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The isolation and properties of crystalline tobacco mosaic virus

Nobel Lecture, December 12, 1946

Although the idea that certain infectious diseases might be caused by invisible living agents was expressed by Varro and Columella about 100 B. C., there was no experimental proof and the idea was not accepted. The cause of infectious disease remained a mystery for hundreds of years. Even the wonderful work of Leeuwenhoek and his description of small animals and bacteria during the years from 1676 to 1683 failed to result in proof of the relationship between bacteria and infectious disease. There was, of course, much speculation and during the latter half of the 19th century great controversies arose over the germ theory of disease. Then, through the brilliant work of Pasteur, Koch, Cohn, Davaine and others, it was proved experimentally, for the first time, that microorganisms caused infectious diseases. The Golden Era of bacteriology followed and the germ theory of infectious disease was accepted so completely that it became heresy to hold that such diseases might be caused in any other way. Thus, when, in 1892 Iwanowski discovered that the juice of a plant diseased with tobacco mosaic remained infectious after being passed through a filter which retained all known living organisms, he was willing to conclude that the disease was bacterial in nature, despite the fact that he could not find the causative bacteria. As a result, his observations failed to attract attention. However, six years later, the filtration experiment was repeated and extended, independently, by Beijerinck, who immediately recognized the significance of the results and referred to the infectious agent, not as being bacterial in nature, but as a *contugium vivum fluidum*. Obviously, Beijerinck was quite aware of the fact that he had discovered a new type of infectious disease-producing agent. It is also obvious, from the terminology that he used, that he thought of this agent as a living entity.

Since the original discovery of this infectious, disease-producing agent, known as tobacco mosaic virus, well over three hundred different viruses capable of causing disease in man, animals and plants have been discovered. Among the virus-induced diseases of man are smallpox, yellow fever, dengue fever, poliomyelitis, certain types of encephalitis, measles, mumps, influenza,

virus pneumonia and the common cold. Virus diseases of animals include hog cholera, cattle plague, foot-and-mouth disease of cattle, swamp fever of horses, equine encephalitis, rabies, fowl pox, Newcastle disease of chickens, fowl paralysis, and certain benign as well as malignant tumors of rabbits and mice. Plant virus diseases include tobacco mosaic, peach yellows, aster yellows, potato yellow dwarf, alfalfa mosaic, curly top of sugar beets, tomato spotted wilt, tomato bushy stunt, corn mosaic, cucumber mosaic, and sugar cane yellow stripe. Bacteriophages, which are agents capable of causing the lysis of bacteria, are now regarded as viruses.

The viruses have been separated as a special group of infectious, disease-producing agents by means of several general properties, no one of which is, however, exclusively characteristic of viruses. Nevertheless, no great amount of difficulty has been encountered in the segregation of the virus group. Viruses are characterized by their small size, by their ability to reproduce or multiply when within the living cells of a given host, by their ability of change or mutate during multiplication and by their inability to reproduce or grow on artificial media or in the absence of specific living cells. The sole means of recognizing the existence of a virus is provided by the multiplication of the virus which is, of course, usually accompanied by manifestations of disease. Viruses spread from diseased to normal susceptible hosts by different methods. Some are transferred by direct contact, as when a diseased leaf is caused to rub against a healthy leaf by a gust of wind, or when a normal person or animal comes into direct contact with a diseased person or animal. Such viruses can usually be spread by indirect contact through the medium of non-specific animate or inanimate objects. Some viruses cannot be transferred by direct contact, but require an intermediate host such as a mosquito, louse or leaf-hopper. In some cases a highly specific intermediate host is necessary, and a more or less definite period of incubation within this host may be required before it can pass on the virus.

Reproduction, mutation and metabolic activity have long been regarded as unique and special properties of living organisms. When viruses were found to possess the ability to reproduce and to mutate, there was a definite tendency to regard them as very small living organisms, despite the fact that the question of metabolic activity remained unanswered. Because of their small size they could not be seen by means of the ordinary light microscope. Although. this fact puzzled some investigators, it was pushed aside and for over thirty years interest in virus research was centered about the discovery of new viruses and on studies of the pathological manifestations of viruses.

Approximate sizes of viruses and reference materials.

	Diameter or width × length in mμ
Red blood cells	*7500*
B. prodigiosus (*Serratia marcescens*)	*750*
Rickettsia	475
Psittacosis	450
Canary pox	260 × 310
Myxoma	230 × 290
Vaccinia	210 × 260
Pleuro-pneumonia organism	*150*
Pseudo rabies	150
Herpes simplex	150
Rabies fixe	125
Influenza	115
Newcastle disease	115
Vesicular stomatitis	100
Staphylococcus bacteriophage	100
Fowl Plague	90
T_2 coli bacteriophage	60 × 80
Chicken tumor I	70
Equine encephalomyelitis	50
T_3 coli bacteriophage	45
Rabbit papilloma (Shope)	44
Pneumonia virus of mice	40
Tobacco mosaic and strains	15 × 280
Latent mosaic of potato	10 × 525
Gene (*Muller's est. of max. size*)	*20 × 125*
Southern bean mosaic	31
Rift valley fever	30
Tomato bushy stunt	26
Poliomyelitis (Lansing)	25
Hemocyanin molecule (*Busycon*)	*22*
Yellow fever	22
Hemocyanin molecule (*Octopus*)	*20*
Louping ill	19
Tobacco ring spot	19
Japanese B encephalitis	18
Alfalfa mosaic	17
Tobacco necrosis	16
Foot-and-mouth disease	10
Silkworm jaundice	10
Hemoglobin molecule (*Horse*)	*3 × 15*
Egg albumin molecule	*2.5 × 10*

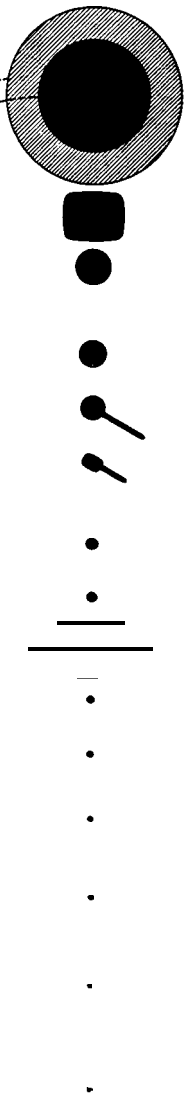


Fig. 1. A chart showing approximate sizes of several viruses and reference materials. (From W. M. Stanley, *Chem. Eng. News*, 25 (1947) *3786.*)

Then, around 1930, Elford began his important work on the filtration of viruses through graded collodion membranes. He demonstrated that different viruses possessed different and characteristic sizes, and that some viruses were as large as about 300 mμ, whereas others were as small as 10 mμ. It was soon realized that the acceptance of a virus 10 mμ in size as a living organism presented certain inherent difficulties, especially with respect to metabolic activity. Grave doubts were expressed that the complicated processes of respiration and digestion and the general metabolic functions usually associated with life could be contained within structures as small as 10 mμ, especially since protein molecules larger than 10 mμ were known. It can be seen from Fig. I, which is a chart illustrating the relative sizes of several viruses and certain reference materials, that the viruses overlap with respect to size, not only with protein molecules, but also, at the other extreme, with accepted living organisms. For example, several viruses are smaller than certain hemocyanin protein molecules, and several viruses are larger than the pleuropneumonia organism, which is an accepted living organism capable of growth on artificial media. The fact that, with respect to size, the viruses overlapped with the organisms of the biologist at one extreme and with the molecules of the chemist at the other extreme only served to heighten the mystery regarding the nature of viruses. Then too, it became obvious that a sharp line dividing living from non-living things could not be drawn and this fact served to add fuel for discussion of the age-old question of "What is life?".

Attempts to learn something about the nature of viruses through studies on their general properties began with Beijerinck's work in 1898 and were continued in different laboratories for over thirty years without too much success. Although Beijerinck and Allard made important contributions, perhaps the most significant work was that of Vinson and Petre during the years from 1927 to 1931 when they showed that tobacco mosaic virus could be subjected to several kinds of chemical manipulations without loss of virus activity. Nevertheless, when the work on viruses, which is recognized by the 1946 Nobel Prize for Chemistry, was started in 1932, the true nature of viruses was a complete mystery. It was not known whether they were inorganic, carbohydrate, hydrocarbon, lipid, protein or organismal in nature. It became necessary, therefore, to conduct experiments which would yield information of a definite nature. Tobacco mosaic virus was selected for these initial experiments because it appeared to provide several unusual advantages. Large amounts of highly infectious starting material were readily

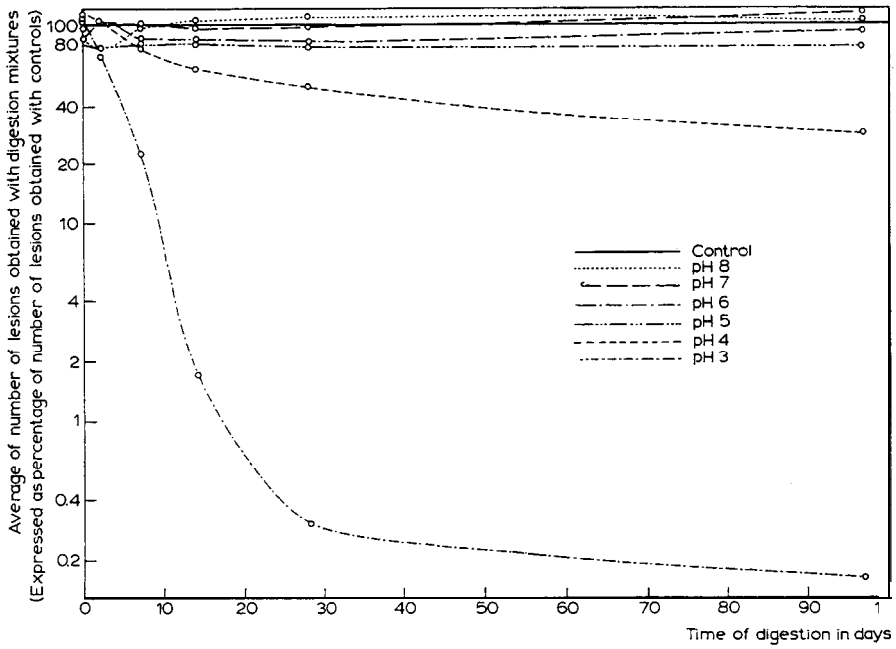


Fig. 2. Graph showing the effect of different hydrogen-ion concentrations on the inactivation of tobacco mosaic virus with pepsin at 37° C. The lines showing the average results obtained at pH 5, 6, 7 and 8 fall very close to the line for the controls, thus indicating little or no inactivation of virus. The line showing the results obtained at pH 3 drops sharply, thus indicating a rapid inactivation of virus. (From W. M. Stanley, *Phytopathology,* 24 (1934) 1269)

available and the virus was known to be unusually stable. Furthermore, it was possible to titrate or measure the amount of this virus in a preparation with ease and rapidity and with great accuracy. The experimental foundation which resulted in the subsequent isolation of crystalline tobacco mosaic virus was laid during the course of two and one-half years. The work was reported in a series of five papers published in Phytopathology in 1934 and 1935 under the general title "Chemical studies on the virus of tobacco mosaic". The subtitles serve to denote the character of the experimental work and are as follows: I. Some effects of trypsin; II. The proteolytic action of pepsin; III. Rates of inactivation at different hydrogen ion concentrations; IV. Some effects of different chemical agents on infectivity; V. Determination of optimum hydrogen-ion concentrations for purification by precipitation with lead acetate.

The experimental results described in the first paper demonstrate that the

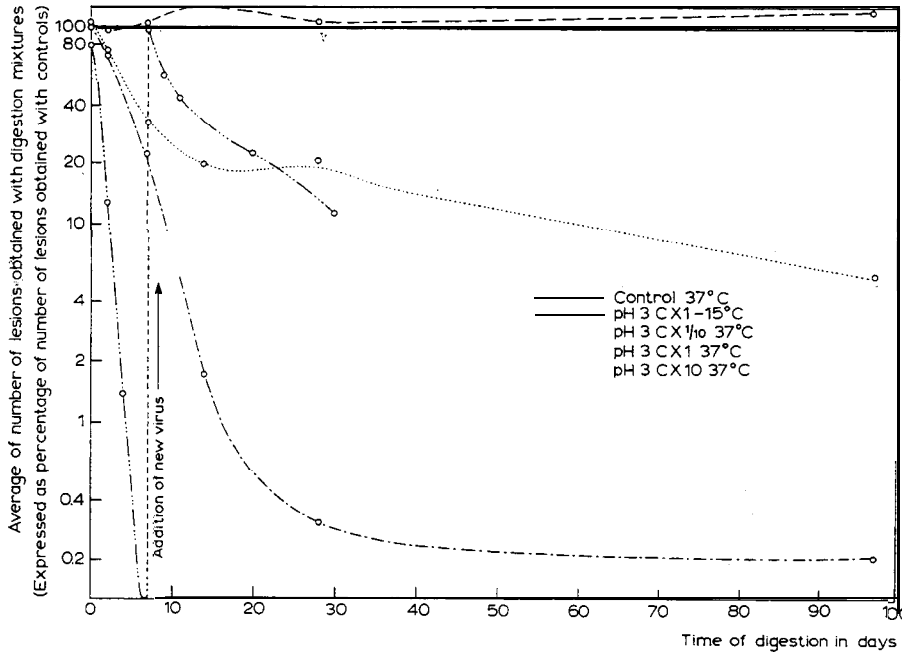


Fig. 3. Graph showing the effect of different pepsin concentrations and of a different digestion temperature on the inactivation of tobacco mosaic virus with pepsin. The line showing the average results obtained with the pepsin concentration (C x 1) used in most of the experiments at 37° C, drops away from the line for the controls sharply, thus indicating a rapid inactivation of virus. The line for the results obtained when the pepsin concentration is reduced to 1/10 (C x1/10) falls off very gradually, whereas the line for the results obtained when the pepsin concentration is increased 10 times (C x 10) falls off almost at right angles. When new or active virus was added to the latter solutions (C x 10) there was but little immediate effect on the infectivity of the added active virus. However on digestion the activity of the added virus decreased as indicated by the extension of the line for the results obtained with pepsin concentration C x 10. The line for the results obtained with the usual pepsin concentration (C x 1) at - 15° C falls very close to the line for the controls, thus indicating little or no inactivation of virus. (From W. M. Stanley, *Phytopathology,* 24 (1934) 1269.)

decrease in infectivity of tobacco mosaic virus on addition of trypsin is not due to the proteolytic activity of trypsin because the loss in infectivity is immediate and can take place over a wide range of hydrogen-ion concentrations, including some at which trypsin is inactive proteolytically. The infectivity of the virus was regained by treatment with heat, by dilution or by digestion and removal of trypsin. It was also found that a similar loss of the infectivity takes place on addition of trypsinogen or globin, proteins which

possess no proteolytic activity, but which, like trypsin, have isoelectric points more alkaline than pH 7. Unfortunately the work with trypsin failed to provide definite information regarding the nature of tobacco mosaic virus. However, in studies with pepsin it was found that this enzyme inactivated the virus only under conditions under which pepsin is active as a proteolytic agent. Pepsin was found to have no appreciable immediate effect on the infectivity of the virus and to have no effect on the infectivity of virus preparations held at pH 7 or pH 8. However, at pH 3 and 37° C the virus activity was found to disappear slowly. Although the rate of inactivation was found to be slower than the rate of peptic digestion of ordinary proteins, it was, nevertheless, proportional to the concentration and activity of pepsin and to the time of digestion. The results of some typical experiments are presented in Figs. 2 and 3. It was not found possible to regain the infectivity of virus inactivated by pepsin. It was concluded in 1934 that "the virus of tobacco mosaic is a protein, or very closely associated with a protein, which may be hydrolyzed by pepsin". At last a definite clue had been obtained and this served to eliminate several kinds of materials from consideration and permitted concentration on proteinaceous materials.

Studies on the rates of inactivation of tobacco mosaic virus at different hydrogen-ion concentrations provided valuable information, for it was found that the virus was extremely stable between pH 3 and pH 8, and fairly stable between pH 1.5 and pH 2.5 and between pH 8 and pH 9. As a result of studies on the effects of over one hundred different chemicals on tobacco mosaic virus, it was found that the only chemicals which had a direct inactivating action could be classified as oxidizing agents, protein-precipitating agents and agents causing a hydrogen-ion concentration known to inactivate the virus. It was concluded that "as a whole, the results are in harmony with the conception that this virus is a protein". As a result of these studies efforts were directed more definitely towards the development of procedures useful in the concentration and purification of proteins. The optimum hydrogen-ion concentrations for carrying out the three principal steps in the lead acetate process for the purification of tobacco mosaic virus proposed by Vinson and Petre were determined. It was found that the optimum hydrogen-ion concentrations for the lead subacetate precipitation, for the neutral lead acetate precipitation and for the elution of the virus from the neutral lead acetate precipitate, were about pH 9, pH 5.5 and pH 7, respectively. Utilization of this process yielded colorless, partially purified solutions having a virus content equal to, or somewhat greater than that of the starting

material. Subsequently, it was found that concentration and purification of the virus could be effected readily by means of a combination of isoelectric precipitation and salting out with ammonium sulfate. A description of this procedure, which yielded a crystalline material as the end-product, is contained in the following paragraph.

Turkish tobacco plants grown in pots or flats in a greenhouse are inoculated when about 3 or 4 inches high by rubbing one or two leaves with a bandage gauze pad moistened with a virus preparation. About 2 or 3 weeks later, and preferably not more than 4 weeks later, the plants are cut and frozen. The frozen plants are put through a meat grinder, the pulp is allowed to thaw, and the juice pressed out. This and all subsequent steps are carried out in a room held at about 4° C, or if this is impossible, the juice and all containers and materials with which it comes in contact are kept as close to 4° C as possible. The juice is adjusted to pH 7.2 ± 0.2 by adding 0.1 to 1 *N* NaOH, recombined with the press cake, well mixed, and again pressed out. The juice will now be at about pH 6.7 ± 0.2. However, if it be desired, sufficient concentrated disodium phosphate to cause the expressed juice to be at pH 6.7 ± 0.2, may be added directly to the pulp. The extract is filtered through a layer of "Standard" celite about ½ inch thick on a Büchner funnel. The celite filter cake is scraped with the flattened end of a spatula from time to time in order to speed the filtration. The filtrate will be found to contain from about 1 to 2 mg total nitrogen per ml, of which about 0.6 to 1.2 mg per ml is protein nitrogen. The globulin fraction is precipitated by the addition of 30 per cent by weight of ammonium sulfate and removed by filtration with filter paper or more rapidly by means of a thin layer of celite on a Büchner funnel. The precipitate is dissolved in 0.1 *M* phosphate buffer at pH 7 or in sufficient water to give about a 1 per cent solution of protein, and then adjusted to pH 7, and again precipitated with ammonium sulfate. It will be found that considerably less ammonium sulfate will be required to precipitate all virus activity. The amount varies somewhat but approaches 11 per cent by weight. The use of smaller amounts of ammonium sulfate permits inactive protein and pigment to be lost in the filtrate. If celite and a Büchner funnel are used, the celite may be removed before precipitation of the protein by filtration at about pH 7 in the presence of less than 11 per cent ammonium sulfate. After two or three precipitations with ammonium sulfate, or when the filtrate from the ammonium sulfate precipitation becomes practically colorless, the precipitate will usually be found to be only slightly colored. If the precipitate has considerable color after several precipitations with salt, much of the color can be

precipitated and removed by treatment of a solution of the precipitate with minimal amounts of lead subacetate. Following an additional precipitation with the salt, the precipitate is dissolved in water and the solution adjusted to about pH 4. This causes precipitation of the protein, which is removed by filtration through a thin layer of celite. The filtrate contains some pigment and inactive, presumably normal, protein. The celite filter cake containing the protein is suspended in water and adjusted to pH 7 and the celite removed by filtration on a Büchner funnel. The filtrate will be opalescent, practically colorless, and contain about 80 per cent of the virus in the starting material.

Fig. 4. Crystals of tobacco mosaic virus (x 675). (From W. M. Stanley, *Am. J. Botany,* 24 (1937) 59.)

The protein in the filtrate is crystallized by adding slowly, with stirring, sufficient of a saturated solution of ammonium sulfate to cause a slight cloudiness, followed by a solution of 10 per cent glacial acetic acid in one-half saturated ammonium sulfate sufficient to increase the hydrogen-ion concentration to about pH 5. The suspension of the crystals has a very characteristic appearance. When stirred it has a satin-like sheen. The crystals, which are shown in Fig. 4, are very small and may best be observed by means of a microscope at a magnification of about 400 times. Within a few months following the publication of a description of the isolation procedure, the isolation of the crystalline material was confirmed in several laboratories in different parts of the world.

Following the isolation of the crystalline material the immediate and important tasks consisted of proving that virus activity either was, or was not, a. specific property of the crystalline material and, in the event of a positive

correlation, of securing a complete chemical, biological and physical characterization of the crystalline material. Much effort has been and continues to be devoted to these two tasks. Needless to say, for a time there was great skepticism that the crystalline material could be tobacco mosaic, due chiefly to the old idea that viruses were living organisms. A wide variety of experimental approaches has been used to test the proposal that the crystalline material represented tobacco mosaic virus. It was found that essentially all of the virus activity present in infectious juice could be isolated in the form of the crystalline material. The virus activity of this material was about 500 times that of the starting material. One ml of a solution containing but 10^{-9} grams of the material has usually proven infectious and occasionally, under favorable conditions, one ml containing only 10^{-14} grams has caused infection. The same crystalline material has been obtained repeatedly from different batches of diseased plants obtained at different times of the year and under different growing conditions. The same material has been obtained from different kinds of plants, such as mosaic-diseased tomato, petunia, spinach and phlox plants. The material has been recrystallized with little or no change in its properties. Fractional crystallization yielded no evidence for inhomogeneity. Fractionation by means of filtration through fine membranes, by means of centrifugation and by means of electrophoresis likewise yielded no evidence for the presence of extraneous material. The crystalline material and virus activity were always directly related on movement in a high-speed centrifuge or in the Tiselius electrophoresis apparatus under a wide variety of conditions. Antiserum to the material was found to give a specific precipitin test and to neutralize virus activity specifically. The virus material was found to bear no serological relationship to material present in extracts of normal tobacco, tomato or phlox plants. When solutions of the material were made more alkaline than about pH 11, or more acid than about pH 2, or were heated to about 75° C the protein was denatured and the virus activity was lost. In these cases, as when tobacco mosaic virus was subjected to strong solutions of urea or of detergents, the rate of denaturation of the protein and the loss of virus activity paralleled each other. The ultraviolet light absorption spectrum of the material was found to coincide with the destruction spectrum of virus activity. Treatment of virus solutions with ultraviolet light, hydrogen peroxide or nitrous acid resulted in loss of activity without accompanying gross chemical and physical changes. The high molecular weight and ability to crystallize were retained. Of special significance was the finding that by treatment with formaldehyde, it was possible to cause measurable

Table I. Composition of tobacco mosaic virus* and some of its strains**

Amino acid	*Strain of virus*								
	TMV	*M*	*J14D1*	*GA*	*YA*	*HR*	*CV3*	*CV4*	*M.D.****
Alanine	5.1	5.2	4.8	5.1	5.1	6.4		6.1	0.2
Arginine	9.8	9.9	10.0	11.1	11.2	9.9	9.3	9.3	0.2
Aspartic acid	13.5	13.5	13.4	13.7	13.8	12.6		13.1	0.2
Cysteine	0.69	0.67	0.64	0.60	0.60	0.70	0	0	
Cystine	0		0		0	0		0	
Glutamic acid	11.3	11.5	10.4	11.5	11.3	15.5	6.4	6.5	0.2
Glycine	1.9	1.7	1.9	1.9	1.8	1.3	1.2	1.5	0.1
Histidine	0	0	0	0	0	0.72	0	0	0.01
Isoleucine	6.6	6.7	6.6	5.7	5.7	5.9	5.4	4.6	0.2
Leucine	9.3	9.3	9.4	9.2	9.4	9.0	9.3	9.4	0.2
Lysine	1.47	1.49	1.95	1.45	1.47	1.51	2.55	2.43	0.04
Methionine	0	0	0	0	0	2.2	0	0	0.1
Phenylalanine	8.4	8.4	8.4	8.3	8.4	5.4	9.9	9.8	0.2
Proline	5.8	5.9	5.5	5.8	5.7	5.5		5.7	0.2
Serine	7.2	7.0	6.8	7.0	7.1	5.7	9.3	9.4	0.3
Threonine	9.9	10.1	10.0	10.4	10.1	8.2	6.9	7.0	0.1
Tryptophan	2.1	2.2	2.2	2.1	2.1	1.4	0.5	0.5	0.1
Tyrosine	3.8	3.8	3.9	3.7	3.7	6.8	3.8	3.7	0.1
Valine	9.2	9.0	8.9	8.8	9.1	6.2	8.8	8.9	0.2

* Tobacco mosaic virus has been found to contain 1.5 per cent of amide nitrogen and 6 per cent of ribonucleic acid.

** The values given in the table represent percentages of the indicated amino acids. In order to facilitate comparison, the values which are considered to differ significantly from those of TMV are underlined.

*** Mean deviation of the values of single determinations from the averages given. Three to five preparations of each strain were analyzed for each amino acid, with the exception of cysteine, and the results were averaged to give the figures presented. (From C. A. Knight, *J. Biol. Chem.*, 171 (1947) 297.

structural changes in the material and loss of virus activity, but by reversing the structural changes the virus activity was regained. As a whole, the results indicated that the crystalline material was, in fact, tobacco mosaic virus.

Attention was therefore directed to the characterization of the crystalline material. The material was originally reported to be a protein and to contain

about 52 per cent carbon, about 7 per cent hydrogen and about 16 per cent nitrogen, and was later found to contain, in addition, about 0.6 per cent phosphorus and 0.2 per cent sulfur. The fact that nucleic acid could be isolated from the crystalline material was reported by Pirie and coworkers in December, 1936, and by the writer a few days later. Although Pirie and coworkers concluded in 1937 that the crystalline virus material was a nucleoprotein, the presence of nucleic acid as a component of the virus was questioned at first by the writer. However, within a few months the writer became convinced that the nucleic acid could not be removed without causing loss of virus activity and there was general agreement that the virus was a nucleoprotein. Later, Loring, working in the writer's laboratory, demonstrated that about 90 per cent of the phosphorus in tobacco mosaic virus could be isolated in the form of pure pentosenucleic acid. This work, as well as the earlier and also subsequent work, has provided ample justification for regarding tobacco mosaic virus as a nucleoprotein. Elementary chemical analyses of the nucleoprotein are not very significant because most proteins have a similar elementary composition. However, the amino acid composition can be used to characterize a protein material somewhat more definitely. Considerable effort has been devoted to a study of the amino acid composition of tobacco mosaic virus and all or most of the virus has been accounted for. The most recent results are given in the first column of Table 1. It can be noted that the virus does not contain histidine or methionine and that there is no special concentration of basic amino acids.

Early experiments involving osmotic pressure indicated that the purified virus material possessed an unusually large particle size. Subsequently a sample of the purified nucleoprotein was sent to Professor Svedberg who, with Eriksson-Quensel, found the nucleoprotein to have a molecular weight, based on a dissymmetry constant of 1.3, of about 17 millions. Later, Lauffer, in the writer's laboratory, conducted extensive studies on the physicochemical and optical properties of solutions of the virus nucleoprotein. Early determinations of viscosity and sedimentation yielded data which permitted the conclusion that the particles of tobacco mosaic virus consisted of rods, about 12 mμ in diameter and about 400 mμ in length, with a molecular weight of about 40 millions. These results were received with some skepticism because they were based on physicochemical theory, certain aspects of which were relatively untested. However, when the electron microscope became available, pictures of purified tobacco mosaic virus were made, first in Germany and later in the writer's laboratory, and these provided direct evidence of the

existence of the rods 15 by 280 mμ in size. A recent study by means of the electron microscope of tobacco mosaic virus from three different parts of the world showed that the most common length of the rods is 280 ± 8.6 mμ. Electron micrographs of these three samples are shown in Fig. 5. Recent studies on the physicochemical and optical properties of solutions of tobacco mosaic virus have yielded data on the size and shape of the virus rods which are in complete accord with the results obtained with the electron microscope. Because of the high molecular weight of tobacco mosaic virus and because adequate high-speed centrifuges have become available during the past few years, a physical method involving differential centrifugation has been developed for the concentration and purification of tobacco mosaic virus and this method is now used extensively.

An interesting and potentially very important property of the rods of tobacco mosaic virus is their ability to aggregate end-to-end. This aggregation appears to be partly reversible and partly irreversible and the degree of reversibility appears to depend in part upon the conditions under which the particles were originally aggregated. Although electron micrographs of the contents of hair cells of tobacco mosaic diseased plants indicate that about two-thirds of the rod-like particles present are about 280 mμ in length, it is possible to secure purified preparations of tobacco mosaic virus in different stages of aggregation. The degree of aggregation depends largely upon the method of purification and preparations having the highest specific virus activity always consist of monomers, that is, of rods about 280 mμ in length. If these are aggregated, the specific activity or activity per mg of protein nitrogen, is decreased. Likewise, preparations obtained from infectious juice by a process designed to yield a preponderance of rods shorter than 280 mμ, or preparations of short rods obtained by sonic treatment of rods 280 mμ in length, always possess a low or negligible virus activity. Although it is impossible to foretell what the future may bring, the experimental data available at present indicate that the rods 15 by 280 mμ in size represent tobacco mosaic virus, despite the fact that preparations of these rods in different stages of aggregation can exist. Studies on the interaction of the virus rods are proving a fruitful field and may yield significant information regarding important processes within living cells. Bernal and Fankuchen have called attention to the fact that, as the rod-like particles of tobacco mosaic virus are brought closer and closer together in solution, the virus rods begin to assume positions parallel to each other even at distances of several hundreds of A. This unusual phenomenon provides evidence for the existence of long

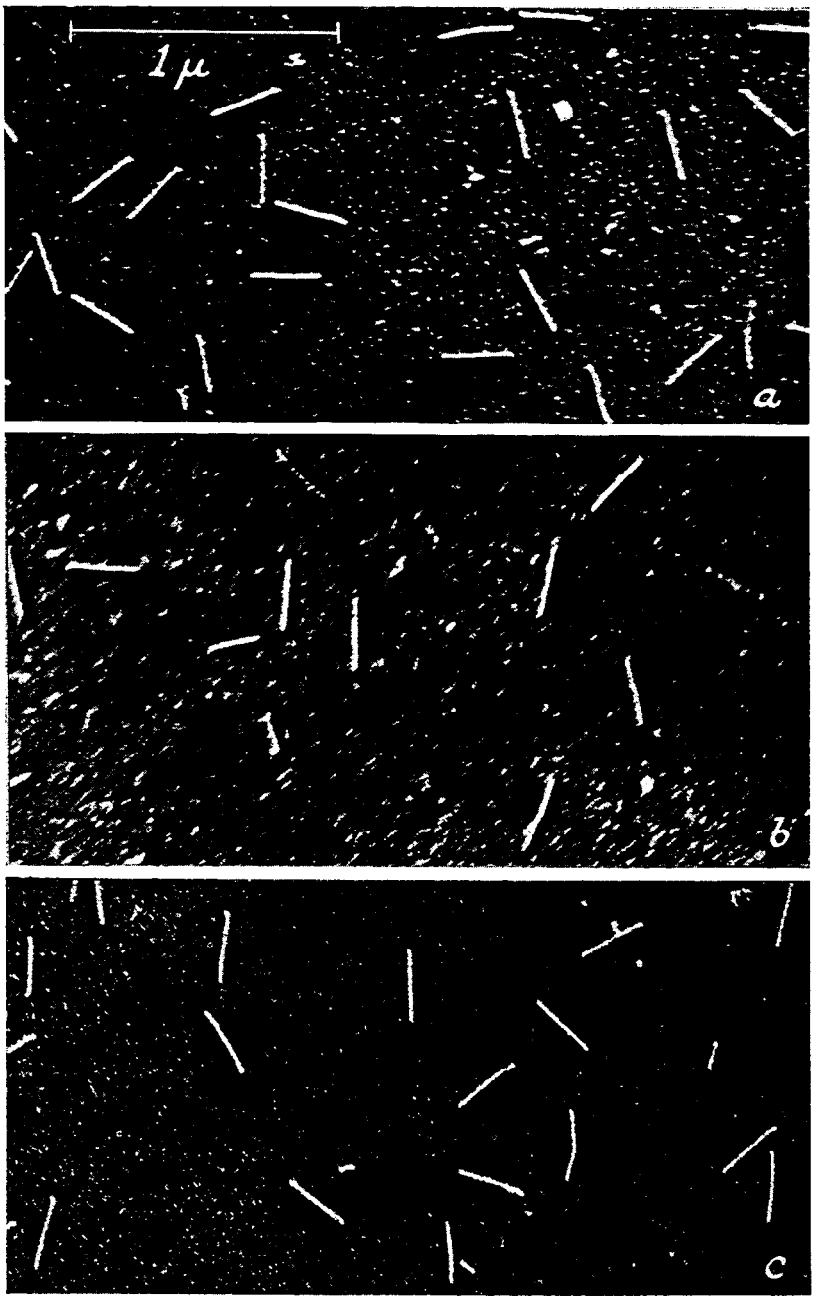
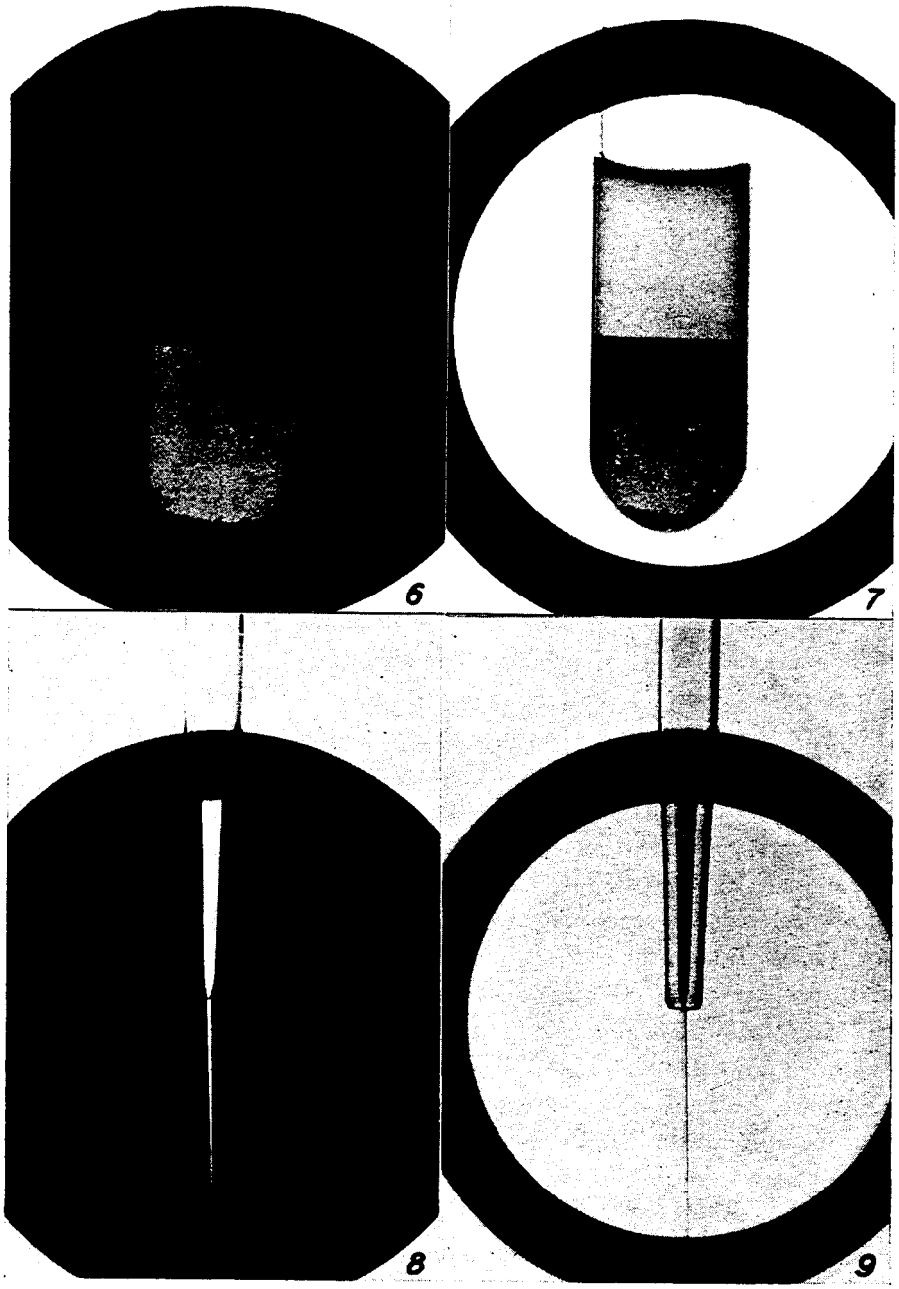


Fig. 5. Typical electron micrographs of the contents of hair cells from leaves of Turkish tobacco plants diseased with the (a) Dahlem, (b) Rothamsted, and (c) Princeton samples of tobacco mosaic virus. Mounts prepared with gold by the shadow-casting technique. (x 34,800). (From G. Oster, C. A. Knight and W. M. Stanley, *Arch. Biochem.*, 15 (1947) 279.)

range forces. These workers have also reported that the needle-shaped crystals of tobacco mosaic virus have no regularity in the direction of their length and hence, that the virus crystals have a two-dimensional, rather than a three-dimensional, type of regularity.

The size and rod-like shape of the particles of tobacco mosaic virus endow its solutions with some unusual properties. If a rather concentrated solution of purified tobacco mosaic virus is allowed to stand it will separate into two distinct layers. As can be seen from Figs. 6 and 7 the line of demarcation between the layers is quite sharp. The upper layer is not birefringent whereas the lower layer is spontaneously birefringent. The lower layer appears to consist of a three-dimensional mosaic of regions arranged at random to each other but in each of which. all of the rod-shaped particles are orientated and are parallel to each other. The concentration of virus is somewhat greater in the lower layer than in the upper layer. Dilution of the lower layer yields a solution having the properties of the upper layer. When solutions of tobacco mosaic virus are caused to flow the flowing stream becomes birefringent, as shown in Figs. 8 and 9. This is due to the fact that the flowing stream causes the rod-like particles to line up parallel to each other and thus to assume a degree of orientation similar to that which obtains in a crystal. When an electric field is applied across a solution of tobacco mosaic virus either positive or negative birefringence may be shown. The electrical double refraction appears to be due to the orientation of the virus rods parallel to the field for the case of positive birefringence and perpendicular to the field for the case of negative birefringence. The double refraction of dilute solutions in an alternating field of 60 cycles appears to be positive for all values of field strength. The results indicate that the two ends of the virus rod differ. This difference may prove to be an important factor in the end-to-end aggregation of the rods of tobacco mosaic virus. When tobacco mosaic virus is dissolved in solvents having a refractive index approaching that of the virus, little or no stream double refraction is obtained. These and other studies indicate that the virus rods have little or no intrinsic double refraction. However, Bernal and Fankuchen have found, by means of X-ray studies, that the individual rods of tobacco mosaic virus have a regular inner structure of such a nature that each rod could be considered to be a crystal. The significance of this detailed structure within the rods of tobacco mosaic virus has not been elucidated. However, as described earlier, the entire rod appears necessary for virus activity, for breakage of the rod into two halves is accompanied by loss of virus activity. It may be of interest that heat treatment of



Figs. 6-9.

solutions of purified tobacco mosaic virus has yielded nucleic acid preparations having very large and asymmetric particles. The size and shape of these particles are such that it has been suggested that a virus rod has eight of these nucleic acid particles running the length of the virus rod. Perhaps it is this combination of amino acid and nucleic acid structure which yields the amazing virus activity. Needless to say, one of the important problems for the future is the elucidation of the nature of virus activity.

Much of the foregoing is a description of the isolation and properties of crystalline tobacco mosaic virus and includes the work on viruses which was recognized by the 1946 Nobel Prize for Chemistry. The isolation of the crystalline tobacco mosaic virus has been followed by very important work and, although there is not sufficient space to describe this subsequent work, it does seem fitting that the more important aspects should be mentioned here. Most viruses are known to exist in the form of different strains. Plants diseased with eight distinctive strains of tobacco mosaic virus were subjected to the same isolation procedure which yielded crystalline tobacco mosaic virus. The eight nucleoprotein preparations which were obtained were found to possess similar, yet distinctive properties. As can be seen from Fig. 10, the particles of the different virus strains were similar in size and shape. Of great importance was the fact that in a study of the amino acid composition of the purified preparations of the eight strains, definite differences, presumably

Fig. 6. A tube of tobacco mosaic virus solution which, upon standing, has separated into two layers. The photograph, taken with the aid of crossed Polaroid plates, shows that the bottom layer material is spontaneously doubly refracting, whereas the top layer material is not spontaneously doubly refracting. Double refraction of flow is shown by the virus in both layers. (From M. A. Lauffer and W. M. Stanley, *J. Biol. Chem.*, 123 (1938) 507.)

Fig. 7. The same system as in Fig. 6 taken between parallel Polaroid plates. The intensity of the light transmitted by the bottom layer is about the same as in the case represented in Fig. 6. (From M. A. Lauffer and W. M. Stanley, *J. Biol. Chem,* 123 (1938) 507.)

Fig. 8. Doubly refracting stream of tobacco mosaic virus solution flowing from the end of a pipette, photographed between crossed Polaroid plates arranged so that each vibration direction of the Polaroid plates makes an angle of 45° with the direction of flow. (From M. A. Lauffer and W. M. Stanley, *J. BioI. Chem.*, 123 (1938) 507.)

Fig. 9. Same system as in Fig. 8 photographed between parallel Polaroid plates. The doubly refracting stream appears to be darker than the background in this case. (From M. A. Lauffer and W. M. Stanley, *J. Biol. Chem.*, 123 (1938) 507.)

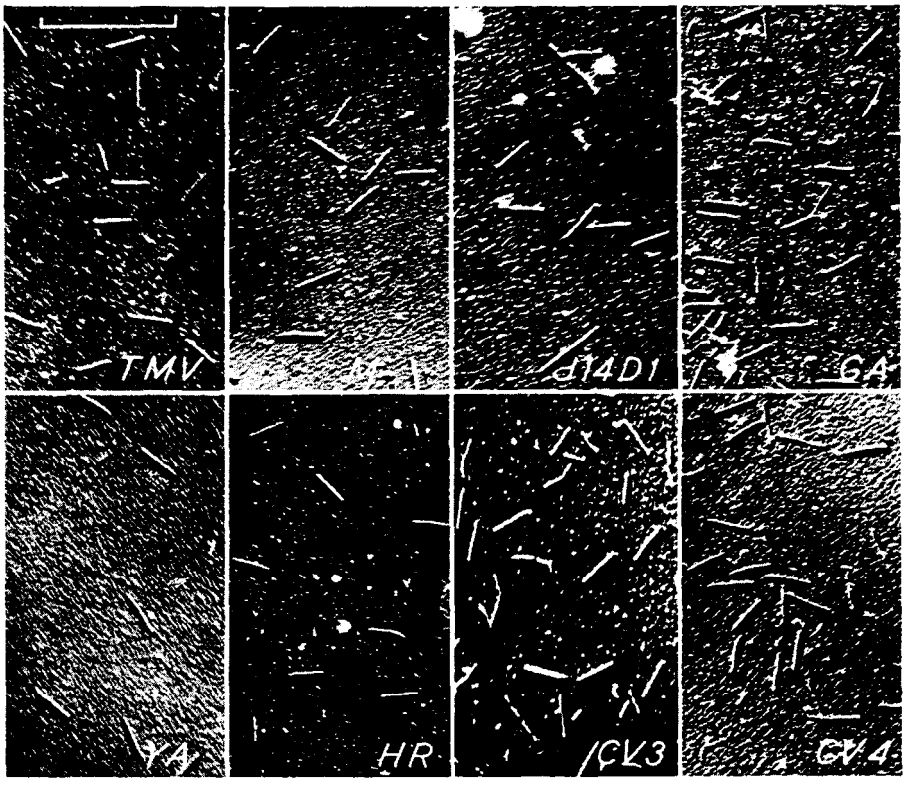


Fig.10. Electron micrographs of 8 strains of tobacco mosaic virus. TMV-ordinary tobacco mosaic virus; M-Holmes' masked; J_{14}DI-derivative of strain obtained by Jensen; GA-green aucuba; YA-yellow aucuba; HR-Holmes' rib-grass; CV_3-cucumber virus 3; CV_4-cucumber virus 4. The micrographs are of contents of hair cells from appropriately diseased Turkish tobacco plants except in the cases of CV_3 and CV_4, which were obtained from hair cells of diseased cucumber plants. Mounts prepared with gold by the shadow-casting technique. Line of the micrograph of TMV represents 1 μ. (From C. A. Knight and G. Oster, *Arch. Biochem.,* 15 (1947) 289.)

correlated with mutation of the virus, were found. The results, which are presented in Table 1, indicate that the mutation of a virus can be accompanied by the elimination of one or more amino acids from the virus structure, by the introduction of one or more amino acids to the virus structure or by a change in the concentration of one or more amino acids present in the virus structure. These results have great significance for biochemistry, for genetics and for medicine.

Chemical derivatives of tobacco mosaic virus possessing full virus activity

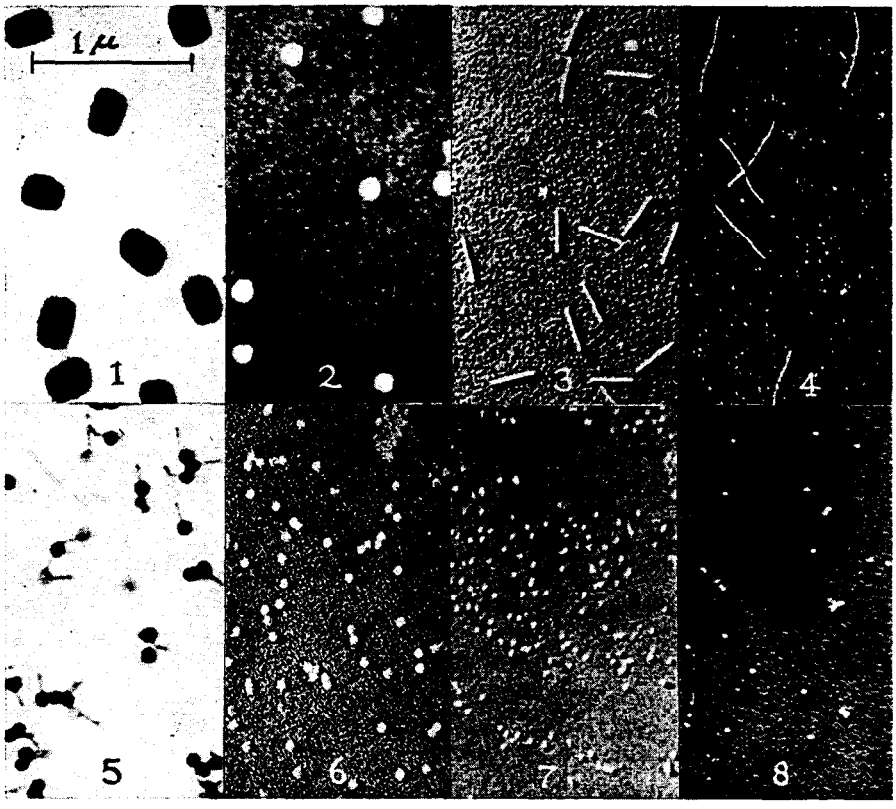


Fig. 11. Electron micrographs of purified virus preparations. All are at the same magnification and all except 1 and 5 were prepared by the gold shadow-casting technique. (1) Vaccinia virus; (2) Influenza virus (Lee strain) ; (3) Tobacco mosaic virus prepared from hair cells; (4) Potato X virus (latent mosaic of potato), hair cell preparation; (5) T_2 coli bacteriophage; (6) Shope rabbit papilloma virus; (7) Southern bean mosaic virus; (8) Tomato bushy stunt virus. (From C. A. Knight, *Symposia on Quantitative Biology, Biological Laboratory, Cold Spring Harbor, New York,* Vol. XII, 115-121, 1947.)

have been prepared. However, the virus produced by inoculation of the chemical derivatives has always proved to be ordinary tobacco mosaic virus. Work on the concentration and purification of different viruses has proceeded in several laboratories. Over a dozen viruses have been obtained in highly purified form, mainly by techniques involving high-speed centrifugation. Electron micrographs of the individual particles of eight of these purified virus preparations are shown in Fig. 11 at the same magnification. Because of the wide array of particle size and shape which they afford, purified virus

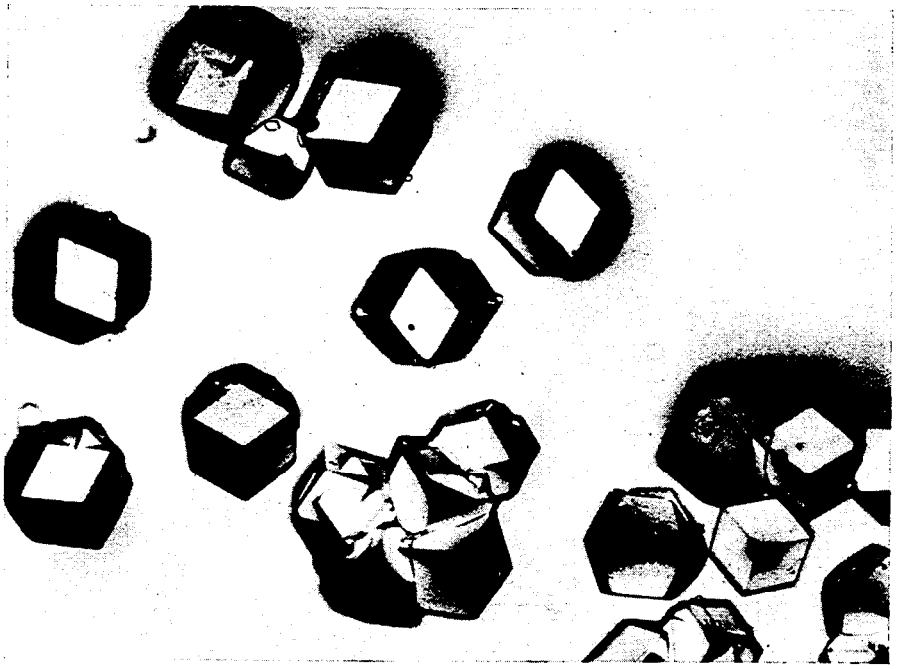


Fig. 12. Crystals of tomato bushy stunt virus (x 224). (From W. M. Stanley, J. *Biol. Chem., 135 (1940) 437.)*

preparations are proving exceedingly valuable in connection with studies on particle interaction and other physicochemical phenomena. Some of these purified viruses are crystallizable nucleoproteins (Fig. 12), either rod-like or spherical in shape; others are nucleoproteins which have, as yet, not been crystallized, and others are large particles consisting of nucleoprotein, lipid, and carbohydrate, and possessing in some cases, a degree of morphological differentiation characteristic of organisms. Still other viruses have, as yet, defied isolation, possibly, in some cases, because of extreme instability. It is obvious that the chemical and physical properties of only a relatively few viruses have been determined. In view of the possibility that these represent the more stable and more readily purified viruses, one cannot be certain that a true picture of the chemical and physical properties of viruses as a whole has been obtained as yet.

The new field of virus research is really in its infancy and much remains to be accomplished. Certain basic and fundamental problems relating to the mode of virus reproduction and mutation have taken definite form. Solution of these problems should yield information of great value to biology, chem-

istry, genetics and medicine. This new field of research has been laid upon a very broad foundation, for the present status of virus research has been achieved by contributions from almost every branch of science. It is likely that a continuation and extension of the application of weapons from many fields of science will be necessary for the proper and full development of the new field of virus research.