Early Alteration of Poliovirus in Infected Cells and Its Specific Inhibition

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SUMMARY

HeLa cells infected with radioactive poliovirus type 2 were disrupted with ultrasonic treatment, followed by addition of a non-ionic detergent. Two types of virus particles were found to sediment at 80 to 90% the rate of native virus. The first of these appeared to be a complex of native virus particles and membrane components, since treatment with 0.2% SDS released infectious native particles. The second was non-infectious and its sedimentation rate was not greatly altered by SDS. One hour after infection this non-infectious particle was the major product of cell-mediated eclipse.

We have confirmed that 10 to 30 μg/ml S-7, a substituted thiopyrimidine, blocks infection of cells by poliovirus in a specific manner. Analysis of cells infected with radioactive poliovirus type 2 in the presence of S-7 showed that the virus particles remained as the complex which can be disrupted with SDS. In addition to blocking cell-mediated eclipse, S-7 stabilizes poliovirus against heat inactivation in vitro at the same concentrations which block infection. This action resembles the effect of 10^{-2} M-glutathione, which is also known to block cell-mediated eclipse of poliovirus.

INTRODUCTION

The early steps of poliovirus-cell interaction have been studied for more than 15 years and although much is now known about this process, there remains some uncertainty about the sequence and nature of the various steps. It seems clear that there is a process by which loosely bound virus becomes resistant to elution by treatment with salts, urea or certain detergents (Fenwick & Cooper, 1962; Holland, 1962; Mandel, 1962; Zajac & Crowell, 1969). The ability to neutralize cell-adsorbed virus with antibodies is lost, at least under some conditions, before the irreversible alteration of the virus particles (Fenwick & Cooper, 1962; Mandel, 1967) and before uncoating of the virus genome (Mandel, 1967). Fenwick & Wall (1973) have provided evidence that one product of cell-mediated eclipse is a particle containing RNA which sediments at 80 to 90% of the rate of native virus and which in this way resembles the fraction of non-infective particles which are eluted during virus-cell interaction (Joklik & Darnell, 1961; Fenwick & Cooper, 1962). Habermehl, Diefenthal & Bucholz (1974) have also recently detected such a particle and have found that more slowly sedimenting empty capsids appear to be formed later in infection.

Fenwick & Cooper (1962) found that 5 to 10 mm-glutathione blocked both the eclipse of infectious cell-associated virus and the ‘sloughing’ of non-infectious altered particles from the cell following infection. This suggests, in retrospect, that both of these processes are related as appears also in the case of human rhinovirus type 2 (Lonberg-Holm & Noble-
Furthermore, ‘penetration’ of poliovirus to a state of antibody resistance was not inhibited by glutathione, suggesting that eclipse can occur independently and subsequently to ‘penetration’. There may, however, not be any physical basis for equating the development of antibody resistance of infectious centres and the passage of adsorbed virus through the cell membrane (as discussed by Lonberg-Holm & Philipson, 1974).

The methylthiopyrimidine S-7 is a specific inhibitor of poliovirus multiplication in cultured cells (Yamazi et al. 1966; Yamazi, Takahashi & Todome, 1970). We have now found that 10 to 30 μg/ml of S-7 protects poliovirus against heat inactivation in vitro and also blocks cell-mediated eclipse. Glutathione shares these properties. This work has confirmed Fenwick & Wall’s (1973) finding of non-infectious particles formed from native virus particles in the infected cell, but also shows that a similarly slowly sedimenting particle containing infectious virus can be produced in the presence of inhibitors. This latter particle may contain tightly bound cellular receptors.

**METHODS**

**Virus and cells.** Poliovirus type 2 (712-Ch-2ab strain) was obtained originally from Dr R. Bablanian of The State University of New York, and poliovirus type 1 (Mahoney strain) was obtained from Dr D. R. Tershak of The Pennsylvania State University. The human rhinoviruses have already been described (Lonberg-Holm & Korant, 1972). EMC virus was obtained from Dr E. Knight, Jun., of this laboratory. All viruses were plaque purified and the identity of each was confirmed by neutralization with standard antiserum.

Purified radioactive poliovirus type 2 was prepared in roller bottles by the procedure already described in detail for human rhinovirus type 2 (Korant et al. 1972) except that both infected cells and medium were saved for virus purification. Poliovirus was also purified by the rhinovirus procedure which entailed sedimentation to a pellet, sucrose gradient rate-zonal sedimentation, and finally isopycnic banding in linear CsCl gradients. The pre-formed CsCl gradients had a density ranging from 1.40 to 1.28 g/ml. The $E_{260}$ of the purified virus in CsCl solution was used to calculate the ratio of virus particles to ct/min (Korant et al. 1972). Heat-inactivated foetal calf serum was then added to the virus in CsCl solution (5 %) and the mixture was dialysed at 0 °C against MEM-spinner (Eagle’s minimum essential medium modified for spinner culture, Grand Island Biological Co., Grand Island, N.Y.).

HeLa cells used to study attachment and eclipse were grown in spinner culture and were washed with MEM-spinner supplemented with 5 % heat-inactivated foetal calf serum as already described (Lonberg-Holm & Korant, 1972).

**Heat inactivation of virus.** A pool of poliovirus type 2 containing $10^9$ p.f.u./ml was diluted 100-fold into MEM-spinner containing 5 % heat-inactivated foetal calf serum either with or without the compound to be tested. Samples of 0.6 ml were heated in $12 \times 75$ mm glass tubes by immersion in a circulating water bath. The samples were then cooled in an ice water bath and titrated for infective virus by plaque assay (Korant et al. 1972). The results are expressed as the logarithm of the ratio of the recovered infectivity to the infectivity of an unheated sample.
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Acrylamide gel electrophoresis. Samples containing radioactive polypeptides were either obtained from sucrose gradients or were purified virus preparations mixed with a comparable volume of the sucrose solutions used for preparation of the gradients. To these samples were added 50 μg/ml of bovine serum albumin and 10% final concentration of trichloroacetic acid. The protein was permitted to precipitate for 16 h at 4 °C, sedimanted and washed twice with 10% trichloroacetic acid (using sedimentation for 10 min at 3000 g) and then washed twice with cold acetone and dried with a stream of nitrogen. The pellet was dissolved in 0.05 M-phosphate buffer (pH 7.3), mixed with 1/10 by volume of a stock solution containing 10% SDS, 5 M-urea and 1% mercaptoethanol and heated for 1 min at 100 °C in a boiling water bath. The dissociated polypeptides were dialysed against 0.01 M-phosphate buffer containing 0.1% SDS and 0.1% mercaptoethanol, separated on 26 cm polyacrylamide gels containing 10% acrylamide and 0.3% (v/v) ethylene diacrylamide, and then fractionated according to the method of Butterworth (1973).

Attachment of radioactive virus to cells and recovery of the products of infection. Suspensions of highly purified radioactive virus particles were mixed with washed HeLa cells in MEM-spinner supplemented with 5% foetal calf serum so that the final concentration of cells was 1 × 10⁷/ml and there were about 10⁴ particles/cell. The mixture was incubated in a shaking water bath at 37 °C for 15 min to permit virus to adsorb to the cells, and then cooled by addition of excess cold medium. The cells were sedimented by centrifuging at low speed and resuspended again to 1 × 10⁷/ml and incubated for an additional 45 min to permit the eclipse of cell-associated virus. After this, the cells were cooled, sedimented, and resuspended in 0.02 M-tris-HCl buffer, pH 8.1, at 0 °C. Infectious virus in the washed cells was titrated after a sample was mixed with an equal portion of 0.8% SDS, held 10 min at 22 to 25 °C and then diluted with 18 vol. of cold medium containing serum. Infectious and non-infectious virus combined were determined by counting a portion of the resuspended cells in a scintillation counter, as already described (Lonberg-Holm & Korant, 1972).

Samples of infected cells to be subjected to sucrose gradient sedimentation were suspended in tris-hydrochloride buffer at 2 × 10⁷ cells/ml and were treated with an ultrasonic generator and with 200 μg/ml DNase and 0.5% Nonidet P40 (NP40) as already described (Lonberg-Holm & Korant, 1972). As noted, 50 μl of 4% SDS was added to 1.0 ml of the sample just before it was layered onto a sucrose gradient.

Sucrose gradients. Linear sucrose gradients contained 10 to 25% sucrose in 1 M-NaCl and 0.02 M-tris-HCl, pH 8.1. These were either 4-5 ml in vol. for the Spinco SW 50.1 rotor (Beckman Instruments, Inc., Palo Alto, California) or 16 ml in vol. for the Spinco SW 27.1. Sedimentation was at 4 °C for 50 min at 42 500 rev/min (SW 50.1) or for 16 h at 15000 rev/min (SW 27.1) and the gradients were collected from the bottom into 2- or 3-drop fractions. The method used for counting the radioactivity and for computing and representing the data has already been described (Lonberg-Holm & Noble-Harvey, 1973).

Compounds. A sample of S-7 (ethyl 2-methylthio-4-methyl-5-pyrimidine carbamoyl) was kindly given to us by the Toyama Chemical Company, Ltd, Tokyo. An additional quantity was synthesized by the condensation of ethyl ethoxymethylene acetooacetate with 2-methyl-2-thio pseudourea (Chi & Wu, 1957). The reaction product was chromatographed on a 'Florisil' column using chloroform as eluant. Fractions which crystallized were combined and dried on an unglazed tile and after recrystallization from hexane melted at 52.5 to 55 °C. The infrared spectrum was identical with that of the sample from Toyama. Reduced glutathione (crystalline, A-grade) was purchased from Calbiochem (La Jolla, California) and solutions were prepared directly before use.
RESULTS

Antiviral properties of S-7

S-7 added to the agar overlay prevented plaque formation by poliovirus type 1 at 30 \(\mu g/ml\) and by type 2 at 10 \(\mu g/ml\). S-7 had no effect on plaque formation by EMC or rhinovirus types 1A and 2 below the cytotoxic level of 100 \(\mu g/ml\). Yamazi et al. (1970) have already demonstrated that S-7 does not inhibit the cytopathogenicity of a few types each of echovirus, Coxsackie A virus and Coxsackie B virus.

Thermal inactivation of virus and its inhibition by S-7

Thermal inactivation of poliovirus type 2 by heat was studied using the medium containing serum also employed to study virus-cell interaction. Fig. 1 shows that control virus lost more than 90% of its infectivity after 3 min at 48 °C while there was no significant loss of infectivity during 25 min heating in the presence of 10 \(\mu g/ml\) S-7. Glutathione at 0.01 M concentration also protected poliovirus against heat inactivation at 48 °C.

Purified radioactive poliovirus type 2 particles, dialysed against medium and containing 5% heat-inactivated foetal calf serum, were heated to 48 °C and were then sedimented in a sucrose gradient together with marker virus containing a second radioisotope. Fig. 2 illustrates the effect of heating \(^{14}\text{C}\)-amino acid-labelled particles for 1 min at 48 °C. The major RNA-containing product sedimented at about 130S relative to 156S (assumed) for the marker; there were also two minor slowly sedimenting components. Other experiments employing \(^{32}\text{P}\)-labelled particles heated under these conditions showed that most of the
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virus particle RNA co-sediments with the protein at 130S. Equivalent results to those of Fig. 2 were obtained when native virus particles were heated for 2 min at 47 °C.

In other experiments which are not shown here, it was found that 30 μg/ml S-7 prevented alteration of 156S native particles after heating for 1 min at 48 °C and that even after 15 min of heating, more than two thirds of the particles remained unaltered. (Heating of native particles for 15 min at 48 °C in the absence of S-7 produced a major product which sedi-
mented at about 120S, significantly slower than the product obtained after 1 min.)

Fig. 3 shows a comparison of the polypeptides of intact virus particles with those of the 130S component produced after [14C]-amino acid-labelled virus was heated for 1 min at 48 °C. The smallest polypeptide, VP4, was lost.

Inhibition of cell-mediated eclipse

Cell-adsorbed poliovirus is irreversibly altered after incubation at physiological temperature; concomitantly, some of the non-infective particles produced during this process are eluted back into the medium. In order to study this process, we have used radioactive infectious virus and have employed 0.4 % SDS to free the non-eclipsed virus from cellular material so that its infectivity can be assayed (Mandel, 1962; Lonberg-Holm & Korant, 1972). The amount of radioactive virus which becomes and remains cell-associated can then be directly compared with the cell-associated infectious virus.

Fig. 4(a) illustrates the normal course of adsorption, elution and eclipse of [14C]-labelled poliovirus under the conditions of these experiments. Virus was adsorbed during 15 min; there was 37 % uptake of [14C]-label, and more than half of the cell-associated virus retained
infectivity. The cells were washed and incubated for an additional period of 45 min during which about 27% of the cell-associated virus eluted and most of the remaining cell-associated virus lost its infectivity.

The antiviral compound S-7 did not inhibit adsorption of poliovirus to HeLa cells. As shown in Fig. 4(b), 10 µg/ml S-7 reduced the elution and prevented eclipse of cell-associated
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Fig. 4. Effect of S-7 on cell-mediated eclipse of poliovirus. Cells were incubated at 37 °C with [14C]-labelled poliovirus type 2 in the absence (a) or presence (b) of 10 μg/ml S-7. At 15 min, excess virus was washed out and the incubation was continued in the absence or presence of drug (see Methods). Cell associated cpm as percent total cpm added at 0 min (○-○) and cell-associated p.f.u. as percent total p.f.u. added at 0 min (●-●) are shown as a function of time in min.

virus. The increased apparent uptake of [14C]-labelled virus after 15 min of adsorption in the presence of S-7 (45% uptake) can be attributed to an inhibition of elution during the period used for adsorption.

Similar inhibition of elution and eclipse was seen when reduced 0.01 M-glutathione was substituted for S-7 (not shown). This confirms the earlier finding of Fenwick & Cooper (1962) and also observations of the effect of glutathione on infection by Coxsackie virus B3 (R. Crowell, personal communication).
Fig. 5. Sucrose gradient sedimentation of [14C]-amino acid-labelled poliovirus type 2 after 1 h interaction with HeLa cells in the absence or presence of S-7. The cell homogenates were not treated with SDS and 16 ml gradients were employed. Sedimentation is from left to right (see Methods). (a) Control infection, the dashed curve shows [14C]-ct/min multiplied by 3.2 for representation; the continuous curve shows [3H]-labelled marker virus added before sedimentation. Of the total [14C]-ct/min added to the gradient, 2.5% was recovered in an alkaline wash of the tube.
(b) Infection in the presence of 30 μg/ml S-7, otherwise as above except that [14C]-ct/min were multiplied by 1.6. Of the total [14C]-ct/min added to the gradient, 3.5% was recovered in an alkaline wash of the tube. Sedimentation is from left to right.
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Fig. 6. Sucrose gradient sedimentation of infective [14C]-amino acid-labelled poliovirus after 1 h interaction with HeLa cells in the presence of S-7. A sample of the cell homogenate analysed in Fig. 5b was sedimented without added SDS and without added marker virus; the arrow indicates the location of marker virus in a parallel gradient. Samples of 20 µl from each fraction were diluted ×100 with medium containing serum and stored at −70 °C for plaque assay, the results of which are expressed as p.f.u./ml of gradient (○—○). Samples of 50 µl from each fraction were also counted and gross cts/min are represented (●—●). The starting virus had an intrinsic infectivity of 4.5 × 10⁴ p.f.u./ct/min. The cell homogenate after treatment with 0.4% SDS had an infectivity of 4.2 × 10⁴ p.f.u./ct/min. Sedimentation is from left to right.

The products of poliovirus-cell interaction

Cells were infected with purified amino acid-labelled virus and were then disrupted without SDS (see Methods) and analysed by sucrose gradient sedimentation. This procedure was originally used for investigations with human rhinovirus type 2 infections (Lonberg-Holm & Korant, 1972).

Fig. 5(a) illustrates a typical gradient of material recovered from cells 1 h after infection. There are two major classes of radioactive particles, both of which sediment more slowly than intact marker virus. Cells infected in the presence of S-7 also released particles sedimenting more slowly than marker virus. Fig. 5(b) shows a typical pattern obtained after 15 min adsorption in the presence of 30 µg/ml S-7 and 45 min additional incubation. A similar pattern was obtained with 10 µg/ml S-7. The major class of particles appeared to sediment at approx. 130S, despite the fact that in this and other experiments, S-7 blocked most or all of cell-mediated eclipse (e.g. Fig. 4b). A similar result was obtained when the cells were infected with poliovirus in the presence of reduced 0.02 M-glutathione (not shown).

The slowly sedimenting particles produced in the presence of S-7 were tested for intrinsic infectivity. In the experiment of Fig. 6, cells were infected and treated as in Fig. 5(b) except that no marker virus was added. Samples were counted for radioactivity or titrated for infectious virus. The initial virus had an intrinsic infectivity of 4.5 × 10⁴ p.f.u./ct/min.
Fig. 7. The effect of 0.2% SDS on the sedimentation of particles produced from poliovirus during infection of HeLa cells for a total of 1 h in the presence of S-7. Gradients of 4.5 ml were employed and sedimentation is from left to right. (a) Cells infected with [14C]-amino-acid labelled virus in the presence of 30 μg/ml S-7 and not treated with SDS sedimented together with [32P]-labelled marker virus. Of the total [14C]-cp/min added to the gradient, 4.5% was recovered in an alkaline wash of the tube. (b) After treatment with SDS as described in Methods. Of the total [14C]-cp/min added to the gradient, 5.6% was recovered in an alkaline wash of the tube.
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Fig. 8. The effect of 0.2 % SDS on the sedimentation of particles produced from poliovirus during infection of HeLa cells for 1 h in the absence of inhibitors. Gradients of 16 ml were employed and sedimentation is from left to right. (a) Cells infected with [14C]-amino acid-labelled virus and not treated with SDS. (b) After treatment with SDS as described in Methods. In (a) 3.4 % and in (b) 11.6 % of the total [14C]-ct/min was recovered in an alkaline wash of tube.

compared to the major radioactive peak at fraction 67, which was 1.8 x 10^4 p.f.u./ct/min (corrected for background radioactivity).

The slowly sedimenting particles isolated from cells infected with [14C]-amino acid-labelled poliovirus type 2 in the presence of S-7 were also analysed by electrophoresis on polyacrylamide gels and found to contain a full complement of all 4 virus polypeptides (data not
shown). It was concluded that the major component isolated in the gradients of Fig. 5(b) and Fig. 6 had a normal composition but reduced infectivity, and that a stronger detergent than NP40 would be required for recovery of particles with full infectivity.

It was found that if 0.2 % SDS was added to the homogenized cells before sedimentation, as described in Methods, all of the cell-associated virus incubated in the presence of S-7 could be recovered co-sedimenting with marker virus (Fig. 7). This suggested that the slower sedimenting particles contain intact virus bound to cellular membrane components, and that 0.2 % SDS is able to free the virus particles. Other experiments showed that 0.1 % SDS was insufficient to disrupt this complex while 0.4 % SDS led to irreproducible results during sedimentation. We were unable to release free 156S virus particles from the 130S component by adding either 1.0 % Triton X-100 or 0.5 % DOC plus 5 mM-EDTA to the cell homogenate containing NP40. Fenwick & Wall (1973) employed DOC and EDTA in their study of early infection with labelled poliovirus, but did not conclude that these reagents removed all cellular material from the 130S component. They also found that 1 % SDS disrupted the non-infectious product of cell-mediated eclipse.

When 0.2 % SDS was added to extracts of cells infected without an added inhibitor of eclipse, a relatively small amount of radioactive material appeared to be released from the 130S region of the gradient and to co-sediment with marker virus (Fig. 8). Most of the infecting virus appears to have undergone irreversible alteration to particles sedimenting at 130 to 135S or at 80 to 90S and both of these are presumed to be non-infectious. We were unable to determine the polypeptide composition of the 130 to 135S particles unambiguously. since after 45 or 60 min total incubation, a significant portion of the polypeptides VP1, VP2, and VP3 had undergone proteolytic cleavage to smaller fragments which obscured the VP4 region of the gel.

It was found in other experiments that most of the radioactive poliovirus particles which are eluted from infected cells also sediment at approx. 130S. Treatment of this fraction with 0.2 % SDS did not greatly alter the rate of sedimentation and released less than 7 % as 156S virus particles.

**DISCUSSION**

The specific anti-poliovirus compound S-7 stabilizes virus against heat inactivation in vitro (Fig. 1). This resembles the action of 10⁻⁴ M-SDS on human rhinovirus type 2 (Lonberg-Holm & Noble-Harvey, 1973). The action of S-7 also resembles rhodanine, a compound which specifically inhibits multiplication of echovirus 12 and also protects this virus against alkaline inactivation in vitro (Eggers, 1970). Since the inhibition of infection by SDS or rhodanine can be attributed to inhibition of cell-mediated eclipse, it is not surprising that S-7 was also found to inhibit irreversible alteration of infectious poliovirus type 2 by the host cell (Fig. 4).

Another thiopyrimidine, 2-thiouracil, also inhibits poliovirus replication. Steele & Black (1967) found that 2-thiouracil interacted irreversibly with virus particles in vitro and reduced infectivity by more than 90 %. The infectivity of the treated virus particles was then stabilized against inactivation at 50 °C. The major distinction between 2-thiouracil and S-7 appears to be the reversibility of the effect of the latter. The free sulphhydryl group of 2-thiouracil might form a mixed disulphide with a capsid polypeptide, while the methylated sulphhydril of S-7 may be unable to form such a bond. The apparent ability of cystine and certain strains of poliovirus to form a complex with reduced infectivity and enhanced thermostability has also been reported (Pohjanpelto, 1958; Hirst, 1960). The site of action of cystine appears to be shared by 2-thiouracil (Steele & Black, 1967). The strain of poliovirus type 2
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which we have employed is not sensitive to stabilization by cystine, as determined in unpublished experiments which compared the thermal stability of virus grown in the absence of added cystine with virus exposed to cystine. The stabilizing effect of S-7 is neither enhanced nor reduced when heat inactivation is conducted in the presence of $10^{-2}$ M-dithiothreitol (L. B. Gosser & K. Lonberg-Holm, unpublished data). This suggests that the effect cannot be attributed to a possible impurity in the S-7 preparation which might contain either a reactive sulphydryl or a disulphide bond capable of reacting covalently with the virus capsid in a manner analogous with that proposed for cystine or 2-thiouracil.

Earlier studies of the mode of action of S-7 showed that it did not inhibit the adsorption of virus to HeLa cells (Yamazi et al. 1966) but that a step prior to the onset of RNA-replication was inhibited (La Colla et al. 1972). However, our results are at variance with those of the latter investigation in which it was concluded that S-7 did not block cell-mediated eclipse of poliovirus type 1. La Colla et al. (1972) did not investigate the ability of S-7 to stabilize virus in vitro, nor did they examine the fate of radioactive virus in the host cell. In the present study we were able to recover almost all cell-associated poliovirus type 2 as intact unaltered particles if cells had been infected for 1 h in the presence of S-7 (Fig. 7b).

Glutathione inhibits cell-mediated eclipse (Fenwick & Cooper, 1962) and also appears to have a stabilizing effect on poliovirus in vitro. Glutathione has also independently been found to inhibit eclipse and, under certain conditions, in vitro inactivation of Coxsackie virus B3 (R. L. Crowell, personal communication), and also heat inactivation of echovirus 6 (Halsted et al. 1970). Hence it does not appear to have the very narrow specificity of S-7, rhodanine or SDS, which each inhibit only certain closely related picornaviruses. It is tempting to attribute the narrow specificity of these agents to their interaction with the capsid surface which is specific in structure by reason of antigenic uniqueness.

When cells are infected with poliovirus in the presence of an inhibitor of eclipse, a component is produced which sediments at approx. 130S. This component contains native virus particles which are probably complexed with membrane material, possibly with poliovirus receptors. We are presently attempting to use this component in a method for the identification of poliovirus receptors. If 'penetration' of poliovirus to a state of antibody resistance can occur before cell-mediated eclipse (Mandel, 1967), then it seems likely from our results that 'penetrated' virus is in a state of intimate contact with membrane components rather than free in the cytoplasm.

A different particle is produced during normal infection and is found sedimenting at 130 to 135S after virus-cell complexes have been disrupted with 0.2% SDS (Fig. 8b). This may be the major portion of the slowly sedimenting component found earlier by Fenwick & Wall (1973) and Habermehl et al. (1974) and may also be related to the non-infectious particles eluted from HeLa cells early in infection (Fenwick & Cooper, 1962; Joklik & Darnell, 1962). Although we were unable to determine whether or not VP4 has been lost from the 130 to 135S particle, it should be noted that both the eluted particles of Coxsackie virus B3 (Crowell & Philipson, 1971) and also the 135S subviral particles produced in cells infected with human rhinovirus type 2 (Lonberg-Holm & Korant, 1972) have both lost VP4. The 130S particles produced by heating poliovirus type 2 at 48 °C for one min (Fig. 2) have also lost VP4 (Fig. 3b). However, the particles produced in vitro may not be identical with those produced in the cell and unpublished evidence suggests that the former are more sensitive to disruption by SDS than the latter.

It is possible that there are a series of particles containing RNA which have properties intermediate between those of the product of in vivo eclipse and the 80S particles which Breindl (1971) prepared by heating poliovirus to 50 °C for 10 min. Breindl also found that
shorter periods of heating produced particles which sedimented faster than 80S (M. Breindl, personal communication) and we have observed that prolonged heating at 48 °C produces particles from poliovirus type 2 which sediment at less than 130S. A method other than heating may be needed to provide an entirely satisfactory in vitro model for in vivo eclipse.

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