INTRODUCTION

The invitation to deliver an opening lecture to this audience has placed me in a somewhat difficult situation. Such a lecture is commonly expected to present a panorama-like view over a field of research. This task, however, is for animal virology at present hardly realizable.

One feels in such a case like a historian who was assigned the task of integrating recent events into valid historical form. He will certainly run into the danger of overestimating some and understimating others in importance. His tract will become therefore more or less speculative or even misleading. Hence an honest historian will postpone such an integration to a later time, when the chaff has already been separated from the wheat by the winds of critical examinations and comparisons. At the moment he will prefer to restrict himself to an exact description and critical evaluation of those events which he could follow himself.

In virology so many new findings have been published during the last few years that it is impossible to study all of them critically. To avoid in this situation mistakes like those just mentioned I would like to focus attention only on those results which have been achieved by our group. It was, I believe, Emerson who mentioned that “concentration is the prudence of life,” and we have tried to follow his advice, concentrating our attention mainly on three animal viruses. The general plan was to purify first the infective particles, the viria, and to study their composition. Valuable information has been obtained by dissecting the viria and studying the composition of their components. Viral anatomy was followed by viral physiological studies in order to clarify the significance of the various structural elements.

I hope that I can convince you that such concentrated action in a rather narrow field of virology can lead to important findings and stimulate new ideas. In presenting the results I function only as the speaker of our group, since no appreciable success would be achieved without the tireless efforts of all members of the group.

STRUCTURE OF THE MODEL VIRUSES

Maus-Elberfeld Virus

The three viruses investigated all belong to the ribonucleic acid (RNA)-containing viruses. The smallest is the Maus-Elberfeld (ME) virus, a member of the Columbia-SK group (9, 14). In purified preparations the shadowed ME virion seems to have a spherical appearance (Fig. 1).

Electron micrographs of replicas of viral crystals suggested, however, that they were like other dwarf or nani-viruses polyhedrons, having a size

1 Based on ONR lecture, presented at the Annual Meeting of the American Society for Microbiology, Kansas City, Mo., 6 May 1962.
of about 24 μ (Fig. 2). Crystallized preparations contained, in addition to protein, more than 20% RNA. It is suggested that the viral RNA is surrounded in some manner by protein, since ribonuclease does not destroy the biological activity of the virion. Further information on the structural details of the ME virion were obtained by investigations with the negative staining technique. In electron micrographs (Fig. 3, 4), the polyhedral shape of the virion is clearly recognizable. Subunits of the polyhedron having a diameter of 40 to 50 Å were observed, although no details of the subunits as contained in the

virion have yet been resolved. It may be, however, that the tiny particles observed in electron micrographs of virus samples purified by density gradient centrifugation (Fig. 4) are released subunits, although their diameter is larger than would be expected. They apparently contain a hole in the center.

More information is now available on the nucleic acid component of ME virus, which was extracted with phenol from a crystallized virus preparation. The fraction obtained was rather pure as indicated by the high E 260/E 280 ratio of 2.05. In an analytical ultracentrifuge the bulk of extracted RNA sedimented with a velocity similar to that of infectious tobacco mosaic virus (TMV) RNA (Fig. 5); thus one can assume that its molecular weight, like that of TMV RNA, is about 2,000,000.

**Fowl Plague Virus**

Much more complex in composition than the ME virus is our next model, the fowl plague virus, which belongs to the influenza A viruses (3, 20, 27–33, 37–41, 52, 55). The size of this virus ranges between 70 and 80 μ (Fig. 6). Chemically, in addition to RNA and protein, carbohydrate and lipids are found. Furthermore, a neuraminidase is present. Assuming that all particles contain an equal amount of nucleic acid, the RNA content per fowl plague virion corresponds to a molecular weight of about 2,000,000. The structural appearance of the fowl plague virion as revealed by negative staining reflects its complex chemical composition (Fig. 7). Like other viruses of the influenza group, it possesses an envelope armed with tiny spikes, which are 30 to 40 Å thick and 100 to 120 Å long. The envelope seems to enclose a coiled filamentous structure.
When the lipids of the virion are removed by ether, two types of subunits can be isolated. One is called "hemagglutinin," since it possesses hemagglutinating activity; it looks like a tiny star (Fig. 8), with the spikes on the surface. Its over-all diameter is 30 to 35 mμ. It is suggested that after removal of the lipid the viral envelope disintegrates into small pieces which roll together like a hedgehog and form thereby the structure just described. This consists of protein and carbohydrate and contains the neuraminidase activity. As in the case of the hemagglutinin envelope, we have also been unable to obtain the inner viral component, called "g or internal s antigen" in an intact state after ether treatment of the virion. The filament, which is about 15 mμ thick, apparently degrades into pieces of various length from 15 to 100 mμ (Fig. 9). Chemical investigation of this fraction showed that in addition to protein it contains the viral RNA. One gains the impression from investigations of negatively stained s antigen with the electron microscope that the RNA strand is surrounded by loosely arranged protein subunits (Fig. 10). Experiments to isolate the viral RNA by phenol treatment of intact viria led to preparations with sedimentation constants not higher than 17 S (Cook, unpublished data), whereas the RNA of ME virus exhibits a sedimentation constant of about 30 S. The reason for this difference is not yet understood. It is conceivable that to fowl plague virion are attached larger amounts of ribonuclease, the action of which cannot be prohibited during the isolation procedure.

Newcastle Disease Virus

The largest model investigated is the Newcastle disease virus (NDV), having a diameter of 120 to 180 mμ (23-26, 35, 42). There are some indications that this virus belongs in the same group as the mumps and parainfluenza viruses. The general building principle of this model resembles that of fowl plague virus. An electron-dense center, and a halo with much lower density which sometimes detaches from the center, can be observed on electron micrographs of unshadowed preparations (Fig. 11). The chemical composition of NDV has not yet been thoroughly explored but seems to be comparable to that of fowl plague virus. It is certain that it contains lipids as well as protein and RNA. Furthermore, it also possesses a neuraminidase. Electron microscopically, Horne
et al. (16) observed with the negative staining technique an analogous spike-armed envelope and an inner component reminiscent of the internal s antigen filament of fowl plague virus but with appreciable differences in fine structure (Fig. 12). Somewhat earlier we had isolated this inner component from NDV by ether treatment (Fig. 13) and found it, like internal s antigen of fowl plague virus, to contain RNA. The fine structure of the NDV inner component (Fig. 13) resembles that of TMV. It is about 170 A thick and contains a central channel 50 A in diameter. There is no doubt that the subunits of this structure are much more tightly packed than those of fowl plague s antigen. The isolated hemagglutinin of NDV, however, is morphologically nearly indistinguishable from that of fowl plague virus (Fig. 14).

The results presented with respect to the structure of the three viruses are not yet sufficient for construction of exact models. But I think that the presentation of very schematic drawings can be risked in order to impress upon our minds those structural principles which are important for our further considerations (Fig. 15).

**Significance of Viral Components**

The significance of the various components of the viria can be evaluated by various methods. Some information can be obtained by biological investigation of the isolated viral components. Important knowledge was gained by using intact viria and following the behavior of their compo-

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**FIG. 6. Fowl plague virus, shadowed.**

**FIG. 7. Fowl plague virus, PTA.**

**FIG. 8. Hemagglutinin of fowl plague virus, PTA.**
extracted with phenol were investigated. The RNA extracted from ME virus, like that of many other viruses, was able to induce host cells to form new viria (9, 13). By following the sedimentation of such active fractions by infectivity tests after ultracentrifugation in a separation cell, it could be demonstrated that the biological activity is coupled to RNA with a sedimentation constant of about 30 S (Table 1). With the 17 S RNA pieces of fowl plague virus, no biological activity was detectable (Cook, unpublished data). Thus, one might suggest that the total complement of RNA contained in a virion is needed to transfer to a host cell the information required for the production of new virus.

Of the two types of subunits obtained from fowl plague virus by ether treatment, neither the

FIG. 9. The g or internal s antigen of fowl plague virus, shadowed.

FIG. 10. The g or internal s antigen of fowl plague virus, PTA.

ments during the reproduction process inside the host cell. Fluorescent antibody and isotopic labeling techniques proved to be very useful for this purpose.

*Biological Investigation of Isolated Viral Components*

Impressive results were achieved, by the first type of approach, when the viral nucleic acids

FIG. 11. Newcastle disease virus, unshadowed.

FIG. 13. The g or internal s antigen of NDV. Shadowed and PTA.

FIG. 14. Hemagglutinin of NDV, PTA.

FIG. 15. Scheme of ME, fowl plague, and NDV viruses.

internal s antigen nor the hemagglutinin was infectious (3, 20, 29–32, 39). From the hemagglutinin, infectivity could hardly be expected since it does not contain nucleic acid. The RNA amount present in the isolated s antigen pieces is apparently not sufficient for induction of the host cell.

Examinations of both types of fowl plague split products revealed furthermore that they do not possess interfering activity (51). Isolated internal s antigen can be recognized as virus-specific material only by serological methods. Its serological specificity is relatively low. Only very slight serological differences could be observed between s antigens of various influenza A strains (3, 20). Thus s antigen possesses a group antigen. However, the antigen of the hemagglutinin subunit, the surface component of the virion, is highly specific. It has apparently no antigenic component in common with the s antigen. In immunization experiments in animals, only the hemagglutinin proved to be fully capable of inducing immunity and neutralizing antibody. Hemagglutinin is, as mentioned earlier, that component of fowl plague virion which contains its hemagglutinating principle.

Corresponding information is not yet available for the isolated subunits of NDV. But in the few areas already examined the situation is similar to that of fowl plague virus.

**TABLE 1. Biological determination of sedimentation velocity of ME virus RNA extracted from crystallized virus**

<table>
<thead>
<tr>
<th>Infectivity of control† (LD50)</th>
<th>Infectivity of supernatant (LD50)</th>
<th>S20</th>
</tr>
</thead>
<tbody>
<tr>
<td>$7.8 \times 10^8$</td>
<td>$1.3 \times 10^8$</td>
<td>34 S</td>
</tr>
<tr>
<td>$3.1 \times 10^9$</td>
<td>$1.0 \times 10^8$</td>
<td>21 S</td>
</tr>
</tbody>
</table>

*Centrifugation was for 20 min at 44,770 rev/min.
†Control not centrifuged.
Investigation of Viral Reproduction

The second approach to clarify the significance of viral components was the investigation of the multiplication process.

Reproduction of ME virus. The concept obtained with respect to the multiplication mechanism of ME virus is still rather incomplete. Nevertheless, the results available from studies with the L-cell system allow us to recognize some important features and problems (11-13). Like other viruses, the ME virus goes into an eclipse phase. Shortly after infection, the ratio of infective virus particles to infected cells falls to about 0.001. The events leading to the loss of infectivity of the penetrating ME virus have not yet been studied. Following the production kinetics of new viral material in synchronously infected L cells, it emerged (Fig. 16) that the phenol-extractable infectious RNA starts to increase between 1 and 2 hr after infection. The production of viral protein, as studied by the complement-fixation test and by pulse-labeling the viral protein with C\textsuperscript{14}-leucine, seems to follow the same time course as that of RNA. There is reason to believe that the subunits of the viral protein shell are synthesized in the cytoplasm; by fluorescent antibody, viral antigen is first detectable at about 3 hr after infection and is found exclusively in this region of the cell (Fig. 17). The nucleus remains free of detectable fluorescence during all stages of infection. The curve representing the appearance of new infective particles increases 1 to 1.5 hr after that of viral RNA and protein. The particles are apparently assembled inside the cytoplasm, because ME virus cannot be neutralized as long as it is associated with the cell, whereas other viruses which are formed at the membrane are neutralized.

The protein and nucleic acid metabolism of the ME-infected system undergoes radical changes at early stages of the cycle. Pulse experiments with C\textsuperscript{14}-leucine suggested (Fig. 18) that the rate of total protein synthesis begins to decrease shortly after infection (Hausen, unpublished data). Most interesting are the changes with respect to RNA synthesis (12). They were detected autoradio-
graphically, using 5-min pulses of tritium-labeled uridine. Normal L cells show, under these conditions, a strong labeling of the nucleus, especially of the nucleoli (Fig. 19), whereas the cytoplasm is practically free of label. In infected cells, however, nuclear RNA synthesis begins to decrease 2 hr after infection. After 3 to 3.5 hr, only a negligible number of cells showed nuclear tritium-uridine incorporation. Instead, increasing amounts of labeled compound were found in the cytoplasm starting at about 3 hr after infection (Fig. 19). Thus, in the RNA metabolism of ME-infected L cells, at least two different processes seem to occur: first, an inhibition of nuclear RNA synthesis, and then a new, rapid RNA synthesis in the cytoplasm. Corresponding controls insured that RNA and not an acid-soluble compound was concerned.

By using small amounts of p-fluorophenylalanine (FPA), which still inhibit viral production, it can be demonstrated that an FPA-sensitive phase precedes the changes observed (Hausen, unpublished data). Adding FPA up to 1 hr after infection inhibits the decrease of nuclear RNA synthesis as well as the increase of cytoplasmic RNA synthesis. After addition at 3 hr postinfection inhibition of RNA synthesis in the nucleus persists but no synthesis of RNA in the cytoplasm occurs. Thus, special virus-induced proteins seem to be necessary before the RNA metabolic changes observed in connection with ME virus multiplication can proceed.

Correlating these findings with the observations described earlier, it seems reasonable to believe that the cytoplasmic RNA is mostly viral RNA. This became somewhat improbable, however, following the chemical characterization of the RNA synthesized later (4 to 6 hr) in the multiplication cycle (Scholtissek, unpublished data). The method used was developed by Scholtissek (44-46) and is hereafter referred to simply as “chemical RNA characterization.” The newly synthesized RNA is labeled by P32. After digestion of the labeled RNA with ribonuclease and separation of the various oligonucleotides by chromatography, the oligonucleotide pattern of the labeled RNA is determined. This pattern is much more characteristic than is the simple base ratio. Examination with this method showed that the total complement of RNA synthesized at 4 to 6 hr after infection in the cell is not identical with viral RNA. Nevertheless, the rapidly synthesized cytoplasmic RNA seems to participate in some manner in the synthesis of viral protein because the latter is produced at nearly the same time at the same cell site. In view of the recently advanced hypothesis of protein synthesis (2), one might speculate that the particular cytoplasmic RNA has a messenger function. At the present time, however, this is nothing more than pure speculation.

The ME virus seems to us to be a very interesting model with respect to its reproduction mechanism. The problems here are based on very impressive and characteristic changes, and are therefore relatively easy to attack.

Reproduction of fowl plague virus and Newcastle disease virus. Somewhat more information is available with regard to the multiplication process of fowl plague virus (1, 4-6, 17, 19, 22, 30, 31, 33, 34, 41, 43, 47-49, 53, 54, 56). The features evaluated here are integrated into a schematic picture. I am fully aware that in presenting this scheme I must neglect somewhat the cate-

FIG. 19. Incorporation of H3-uridine in L cells: (top) normal cells; (bottom) ME-infected cells, 5 hr after infection.
gorical imperative postulated initially. To bring the scheme into a reasonable form, I must substitute some missing links by speculation.

As demonstrated in the scheme (Fig. 20), the fowl plague virion attaches with the spikes of its shell onto the cell surface. The possible function of the neuraminidase probably contained in the spikes during the penetration of viral material into the cell is still open to discussion. After adsorption onto the cell, the fowl plague virion goes into eclipse. The ratio of infective virus to the number of cells acting as infective centers falls to a value smaller than 0.01. Investigations showed that shortly after infection homogenized suspensions of the cells contained a considerable amount of $^{32}$P carriers which could be precipitated by highly specific s antigen antisem. This serum does not react with intact viria. After precipitation of s antigen, labeled RNA was still contained in the supernatant. This seems to be viral RNA, since no significant transfer of viral $^{32}$P to cell substances was observed. No convincing experimental evidence is available at present as to how the hemagglutinin shell of the virion behaves during the degradation procedure.

Before the production of detectable amounts of new viral material begins, a substance has to be formed the occurrence of which could be demonstrated only indirectly using FPA as inhibitor, as in the case of ME virus (Fig. 21). The dose of FPA used did not disturb markedly the metabolism of cell RNA, the incorporation of C$^{14}$-leucine into cell protein, or the eclipsing of fowl plague virus after infection. When added early in the multiplication cycle, however, it inhibited the appearance of viral RNA and other viral components, as far as they can be identified by the available tests. When FPA was added after the critical time of about 1 hr postinfection, a material behaving serologically like s antigen and containing viral RNA was produced in amounts comparable to those occurring under normal conditions. Further effects of FPA will be considered later on.

With regard to the functions of the early occurring FPA-sensitive material, which is very probably protein, no fully convincing explanation can be offered at present even in this somewhat more intensively investigated case. Since, however, FPA does not markedly disturb synthesis of normal cell RNA and since fowl plague viral RNA does not contain abnormal nucleotides, the proteins in question may not be particular enzymes needed for viral RNA synthesis. Moreover, the hypothesis comes to mind that in the case of fowl plague virus the particular protein works in stabilizing the viral genetic material; once this has occurred, synthesis of viral RNA and of protein behaving serologically like s antigen protein can no longer be stopped by FPA, as the normal cell RNA metabolism might not be stopped by FPA, since corresponding stabilized templates are already established in the cell.

During the normal viral multiplication cycle, new viral RNA and s-antigen protein can be first detected 2 to 3 hr after infection, just after establishment of the FPA-sensitive factor. The over-
whelming amount of initially appearing viral RNA can be precipitated by s antigen antiserum. This implies that either both components of the viral inner component are assembled immediately after their synthesis or they are formed as a unit. That the s antigen appearing inside the cell is indeed identical with the inner component of the virion can be shown by isolating this component from cell homogenates and comparing it with s antigen liberated from viria (Fig. 22). According to our investigations with specific fluorescent antibody, which have already been reported on numerous occasions, s antigen is produced in the cell nucleus and seems to be transported later into the cytoplasm. The alternative sometimes discussed, that the later appearance of s antigen in the cytoplasm occurs by the creation of new cytoplasmic s antigen synthetic centers, appears rather improbable in light of the results of the experiments performed with FPA. After adding this compound 2 hr after infection, s antigen accumulated in the nucleus and caused remarkable swelling of this cell organelle (Fig. 23). This finding not only supports the first theory but also indicates that an FPA-sensitive compound participates in the transport of the s antigen from the nucleus to the cytoplasm.

Somewhat after the appearance of s antigen, hemagglutinin can be detected. In contrast to s antigen, hemagglutinin antigen can be found with fluorescent antibody exclusively in the cytoplasm. Attempts to isolate the respective component from cell homogenates by adsorption on and elution from red cells led to preparations which contained structures that were quite different from those we expected to find (22, 31, 43). Although they possessed hemagglutinating, neuraminidase, and virus-specific antigenic activity and were lacking infectivity, as was to be expected from the hemagglutinin component, they resembled in structure and chemical composition microsomes of normal cells (Fig. 24). The microsomal nature of these elements was further stressed by the finding of glucose-6-phosphatase activity and by isolation of ribosome-like particles from them with fluorocarbon (Fig. 25). No convincing evidence was obtained that these ribosomes contain virus-specific material. Such material was isolated, however, quite recently by another method used to degrade the lipoprotein membrane of the microsomes and seems to behave biologically and physically like the hemagglutinin spikes of the

FIG. 22. The s antigen of fowl plague isolated from infected tissue.

FIG. 23. Localization of s antigen by fluorescent antibody in chick embryo lung cells. a, c, e, g: no FPA; b, d, f, h: FPA 8 hr after infection. Time of staining; a and b, 4 hr after infection; c and d, 7 hr; e and f, 10.5 hr; g and h, 13 hr.
Comparing the over-all RNA metabolism of the virus-chick embryo cell system with that of corresponding normal cell cultures, it emerged (49) that the rate of total RNA synthesis increases at first in the infected system, reaching a maximum at 3 hr, and declines later (Fig. 26). Chemical characterization of the nucleic acid produced showed that the early increase coincides with and is caused by the additional virion (Maes, unpublished data). The microsomal elements containing this viral compound are now called "viromicrosomes." It was convincingly shown that other hemagglutinating noninfectious particles, known as "incomplete forms of v. Magnus-type" have another origin and are really incomplete viria lacking internal s antigen (21, 22). The fact that hemagglutinin-containing microsomes can be isolated from infected cells lends further support to the concept that the surface component of the fowl plague virus is completed in the cytoplasm.

The sequence of appearance of s antigen and hemagglutinin and their different localization inside the cell suggested that both are completed separately. The experiments with FPA strengthened this suggestion. By adding FPA 2 hr after infection, it is possible to achieve the production of s antigen without corresponding appearance of hemagglutinating and infectious material (Fig. 21). With later addition of FPA, the inhibition of hemagglutinin and infectious virus production declines gradually.

During the further course of the normal multiplication cycle, both viral subunits are transferred to the cell periphery where they are assembled into new viria near the cell membrane or mostly in protrusions of it. Electron microscopic studies suggested that they are coated at this occasion with lipid originating from the cell wall. Cellular origin of the viral phospholipids was indeed demonstrated, using an isotopic tracer (53). The assembly of s antigen and hemagglutinin seems to be a rather selective process, since even s antigen which accumulates in the presence of FPA is not incorporated to an appreciable extent into virus that is formed after release of the inhibition.
synthesis of viral RNA, which is, according to earlier experiments of Wecker (53), produced de novo. Besides viral RNA and normal cell RNA, no further RNA was observed to be produced during the infectious cycle, not even during that period when hemagglutinin appears. Thus it is not very probable that a RNA different from viral RNA is engaged in the production of fowl plague viral protein. Unfortunately, chick embryo cells were unsuitable for autoradiographic studies which we would like to perform to settle this topic somewhat more.

The contribution of our group to the understanding of NDV reproduction is still of a preliminary nature. It may be mentioned briefly that a material resembling the viral inner component (24), and something like viromereosomes (Rott and Reda, unpublished data), have been isolated. Therefore, one might suggest that at least some parallels exist in the reproduction mechanism of fowl plague and ND viruses.

Summary

The observations described with respect to the significance of the various virus components support the concept, already widely accepted, that the viral nucleic acid which is situated in the interior of the viria possesses all the information the host cell needs to produce new virus. With ME virus, this could be proved rather convincingly. With fowl plague virus, at least some indication was obtained in this direction. The main tasks of the materials surrounding the nucleic acid are to protect this component and to facilitate its penetration into the cell. Thereby the "foreign gene" viral nucleic acid becomes a "strolling gene" and therewith an infectious agent. The surface material contains, on the other hand, those components of the virus which induce immunity in the organism, and the sites where neutralizing antibodies attack. During the multiplication procedure, the viral nucleic acid or the nucleic acid containing inner component and the coating material seems to be generally produced separately and to be assembled later to form the new virion.

This principle is well-established now in the case of fowl plague virus and can also be suggested from the results with ME virus. Further evidence for such a concept is delivered by the genetic studies of Hirst (15), who found "phenotypic mixing" with several animal viruses. In the myxoviruses, lipids—probably of cellular origin—serve to hold the viral components together in the completed virion.

More thorough investigation of the viral reproduction procedure led to the detection of some factors which do not belong to the equipment of the virion itself. They seem to be, however, essential for establishing the virus-synthesizing capacity inside the host cell. First, there may be one particular substance of the host cell which undresses the virion initially in order to liberate its nucleic acid. That an undressing of the virion precedes its reproduction process could be rather convincingly shown with fowl plague virus. Furthermore, virus systems as different as ME and fowl plague produce, apparently early in the reproduction cycle, particular proteins which are reminiscent of the "early protein" of phages. According to Levintow and co-workers (18), such early protein seems to occur also in the polio system. With regard to the function of these proteins in the animal viral systems, no more is known at present than that they are necessary for the normal progression of viral reproduction. A more thorough study of them might, however, shed some light on the early steps which lead to the realization of viral information inside the cell.

A most interesting problem is posed, finally, by the drastic RNA metabolic changes observed by our group in ME virus-infected cells and by Franklin and Rosner (7) in Mengo virus-infected cells. Of special interest is the establishment of an efficient RNA-synthesizing system in the cytoplasm at a time when viral protein appears there. It may well be that the rapidly synthesized cytoplasmic RNA is concerned with the production of virus protein.

Application of Some Features Evaluated to Problems of Practical Importance

Although the survey presented offers many more problems than well-established facts, some of the latter may lead to implications of practical importance.

The finding that the immunizing capacity of the fowl plague virus resides in its hemagglutinogen shell material led to the proposal to use, instead of inactivated total virus, the isolated hemagglutinins of influenza and possibly other myxoviruses as vaccines. The potency of such a
vaccine may be demonstrated only by one result (3). An injection of 0.003 mg of purified swine influenza hemagglutinin, prepared with Freund adjuvant (10), initiated in a rabbit after 6 weeks a hemagglutination-inhibition titer of 1:32,000. With such vaccines, the occurrence of undesirable reactions would certainly fall to a minimum, since in contrast to intact virus isolated hemagglutinin is practically free of normal host-cell components.

Where the isolation of the immunizing viral shell material is not yet possible, one should inactivate the respective viria for vaccine production with a chemical which does not change the antigenic material but acts exclusively on the viral genetic substance, the nucleic acid. In this respect, hydroxylamine is clearly superior to the still commonly used formaldehyde. NH2OH seems to react exclusively with the nucleic acid, where, according to Schuster (50), it changes the pyrimidine bases as indicated in Fig. 27.

Small RNA-containing viruses, like ME, polio, and EE, as well as the larger influenza viruses, could be inactivated by this compound. The deoxyribonucleic acid-containing Herpes

![RNA chain](image)

![2 Molecules NH2OH reaction](image)

![Probable further reaction](image)

**FIG. 27. Reaction of NH2OH with RNA (Schuster).**

<table>
<thead>
<tr>
<th>Time of treatment (hr)</th>
<th>Dilution†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10⁻¹</td>
</tr>
<tr>
<td>0</td>
<td>110</td>
</tr>
<tr>
<td>1.5</td>
<td>35</td>
</tr>
<tr>
<td>3</td>
<td>64</td>
</tr>
<tr>
<td>6</td>
<td>48</td>
</tr>
<tr>
<td>7.5</td>
<td>150</td>
</tr>
<tr>
<td>9</td>
<td>251</td>
</tr>
</tbody>
</table>

* Treatment was with 0.2 M NH2OH. Results are expressed as the number of plaques formed per culture.
† The numbers in parentheses correspond to the number of virus particles (original PFU's) present in each dilution. Number of cells per culture: ~10⁶.

**FIG. 28. Inactivation of ME virus by NH2OH.** O, 0.1 M; ●, 0.5 M NH2OH, pH 7.0, 20 C.

virus and, surprisingly, the NDV and mumps virus strains examined proved to be resistant (8, 36), although the latter viruses are similar in their over-all structure to the influenza agents. In this connection it was of some interest that TMV was also resistant, but extracted TMV-RNA sensitive to NH2OH. Thus, differences in coating of viral RNA may play a role in causing differences in their behavior to NH2OH.

The inactivation kinetics (36) of ME virus with NH2OH were strictly exponential over the course examined (Fig. 28). An irregularity was observed with fowl plaque virus. After inoculating tissue cultures with multiplicities higher than one of
incompletely inactivated fowl plague virus samples, more plaques appeared than were to be expected from the number of those appearing at higher dilutions (Table 2). In this connection, multiplicity is referred to the plaque-forming units originally present. This phenomenon is caused by multiplicity reactivation (Cook, unpublished data). In preparing vaccines, the difficulty resulting therefrom can be overcome by an intensive treatment with NH$_2$OH, that is, by causing many hits in the nucleic acid strand. Even after 4 days of incubation at 20°C with 1 M NH$_2$OH, neither influenza nor fowl plague virus lost antigenic potency (Fig. 29). Respective vaccines were at least as potent, usually even somewhat more potent, in the animal than corresponding cautiously prepared formaldehyde-treated vaccines (Fig. 30). Thus NH$_2$OH might be used in future to prepare safe and potent vaccines of various viruses.

Finally, it may be stressed briefly that each advance in knowledge of the structural and chemical behavior of viruses is strongly needed in order to place viral taxonomy onto a more solid foundation. The simple differentiation by NH$_2$OH, for example, can be of some value in this sense.

**Concluding Remarks**

I hope I have been able to demonstrate that the concentrated action of our group on a very small sector of animal virology was not quite unsuccessful but has created at least some ideas of possibly a more general importance for the fight against virus diseases. At the present time, when concentrated scientific activity is often directed toward the destruction and not toward the preservation of life, one might fear that the phrase “concentration is the prudence of life” will some day be inverted into “concentration is the stupidity of life.” To avoid such an occurrence, scientific workers all over the world would do well to return to the ethics of the stoicist Seneca, who noted in his *Naturales Quaestiones* that “by exploration of the facts natural sciences base the moral life of man on a solid foundation; they liberate him from fear and let him recognize the glory and magnitude of divine creation.”

**Acknowledgments**

I wish to thank R. Rueckert for assistance in the translation of the manuscript.

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28. Schäfer, W. 1955. Vergleichende sero-im-


