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ENTEROVIRUS ENTRANCE INTO SPECIFIC HOST CELLS, AND SUBSEQUENT ALTERATIONS OF CELL PROTEIN AND NUCLEIC ACID SYNTHESIS

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INTRODUCTION

It is the purpose of this review to recapitulate and discuss recent studies of enterovirus infection by the reviewer and his colleagues, and related work from other laboratories. Much of the work on enterovirus receptors was performed in collaboration with L. C. McLaren, B. H. Hoyer, and the late J. T. Syverton at the University of Minnesota. Subsequent work was carried out at the University of Washington. Studies of cell, tissue, and species specificity, and of receptor determinants of enterovirus adsorption and penetration, were recently reviewed in detail (30), so only general consideration will be given to these areas. Unless otherwise stated, all references to virus or virus infection pertain to poliovirus, although many of the phenomena reported have been shown to be general among enteroviruses by inclusion of Coxsackie viruses at various phases of the investigations.

POLIOVIRUS HOST SPECIES SPECIFICITY

Poliovirus host range has long been known to be restricted to man and certain other higher primates. Poliovirus was once thought to multiply only in cells of the central nervous system and in certain lymphoid and brown fat cells. The early cell culture work of Enders et al. (14) and subsequent work of others showed that cells from nearly all nonnervous tissues of humans are susceptible to poliovirus when cultivated in vitro. Only a few strains of poliovirus are capable of multiplying in chick embryos, mice, and rats in vivo after extensive adaptation (41, 69), but no convincing reports of significant poliovirus multiplication in vitro in cultured nonprimate cells are known to the reviewer. The purpose of our studies was to determine the basis for this strict species, tissue, and cell specificity of poliovirus.

ADSORPTION OF POLIOVIRUS TO PRIMATE AND NONPRIMATE CELLS CULTURED IN VITRO

In view of what was known of phage-bacterium adsorption specificities, it seemed logical to determine whether nonprimate cells were insusceptible to poliovirus merely because they failed to adsorb virus and allow penetration, or whether they were metabolically incapable of supporting virus replication. It was found that all susceptible human and monkey cell cultures adsorbed poliovirus efficiently with first-order kinetics, whereas all nonprimate insusceptible cells (dog, cat, chick, pig, mouse, rabbit, guinea pig, etc.) failed to adsorb detectable amounts of poliovirus in vitro (44). The factors controlling ability of primate

and inability of nonprimate cells to adsorb poliovirus were investigated next. It was demonstrated that all susceptible cells released a virus-binding receptor-like material, whereas no such component could be found in insusceptible nonprimate cells from any species tested (31). It could be shown that poliovirus adsorbed firmly but reversibly to these receptors, and that receptors of disrupted cells had the same cofactor requirements for virus adsorption as did receptors of intact cells (45). Studies of the nature of cell receptors for enteroviruses established that they are protein, or contain protein, for all treatments which degrade or denature proteins destroyed receptor activity (e.g., proteolytic enzymes, heat, detergents, urea, phenol, formaldehyde, etc. (32)). By examining the kinetics of virus-binding to subcellular fractions, it was established that most enterovirus receptors were associated with insoluble lipoproteins of the microsomal membranes. All attempts to solubilize poliovirus receptors (i.e., to free them from insoluble lipoprotein membranes) have failed (32), and it is our current hypothesis that more than one molecular species contributes to the patterns of charge distribution on the cell membranes that are responsible for complementarity to virus capsid surfaces. If this were so, any treatment which perturbed membrane subunit organization would be expected to destroy poliovirus receptor activity.

Infection of Naturally Insusceptible Cells With Virus Ribonucleic Acid (RNA)

If, as the above findings indicated, poliovirus host species specificity is determined simply by presence or absence of receptor, then it should have been possible to bypass this specific interaction between virus capsid surface and cell surface by exposing insusceptible cells to infectious RNA from poliovirus. It was, in fact, demonstrated that poliovirus RNA could infect the normally insusceptible cells of every nonprimate species tested (33). The progeny virus produced by infection of insusceptible cells was complete intact poliovirus, serologically identical to parent virus from which the RNA was extracted. Furthermore, progeny virus was identical to parent virus in host cell specificity, and adsorbed only to primate cells and not to nonprimate cells of the type in which they had reproduced. Mountain and Alexander (46) and DeSommer et al. (11) showed independently that poliovirus RNA could infect chick embryos and rat tumor cells.

Although we were able to infect cells of all warm-blooded species tested, we never succeeded in infecting cells of lower animals or plants (30, 33). Fish cells, frog cells, plant cells, protozoa, and bacterial protoplasts consistently failed to replicate infectious RNA at a variety of temperatures. It seems unlikely that this merely represents failure to introduce RNA into these cells, for exposure of HeLa cells to infectious tobacco mosaic virus RNA and phage RNA, under conditions optimal for poliovirus RNA, did not result in production of infectious virus (unpublished experiments with M. P. Gordon and N. B. Groman). Thus, it appears that in warm-blooded animals poliovirus cell species specificity is determined by presence or absence of specific cell-surface receptors, but there also appears to be a limit to the host range of infectious RNA from small RNA viruses. Studies of the factor(s) preventing replication of viral RNA in certain species should prove interesting.

Poliovirus Cell and Organ Specificities in the Primate Body

It was of obvious interest to extend the above findings concerning poliovirus species specificity to tissue and organ specificities in humans and monkeys. Poliovirus apparently replicates and causes pathology within central nervous system cells, brown fat cells, and certain pharyngeal and intestinal cells (8), but not within most other cells of the body. As had been expected, homogenates and microsomes of susceptible human and monkey tissues (brain, spinal cord, intestine) exhibited receptor activity resembling that of HeLa cells, whereas homogenates of insusceptible human and monkey tissues and organs (kidney, heart, lung, skin, skeletal muscle, etc.) failed to bind detectable amounts of poliovirus (22). There were several unexplained exceptions (such as the apparent presence of receptor activity in certain liver homogenates), but the correlation between virus tissue tropisms and virus binding by tissue homogenates was generally excellent. Kunin (38, 39) independently arrived at the same conclusion.

Sabin (55) reported that the attenuated LSc oral vaccine strain of type 1 poliovirus was not bound by brain tissue under conditions where

virulent strains were bound. We confirmed this observation, and further found that the LSc strain was bound by intestinal homogenates despite its failure to attach to central nervous system tissue. This observation might at least in part explain the ability of this oral vaccine strain to multiply in the gut without causing central nervous system infection, and it implies that receptors in various cells differ from each other (22). Differences in receptor affinities for various strains of poliovirus could also explain the differential susceptibility of brainstem and lumbar cord neurons to different virulent virus strains (55). Kunin (39) reported that the attenuated LSc strain of poliovirus is bound by brain homogenates. The reason for this discrepancy is not clear.

ACQUISITION OF VIRUS SUSCEPTIBILITY BY HUMAN TISSUES CULTURED IN VITRO

Since Enders et al. (14) demonstrated poliovirus infection of cultured human cells, it has become apparent that nearly all human cells are susceptible to poliovirus in vitro even though they are insusceptible in the body. Evans et al. (15) showed in 1954 that despite the multiplication of poliovirus in monkey testicular fibroblasts cultured in vitro there was no in vivo multiplication of virus after intratesticular inoculation. Kaplan (36) showed further that direct inoculation of poliovirus into monkeys did not lead to multiplication, whereas cells from the same monkey kidney after cultivation were completely destroyed by poliovirus. Again, when we examined this phenomenon from the standpoint of virus entrance into cells, it was found that acquisition of virus susceptibility was paralleled by acquisition of receptor activity (22). Human amnion cells or human kidney cells freshly removed from tissue did not support virus growth and did not exhibit receptor activity, but the same cells, after cultivation in vitro for several days as monolayers on glass, contained large quantities of receptor and were completely destroyed by poliovirus. Since in vitro cultivation of human amnion cells in their normal tissue relationships on the amniotic collagen membrane failed to trigger receptor production and virus susceptibility, it must be concluded that it is not the cell culture medium, but rather disturbance of normal contact relationships, that leads to receptor synthesis (or receptor unmasking) and virus susceptibility.

Quersin-Thiry (51) reported that both grafted (transplanted) monkey kidney cells and cultured monkey kidney cells acquired poliovirus binding activity, and Hermodsson and Wesslin (21) demonstrated extensive poliovirus multiplication in heterotransplanted human tissues. Barski et al. (7) found that respiratory epithelial cell cultures as explants in vitro retained their resistance to poliovirus and adenovirus as long as they retained cilia and other differentiated characteristics. Evans and Hoshiwara (16) showed that poliovirus multiplied to some extent in healing lesions of monkeys with skin wounds. It would appear, then, that in vitro culture conditions which allow cells to maintain their normal tissue relationships with other cells prevent appearance of receptor activity and poliovirus susceptibility, whereas procedures which greatly disturb the normal cell contact relationships with other cells or with intercellular ground substances can lead to receptor acquisition and virus susceptibility, both in vitro and in vivo. But, as Evans et al. (15) showed, disruption of tissues in vivo does not necessarily result in appearance of virus susceptibility.

ROLE OF CELL RECEPTORS AFTER VIRUS ADSORPTION

Since enterovirus receptors appear to play such a large role in determining cell susceptibility, it seemed unlikely that their sole function in facilitating infection was virus adsorption to the cell surface. It had early been found that about 1% of a poliovirus inoculum was nonspecifically bound to insusceptible cell surfaces so that it could not be washed off (44). It might have been expected then, that exposure of insusceptible cells to massive inocula of poliovirus might enable about 0.1% or more of the inoculum to replicate, if mere binding of virus to the cell surface were the only requirement for initiating infection. A careful test of this hypothesis showed, however, that only about 1 plaque-forming unit (PFU) of 10 million actually succeeded in infecting insusceptible nonprimate cells exposed to massive inocula of poliovirus (33). Thus, it appeared that receptors perform more of a function than simply adsorbing poliovirus. In fact, Vogt and Dulbecco (66) showed that a line of HeLa cells selected for poliovirus resistance adsorbed virus efficiently, and Darnell and Sawyer (10) later showed that such cells were as susceptible as sensitive HeLa cells to infectious RNA from poliovirus.

Detailed study of the fate of poliovirus adsorbed to HeLa cells indicated that a large proportion of the adsorbed virus particles lost infectivity irreversibly (were eclipsed) at the cell surface at 37 C but not at 0 C (24). Loss of antiserum sensitivity paralleled this eclipse. HeLa cell lines selected for poliovirus resistance failed to eclipse adsorbed virus, and most of the adsorbed virus remained at the cell surface and retained antiserum sensitivity during prolonged incubation at 37 C. Finally, it was found that virus receptors on the plasma membrane of sensitive cells differed from microsome membrane receptors in being able to eclipse poliovirus infectivity irreversibly. Plasma membranes isolated from disrupted HeLa cells by the method of Neville (47) adsorbed and eclipsed poliovirus, and microsomal membranes adsorbed but did not eclipse virus (24).

Incubation of virus-plasma membrane receptor complexes at 37 C apparently resulted in a reorientation of capsid subunits. This capsid change did not result in release of viral RNA, nor did it render the RNA accessible to added ribonuclease, but it did cause the virus to lose infectivity and antiserum sensitivity (30). Furthermore, the capsid became sensitive to proteolytic enzymes so that RNA could be released. It was suggested, therefore, that plasma membranes play an essential role in triggering the first step in release of the viral RNA genome from its resistant protein coat (30). After this "eclipse" has occurred, the altered virus might be pinocytosed and its RNA released by proteolysis, by pH conditions within a pinocytosis vacuole, or by some other means. Mandel (43) presented evidence that poliovirus may enter HeLa cells at 25 C before it is eclipsed, but in this case virus might be eclipsed as a result of the activity of receptor present on the membrane bounding a pinocytic vacuole. In any case, Philipson and Bengtsson (50) recently confirmed our findings of a temperature-dependent eclipse occurring with exponential kinetics on HeLa cell membrane receptors. Furthermore, they extended these studies to show similar eclipse of hemagglutinating enteroviruses on cell membrane receptors of red blood cells. In the case of red blood cells, as with isolated HeLa cell plasma membranes, there can be no possibility of engulfment by the cell, so alteration of the virus particle must have occurred at cell surface receptor sites.

It can be concluded that receptors determine cell, tissue, organ, and species specificities of enteroviruses not only because they adsorb virus but because they cause an alteration of the virus capsid which is a necessary prelude to release of the virus RNA. This alteration (eclipse) shows some of the characteristics of an enzyme-mediated process.

ALTERATIONS IN RNA SYNTHESIS DURING POLIOVIRUS INFECTION OF HELA CELLS

The above results demonstrated that enterovirus host species, cell, and tissue specificities depend upon receptor facilitation of viral genome entrance into cells. The remainder of this review will deal with metabolic events which transpire once the virus RNA has been introduced successfully into the cell. Early studies of poliovirus effects on host cell RNA failed to detect major changes during infection. Salzman et al. (56) reported that RNA, deoxyribonucleic acid (DNA), and protein synthesis were inhibited within 6 hr after poliovirus infection of HeLa cells. Ackermann et al. (1) reported that the RNA of infected HeLa cells had the same base ratios as control uninfected cells. However, when we examined the base ratios of newly synthesized RNA in poliovirus-infected HeLa cells by labeling RNA with P^{32} after infection, it was found that the base ratios shifted strikingly (23). The base composition had shifted from the high guanine-cytosine content characteristic of HeLa cell ribosomal RNA to high adenine values similar to the base ratios of RNA from purified poliovirus, as characterized by Schaffer et al. (58).

The fact that these base-ratio shifts in newly synthesized RNA took place without significant changes in the rate of RNA synthesis suggested that poliovirus was inhibiting host cell RNA synthesis as well as directing synthesis of a viral type of RNA. It was subsequently found that host cell RNA synthesis was, in fact, drastically inhibited within several hours after infection by poliovirus (27). This was demonstrated by infecting HeLa cells for several hours with poliovirus, then specifically inhibiting virus-directed RNA synthesis by adding 10^{-3} M guanidine. Guanidine at low concentrations prevents poliovirus multiplication and cytopathic changes, and HeLa cells are able to grow in medium levels that inhibit poliovirus (9, 42, 54). It was shown

that guanidine inhibits poliovirus RNA synthesis at levels which do not affect normal HeLa cell RNA synthesis (27). When guanidine was added immediately after infection with moderate multiplicities of poliovirus, no virus-directed RNA synthesis took place (no infectious RNA was replicated, and no detectable shift in base ratios occurred), and host cell RNA synthesis continued at nearly normal rates. But when poliovirus infection was allowed to proceed for several hours before addition of guanidine, it was found that nearly all RNA synthesis ceased. Apparently, virus infection inhibited host RNA synthesis, and guanidine prevented poliovirus-directed RNA synthesis from replacing host RNA synthesis as would normally occur (27). Franklin and Baltimore (18) reported that Mengo virus (another small RNA virus) inhibits mouse cell RNA synthesis, and Scholtissek et al. (61) observed similar inhibition with a related virus, ME virus.

When actinomycin D was added to HeLa cells to inhibit RNA synthesis directed by host genes (53), poliovirus infection caused a marked stimulation of RNA synthesis, and the RNA formed had base ratios nearly identical to those of purified poliovirus RNA (25). Fenwick (17) and Zimmerman et al. (71) showed that most of the RNA produced in infected cells is about 35 *S* in size. This is about the same size (37 *S*) that we reported for the RNA from purified poliovirus (34). This finding indicated that only one major molecular species of RNA was produced in infected cells, and Zimmerman et al. (71) showed that this large RNA molecule had base ratios identical to that of RNA from purified poliovirus. We confirmed this finding (35) and spent considerable time searching in vain for evidence of a molecule complementary to the single strand of poliovirus RNA. It might be predicted that at some stage of poliovirus replication there must be synthesized a complementary molecule which acts as a template for synthesis of viral RNA. Such a molecule would have base ratios high in uracil, but no such RNA has yet been found at any stage of infection, nor has it been possible thus far to find a double-stranded "replicative form" of poliovirus analogous to those found during replication of single-stranded DNA (62). It is likely that a complementary RNA or double-stranded replicative form is present, but in amounts too small to be detected by the means employed thus far.

Effect of Poliovirus on Host Cell DNA Synthesis

It was shown by Simon (63) and by Reich and Franklin (52) that neither DNA synthesis nor integrity of pre-existing DNA is necessary for replication of the small RNA viruses. We have shown, furthermore, that the DNA which is synthesized during poliovirus infection has base ratios identical to normal cell DNA (25). However, poliovirus infection does cause progressive inhibition of DNA synthesis in poliovirus-infected cells (25, 56, 71) and in cells infected by other small RNA viruses (18, 61). To the best of the reviewer's knowledge, no definitive investigation of the mechanism of this inhibition has yet been carried out, but Simon (64) found no evidence that DNA is degraded during poliovirus replication. There is no evidence that the RNA of small animal viruses is complementary to regions of host cell DNA; in the case of RNA phage, Doi and Speigleman (12) presented good evidence that it is not.

Host Cell Protein Synthesis During Poliovirus Infection

Salzman et al. (56) reported that net synthesis of protein by HeLa cells was inhibited within 6 hr after poliovirus infection. Zimmerman et al. (71) subsequently showed that the rate of protein synthesis drops off rapidly after poliovirus infection, and we observed the same effect (35). Franklin and Baltimore (18) and Scholtissek et al. (61) observed similar depression of protein synthesis in mouse cells infected with Mengo virus and ME virus. Because poliovirus infection also inhibits synthesis of RNA, it seemed possible that inhibition of protein synthesis might merely be a result of depletion of messenger RNA without replenishment by synthesis. This was tested indirectly by determining the effect of actinomycin D on HeLa cell protein synthesis.

Despite the fact that actinomycin D quickly inhibited nearly all HeLa cell RNA synthesis, it was found that protein synthesis was depressed very slowly. Protein synthesis decreased exponentially with time after actinomycin treatment, and exhibited a half-life of approximately 5 to 6 hr (35). It appears that messenger RNA in these mammalian cells has a much longer average

half-life than does messenger RNA in bacteria. Poliovirus infection caused a considerably more rapid decline in HeLa cell protein synthesis than did actinomycin, even though there was a lag of several hours before RNA synthesis was affected by virus infection. Thus, it seems that poliovirus infection inhibits host cell protein synthesis more directly than by inhibition of messenger RNA synthesis.

Penman et al. (49) reported that poliovirus infection of HeLa cells caused a drastic reduction in the number of polyribosomal aggregates present in HeLa cells, with appearance of many free ribosomes. They suggested that a ribonuclease might cause this by degrading the messenger RNA linking the ribosomes. However, they also found that very large polyribosomes were formed in infected cells, and these appeared to be aggregates of ribosomes on the large 37 *S* viral RNA molecule. It is not clear why these should not have been attacked by ribonuclease if host cell polyribosomes were. Scharff et al. (60) obtained similar results, and found that immunologically identifiable viral protein was formed on these polyribosomes. Warner et al. (67) had previously shown that RNA from purified poliovirus stimulated *Escherichia coli* ribosomes to produce protein immunologically related to poliovirus protein.

RNA Synthesis by Cell-Free Extracts of Normal and Infected HeLa Cells

In an effort to study the mechanism of inhibition of host cell RNA synthesis during poliovirus infection, cell-free polymerase systems were investigated. Mammalian cell RNA polymerase has not yet been purified, but Weiss (70) studied a DNA-protein aggregate from liver cells which he named "aggregate enzyme" and which was capable of catalyzing RNA synthesis in a cell-free system with DNA as the template. Goldberg (19) defined the optimal conditions for assay of this "aggregate enzyme" RNA polymerase in vitro. We found that "aggregate enzyme" from infected HeLa cells was much less active in synthesizing RNA than was a similar preparation from normal cells (26). Baltimore and Franklin (3) observed the same effect in mouse cells infected with Mengo virus.

Attempts to solubilize and purify mammalian cell RNA polymerase were not successful, so it was not possible to determine whether poliovirus infection inhibits RNA polymerase by destroying the enzyme. However, Simon (64) found that poliovirus infection does not cause detectable DNA degradation, and we showed that DNA from infected cells and DNA from normal cells are equal in their abilities to serve as templates for DNA synthesis with purified *E. coli* RNA polymerase (26). There was no evidence that deoxyribonucleoprotein from infected cells or normal cells differed in template activity (26). Extracts of infected cells failed to depress RNA polymerase activity of aggregate enzyme extracted from normal cells (26).

Eason et al. (13) reported that crude extracts of mouse cells infected with encephalomyocarditis virus contained as much RNA polymerase activity as similar extracts from normal cells. This seems to contradict the above findings with "aggregate enzyme." However, we were able to repeat their findings with the HeLa cell-poliovirus system. When HeLa cell extracts were prepared by ultrasonic disruption, RNA polymerase activity was found in small but equal amounts in both normal and infected cells (35). Such extracts, prepared in different ways, were stimulated weakly or not at all by added DNA template. Furthermore, only a small fraction of the total RNA polymerase activity originally present as "aggregate enzyme" could be found in these extracts prepared by sonic treatment. Despite the very low level of RNA polymerase activity found in such extracts, and despite the fact that the polymerase and its product are not well-characterized, these results may suggest that the RNA polymerase level remains constant throughout infection, but that the polymerase is in some manner prevented from interacting normally with its DNA template. We showed that continued protein synthesis is not required to maintain HeLa cell "aggregate enzyme" RNA polymerase levels constant for 6 hr. Inhibition of protein synthesis in HeLa cells with puromycin did not cause much depression of RNA polymerase within 6 hr, indicating that the enzyme is quite stable and need not be constantly replenished (28).

A very important enzyme was recently identified in mouse cells and human cells infected with Mengo virus and poliovirus, respectively, by Baltimore and Franklin (4, 5). This is an RNA polymerase which appears in the cytoplasm of cells infected with these small RNA viruses.

This enzyme is part of a particulate complex which seems to carry its own template nucleic acid. It appears that this enzyme is the polymerase responsible for replicating poliovirus RNA, because it is not found in normal cells, and its synthesis is inhibited by guanidine and other specific inhibitors of poliovirus synthesis (6).

We were able to confirm the appearance of this enzyme in poliovirus-infected cells, but found that infectious RNA is not replicated in vitro in this system (35). However, this was to be expected, since the RNA synthesized in vitro by this enzyme was considerably smaller than the 37 *S* infectious poliovirus RNA molecule. Presumably there are too many nucleases present in this system to achieve replication of infectivity, even if net RNA synthesis could be achieved. Further characterization of this enzyme and its template should prove invaluable for understanding of the biology of poliovirus replication.

Amino Acid Incorporation by Cell-Free Extracts of Normal and Virus-Infected Cells

Because of the rapid and drastic inhibition of host cell protein synthesis observed in poliovirus-infected cells, it would be expected that in vitro amino acid-incorporating systems from infected cells would also be greatly reduced in activity. However, Attardi and Smith (2) found that microsomes and ribosomes from poliovirus-infected cells incorporated amino acids at nearly the same rate as did preparations from normal cells. Kerr et al. (37) observed a slightly reduced amino acid incorporation by ribosomes from mouse cells infected by EMC virus as compared with normal cell ribosomes. They presented evidence that the deficiency of infected cell extracts lay in the ribosomes rather than in the cell sap which was used as the source of incorporating enzymes. We confirmed these findings with a large number of extracts from normal and poliovirus-infected HeLa cells, and usually found only slightly lower amino acid incorporation by infected cell extracts, even though there was very great depression of protein synthesis in intact infected cells (35). As had been found by Kerr et al. (37), where poliovirus infection did cause a measurable inhibition of protein synthesis in cell-free extracts, the defect appeared to be due to an alteration of the ribosomes rather than the cell sap.

Any hypothesis invoked to explain poliovirus suppression of host cell protein synthesis should take into account the apparent failure of the control mechanism to operate in vitro as it does in vivo.

Inhibition of Host Cell RNA and Protein Synthesis by Very High Multiplicities of Poliovirus

It is obvious from the work reviewed above that, in spite of extensive studies in intact cells and in cell-free systems, little is known of the mechanisms by which poliovirus or other small RNA viruses depress host macromolecular synthesis. Nomura et al. (48) provided evidence that phage T_4 synthesizes a specific protein which inhibits host RNA synthesis. Franklin and Baltimore (18) reported that puromycin partially prevents the inhibition of host cell RNA synthesis normally effected by Mengo virus infection. Therefore, they suggested that a protein product is the agent causing depression of host RNA synthesis. However, this interpretation is complicated by the fact that the small RNA viruses cannot even carry out the initial stages of replication without protein synthesis (30, 59, 68). Baltimore and Franklin (4) reported that puromycin treatment of infected cells prevents appearance of the viral RNA polymerase, so that no viral RNA synthesis occurs after puromycin treatment either. In an effort to avoid the complex situation occurring during virus replication, and to determine whether a component of the mature poliovirus particle is responsible for inhibition of macromolecular synthesis, we infected HeLa cells with high multiplicities of purified poliovirus under conditions preventing poliovirus replication. It was found (29) that multiplicities of 10,000 PFU per HeLa cell (over a million physical particles of poliovirus per cell) caused rapid inhibition of host cell RNA and protein synthesis even in the continuous presence of 0.0025 M guanidine (which completely prevents poliovirus replication). Cells treated in this way showed cytopathic effects and detached from the glass and died within 16 to 24 hr even though no new virus or infectious RNA was produced. On the other hand, multiplicities of 10 PFU per cell did not depress HeLa cell RNA or protein synthesis or cause rapid death when guanidine was present throughout infection.

Even when puromycin or *p*-fluorophenylalanine (FPA) was added along with guanidine,

virus multiplicities of 10,000 PFU per cell depressed host cell protein synthesis to the same degree and within the same time as in cells not treated with puromycin or FPA. (Puromycin and FPA could not be kept on the cells for the entire infection period, of course, but were removed and their effects reversed before examining the ability of treated cells to incorporate C^{14} amino acids.) Thus, it appeared that a component(s) of the mature virus particle was able to inhibit host cell RNA and protein synthesis and cause cytopathology and cell death under conditions preventing virus RNA synthesis (29).

Since mature poliovirus particles apparently contain only capsid protein enclosing a single-stranded RNA molecule, we attempted to determine which component was responsible for inhibition of host synthesis under conditions preventing replication. Poliovirus grown in the presence of proflavine (40) seemed an ideal tool. Schaffer (57) showed that such virus contains over 100 dye molecules bound within each virus particle. As long as this proflavine virus is kept in the dark it is fully infectious, but light causes photo-oxidation of RNA components and renders the virus (and its RNA) noninfectious, apparently without affecting the viral protein (57). We found that very high multiplicities of proflavine virus caused drastic inhibition of host cell synthesis in the presence of guanidine when the virus was kept in the dark. Exposure to light before, or immediately after, adsorption destroyed the ability of proflavine virus to inhibit host cell synthesis or to cause rapid cell death in the presence of guanidine (presumably by causing oxidation of RNA nucleotides; 57).

It appears, therefore, that poliovirus RNA is capable of suppressing host cell RNA and protein synthesis and causing cell death under conditions in which it cannot replicate RNA. Two possible mechanisms were proposed by which the mere physical presence of poliovirus RNA might depress host synthesis. (i) It could tie up RNA polymerase, thereby suppressing RNA synthesis. (ii) It could in some way directly interfere with host protein synthesis (and indirectly ribosomal RNA synthesis; 28). The possibility cannot be excluded that viral RNA produced protein which inhibited host cell synthesis under the above conditions, but it appears to be the viral RNA which causes depression of host cell synthesis, whether directly, or by coding a protein. It is interesting that Reovirus, which has a double-stranded RNA genome, does not inhibit host cell RNA and protein synthesis during replication, but does inhibit DNA synthesis (20). It would be predicted that double-stranded RNA would not compete as well for RNA polymerase as does single-stranded RNA (65).

Much more work is necessary before the complex macromolecule alterations transpiring in cells infected by poliovirus can be resolved into a coherent picture.

SUMMARY

Evidence is reviewed which suggests that host cell receptors determine the host cell, tissue, organ, and species specificity of enteroviruses. These receptors are necessary for efficient infection, not only because they adsorb virus, but also because they cause a temperature-dependent alteration of the virus capsid which seems to be a necessary first step in release of the virus RNA genome.

Recent studies of the alterations in host cell nucleic acid and protein synthesis caused by small RNA animal viruses are reviewed. Poliovirus infection causes rapid inhibition of host cell RNA and protein synthesis, and replaces it with virus-directed synthesis. Evidence is reviewed which suggests that poliovirus single-stranded RNA is the component responsible for inhibition of host cell RNA and protein synthesis, and for the cytopathic effects and cell death which result from poliovirus infection, whether it acts directly, or indirectly by coding production of a protein inhibitor.

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