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Techniques for the Quantitative Study of Mutation in Plant Viruses*

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Summary. Hardly any other virus is chemically and ultramicroscopically as well known as TMV. It is not possible to perform genetic recombinations with this object. The phenomenon of mutation is, however, known and an analysis of the dosis-effect relationship was possible by using the characters "chlorotic" versus "necrotic" primary symptoms. Taking into account the phenomenon of interference (mutual exclusion), i.e., comparing the induced mutation frequency with that of a control virus sample diluted to the same level of infectivity, one can perform quantitative analyses. In this way the first chemical mutagenesis in the test tube was demonstrated 10 years ago with nitrous acid as mutagenic agent. The criticism raised by BAWDEN to the first publication of MUNDRY and GIERER was already inappropriate at that time. In the meantime it has been demonstrated by WITTMANN-LIEBOLD and WITTMANN through analysis of amino acid exchanges in spontaneous mutants and in those isolated after incubation with HNO_2 that the "difference" between spontaneous and induced mutants demanded by BAWDEN, which cannot be postulated for symptoms in plants, lies, as expected, in amino acid exchanges of the protein coat.

1. Introduction

Quite a number of viruses has, during the last two decades, made it possible to set up useful systems for the investigation of problems in the field of molecular genetics. This is true primarily of bacterial viruses (bacteriophages). The most important requirements for quantitative studies are good countability of host cells, bacteria, and infecting particles, as well as of phage yield of single cells. A plating efficiency of nearly 1 simplifies working with these systems. Host range and plaque-type mutants offer some good characters. A mixed infection of single host cells by different types of phages that results in the recombination of characters is possible. Under these circumstances it is understandable why the genetics of bacteriophages has become an important and often decisive part of modern genetics.

Animal viruses too are somewhat suitable for the quantitative handling of genetic problems, since, in a monolayer of animal cells, conditions prevail that are more or less similar to those in a layer of bacteria.

In the case of phytopathogenic viruses, the conditions for quantitative genetic studies are far more complicated. None of these viruses is endowed with a mechanism for infecting a host cell. They reach the cells only through wounds produced either by mechanical means or by insect pricks. The host cells cannot be counted as easily as the bacteria or the animal cells of a tissue culture. The plating efficiency lies several orders of magnitude below 1. Mixed infection of single cells and a subsequent recombination of characters has not been proved with sufficient reliability. If it occurs at all, as has been claimed by a few authors (BEST and GALLUS, 1955, BEST, 1961, 1964; WATSON, 1960; AACH, 1961), the phenomenon is so rare that it has not been utilized for genetic studies.

The substrate for the multiplication of phytopathogenic viruses, e. g., the leaf of a plant, is much more complex than a bacterial colony or even a monolayer of animal cells. It is an organ in which the cells in several layers, above and beside one another are differentiated in different ways. The behavior of a cell or a group of cells is expected to be influenced by what is happening in the other cells, primarily, of course, in the neighboring cells. Since this type of organ contains special tissues for the transport of materials, a certain influence is exerted not only upon the neighboring cells but also upon those at a distance. The exact nature of this influence is, however, not easy to visualize. Owing to its anatomical as well as its physiological specialities, the conductive system of a leaf promotes the transport of materials and the spread of viruses, possibly as intact virus particles, whereas the movement of viruses from cell to cell in the parenchyma may be possible in the form of free RNA.

In view of this situation, it is no wonder that quantitative work with phytopathogenic viruses is quite difficult.

Since the tobacco mosaic virus (TMV), because of its stability and relatively clear morphology and chemical constitution, almost always marched at the head of all chemical and physical investigations on the structure of viruses, it was desirable to set up for this virus the conditions necessary for quantitative genetic studies. Thus for the first time in the history of genetics it was possible to isolate the genetic material (RNA) of TMV as a pure chemical substance — which was successful quite a number of years earlier with DNA in pneumococci — and also to bring about in the test tube in a chemically intelligible way a few alterations in this pure substance, available in large quantities, which could lead to mutation or perhaps might represent the process of mutation itself. It is not the task of this paper to describe and judge the consequences of the experiments that achieved this goal, a goal that was at best only dreamed of by older geneticists.

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The aim here is to describe only one of the methodological requirements for the attainment of the goal.

Principally two requirements had to be fulfilled according to the publications of GIERER and MUNDRY (1958) and MUNDRY and GIERER (1958), who for the first time performed true chemical mutagenesis. One of the requirements, which was fulfilled by chemistry, has often been described and properly appreciated. This constitutes the isolation of the RNA of TMV (SCHRAMM et al., 1955; GIERER and SCHRAMM, 1956; FRAENKEL-CONRAT, 1956), together with the study of chemical changes in RNA through the action of HNO_2 and the kinetics of inactivation by HNO_2 (SCHUSTER and SCHRAMM, 1958). The other requirement that was equally important for the decisive investigations of 1958 was present in the studies that (1) proved that mutations occur in phytopathogenic viruses, particularly in TMV, (2) led to the isolation of mutants, thus making possible quantitative work, and (3) secured the quantitative investigations concerning disturbances and sources of errors that are not present or that hardly exist with other viruses, e. g., bacteriophages.

II. Proof of Mutability of Phytopathogenic Viruses

The advanced secondary symptom, which is caused by TMV on the tobacco leaves, consists, as the name of the disease tells, of a mosaic, usually a mosaic of light- and dark-green areas in the case of the most widely distributed strain *vulgare*. Transmission experiments in which the lighter or the darker areas are used as inocula do not generally produce different symptoms in the subsequent transfers. Obviously the color differences of neighboring sectors of the mosaic do not owe to a possible occurrence of different types of viruses in the visibly different areas of the leaf, but for some unknown reason the leaf tissue in neighboring sectors reacts in different ways to the same virus, perhaps because of variations in the concentration of virus. The result is, however, different if one transmits the virus from the sharply bordered rarer "yellow spots". One then gets in the next passages, and sometimes in the passage that immediately follows a symptom different from that of *vulgare*, *e. g.*, a true "yellow strain". In the yellow spots, therefore, a virus is present that markedly differs from that in the neighboring light- and dark-green checkered areas. This finding indicates the mutative origin of the yellow strain from *vulgare*, but it does not prove it conclusively. It does not seem impossible that the strains, *vulgare* and *flavum* (one particular yellow strain) occur mostly in close association and only rarely alone. The possibility of this interpretation has, however, already been excluded by the experiments of JENSEN (1933, 1936, 1937), MCKINNEY (1935, 1937), and NORVAL (1938). The arguments for a mutative origin of yellow strains and of other spontaneous mutants are the following:

1. If one inoculates systemically reacting plants with such a low concentration of virus that at best only one out of every five plants becomes infected, then it can be assumed on the basis of "Poisson distribution" that the plants which get infected at all owe their infection very probably to a single particle. However, in these plants the yellow spots appear again and again, indicating that most probably they originate after infection and do not enter the plants during inoculation.

2. One can inoculate with highly diluted virus solutions plants that react by producing primary local lesions. The spots originating thereafter can with a high probability be traced back to single infective particles (ZIMMER, 1943). The yield of one single lesion can be again diluted so far that only very seldom do lesions appear at all. One can repeat this experiment as often as desired. This and similar experiments were performed by the authors cited above, and the yellow spots, from which the yellow strains could be isolated, appeared spontaneously again and again.

The existence of the phenomenon of mutability in phytopathogenic viruses has been proved since the 1930's.

III. Quantitative Tests for Mutability

To a certain extent it is possible to do quantitative studies on the frequency of yellow spots and also to compare the "back mutation" of yellow strains with that of the normal green strains. Several experiments were performed on the basis of this or similar tests (MCKINNEY, 1937; PFANKUCH *et al.*, 1940; FRIEDRICH-FREKSA *et al.*, 1946; MELCHERS, 1948; MUNDRY, 1957b). However, such a test suffers from several drawbacks: (1) yellow spots can be of very different sizes, and accordingly, in the assessment of particularly small and not very distinct spots, a personal factor may easily be introduced; (2) it is very difficult to correlate conclusively the variations in the frequency of yellow spots with changes in the mutation rate and to distinguish these events from selection phenomena (FRIEDRICH-FREKSA *et al.*, 1946; MUNDRY, 1957b); and (3) the test demands a large area of greenhouse space. It is, therefore, understandable that in the days when biology was under the strong influence of mutation research in *Drosophila* there was an endeavor to find simple systems for experiments with mutants of phytopathogenic viruses. The laboratory jargon in those days was, "We also need a CIB method for TMV".

Such a method should offer the following features: (1) easy and assured differentiation between the starting material and the mutants; (2) good countability; (3) independence of the starting material and the mutants from one another with respect to the expression of characters and their number; (4) low space requirements. To this end a compromise might have to be made in that one would register only a relatively narrow section of the many possible mutations.

Under these circumstances it was natural to search among primary symptoms. The yellow strains are additionally characterized by the chlorotic spots that appear on the primary infected leaf, and these areas are easier to recognize than the chlorosis induced by the green strains (*vulgare*). The chlorotic areas of *vulgare* are, however, often so faint that they cannot be counted easily. Very often it is difficult to differentiate between the chlorosis of *vulgare* and that caused by not very brilliant yellow strains.

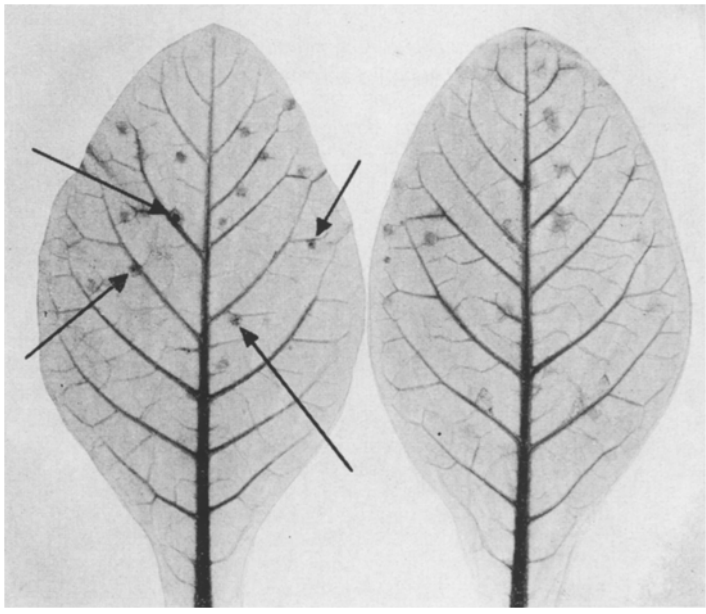

Fig. 1. Two leaves of *Nicotiana sylvestris* (right, with standard TMV; left, with TMV which was previously propagated at high temperature) infected several days before photography. Chlorophylls and carotenoids were removed in the morning with hot alcohol and the leaves were placed in iodine potassium iodide solution. The primary lesions are colored blue, since at the positions enzymatic starch hydrolysis was blocked in the night. On the right leaf inoculated with control virus only chlorotic lesions are observed, these are difficult to recognize in the intact leaf. On the left leaf there occur, in addition, four necrotic lesions from mutant TMV (arrows). [Photo from unpublished experiments, 1940 (first published by MELCHERS, 1960).]

The objection concerning insufficient differentiability between the starting strain and mutant applies also to the size of necrotic spots.

On *Nicotiana glutinosa* and on such *N. tabacum* varieties as carry the gene *N* of *N. glutinosa,* all strains of TMV and—as far as is known — all mutants of TMV react with primary necrotic symptoms in an average temperature range (JOCKUSCH, 1966). Actually, the necrotic spots of many mutants are on the average clearly smaller than those of *vulgare*. In an individual case, however, it is often difficult or even impossible to decide whether the lesion is produced by the original strain or by such a mutant.

Nicotiana sylvestris and several *N. tabacum* varieties (*e. g.*, 'Java') respond to the *vulgare* strain of TMV and to one group of strains and mutants with primary chlorosis and systemic spreading, but they respond with primary necrosis alone after infection with *dahlemense* and some other strains and mutants (cf. MELCHERS *et al.*, 1966). This system is really useful for quantitative studies.

A quantitative measure of the number of primarily inoculated sites, which is a function of the concentration of inoculum and the plating efficiency of the system, can also be obtained in that the otherwise hardly visible chlorotic spots are made distinct by the starch accumulation (Fig. 1). Sometimes it is sufficient to count the chlorotic spots directly or to gain at least a certain quantitative evidence by assay of aliquots of the infection sample on test plants of the constitution NN that respond with primary necrosis alone. The counting of mutants (necrotic primary lesions) is then no problem at all. Only totally unpracticed persons (*e.g.*, YAMAFUJI *et al.*, 1947) might confuse true necrotic primary lesions with the small wounds that are not completely avoidable during mechanical inoculation using abrasive like carborundum.

If one tests the mutants, which are primary necrotic on Java tobacco, for other characteristics, e. g., secondary symptoms on a systemically reacting host like 'Samsun' tobacco, or for amino acid exchanges in the coat protein, one recognizes a very large variability among the mutants (MUNDRY and GIERER, 1958; WITTMANN, 1962). Chlorotic versus necrotic lesions on the directly inoculated leaf are nothing more than one difference in phenotype, which is particulary amenable to quantitative treatment.

The finding of this mutation type and its application in the correct way were, as mentioned, as important for the discovery of chemical mutagenesis in the test tube as was the ability to meet the chemical requirement. If, as had been seriously suggested by chemists, the phenotypic difference "more or less acidic or basic amino acids in the outer layers of coat protein" (distinguishable in their electrophoretic behavior) were chosen, one would not have been able to learn even today anything exact about HNO_2-induced mutations and the consequent nucleic acid protein correlation in phytopathogenic viruses. One of the aids for the deciphering of the genetic code would not have been available.

IV. Risks of the Test System for Chlorotic Versus Necrotic Primary Symptoms

As mentioned earlier, the substrate, namely the leaf of a host plant, is more complex for the observation and quantitative assay of primary symptoms than the nutrient media of fungi and bacteria and the bacterial layers used in studies on the genetics of bacteriophages.

When leaves of plants are inoculated with a mixture of closely related viruses, each of the viruses influence the frequency of appearance of the other's primary symptoms. This phenomenon is called "interference" or "mutual exclusion". It was, for example, the pitfall in an experiment (GOWEN, 1941) that was strongly influenced by the then prevalent mutation research on *Drosophila*, which at that time stood for the comparison of mutability of higher organisms and viruses. In this study the phenotypic difference, systemic versus primary necrotic, was in fact utilized using *N. sylvestris* as test plant, but without attention to the phenomenon of interference. In this investigation the concentration of infectious particles was drastically reduced by x-ray irradiation of the virus. The results of all experiments except one can be interpreted to mean that there is no influence of irradiation on the systemic → necrotic or necrotic → systemic mutation frequency by assuming only that the material reacting systemically was not absolutely free from necrotic materials, and vice

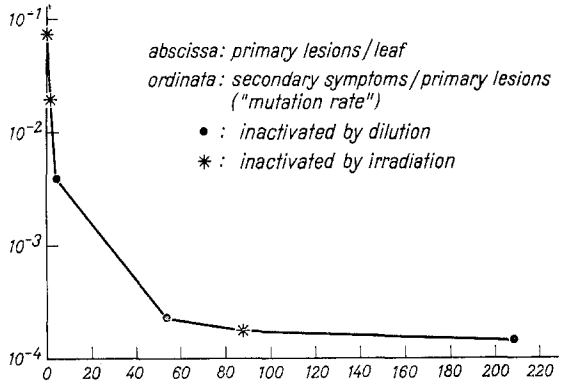

Fig. 2. Dependence of the ratio of secondary symptoms to primary symptoms (apparent mutation rate) produced on Java tobacco from a mixture of virus necrotic and chlorotic on Java, on the reduction of total infectivity by dilution and by irradiation. First presented at the meeting of the British Association for the Advancement of Sciences, Bristol, 1955, by MELCHERS. (From MUNDRY 1957a, data of Table 9.)

versa. The error committed by GOWEN was that he compared his experimental groups, which were very poor in the number of active particles, with control groups of high unchanged original activity. His results could also be explained through a reduced interference in the experimental groups by a lowering of the total infectivity. We were aware of this mistake (MELCHERS, 1949), and it was clearly demonstrated in experiments of MUNDRY (1957a) that were designed for this purpose* (Fig. 2). This mistake was carefully avoided in the experiments on chemical mutagenesis (MUNDRY and GIERER, 1958) because, having been discovered by us it was well known to us. It remains, therefore, incomprehensible, how BAWDEN (1959), who was familiar with our earlier works, but which he nevertheless mentioned insufficiently, formulated his incorrect criticims—as if the danger of interference was not known to us. MUNDRY (1959) refuted the objections of BAWDEN with precision. This was in fact not at all necessary, in view of the publications available up to that time. However, since BAWDEN upheld his criticism, though partially, even in 1964, it may be refuted here once more. Incorrect is the assumption that: "Only BAWDEN (1959) has questioned whether this (the increased number of Java-necrotic material after HNO_2 incubation) could be because the treatment allows already existing variants to be more readily selected rather than it produces them." Since the appearance of the paper of GOWEN (1941), we have not only discussed this question critically but have also stressed in several cases the possibility of selection of preexisting mutants and even of contaminants (FRIEDRICH-FREKSA *et al.*, 1946; MELCHERS, 1949; MUNDRY, 1957a). MUNDRY and GIERER (1958) have also discussed this point for their experiments, but they reached the conclusion, with full justification, that the great increase in the chlorotic → necrotic mutation frequency found by them cannot be interpreted on the basis of selection of preexisting mutants.

BAWDEN demands several times that after incubation with HNO_2 one should find "other" mutants than those that are isolated as spontaneous mutants. One should actually expect to find a broader spectrum of spontaneous mutants than after the two "induced" transitions adenine → guanine and cytosine → uracil. In all cases where the corresponding change in protein is unknown, nothing can be said as yet. The investigations of WITTMANN-LIEBOLD and WITTMANN (1965) on the amino acid exchange in coat protein of TMV have shown that, according to the code valid at that time out of 31 exchanges which were isolated after HNO_2 incubation and localized in the sequence of amino acids only one (the mutant PM 2 isolated by SIEGEL and his coworkers, 1962) necessitates the assumption that the exchange could not have originated through deamination by HNO_2. However, among nine exchanges in spontaneous mutants, as many as five represent such amino acids exchanges as are not due to the nucleotide transitions A → G or C → U. The demand of BAWDEN, which could not be met by a mere comparison of symptoms between spontaneous mutants and mutants isolated after HNO_2-incubation, and which was consequently at that time unfair, is now fulfilled, therefore, to complete satisfaction through the comparison of amino acid exchanges in the coat protein. In fact after nitrite incubation the WITTMANNs found "other mutants" (that means not so many different changes of nucleotide bases) than they found spontaneously.

When BAWDEN cites the experiment from Berkeley (e. g., FRAENKEL-CONRAT, 1961) in which after treatment with other chemicals e. g., dimethyl sulfate whose mechanism of action is by no means as clear as that of HNO_2 and compares them with the nitrite experiments done in Tübingen, he does not mention that after consideration of the controls of correctly reduced infectivity no mutagenic effect of dimethyl sulfate was found in Tübingen (SCHUSTER *et al.*, 1960).

Notwithstanding the incorrect claim that „BAWDEN alone" — or at least as the first authority — had taken a critical approach to the question "selection or mutation" after treatment with mutagenic agents, it should be mentioned once more at the end of this paper what we, since we have been engaged in this question, have published starting in 1946. It is more difficult to differentiate between the selection of preexisting and the induction of new mutants in the case of phytopathogenic viruses than in the case of other objects of genetics, including bacteriophages and animal viruses. However, by the introduction of controls of the same reduced infectivity, it is possible to perform precise quantitative experiments, and such experiments were performed in sufficient number and were also published.

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* The question of the temperature dependency of the rate of mutation, which could not be verified unambiguously in the case of TMV using the mutations green to yellow strains and vice versa because we had to reckon with disturbances through selection (MUNDRY 1957b), was tested with bacteriophages (WITTMANN, 1957) in order to avoid this source of error.

Zusammenfassung

Kaum ein anderes Virus ist chemisch und ultramikroskopisch so gut bekannt wie das TMV. Rekombinations-Genetik ist nicht möglich. Das Phänomen der Mutation ist aber bekannt, und eine Analyse der Dosis-Effekt-Beziehung wurde möglich durch Benutzung der Symptomcharaktere „chlorotische" versus „nekrotische" Primärsymptome. Bei Berücksichtigung des Phänomens der Interferenz (mutual exclusion), d. h. wenn man die induzierte Mutationsrate mit der auf gleiche Infektiosität durch Verdünnen der Viruslösung gebrachten als Kontrolle vergleicht, kann eine quantitative Analyse durchgeführt werden. So wurde vor 10 Jahren die erste Chemomutagenese im Reagenzglas mit salpetriger Säure als mutagenes Agens nachgewiesen. Die an der ersten Veröffentlichung von MUNDRY und GIERER von BAWDEN geäußerte Kritik war schon damals unzutreffend. Inzwischen ist durch die Analyse der Aminosäureaustausche von spontanen und nach Inkubation mit HNO_2 isolierten Mutanten von WITTMANN-LIEBOLD und WITTMANN gezeigt worden, daß die von BAWDEN geforderte „Verschiedenheit" spontaner und induzierter Mutanten, die für Symptome an den Pflanzen nicht postuliert werden kann, in den Aminosäureaustauschen des Hüllproteins wie zu erwarten vorhanden ist.

References

1. AACH, H. G.: Interferenz zweier nahe verwandter Stämme des Tabakmosaikvirus. Ber. Dt. Bot. Ges. **74**, 433–435 (1961). — 2. BAWDEN, F. C.: Effect of Nitrous Acid on Tobacco Mosaic Virus: Mutation or Selection? Nature **184**, 27–29 (1959). — 3. BAWDEN, F. C.: „Plant Virus and Virus Diseases", 4 th ed. New York: Ronald Press 1964. — 4. BEST, R. J., and A. GALLUS: Further Evidence for the Transfer of Character-determinants (Recombination) between Strains of Tomato Spotted Wilt Virus. Enzymologia **17**, 207–220 (1955). — 5. BEST, R. J.: Recombination Experiments with Strain A and E of Tomato Spotted Wilt Virus. Virology **15**, 327–339 (1961). — 6. BEST, R. J.: Antagonism between strains of plant viruses — interpretation of the phenomenon and its practical significance in plant virology. Proc. 10 th Internat. Congr. Bot. Edinburgh, p. 76–77 (1964). — 7. FRAENKEL-CONRAT, H.: The role of the nucleic acid in the reconstitution of active tobacco mosaic virus. J. Am. Chem. Soc. **78**, 882 (1956). — 8. FRAENKEL-CONRAT, H.: Chemical modification of viral ribonucleic acid. I. Alkylating agents. Biochim. Biophys. Acta **49**, 169–180 (1961). — 9. FRIEDRICH-FREKSA, H., G. MELCHERS und G. SCHRAMM: Biologischer, chemischer und serologischer Vergleich zweier Parallelmutanten phytopathogener Viren mit ihren Ausgangsformen. Biol. Zbl. **65**, 187–222 (1946). — 10. GIERER, A., und G. SCHRAMM: Die Infektiosität der Nukleinsäure aus Tabakmosaikvirus. Z. Naturforsch. **11b**, 138–142 (1956). — 11. GIERER, A., and K. W. MUNDRY: Production of Mutants of TMV by Chemical Alteration of its Ribonucleic acid in vitro. Nature **182**, 1457–1458 (1958). — 12. GOWEN, T. W.: Mutation in Drosophila, Bacteria and Viruses. Cold Spring Harb.-Symp. Quant. Biol. **9**, 187–193 (1941). — 13. JENSEN, J. H.: Isolation of yellow mosaic from plants infected with tobacco mosaic virus. Phytopathology **23**, 964–974 (1933). — 14. JENSEN, J. H.: Studies on the origin of yellow mosaic viruses. Phytopathology **26**, 266–277 (1936). — 15. JENSEN, J. H.: Studies on representative strains of tobacco mosaic virus. Phytopathology **27**, 69–84 (1937). — 16. JOCKUSCH, H.: The Role of Host Genes, Temperature and Polyphenoloxydase in the Necrotization of TMV-infected Tobacco Tissue. Phytophath. Z. **55**, 185–192 (1966). — 17. MCKINNEY, H. H.: Evidence of Virus Mutation in the Common Mosaic of Tobacco. J. Agr. Res. **51**, 951 (1935). — 18. MCKINNEY, H. H.: Virus Mutation and the Gene Concept. J. Heredity **28**, 51–57 (1937). — 19. MELCHERS, G.: III. Phytopathogene Viren. FIAT Rev. Germ. Sci. **52**, Part. I, 111–129 (1948). — 20. MELCHERS, G.: Über Mutationen beim Tabakmosaikvirus. Proc. 8th Internat. Congr. Gen. Stockholm, Hereditas, Suppl. Vol; 626–628 (1949). — 21. MELCHERS, G.: Warum interessiert den Biologen das Tabakmosaikvirus? Jahrbuch der Max-Planck-Gesellschaft 1960, 85–113 (1960). — 22. MELCHERS, G., H. JOCKUSCH and P. v. SENGBUSCH: A Tobacco Mutant with a Dominant Allele for Hypersensitivity against some TMV-Strains. Phytopath. Z. **55**, 86–88 (1966). — 23. MUNDRY, K. W.: Zur Frage des Einflusses von Röntgen- und UV-Strahlen auf die Mutationsrate des Tabakmosaikvirus nach Bestrahlung reiner Präparate. Z. Vererbungsl. **88**, 115–127 (1957a). — 24. MUNDRY, K. W.: Die Abhängigkeit des Auftretens neuer Virusstämme von der Kulturtemperatur der Wirtspflanzen. Z. Vererbungsl. **88**, 407–426 (1957b). — 25. MUNDRY, K. W.: The effect of nitrous acid on TMV: Mutation, not selection. Virology **9**, 722–726 (1959). — 26. MUNDRY, K. W., and A. GIERER: Die Erzeugung von Mutationen des TMV durch chemische Behandlung seiner Nukleinsäure in vitro. Z. Vererbungsl. **89**, 614–630 (1958). — 27. NORVAL, J. P.: Derivatives from an unusual strain of tobacco mosaic virus. Phytopathology **28**, 675–692 (1938). — 28. PFANKUCH, E., G. A. KAUSCHE und H. STUBBE: Über die Entstehung, die biologische und physikalisch-chemische Charakterisierung von Röntgen- und γ-Strahlen-induzierten „Mutationen" des TMV-Proteins. Biochem. Z. **304**, 238–258 (1940). — 29. SCHRAMM, G., G. SCHUMACHER and W. ZILLIG: An infectious nucleoprotein from Tobacco Mosaic Virus. Nature **175**, 549–550 (1955). — 30. SCHUSTER, H., und G. SCHRAMM: Bestimmung der biologisch wirksamen Einheit in der Ribosenukleinsäure des TMV auf chemischem Wege. Z. Naturforsch. **13b**, 697–704 (1958). — 31. SCHUSTER, H., A. GIERER und K. W. MUNDRY: Inaktivierende und mutagene Wirkung der chemischen Veränderung von Nukleotiden in Virusnukleinsäure. Abh. Dt. Akad. d. Wiss. Berlin, Erwin-Baur-Gedächtnis-Vorlesungen I, 76–85 (1960). — 32. SIEGEL, A., M. ZAITLIN and O. P. SEHGAL: The isolation of defective tobacco mosaic virus strains. Proc. Nat. Acad. Sci. US **48**, 1845–1851 (1962). — 33. WATSON, M. A.: Evidence for interaction or genetic recombination between potato viruses Y and C in infected plants. Virology **10**, 211–232 (1960). — 34. WITTMANN, H. G.: Untersuchungen über die Bedeutung von Temperatur und genetischer Konstitution für die Mutabilität von Bakteriophagen. Z. Vererbungsl. **88**, 128–144 (1957). — 35. WITTMANN, H. G.: Proteinuntersuchungen an Mutanten des TMV als Beitrag zum Problem des genetischen Codes. Z. Vererbungsl. **93**, 491–530 (1962). — 36. WITTMANN-LIEBOLD, B., und H. G. WITTMANN: Lokalisierung von Aminosäureaustauschen bei Nitritmutanten des TMV. Z. Vererbungsl. **97**, 305–326 (1965). — 37. YAMAFUJI, K., and T. FUJIKI: Experimentelle Erzeugung des Tabakmosaikvirus. Biochem. Z. **318**, 101–106 (1947). — 38. ZIMMER, K. G.: Zur Deutung der Beziehung zwischen Konzentration von Viruslösungen und Infektionshäufigkeit. Biol. Zbl. **63**, 142–152 (1943).