INTERRUPTION OF POLIOVIRUS RNA SYNTHESIS BY p-FLUOROPHENYLALANINE AND PUROMYCIN

M. D. Scharff, M. M. Thorén, N. F. McElvain, and L. Levintow

National Institute of Allergy and Infectious Diseases
National Institutes of Health
Bethesda 14, Maryland

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Poliovirus belongs to the group of small, spherical viruses which are comprised of a single molecule of RNA surrounded by a protein coat. In the infected cell, the viral RNA specifies its own replication, and that of the viral protein as well, by mechanisms which neither require DNA synthesis nor involve the cell's DNA (Salzman, 1960; Simon, 1961). Thus, the antibiotic actinomycin, which blocks the synthesis of DNA-directed cellular RNA, has no effect on the replication of poliovirus (Shatkin, 1962; Darnell, 1962).

The synthesis of poliovirus protein and RNA are not only coordinated in time, but appear to be functionally interrelated to the extent that agents which inhibit the former also interfere with the synthesis of the latter. Concentrations of the amino acid analogue p-fluorophenylalanine (FPA) sufficient to inhibit the appearance of antigenically identifiable viral protein inhibit the formation of viral RNA as well (Wecker et al., 1962; Scharff and Levintow, in press). Also, the addition of puromycin to an infected culture during the latent period prevents the initiation of synthesis of viral RNA, and its addition after RNA synthesis has begun leads to interruption of synthesis after about an hour (Levintow et al., 1962).

In the experiments just described, viral RNA was measured by plaque assays of its infectivity. The fact that actinomycin allows viral replication while inhibiting the synthesis of cellular RNA provides a sensitive means of examining the formation of virus-directed RNA from a chemical point of view, and this approach has been utilized in the present experiments. The effect of puromycin and FPA, both of which primarily affect protein synthesis, has now been re-examined in this manner.

Methods

Strain S_3 HeLa cells were suspended at 4 x 10^6 cells per ml in chemically defined medium containing 5 μg of actinomycin D per ml, and infected 30 minutes later with Type 1 poliovirus according to methods which previously have been given in detail (Levintow and Darnell, 1960). We are indebted to Dr. Karl Folkers of Merck, Sharpe and Dohme Research Laboratories for providing the actinomycin. Phenylalanine was omitted from the medium in experiments with FPA. Uridine-2-C^{14} was added at the time of infection, thereby labelling the intracellular nucleotide pool which is the source of precursors of viral RNA (Darnell et al., 1961).

Portions of the infected culture were withdrawn at intervals and made 5% w/v with respect to trichloroacetic acid. The resulting precipitates were washed twice with 5 ml portions of cold 5% trichloroacetic acid, and extracted with 5% trichloroacetic acid for 15 minutes at 90°. After removal of the trichloroacetic acid with ether, portions of the extract were plated for the determination of radioactivity with a low-background thin-window counter. After subtraction of small blank values obtained with uninfected, actinomycin-treated cultures, the amount of radioactivity was taken as a measure of the amount of virus-directed RNA formed.

Results

Addition of 0.5 or 1.0 mM FPA to a culture at the time of, or shortly after, infection prevents the formation of appreciable amounts of either poliovirus antigen or RNA (Wecker et al., 1962; Scharff and Levintow, in press). As Fig. 1 indicates, inhibition of RNA synthesis was virtually complete when 1.0 mM FPA was added as late as two hours after infection, or about a half hour before synthesis is initiated in the absence of FPA. Addition of FPA at 2-1/2 hours, or at later times during the maturation cycle, permitted RNA synthesis to continue for about 3/4 of an hour, after which it was abruptly cut off. Addition of the inhibitor at 4 hours permitted completion of the usual 5-hour cycle of RNA synthesis. Similar results were obtained with 0.5 mM FPA.

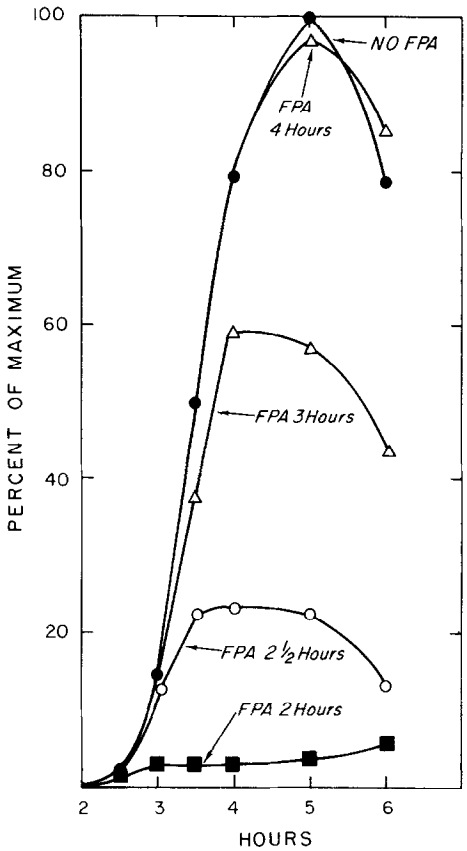

Fig. 1. Inhibition of poliovirus RNA synthesis by FPA. 4.6 x 10^8 cells were pretreated with actinomycin as described in the text, and 20 μc of uridine-C^{14} (3.3 μc/micromole) was added to the culture. A portion of the culture was infected, and the remainder used for an uninfected control. Aliquots of the infected culture were withdrawn at intervals, and 1.0 mM FPA added. Samples were withdrawn and treated as described in the text.

The results of the experiment shown in Fig. 2 indicate that, under these conditions, FPA does not act by an irreversible mechanism. Addition of 4 mM phenylalanine, after a delay of approximately one hour, specifically reversed the inhibitory effect of FPA on viral RNA synthesis. Only 50% of the normal yield of RNA was obtained after reversal of the inhibition, possibly because of inability of the cells under these conditions to support synthesis of viral macromolecules beyond 6 hours (Ackermann et al., 1954).

In the preceding experiments, the total amount of radioactivity incorporated into acid-precipitable RNA was measured, and the results did not exclude the possibility that the apparent inhibition of RNA synthesis in the presence of FPA

actually reflected only an increased rate of breakdown. Accordingly, the incorporation of radioactivity after a short period of exposure to labelled precursor was studied (Table 1). Although the inhibition of RNA synthesis in this experiment was not complete (cf. Fig. 2), the results leave little doubt that RNA synthesis was primarily inhibited.

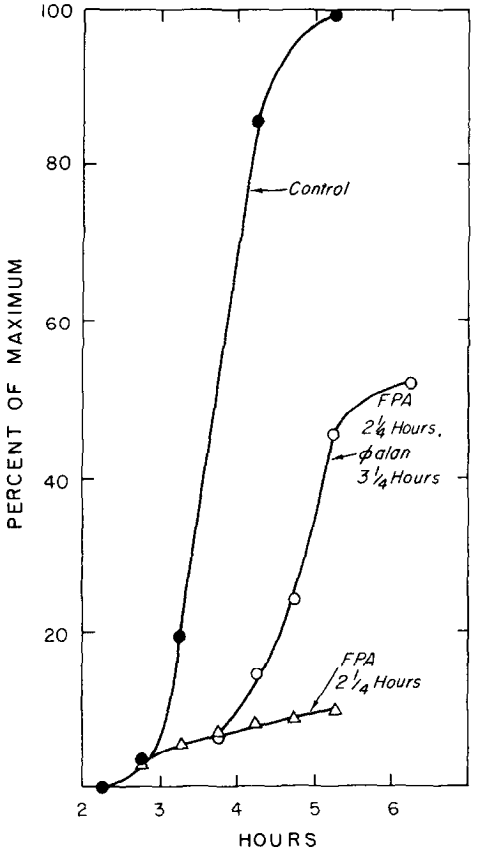

Fig. 2. Reversal of FPA inhibition by phenylalanine. 2.2 x 10^8 cells were pretreated with actinomycin, labelled and infected as in Fig. 1. 0.5 mM FPA was added to a portion of the infected culture at 2-1/2 hours; this portion was in turn divided at 3-1/4 hours and 4 mM phenylalanine added to one half. Samples were withdrawn and treated as described in the text.

Puromycin, at a concentration of 0.05 mM, interrupts virus directed RNA synthesis in a manner similar to 0.5 mM FPA, and after a similar lag, leading to data like those shown in Fig. 1. No compound capable of specifically reversing the effects of puromycin is known, but if the drug is removed by washing and

TABLE 1

Measurement of Poliovirus RNA Synthesis After Onset of Inhibition by FPA

	c.p.m. Incorporated in 15 minutes	45 minutes
Control Culture	68	536
Inhibited Culture	0	36

Experimental conditions were as described for Fig. 2, except that uridine-C^{14} was not added until 3-1/4 hours. The non-linearity of the data reflects incomplete equilibration of the precursor and the intracellular pool at the earlier time point (Darnell et al., 1961).

resuspending the infected cells, RNA synthesis resumes after a lag, in this case, of about 2 hours.

Discussion and Summary

Granting that (a) FPA and puromycin exert their effects by interfering with protein synthesis and (b) only virus-directed RNA is synthesized in the presence of actinomycin, the present experiments permit the conclusion that formation of poliovirus RNA in the infected cell is dependent on the continuing synthesis of a specific protein. This conclusion bears out earlier impressions derived from study of the formation of infectious RNA (Levintow et al., 1962). Furthermore, the fact that this protein is made in the presence of actinomycin implies that its synthesis is directed by the viral genome.

If, according to current views, the virus directs the synthesis of a specific polymerase for the replication of its RNA (Baltimore and Franklin, 1962), the present results imply that the functional life of this enzyme, early or late in the maturation cycle, is less than one hour. Alternative explanations for the interruption of poliovirus RNA synthesis after interference with protein synthesis have no apparent parallel in non-viral systems which have been investigated.

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