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INTERACTIONS BETWEEN PATHOGENIC VIRAL TYPES AND THEIR HOST CELLS

[The following is a translation of an article by W. Schafer* which appeared in the German language periodical, Path. Microbiol (Pathology and Microbiology), Vol. 28, pages 917-935, (1965)]

The fact that proliferating animal-pathogenic viruses are dependent upon the metabolic apparatus of the cell has been known since the time of Loffler and Frosch; also the fact they are effective in many different ways was immediately apparent to clinicians and lead to the first histologic and histochemical investigations. However more precise studies became possible only when more sophisticated techniques were introduced. Only after the application of tissue-culture techniques could reproducible experimental results be achieved, the application of isotopes leading to great accuracy and sensitivity.

The study of the interactions between two partners can only lead to worthwhile results if the properties of each partner are well-known. For this reason only a few animal virus types which have been analyzed in detail, have so far been chosen. As the field of experimental virology is still in a state of flux I should like to limit my discussion to the studies performed by our own group. My collaborators in this work were P. and H. Hausen, R. Rott and C. Scholtissek (See references 1,2,3 and 4).

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Viruses and Host systems studied.

In recent years our sphere of interest has involved three different types of carefully chosen viruses. They are representative of three groups of viruses which have practical importance and which can be easily handled experimentally. The chosen types were: a Columbia SK strain, the ME virus, as representative of the Pikorn viruses; the classical Poultry-plague virus (FP) of the Myxoviruses as representative of the influenza viruses and finally, the Newcastle disease virus (NDV) as representative of the Para-influenza viruses.

Over-simplified diagrams of these viruses are given in Figure 1.

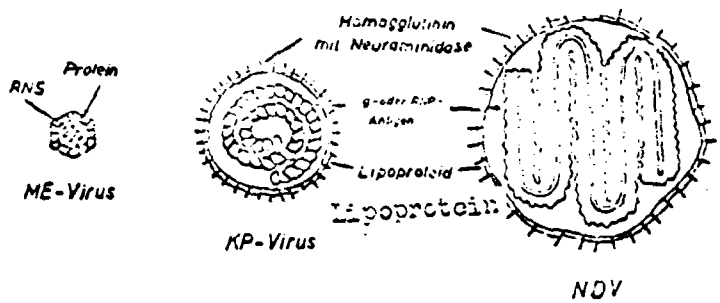

Figure 1. Diagram of three model viruses.

All three types of viruses contain single-stranded nucleic acids of the ribose type (RNA); in ME- and KP-virus the amount corresponds to a molecular weight of about 2×10^6, in NDV it appears to be somewhat greater. In ME-virus the RNA is surrounded by a relatively simply structured protein shell. According to the latest results of R. R. Rueckert (5,6), this consists of two, or even three, polypeptides. In KP-virus and NDV the nucleic acid together with a special protein forms a thread-like inner component, the g- or RNP-antigen, whose structure differs for each virus. In the Myxoviruses the RNP-antigen is surrounded by a spiny shell, the Hemagglutinin, whose distinctive characteristic is the fact that it is the carrier of the hemagglutinising properties of the virus particle. Neuraminidase also appears to be contained within this component. Studies of KP virus show that the hemagglutinin is a carbohydrate protein complex. The entire structure is held together with lipoproteins which, as will be shown later, were originally components of the cell wall.

Of great experimental significance was the possibility of sero-
[illegible] dissection of the complex structure of the myxoviruses to obtain
their RNP-antigen - and hemagglutinin-complex in an essentially pure
form (see Figure 2). It was also possible to prepare potent component-
specific antisera which we used for the localisation of the various
viral components of the cell or for their isolation.

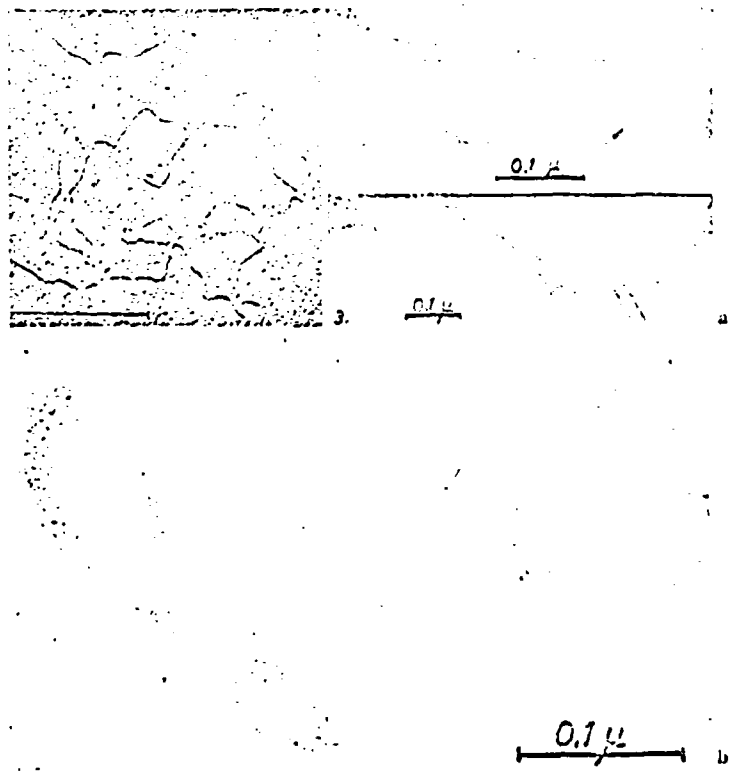

Figure 2. (a) RNP-antigen of NDV.
Left: evaporated; Right: negative
contrast obtained with Phospho-Tungstic
acid (PTA). (b) Hemagglutinin of LP-
virus (PTA).

As host cell, we used mouse fibroblast of strain L for the NE virus, and primarily chicken fibroblasts for the myxovirus.

Virus-Host cell interactions.

In the study of the interactions occurring between the virus and host-cell we first followed the behavior of the virus-specific material and then attempted to correlate this with changes in the metabolic behavior.

a) Behavior of the virus-specific material.

The following picture for the NE-virus-L-cell system resulted from studies of the behavior of the virus material: significant changes become apparent shortly after contact occurs between host cell and the infecting virus particle. It enters a phase which can be designated the "Eclipse" phase in which its infectiousness can no longer be detected by the usual means. According to accepted opinion this indicates that the virus particle has liberated its genetic material, nucleic acid. Apparently the NE-virus requires no special enzyme for this. By using highly-purified crystallized NE-virus we found that in 0.07 m. NaCl solution at pH 6.0 and 37°C its shell rapidly breaks up into fragments. In addition we observed, in electron-optic investigations, that substructures can detach from the virus shell at the weak points (without further preparation during evaporation on the slide) from which the nucleic acid threads emerged (Figure 3). The NE-virus shell is therefore a relatively unstable system which under quasi-physiological conditions can be split without enzymic participation, causing release of its nucleic acids. Drews made similar observations with polio-virus (Personal communication).

Under the direction of the liberated RNA, virus-RNA and -protein are formed on the new virus material in the host-cell (Figure 4). The RNA was found to be phenol-extractable, ribonuclease labile, and the infectious component. The kinetics of the formation of . . . . . [page 922 is missing]

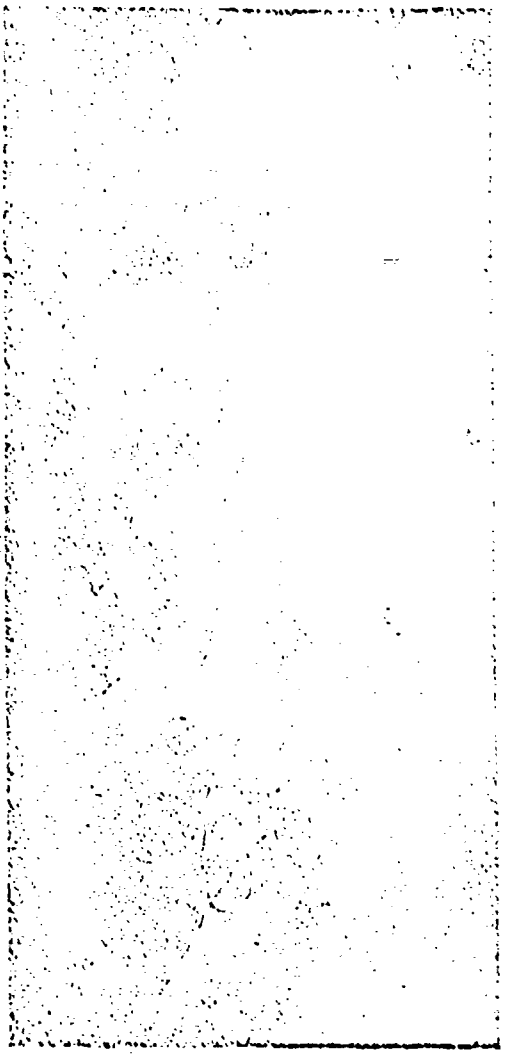

Figure 3. Partially broken ME-virus (evaporated). Liberation of Nucleic acid.

[p. 925] . . . . virus proteins were followed serologically and with C^{14}-leucine labelling. Naturally we were also interested in seeing whether double-stranded RNA may play a role in the formation of new single-stranded viral RNA. During these investigations we were able to isolate double-stranded RNA from the infected cells, as Montagnier and Sanders (7) were able to do for EMC-virus and P. Hausen (8) for ME-virus (Figure 5). Hausen showed that its production essentially paralleled that of the single-stranded virus RNA. "Chase" experiments with radioactively labelled nucleic-acid precursors indicated that a part of the double-stranded RNA is continually replaced with newly-synthesized material. Melting and purification experiments in which the "hot +" portion of the double strand is replaced by "cold" RNA isolated from the virus particle showed that the +-portion is continually renewed. All these findings can be understood most easily by assuming that the formation of ME-virus RNA follows the principle of asymmetric semiconservative proliferation (cf. 9) (Figure 6).

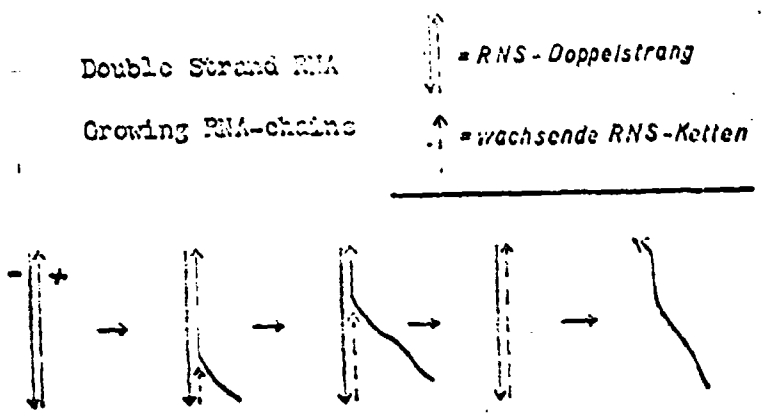

Figure 6. Schematization of the asymmetric, semiconservative proliferation of virus-RNA.

Topographically, according to the autoradiographic experiments described below, the proliferation of ME-virus-RNA appears to take place in the cytoplasm. In the same place one also finds virus-protein with fluorescent antibodies (Figure 7). As soon as sufficient quantities of both components are applied they combine to form the new infectious virus elements (Figure 4). Their liberation occurs

as the cell decays. More than 100,000 virus particles are formed per cell.

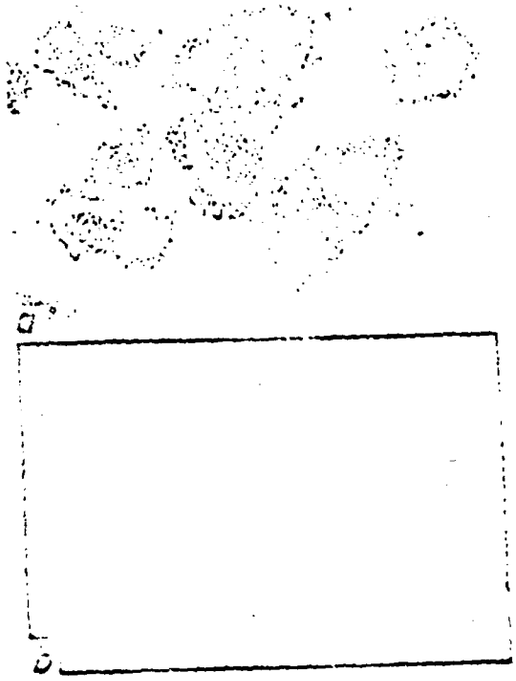

Fig 7. (a). Autoradiograph of RNA-synthesis in ME-infected-L-cells. 5 hours post-injection. 5 minutes H^3-uridine. (b). Demonstration of ME-antigen in infected L-cells, using fluorescent antibodies. (4 hours post-injection).

Since the proliferation of KP-virus in chicken fibroblasts has been dealt with frequently. I will summarize it in a diagram (see Figure 8).

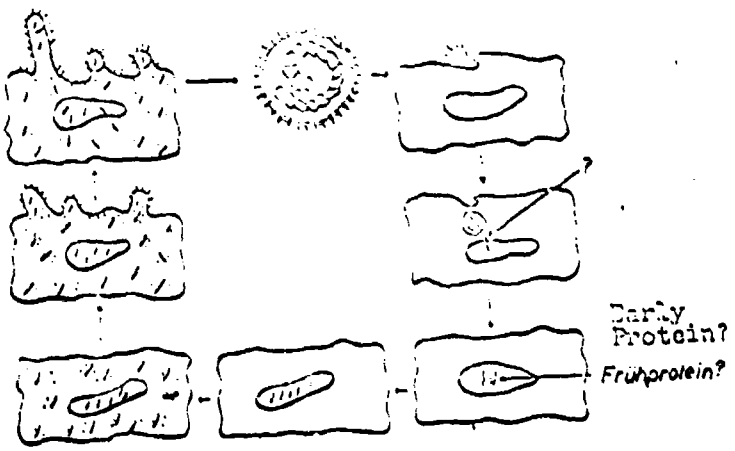

Figure 8. Diagram of the KP-virus. ⋮ = RNP-Antigen, ⋮ = RNA; and / = Hemagglutinin.)

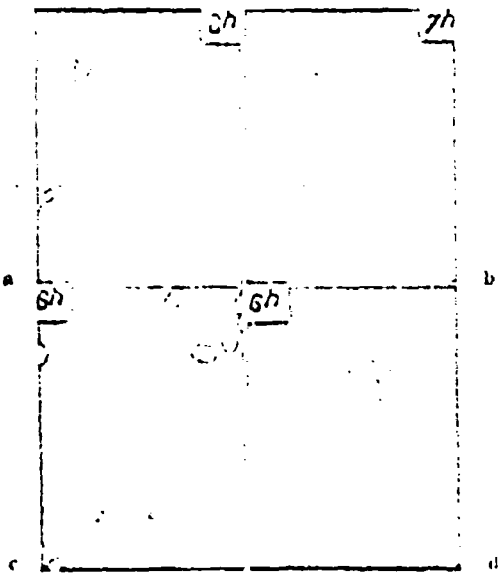

Figure 9. Diagram of NDV-antigen in infected cells with component specific fluorescent antibodies. (a) Chicken fibroblast 8 hours post-infection (p.i.). RNP-antigen-antibodies. (b) Chicken fibroblast 7 hours p.i., Hemagglutinin-antibodies. (c) Chicken embryo-lung-cells 6 hours p.i., RNP-antibodies. (d) Chicken embryo-lung-cells 6 hours p.i., Hemagglutinin-antibodies.

The first components can be found soon after infection with KP-virus, i.e. virus-RNA which enables synthesis of RNP-antigen in the nucleus. Shortly afterwards new hemagglutinin appears in the cytoplasm. As the infection proceeds both components move towards the cell periphery where, using the cell wall material, they combine to form the new infectious elements. Release of mature virus occurs continuously under the action of virus-neuraminidase. The total quantity of virus which a single cell can produce is significantly less than in the KP-virus-L-cell system.

The proliferation cycle of NDV, the other myxovirus, shows certain similarities with that of the KP-virus but also characteristic differences. These refer primarily to the localization of the RNP-antigen and therein to the Virus-RNA-synthesis. While the RNP-antigen appears in the cell nucleus with KP-virus, with NDV in the cytoplasm it is found on the nucleus (Figure 9). Furthermore the proliferation of KP-virus is stopped by actinomycin, however that of NDV is not. The meaning of this is not yet clear.

b) Metabolic behavior of the infected host cells.

How does this phenomenon affect metabolism of the host cell? The emphasis of our studies to-date has been on RNA- and protein metabolism which was followed using P^{32}- C^{14}- and H^3-labelled compounds. The kinetics were studied using Geiger- and Scintillation-counting, the topography by autoradiographic methods.

The studies showed that infection of L-cells with KP-virus leads to marked changes in metabolism. Soon after infection the autoradiographic picture shows extensive interference of RNA-synthesis in the cell nucleus, which at the time of the virus-RNA is followed by marked RNA-synthesis in an unusual location i.e., the cytoplasm (Figure 10). This phenomena was studied quantitatively by labelling the RNA with C^{14}-uridine and determining the rates of incorporation using a scintillation counter. The results are graphed in Figure 11.

As the incorporation studies with radioactively labelled leucine showed, an inhibition of protein synthesis parallels the reduction of normal RNA synthesis in the nucleus which is, however, interrupted at the time when RNA synthesis takes place in the cytoplasm (Figure 11). Cessation of cell-DNA-synthesis was also demonstrated using Tritium-labelled thymidine.

If one correlates these results with those described above then one can conclude, with greater certainty, that the RNA which appears in the cytoplasm is virus-RNA and that the protein formed

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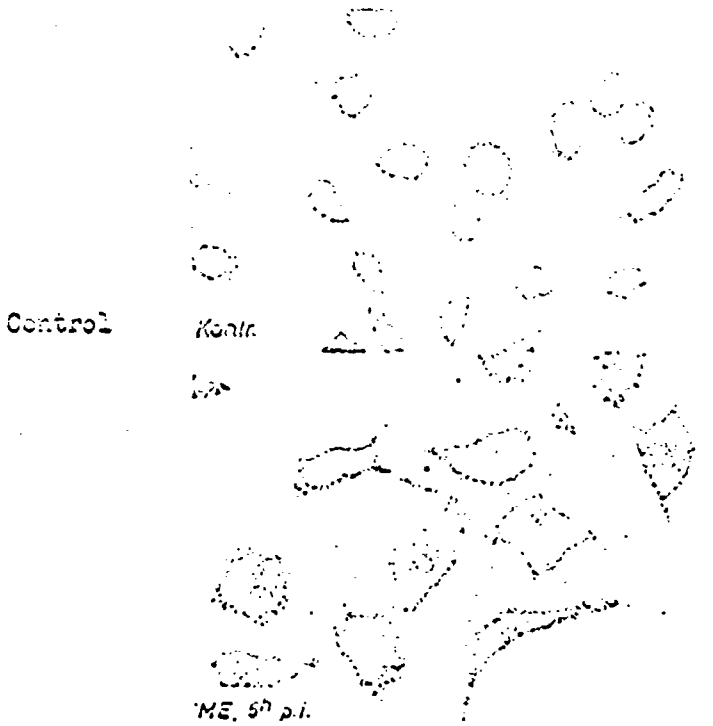

Figure 10. Autoradiograph of RNA-synthesis in ME infected L-cells (H^3-uridine, 5-minute pulse).

simultaneously is virus protein. Pulse experiments confirm that RNA which later appears in the mature virus elements is actually identical to the RNA produced in the cytoplasm 3 to 6 hours p.i. The labelled protein which is formed at this time can be characterized as virus-specific protein with the aid of a precipitating antibody.

In the quest for the reason for the marked changes in RNA- and protein synthesis we came upon substances which appear very early in the proliferation cycle and apparently have an albumin-like nature. Even though they are produced under the direction of the virus - RNA they still cannot be detected by virus-specific tests. They can only be demonstrated by indirect methods using substances which interfere with protein synthesis (Figure 12). These are designated as "early proteins." Previous results with ME-virus and other virus-types of the same group suggest that there are at least three different types of early protein in the corresponding systems i.e.:

1. an inhibitor of normal RNA synthesis;
2. a substance which obviously independently inhibits normal protein synthesis;
3. a virus-RNA-polymerase.

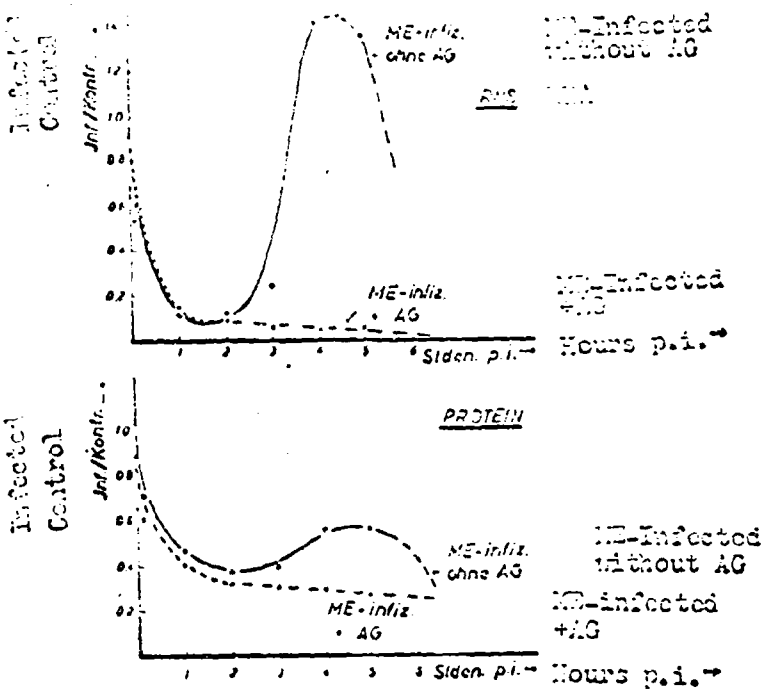

Figure 13. Action of 8-Azaguanin (AG) or RNA- and protein-metabolism of ME-infected L-cells. AG was added at time 0.

It was interesting to find that both inhibitors are already formed at a time at which no perceptable proliferation of virus-RNA has occured. Even though the proliferation is suppressed with 8-Azaguanine, which inhibits the synthesis of virus-RNA without significantly distrubing that of cell-RNA, the "early-proteins' will still be formed (Figure 13). We are therefore inclined to assume that their synthesis is induced immediately by the RNA of the infiltrated virus particle.

Figure 14 summarizes the total picture of the interactions between ME-virus and its host cell.

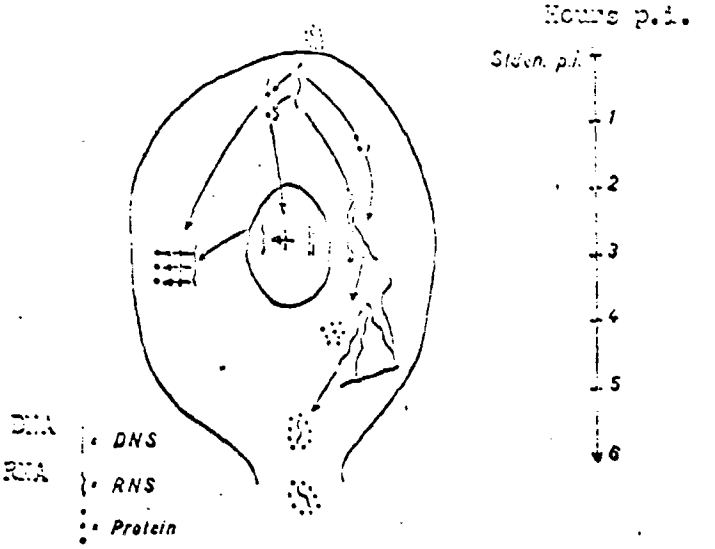

Figure 14. Diagram of the interactions between ME-virus and L-cells. On the right is indicated the time (after infection) at which the corresponding processes occur in the cell.

1: Inhibitor of normal protein synthesis.
2: Inhibitor of normal RNA synthesis.
3: RNA-polymerase.

Unfortunately, an abridged and partially hypothetical picture, similar to the one for ME-virus, can not yet be presented for both myxoviruses. Early proteins also occur. We can only state with certainty that at least one of them is necessary for virus replication to proceed normally. It would be interesting to ascertain whether the transport of RNP-antigen in KP-virus, from the nucleus into the cytoplasm, is inhibited by the amino acid analog parafluorophenylaniline (PFPA) (see Figure 15). It is possible that a special protein is again required for this process.

Striking differences come to light if one compares the influence of ME-virus on the one hand, with that of both myxoviruses on the other on the RNA- and protein-metabolism of the host cell. In contrast to the ME-virus neither the KP- nor the ND-virus intervenes to any great extent in the early stages of proliferation. NDV may be used as an example. In NDV normal RNA synthesis remains so intense that it masks the synthesis of virus-RNA in the autoradiograph (Figure 16). The latter can only be clearly seen in the auto-

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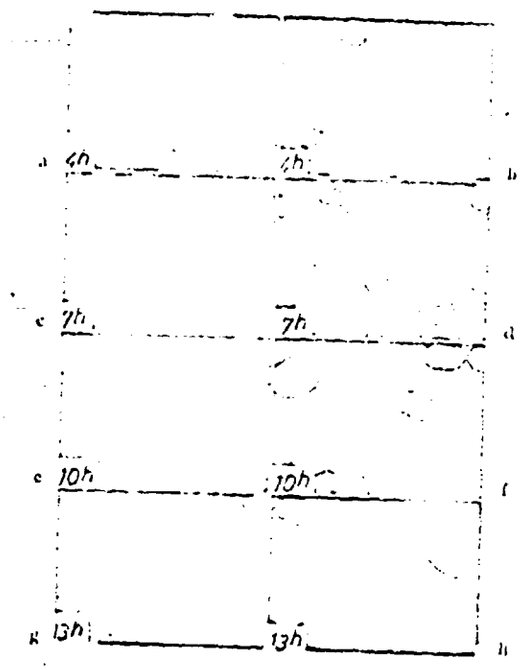

Figure 15. Localization of the RNP-antigen of KP-virus without (left row) and after addition (right row) of PFPA (2 hours p.i.). Stained with fluorescent RNP-antigen-antibody. (a)(b) 4 hours p.i. (c) (d) 7 hours p.i.: (e)(f) 10 hours p.i.; (g) (h) 13 hours p.i.: Chicken embryo lung cells.

radiograph if the production of normal RNA is suppressed by actinomycin.

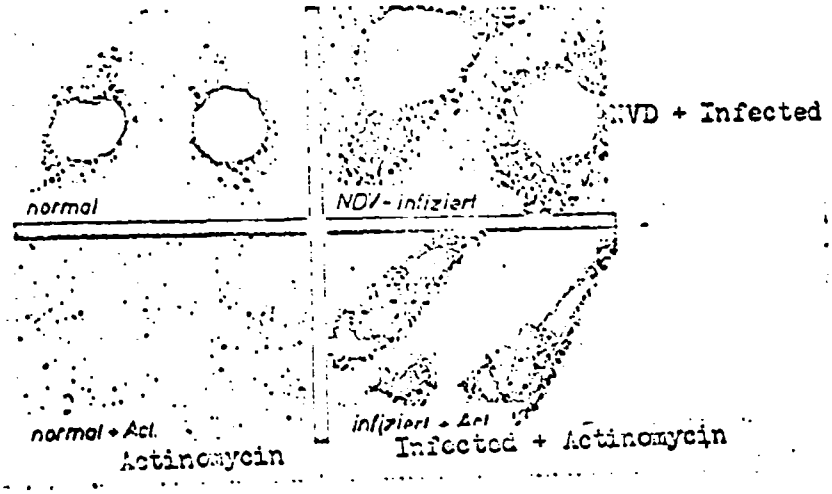

Figure 16. Autoradiograph of RNA synthesis (H^3-uridine incorporation) in normal and NDV-infected KB-cells. Upper row without, lower with actinomycin addition.

Concluding remarks.

As these few examples show, virus types which contain the same type of nucleic acid can themselves be synthesized in the host cell and can affect its metabolism in many different ways. Whereas in KP-virus the synthesis of virus- RNA occurs in the cell nucleus, in ME-virus as well as in NDV, which in several respects resembles KP-virus, synthesis apparently occurs in the cytoplasm. Also, whereas there is an immediate, drastic, reduction of normal RNA-

and protein-metabolism with ME-virus, these are disturbed relatively little in the most important phase of the proliferation cycle in both myxoviruses.

Unfortunately there have been no analogous studies of tumor-producing, RNA-inhibiting viruses to date. It is most likely that these viruses, which somewhat resemble myxoviruses morphologically and to which even the PR 8-influenza virus seems to belong, according to the very interesting observations of von Leuchtenberger et al (10), change the normal metabolism of the cell to a lesser extent than ME-virus or NDV. It appears that they have found a modus vivendi with the cell which the latter however must repay through unlimited proliferation. How this occurs will only be clarified by an extensive investigation of the interaction between both partners.

When we are faced, on the biochemical level, with the various activities of viruses, we must ask how information may be obtained from such minute organisms. The latest results of molecular biology show that even the smallest viruses contain large quantities of genetic material, in contrast to expectations. One-third of the RNA contained in RNA-virus suffices to transfer the information of the virus protein. The remaining two-thirds is available for other information. The range of variation thereby possible would explain why the quest for chemotherapeutic substances having a wide range of action has thus far been unsuccessful. We must also consider the fact that the only substances worthy of investigation are those which leave the metabolism which is normally a part of virus synthesis, substantially unchanged. In animal studies we observed that a compound X may have a marked effect on a certain strain of virus, but no effect whatsoever upon a closely related variety. On the other hand, a compound Y may have a relatively wide range of effects in the experimental animal. Closer analysis of its mechanism of action showed that it seriously interferes with normal RNA-metabolism of the cell.

However I should like to discuss not only the negative aspects of our findings but a positive aspect as well. Using ME-virus as an example it can be shown that there are viruses capable of inducing the production of materials which regulate the genetic functions of the host cell. In my opinion the possibility exists that such "virogene regulators" will prove to be of therapeutic value - e.g. in tumor treatment - and the viruses now known only as pathogenic agents, will be found to serve as transmitters of the desired information.

It is natural to assume that we will succeed in making virus particles that contain a limited amount of information. Experimental

solutions to this problem already exist (11).

These studies were supported by the German Research Foundation.

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DISCUSSION

F. Burke: According to your findings NDV contains essential elements of the host cell in the shell; nevertheless it is a good immunogen. According to Baker, Herpes viruses are not immunogens because they have too much host material in their shell. Could you make some statement concerning the shell material of Herpes virus?

Do you consider the late fluorescence in the nucleus after infection with paramyxoviruses to be specific?

W. Schafer: 1. The finding of RNP-antigen in the nuclear region of NDV-infected KB-cells can be explained by the fact that in later stages of the proliferation processes of degeneration phenomena appear in the nuclear membrane and the antigen passes through the membrane. However it is more likely that at this time RNP-antigen groups are demonstrable in the thin layer of plasma above or below the nucleus. Our studies were made on intact cells.

2. I would imagine that there are gradual differences in the coating of virus particles with normal host material. In influenza - and parainfluenza - viruses, large quantities of virus-specific material penetrate the host-shell; perhaps these are parts of the hemagglutinins which are present. - the situation appears to be different in myeloblastose virus which is of construction similar to that of the myxoviruses. The portion of normal host material is apparently much larger in myloblastose virus. It contains demonstrable amounts of ATPase derived from the cell membranes and can carry along cartilagenous material when it is produced by cartilage cells. We were able to show that when this virus is injected into rabbits, in which it does not proliferate, one finds that antibodies for host material predominate in the serum. These surround the virus-specific material to such an extent that the immunogens can no longer act upon it. - Herpes virus can behave in a manner similar to that of myeloblastosevirus in this instance.