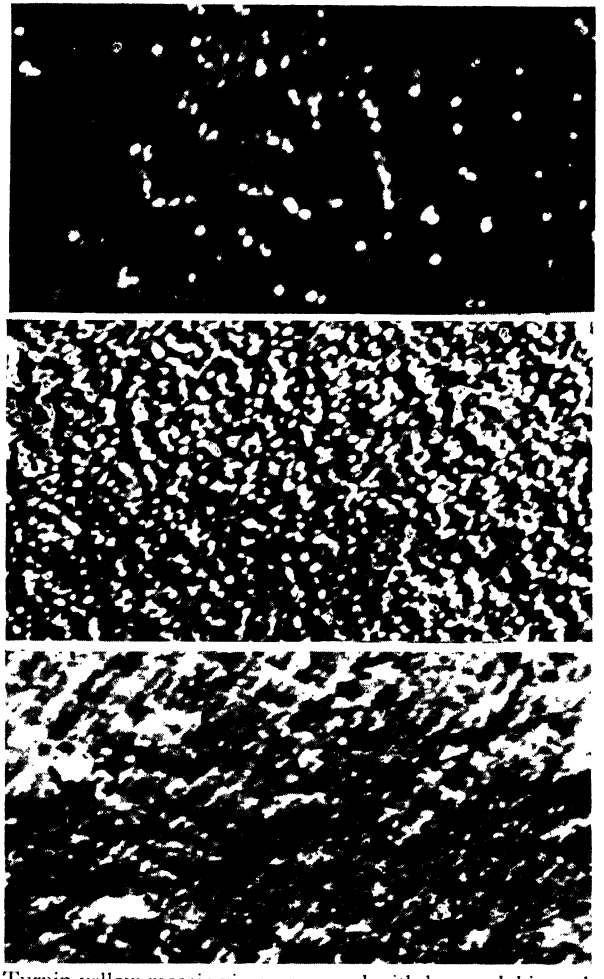
CLASSIFICATION OF VIRUSES

There have now been described about one hundred and fifty distinct viruses, not counting virus strains, and many new ones are constantly being added to this number. It is evident, therefore, that some method of classification and nomenclature is urgently needed. Several attempts have been made to classify and name the plant viruses and none has proved very successful or acceptable to virus workers as a whole. The first to attempt this was James Johnson,[47] who gave the name of the first-described or most important host plant to the virus, followed by a number; thus tobacco mosaic virus became Tobacco Virus 1, and so on. This system of nomenclature was adopted in a modified form in the writer's textbook of plant virus diseases,[78] the Latin name of the plant being substituted for the English one to give the system an international application; under this system Tobacco Virus 1 becomes *Nicotiana Virus 1*. There are obvious disadvantages in a system of this sort and they can be briefly stated as follows: first, the simplicity of the numbering system may prove one of its sources of greatest confusion.

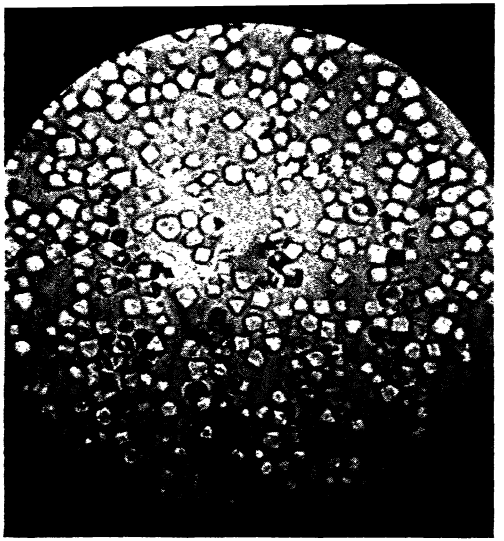
Numbering viruses is so easily accomplished that it encourages frequent changes in numbering and the too easy numbering of viruses insufficiently identified as new. Secondly, a number means nothing in respect to any characteristic of a virus or of the disease it causes. Because of this it is difficult to remember numbers in association with specific viruses. Thirdly, numbers do not permit the desired degree of mobility in the organization of viruses according to different conceptions of relationships. For example, the curly-top virus of North America is known as *Beta Virus 1*, but there is another curly-top virus recently discovered in South America with a different specific insect vector and there is no certainty that the two viruses are identical. The second curly-top virus must therefore either be *Beta Virus 1* also or else *Beta Virus 6*, since the intervening numbers have already been assigned to other beet viruses, some of the mosaic type.[14]

Bennett [14] has suggested a modification of this system whereby names are substituted for numbers; under this system Tobacco or *Nicotiana Virus 1* would become *Nicotiana Virus altathermus*, the last name referring to some characteristic of the virus, in this case to its high thermal inactivation point.

In 1939 Holmes [42] put forward a scheme of classification and nomenclature based on a Latin binomial-trinomial system. This certainly has a more scientific appearance than the foregoing system, but closer investigation reveals serious drawbacks. The chief fault is that the system is based mainly on symptomatology. For example, there are the three genera, *Marmor*, the mosaic viruses, *Annulus*, the ringspot viruses, and *Lethum*, the streak viruses. Now it is easy enough to find one virus, that of tomato spotted wilt, which will fit equally well into all three genera according to the type of host plant it happens to be affecting. Another drawback to the system is the size of the genus *Marmor*, which contains a vast number of totally unrelated and



Turnip yellow mosaic virus compared with haemoglobin and *Helix* haemocyanin : electron shadowgraphs by G. Crowe.
× 100,000



(*a*) Octahedral crystals of turnip yellow mosaic virus: photographed under the 1/12 oil immersion lens

(*b*) Fragmented crystals of tobacco necrosis virus: this virus crystallizes in very thin plates which easily break up as shown

dissimilar viruses which are lumped together chiefly because they happen to produce a mosaic mottling on some hosts. This grouping ignores the fact that the viruses themselves differ fundamentally in all their main properties.

There seems no good reason for including tobacco necrosis virus and dahlia mosaic virus in the same group, since the first is a sap-transmissible, crystallizable virus which has no insect vector and never under any circumstances causes a mosaic disease, and the second is a non-sap-transmissible mosaic virus with an aphis vector.

In addition to the several systems outlined above, other suggestions have been made by Valleau,[94] Fawcett,[33] and others.

Obviously the ideal system of virus classification would be one based on the fundamental properties of the viruses and particularly on their serological relationships. This has been advocated by Bawden,[4] who has drawn up a list of viruses related in this manner. These form twelve groups in which the viruses in each group are precipitated by each other's antisera but not by antisera prepared against viruses in other groups.

This is admirable so far as it goes, but it does not go nearly far enough, and there remain a great number of viruses which are not amenable at present to serological treatment.

This, then, is the situation at the present moment: a few virus workers in North America use Holmes' method and a few prefer the numbering system advocated in the writer's textbook. The majority, however, still name the virus after the disease symptoms produced, and since there is no unanimity among plant virus workers, the present confused situation is likely to continue.

A useful supplement has recently been published by the Imperial Mycological Institute as Part 13 of the

Review of Applied Mycology, vol. 24, 1945, in which the common names of all plant virus diseases are listed, together with the numerous names and synonyms of the viruses given by various authors.

CHAPTER VII

CONTROL OF PLANT VIRUS DISEASES

THERE are various methods of approach to the question of the control of plant virus diseases, but they are not of course all applicable in the same degree to the various diseases. These methods can be classified roughly under five headings and the application of each of them to specific virus diseases will be briefly discussed.

(1) Elimination of the sources of virus infection.
(2) Avoiding the insect vectors.
(3) Direct attack on the insect vectors.
(4) Breeding resistant varieties of crops.
(5) Cure of virus-infected plants.

To these may perhaps be added the ' vaccination ' of a plant with an avirulent or masked strain of a virus which thereby immunizes the plant against a more severe strain of the same virus but not of course against a different virus (see Chapter II). This method is at present purely academic.

ELIMINATION OF THE SOURCES OF VIRUS INFECTION

This procedure is of course applicable in a greater or lesser degree to the control of all plant virus diseases, but it is especially important in dealing with the virus diseases affecting the potato and sugar-beet crops, most of which are aphis-borne. The foundation of good crops of potatoes is the use of good quality ' seed '; it is essential to start the crop with as little virus in it as possible. Having obtained a stock of clean ' seed ', it is extremely important to grow it as far as possible

from second-rate potatoes. All the advantages of virus-free ' seed ' will be lost if the crop is grown alongside home-saved ' seed '.

Another important point in the elimination of sources of infection is careful attention to roguing out any obviously virus-diseased plants and also any ground keepers which are usually a prolific source of virus infection.

Roguing should be done as early as possible while the plants are still small; there are several reasons for this. There will have been less time for the virus to have spread from the infected plants, no tubers will have been formed and small plants are easier to dispose of.

With sugar-beet it is easy enough to start with a virus-free crop since the root crop arises from the true seed and few viruses are seed-transmitted. The virus infection is thought to come mainly from two sources, the seed crop and the volunteer beets. It is therefore important to grow the root crop as far away as possible from the seed or mother beets which, being two-year plants, are always heavily infected with virus. Volunteer beets growing on headlands, in corn crops or elsewhere in the neighbourhood should be rogued out so far as is practicable.

AVOIDING THE INSECT VECTORS

The growing of seed potatoes in particular areas of Scotland is a practical illustration on a large scale of the control of potato virus diseases by avoiding the insect vectors.

The climate of the Scotch seed-growing districts is unfavourable to the aphides which spread the viruses. These insects will not fly if there is a wind blowing more than 4 m.p.h.; if the temperature is lower than about 65° F. and if relative humidity is more than 75 per cent. There are also humid areas in England

which fulfil these conditions and efforts are now being made to grow seed potatoes in some of these areas.

Attention to the life history of the chief potato aphis, *Myzus persicae*, shows that this insect can be partially avoided even in England. There are three possible ways in which the aphis can pass the winter, as an egg on the peach tree, in glasshouses and in mild winters out of doors on brassica crops such as cabbages and brussels sprouts. Whenever practicable, therefore, potato crops should be grown in areas where *Myzus persicae* cannot find these facilities for overwintering.

Sometimes it is possible to avoid a bad infestation of an aphis vector by early sowing. Thus early sowing of the sugar-beet commercial crop is recommended because it avoids the infestation by *Myzus persicae* of the very young beet plants and allows them to be more advanced before the aphis appears.

A more positive method of avoiding the insect vectors of viruses is to ward them off the crop by means of screens. This has been done in the U.S.A. against the leaf-hopper which spreads a virus disease of asters known as ' aster yellows '. Two types of shield have been tested. In one type, cloth-covered side walls or ' fences ' 6 feet high with tops uncovered were made. These combined with roguing reduced the incidence of yellows but were not found to be commercially satisfactory under local conditions. In the second type, cloth-covered cages or houses were employed. The tops and sides of the enclosures were completely covered with cloth not coarser than 22 by 22 threads per inch. These were found satisfactory for the practical control of the disease.

A similar form of protection has been employed in production of virus-free potato ' seed ' in the U.S.A. Certified tubers were grown under large cages made of the ' aster cloth ' above mentioned. The experiments were made with two cages, covering 32 and 4 square rods, respectively. The yield rate under the cloth

was 140 barrels (385 bushels) per acre, this being about the same as in the open field. The cloth had about 21 meshes to the inch and cast some shade but not enough to change the appearance of the plants, as do small cages of heavier cloth. On the whole, the cages seem to have been fairly efficient in excluding the aphis vectors of potato viruses, but the method is likely to prove too expensive for general use, at all events in this country.[34]

DIRECT ATTACK ON THE INSECT VECTORS

This is perhaps the least hopeful of the methods of combating plant virus diseases, because to control the spread of the virus the kill of the insect vector concerned must be so high; very few insects are necessary for the efficient spread of a virus. However, the possibility is mentioned here in view of the development of new insecticides; but D.D.T. does not promise well against aphides owing to the slowness of its action, and the need for some persistent insecticide is still very great. Some success has been achieved in the control of the strawberry aphis, which spreads the virus diseases of that plant, by means of rapid nicotine fumigation. This is carried out by means of a low canvas tent towed slowly over the crop while nicotine vapour is pumped in by the towing vehicle.

BREEDING RESISTANT VARIETIES

One of the promising methods of control lies in the production of virus-resistant varieties of plants, and for this we must look to the plant breeder. Some success in this direction has already been achieved. Several good varieties of mosaic-resistant sugar-cane have been produced, known as the P.O.J. strains, and the substitution of these for susceptible varieties in most of the sugar-growing areas has reduced the disease to one

of small importance, although at one time it threatened the very existence of the sugar-cane industry. Similarly with the sugar-beet in the U.S.A.; at one time the curly-top disease seriously threatened the whole industry and in certain parts of the Union the growing of sugar-beet had to be abandoned. However, by the combination of a number of strains selected for resistance, varieties have been produced (U.S. Nos. 1, 33, and 34) which have a fair degree of resistance to curly-top and are reasonably satisfactory as regards sugar content, &c.

In England, the most serious virus disease of sugar-beet is known as ' virus yellows ', and here the situation is less satisfactory because no varieties of sugar-beet seem to exist which show any kind of natural resistance to yellows which might be used as breeding material.

Strains of cotton of Sakel type resistant to the leaf-curl disease have been evolved, and these seem to combine vigour and fruitfulness with a high degree of resistance.

As regards the production of virus-resistant potatoes, the position is complicated by the number of viruses which commonly attack the potato. The choice before the plant breeder is to develop either tolerant or carrier types or intolerant varieties. The drawbacks to the carrier or tolerant types are, firstly, their liability to infect other more susceptible varieties, and secondly, the fact that a second virus infection added to the carried virus produces a more severe disease than would otherwise be the case. The aim behind the development of intolerant varieties is to make them so susceptible to the virus or viruses in question that they are killed outright. Such varieties are said to be ' field-immune ' since the virus is destroyed with its host and cannot spread further.

In the U.S.A. a potato seedling No. 41956 has been produced which is not only resistant but appears to be actually immune from infection with potato virus X,

the most widespread of all potato viruses. Breeders of tobacco plants for resistance to the tobacco mosaic virus have two types of resistance to work with. In one type it is necessary to breed a variety of tobacco with the gene that localizes the virus in necrotic lesions on the leaf, and so prevents the spread of the virus from plant to plant. This has already been achieved to a certain extent by transferring the gene from *Nicotiana glutinosa* to the tobacco plant.[41] The other possibility is to breed from the tobacco variety Ambalena, which shows a natural resistance to the virus.

CURE OF VIRUS-INFECTED PLANTS

The most successful method of curing a virus-infected plant is by means of heat, and this has a limited application since it can only be used against viruses with a low thermal inactivation point. This treatment has been applied by Kunkel [53] to peach trees affected with such virus diseases as peach yellows, little peach, red suture and rosette. The trees were kept at a temperature of about 35° C. for a fortnight or more and the time necessary was longer for large trees than for small, and it was easier to destroy the virus in the top of the tree than in the roots. There seems no doubt that the trees were actually cured of the viruses since a scion from the treated tree produced no disease when grafted to a healthy one. Moreover, the trees could be reinfected with the viruses, which shows that there was no question of attenuation or masking of the disease. Later Kunkel [56] showed that the virus of aster yellows could also be destroyed by heating the host plant, but the treatment could only be applied to certain plants such as periwinkle, *Vinca rosea* and *Nicotiana rustica* which could survive being grown at 40° C. for two weeks.

Other treatments against viruses in their hosts are irradiation and the use of chemicals. The writer and

his colleagues have attempted to free tubers of King Edward potato from the paracrinkle virus by irradiation, but without success. Any tubers which survived the various doses still contained the virus.

So far as chemotherapy is concerned, the only attempt seems to be that of Stoddard,[90] who states that he cured buds from peach trees affected with the X-disease by soaking them in water solutions of quinhydrone, urea and sodium thiosulphate.

A virus with a very low thermal inactivation point is that of tomato spotted wilt, which is inactivated by ten minutes' exposure to a temperature of 42° C. Since this virus is of considerable importance to horticulturists, and particularly to growers of dahlias, it is worth considering whether cures of dahlias might not be effected by submitting the tubers to heat treatment.

CHAPTER VIII

NATURE OF VIRUSES

IN trying to arrive at some conception of the nature of viruses, it may be helpful to make a brief survey of the various theories which have been held from time to time since before the first scientific demonstration of a virus in 1892. In spite of his own showing of the filter-passing ability of the tobacco mosaic virus, Iwanowsky [45] seemed convinced that the disease was caused by a small bacterium, a theory first suggested in 1886 by Mayer, but this theory was disproved for the time being in 1899 by Beijerinck,[11] who a year previously had put forward his idea of a *contagium vivum fluidum*. Then came the suggestion that virus diseases were caused by enzymes, and this was followed for a time by the protozoan theory which was fostered by the discovery of the intracellular inclusions which bear a superficial resemblance to protozoa.

All these ideas and theories were gradually dissipated by improving methods of study and the final death-blow to them was given by Stanley's isolation of the tobacco mosaic virus protein.[89] The application of electron microscopy and its shadow technique to the study of viruses have enabled us to form an excellent conception of what many viruses look like, but as regards their origin and nature we have not advanced much beyond Beijerinck's 'living infectious fluid', whatever that may mean.

A question invariably put by the layman and by some scientists who should know better is whether viruses are living organisms. This is a question which cannot be answered since there is no criterion of what is a living organism. It may, however, help to clarify

matters slightly if we recapitulate briefly some of the outstanding characteristics of viruses. There is first the characteristic of all living things, the power to multiply, though as to the mechanism of this reproduction we have not the slightest conception. Secondly, there seems little doubt that viruses, both plant and animal, mutate, or at any rate give rise to closely related strains, variants or bio-types of the parent strain. Both these properties are characteristic of living organisms. On the other hand, there is the undoubted chemical behaviour of many viruses and their isolation in a crystalline form. Furthermore, several of the plant viruses and one or two animal viruses are apparently so small as to be of molecular size and it is difficult if not impossible to conceive of these as organized.

Another characteristic of viruses is their close affinity with the living cell, an affinity which apparently precludes the multiplication of viruses in any medium except that of a susceptible cell.

Various theories on the origin of viruses have been suggested, one of which was put forward independently by Green[37] (1933) and Laidlaw.[59] (1936) This theory supposes the viruses to be parasites which have developed parasitism to the highest possible degree. Laidlaw suggests that parasites through the indolence of living a ' borrowed life ' gradually give up making substances essential for their growth because they are always at hand in the cells of the host.

On this assumption the larger viruses would be organisms which had lost the power to synthesize some factor or factors essential for their growth. The intermediate-sized viruses would have lost several essential ferment systems and the smallest-sized viruses would have lost all ferments and all auto-synthetic potentialities, retaining only the irreducible minimum required to transmit the characters of the species. In other words, the diminution in size may be a rough measure of the number of ferment systems lost. This

is certainly a plausible and ingenious theory, though personally the writer finds it difficult to visualize the extremely small plant viruses as all that is left of a parasitic organism. Moreover, it is logical to suppose that the smaller the virus has become the more ferment systems it will need to exist and multiply and the more demands it will make on its host. The smallest viruses therefore on this theory are the ones which would do the most damage and there seems no evidence to support this.

Another school of thought rejects the conception of a highly evolved parasitic organism and visualizes an 'auto-catalytic protein'. This theory presupposes that there is some constituent in the host's cell which can be readily converted into the virus protein, in other words, a precursor of the virus. The difficulty here is to conceive that in the cells of a given host there exist precursors to all the different viruses to which it may be liable. It is no uncommon thing for a plant, tobacco for example, to be susceptible to more than a dozen unrelated viruses.

The question of the heterogeneous or spontaneous origin of viruses [89] is one which is of interest to all virus workers, though at the present time few workers accept the theory of spontaneous origin, the majority preferring to give no opinion but to await further developments. One suggestion on these lines is that the virus may be of the nature of a 'free' or naked gene which had escaped from the nucleus and was multiplying independently of it. It has been suggested by Bawden [4] that it would be more reasonable to compare the viruses with groups of genes or with fragments of chromosomes rather than with single free genes. There are undoubtedly certain resemblances between genes and viruses, both have the property of synthesizing replicas of themselves from the cell fluids of appropriate hosts and both seem to belong to the same physico-chemical category of particles. The fact, however, that the

nucleoprotein of viruses seems to differ from the nucleoprotein of nuclei in containing nucleic acid of the ribose type rather than thymus nucleic acid does not support very strongly a chemical relationship between viruses and chromosomes.

Another suggestion concerning the origin of plant viruses has recently been made;[100] in this they are considered to have arisen from mitochondria and their derivatives. Put very briefly, the theory seems to be as follows; there are some non-infectious variegations in plants which are to a certain extent under plastid control, but the chief difference between ordinary variegations and virus diseases seems to lie in the migration or movement of the causal agents. Now in this theory it is suggested that there is a limited movement of the plastids (or chondriosomes) from cell to cell by means of the plasmodesmata as opposed to distribution by cell division. This evidence for limited invasion by the chondriosomes allows of a comparison between a variegation and one of the slow-moving viruses causing an infectious variegation such as *Abutilon* mosaic. From this it is argued that mutations within the chondriosomes coupled with the natural selection of more stable, actively multiplying and invasive types may have resulted in the appearance of viruses. Thus certain viruses may be considered to be derivations of the chondriosome rather than to have evolved from parasitic micro-organisms. Further evidence of such evolution would consist in the demonstration that the plastid protein complex of higher plants contains a ribose nucleoprotein such as the known virus-ribose nucleoprotein. Various reactions characteristic of ribose-nucleoprotein are said to be given by a protein fraction of the chromoprotein complex of purified plastids of *Nicotiana tabacum*.

Darlington[29] considers that viruses and plasmagenes The particles in the nucleus are genes; those in the plastids or cytoplasm may perhaps be treated more

rigorously if we also think of them as genes, plastogenes and plasmagenes '[28]) are derived from cell proteins. In his view these undifferentiated proteins may come to acquire the properties either of infection or, alternatively, of inheritance by a process of adaptation (see Fig. 3). In support of his theory Darlington quotes the paracrinkle virus in the potato King Edward (see

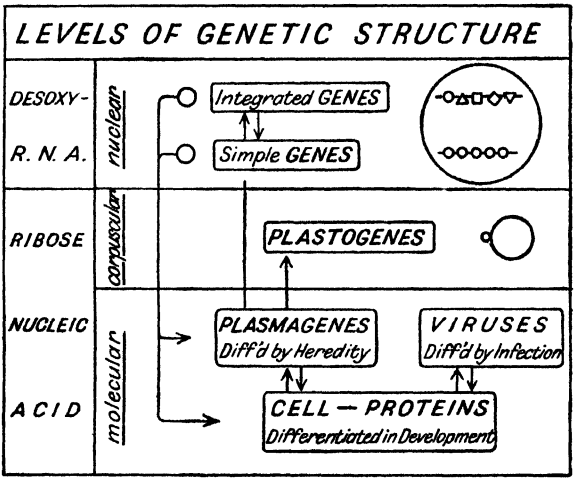


FIG. 3.—Diagram showing the suggested origin and relationship of plasmagenes and plant viruses.
(After Darlington, *Nature*, 1944.)

Chapter II) which he supposes is manufactured by the potato plant itself and, in his words, ' what is a stable and presumably useful cell protein with one plant genotype acts as a destructive agent with another '.

This theory of the heterogenesis of viruses seems to depend upon the following statement which is quoted from Darlington [30]—' Cell proteins characteristically produced by a nucleus of a particular new genotype, may have the capacity for indefinite self propagation

even when subject to other nuclei. Such proteins will sometimes have the potentiality of developing, either into plasmagenes or into viruses, according to their distribution in development or their opportunities in infection '.

A similar theory has been put forward by Haddow [38] on the origin of the viruses causing the Rous sarcoma in fowls and mammary cancer in mice.

At the time of the first isolation of a virus in crystalline form the criticism was frequently made that the crystals were not the actual virus itself which was said to be adsorbed to, or a contaminant of, the crystals. Although this criticism is no longer considered valid, it may be helpful to the reader at this juncture to recapitulate briefly the reasons for supposing that some plant viruses can be obtained in a pure crystalline condition. First, the virus nucleoprotein can only be obtained from the virus-infected plant; if the purification technique is carried out on a healthy plant nothing resembling the nucleoprotein can be found. Furthermore, the same nucleoprotein can be obtained from plants botanically unrelated, but only if they are virus-infected, as, for example, the tobacco and phlox. The particular type of nucleoprotein which can be isolated from a virus-infected plant depends entirely upon the particular virus with which the plant is infected. Moreover, the infectivity of the virus protein is extremely high, as little as 10^{-8}–10^{-10} gm. being sufficient to infect a susceptible plant. If the virus protein could be shown to be really pure, then it must be the virus itself, and the results of all the physico-chemical studies carried out on virus proteins suggest that they are, in fact, pure nucleoproteins. Any procedure which removes or degrades the protein decreases infectivity. The temperature or degree of alkalinity which destroys the protein also destroys infectivity and all attempts to separate the virus from the protein have failed. Again, the virus protein

gives the sharp boundary in the ultracentrifuge characteristic of a single substance and electron micrographs of the tomato bushy stunt and tobacco necrosis viruses show the particles to be uniform in size and shape. If, therefore, there is a second substance present besides the virus, it must be one with the same molecular weight and must in fact have identical physical and chemical properties. It seems unnecessary to postulate the existence of two almost identical substances when one will suffice.

There is one property of some of the crystalline viruses which in the writer's personal opinion is interesting and seems to require explanation, and that is the loss of infective power by the crystals. This can be brought about, of course, rapidly by certain treatments such as change of pH and by treatment with heat or nitrous acid without denaturing the protein or affecting antigenicity and ability to crystallize. If, however, the crystals of turnip yellow mosaic virus are left at room temperature for three months, they are no longer infectious. Superficially the crystals are unchanged and probably they are still serologically active, but the fundamental attribute of the virus, the power to multiply, is lost. What, then, has happened? Presumably some chemical change has taken place, but it would be interesting, to say the least of it, to know exactly what has occurred.

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