RNA WITH A HIGH CYTOSINE AND A LOW GUANINE CONTENT IN POLIOVIRUS INFECTED CELLS

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Infection of animal cells by poliovirus causes a decrease in the rate of total RNA synthesis (Holland, 1962). Experiments on the mechanism of this inhibition (to be reported elsewhere) have shown that within two hours after infection of human cells by poliovirus the synthesis of messenger RNA and especially of ribosomal RNA precursor is impaired. Fractionation of ^{32}P-labeled RNA isolated from infected cells on a methyl-esterified albumin column (Kubinski et al., 1962) and subsequent analysis of base composition of labeled RNA enabled us to identify an RNA distinguishable from viral and cellular RNAs. This RNA can be recognized before the onset of virus RNA production; it has a molecular weight comparable to viral RNA and a nucleotide composition with a high cytosine (C) and low guanine (G) content. This RNA possesses properties comparable to an RNA fraction which is supposed to act as messenger in non-infected animal cells (Scherrer and Darnell, 1962; Georgiev et al., 1963). In the presence of moderate concentrations of actinomycin, the C-rich, G-poor RNA (Cr-RNA) is synthesized in virus infected cells with little or no contamination by ribosomal precursor RNA.

Amnion cells (or HeLa-cells) incubated in suspension were centrifuged, washed and resuspended (4×10^6 cells/ml) in phosphate-free Eagle's medium with and without actinomycin (2 ug/ml). The cells were infected with 50 PFU of virus and 40 minutes later the medium was supplemented by radiophosphate. At various times samples were withdrawn, RNA was extracted with hot phenol and fractionated on a methyl-esterified albumin column. The ^{32}P-nucleotide composition of the RNA fractions was determined. The methods used have been described in detail (Kubinski et al., 1962; Koch et al., 1964).

Figure 1a shows the elution diagram of RNA obtained from an infected cell culture, incubated for 3.5 hours without actinomycin. The radioactive label enters predominantly two high molecular weight RNA fractions, as reported previously (Kubinski and Koch, 1963 b). Fraction I was never found to contain infective virus RNA. Fraction II corresponds in size to infective poliovirus RNA. The base composition (Table 1) of ^{32}P-labeled RNA in fraction I is similar to that of ribosomal RNA. The ^{32}P-RNA in fraction II has a base composition which distinctly differs from both polio RNA and "cellular messenger" RNA (Table 1).

In the presence and in the absence of actinomycin D, the Cr-RNA is formed. With actinomycin, however, the overall inhibition of RNA labeling was as high as 97% (figure 1b) and no synthesis of ribosomal RNA was detected.

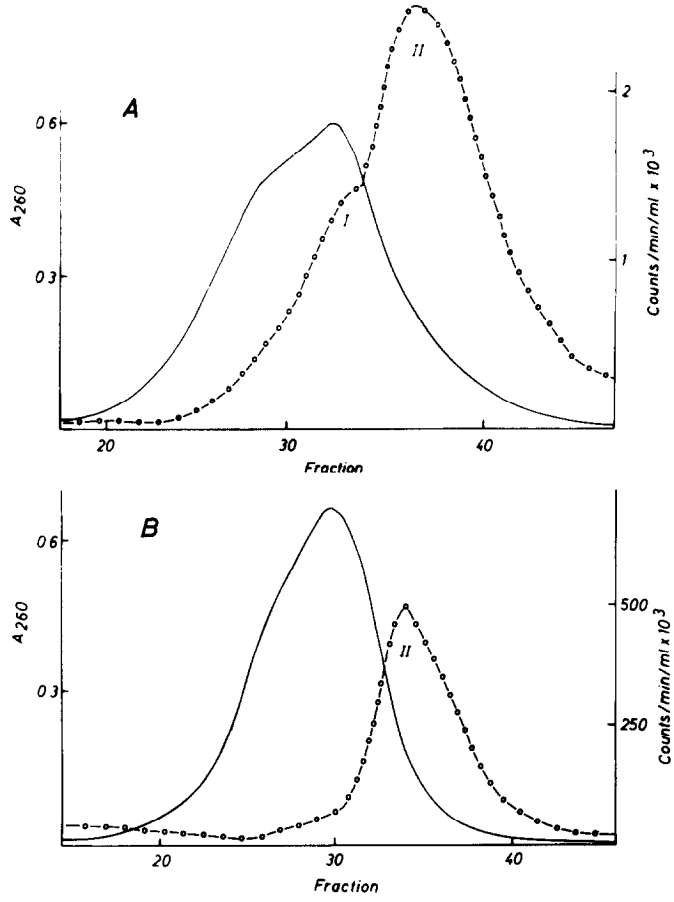

FIGURE 1

Chromatographic separation of RNA obtained from amnion cells infected with 50 PFU/ml of poliovirus and incubated for 3.5 hours at 36°C.

1a) without actinomycin; 8 uC of ^{32}P/ml

1b) 2 ug actinomycin D added at time of infection; 12 uC of ^{32}P/ml

_____ UV absorbance: A 260

-o-o-o ^{32}P (please note that the scale differs in fig. 1a and b)

The Cr-RNA is synthesized exclusively within the eclipse period. After the eclipse only poliovirus RNA is synthesized. Cr-RNA starts to elute from the column at lower

TABLE 1

% Distribution of ^{32}P-nucleotides in various RNA fractions

	C	A	U	G
ribosomal*	29.9	17.2	20.0	32.9
fraction I, figure 1a	29.8	19.4	22.2	28.5
fraction II, figure 1a	32.0	26.9	20.0	21.2
polio*	22.5	28.3	25.7	23.6
"cell messenger"*	21.8	26.1	28.0	23.2
"Cr"*	34.0	28.1	20.1	17.8

* mean values obtained in several experiments.

salt concentrations, corresponding to fraction I in figure 1a and to the unstable RNA fraction observed earlier in polio-infected cells (Kubinski and Koch, 1963 b).

The role of Cr-RNA in the metabolism of virus infected cells is not known. It seems unlikely that its formation is directed by the virus RNA since actinomycin has an inhibitory effect on its synthesis. Moreover, the base composition of Cr-RNA is not complementary to poliovirus RNA in spite of the fact that both RNAs are of the same size, since they elute from the column at the same salt concentration. A more plausible explanation seems to be that the production of Cr-RNA is directed by the host cell. The properties of Cr-RNA suggest that it is related to the cellular "messenger" RNA. It was recently shown (Georgiev et al., 1963; Kubinski and Koch, 1963 a) that the synthesis of messenger RNA is less reduced by moderate concentrations of actinomycin than that of ribosomal RNA precursor.

Two possibilities might be considered: (1) the synthesis of some G-rich, C-poor high molecular weight cellular RNA is preferentially inhibited by virus infection so that the newly formed RNA is more strongly labeled in C than in G; or (2) the infection can induce the synthesis of a special RNA during the eclipse period. This second possibility is supported by the finding that some virus infections trigger the synthesis of a new protein, viz. interferon (Isaacs and Lindenmann, 1957). It is known that the production of interferon is inhibited by actinomycin (Heller, 1963). One could expect, therefore, that the virus infection induces the formation of a new messenger RNA for interferon.

Further investigations are called for to clarify the role of Cr-RNA in poliovirus infected cells.

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