The Kinetics of the Appearance of Poliovirus Ribonucleic Acid

By P. D. COOPER*

*Virus Culture Laboratory, Medical Research Council Laboratories,
Carshalton, Surrey*

(Received 11 June 1964)

SUMMARY

One-step growth conditions were used in which cells began replication of poliovirus RNA almost synchronously, and in which factors limiting the rate of replication appeared to be absent. RNA of uneclipsed virus was destroyed by using light-sensitive inocula. Under these conditions, ribonuclease-sensitive infective RNA was detectable in unchanged amount for the first 2 hr of infection, at which time replication began abruptly; the increase of poliovirus RNA was geometrical for a further 1-2 hr.

INTRODUCTION

Where the kinetics can be determined, the rates of increase of several viral nucleic acids (measured chemically or by infectivity) appear in one-step growth experiments to be more or less constant with time. However, in phage T₂ (Hershey, Dixon & Chase, 1953) a genetic experiment (Luria, 1951) showed that DNA replication depends on a geometric mechanism. Rate-limiting factors may, therefore, have transformed a presumptively exponential rate of increase into an apparently linear one. Another complication having the opposite effect is that individual cells may not begin nucleic acid replication synchronously. In this case, an exponential rate of increase in the proportion of cells which begin replication could transform a presumptively linear rate of increase of nucleic acid into one which was apparently exponential in the initial phase.

This paper reports experiments on the rate of increase of poliovirus RNA under conditions lacking these complications. Random delays were largely absent so that maturation began almost synchronously (Cooper, 1964). Cells were infected with a light-sensitive inoculum (Wilson & Cooper, 1962). Illumination after a short 'pulse' of penetration gave additional synchrony and destroyed the RNA of virus uneclipsed during this period (Wilson & Cooper, to be published): the very early kinetics could be examined without their being obscured by excess of non-productive RNA. The appearance of new infective RNA began sharply at 2 hr; the increase was exponential for the next 2 hr, so that rate limiting factors were also absent.

* Present address: Department of Microbiology, John Curtin School of Medical Research, Australian National University, Canberra, Australia.

METHODS

Virus. Poliovirus type 1 (strain Brunenders) was used, grown and assayed in ERK cells (Cooper, Wilson & Burt, 1959; Cooper, 1961). Infective RNA was extracted and assayed by the method described by Cooper (1962); all RNA assays used two or three 8-fold dilutions, 4 in. Petri dishes and four replicates per dilution.

One-step growth method. The method used was described by Cooper (1964); mono-disperse cells were infected to an adsorbed multiplicity of 10 at 0°, washed and added to medium at 37° at zero time. Serum was omitted from the medium. The cells were maintained in suspension by a rotary shaker. Inoculum virus was grown in neutral red, 4 µg./ml. and all operations including addition to warm medium were performed under a red light (Wilson & Cooper, 1962, 1963); after 5 min. at 37° the cultures were strongly illuminated for 10 min. with white light (proportion of virus surviving under these conditions equals 10⁻³). Samples were taken for infective cell and free virus assay, and at intervals into 2 mg. deoxycholate/ml. for cell associated mature virus assay (no free virus was present, and no cell associated virus was found up to 2.8 hr after infection), and into aqueous phenol for extraction of RNA.

For all cultures the eclipse periods (time after infection of appearance of 1 intracellular p.f.u. per infective cell) were 2.6–2.7 hr and maturation was rapid and exponential. These criteria were regarded as essential to indicate the absence of random delays and asynchrony (Cooper, 1964).

Fig. 1

Fig. 1. One-step growth curve of poliovirus infective RNA, plotted semilogarithmically (○, left-hand ordinate) and arithmetically (●, right-hand ordinate). Uneclipsed inoculum virus was destroyed by use of light sensitive inocula, and virus maturation was approximately synchronous.

Fig. 2

Fig. 2. Two replicate one-step growth curves of poliovirus infective RNA, expressed as a percentage of the 4-hr yield. Conditions as for Fig. 1.

RESULTS

An intensive examination was made of the rate of increase of poliovirus RNA between 1 and 3 hr after infection (Fig. 1). The eclipse period, as determined by assays of mature virus and infective centres (not shown), was 2.6 hr. This indicates that maturation was approximately synchronous (Cooper, 1964), and hence that all cells must have begun replication of viral RNA well before this time. More than 60% of the RNA found up to 2 hr was sensitive to ribonuclease when the cells were disrupted and treated with the enzyme before phenolic extraction. Infection with virus grown in 4 µg. neutral red/ml. followed by white light has destroyed 99.9% of the RNA of uneclipsed virus (Wilson & Cooper, to be published). Therefore this initial RNA represents input nucleic acid which was uncoated but not degraded or eclipsed. RNA synthesis began sharply 2 hr after infection and increased exponentially up to 4.0 hr (Fig. 2). Increases in infective RNA generally continued up to 5 hr in this system; the increase in infective RNA was 10^3 to 10^4 times the initial (2 hr) value, whereas the yield of virus rarely exceeded 300 p.f.u./cell. It is presumed that this excess of infective RNA reflects the RNA built into particles which do not register as plaque-forming units, together with any RNA which is not matured.

DISCUSSION

These data indicate that poliovirus RNA can be shown to increase geometrically, provided that adequate technical safeguards are taken. This may reflect a truly geometric mechanism, in which each progeny RNA molecule is able to act as a template for new molecules. This conclusion is supported by the observations that the proportion of poliovirus recombinants increases during the growth cycle (Ledinko, 1963), and that more than one molecule per cell of the RNA of the closely similar EMC virus can exist in double-stranded form (Montagnier & Sanders, 1963). The underlying mechanism, however, may be no more than pseudogeometric. For example, each progeny RNA molecule may induce the synthesis of some substance which increases the rate of replication, conceivably viral RNA polymerase.

I am grateful to Mr H. Cumming and Miss J. Constable for skilful technical assistance.

REFERENCES

- COOPER, P. D. (1961). An improved agar cell-suspension plaque assay for poliovirus: some factors affecting efficiency of plating. *Virology*, **13**, 153.
COOPER, P. D. (1962). Studies on the structure and function of the poliovirion: effect of concentrated urea solutions. *Virology*, **16**, 485.
COOPER, P. D. (1964). Synchrony and the elimination of chance delays in the growth of poliovirus. *J. gen. Microbiol.* **37**, 2.
COOPER, P. D., WILSON, J. N. & BURT, A. M. (1959). The bulk growth of animal cells in continuous suspension culture. *J. gen. Microbiol.* **21**, 702.
HERSHEY, A. D., DIXON, J. & CHASE, M. (1953). Nucleic acid economy in bacteria infected with bacteriophage T2. I. Purine and pyrimidine composition. *J. gen. Physiol.* **36**, 777.
LEDINKO, N. (1963). Genetic recombination with poliovirus type 1. Studies of crosses between a normal horse serum-resistant mutant and several guanidine resistant mutants of the same strain. *Virology*, **20**, 107.

- LURIA, S. E. (1951). The frequency distribution of spontaneous bacteriophage mutants as evidence for the exponential rate of phage reproduction. *Cold Spr. Harb. Symp. quant. Biol.* **16**, 463.
- MONTAGNIER, L. & SANDERS, F. K. (1963). Replicative form of encephalomyocarditis virus ribonucleic acid. *Nature, Lond.* **199**, 664.
- WILSON, J. N. & COOPER, P. D. (1962). Photodynamic demonstration of two stages in the growth of poliovirus. *Virology*, **17**, 195.
- WILSON, J. N. & COOPER, P. D. (1963). Aspects of the growth of poliovirus as revealed by the photodynamic effects of neutral red and acridine orange. *Virology*, **21**, 135.