ALTERED BASE RATIOS IN HeLa CELL RNA DURING POLIOVIRUS INFECTION*

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Ackermann et al. (1959) reported that resting (non-dividing) HeLa cells infected with poliovirus produced large increments of protein and ribonucleic acid (RNA) as compared to uninfected controls. However, the incremental RNA exhibited base ratios similar to normal HeLa cell RNA, indicating that the bulk of the new RNA was not virus RNA. Salzman et al. (1959) found no significant increase in RNA of HeLa cells infected with poliovirus when the cells were actively multiplying, rather than resting.

The present report shows that the RNA in actively-multiplying HeLa cells infected with poliovirus has greatly altered base ratios. This shift in base ratio was detected by measuring P^{32} incorporated into 2', 3' nucleotides of RNA during the period of virus replication. The shift is in the direction of the base composition of RNA obtained from purified poliovirus (Schaffer et al., 1960).

Methods: HeLa cells were grown as monolayers in glass bottles in a medium consisting of 10% calf serum and 0.1% yeast extract in Hanks balanced salt solution. Cells were infected by a 10 minute exposure to a high multiplicity of type 1 poliovirus (Mahoney) (effective multiplicity >10).

P^{32} labelling was accomplished by incubation in Eagles' medium containing 10% dialyzed calf serum and 50 microcuries/ml. P^{32}-labelled orthophosphate as the sole added source of phosphate. One ml. of P^{32} medium was added to bottles containing about 10^7 HeLa cells which had been rinsed with phosphate-free Eagles' medium. Bottles were incubated 3 hours at 37°C. with frequent

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agitation to keep the cell monolayer bathed in medium. After 1.5 hr., 2 ml. of fresh phosphate-free Eagles' medium was added to feed the cells. After 3.5 hr. the cells were thoroughly washed with 0.15 M NaCl to remove excess P^{32}, and RNA was extracted with phenol. Because HeLa cells are slow in incorporating P^{32} into nucleotides and RNA, it was necessary to use several hours' incubation in a high concentration of P^{32} in order to obtain the desired labelling of RNA. The bulk of P^{32} incorporated into RNA during 3.5 hours' incubation was incorporated during the final hour.

HeLa cell RNA was extracted at 4°C. into 0.02 M phosphate buffer pH 7.2 by the phenol method of Gierer and Schramm (1956). RNA was precipitated about 10 times with ethanol from aqueous solution until only traces of inorganic P^{32} remained. RNA was hydrolyzed to 2' 3' nucleotides by incubation in 0.3 N KOH for 18 hrs. at 37°C. (Davidson and Smellie, 1952), and individual nucleotides were separated by paper ionophoresis at pH 3.5 (Markham and Smith, 1952; Davidson and Smellie, 1952). Nucleotides were eluted into 0.01 N HCl and molar concentrations determined by UV spectrophotometry (Beaven et al., 1955). The identity of each band was confirmed by checking its UV spectrum (Beaven et al., 1955), and its radioactivity was determined with a Nuclear-Chicago D-47 gas flow counter with micromil window.

Results and Discussion: Table 1 shows that the P^{32} base ratios of cell RNA labelled from 1 to 3 hrs. after poliovirus infection were still similar to control RNA from uninfected cells. However, after 4.3, 5, 6, or 7 hrs., the base ratios of labelled RNA shifted from values characteristic of uninfected cells to ratios resembling those found in RNA from purified poliovirus. RNA labelled up to 9 hrs. after infection no longer resembled virus RNA in base ratio, but had reverted toward the ratios of normal cell RNA. Base ratios obtained from RNA 9 hrs. after infection were not reproducible and may reflect abnormal RNA synthesis by dying cells. P^{32} incorporation into RNA at this late stage of infection was often greatly reduced. Salzman et al. (1958) reported apparent breakdown and extracellular release of RNA 6 hours after poliovirus infection of HeLa cells.

Table 1

Molar Base Ratios of 2', 3' Nucleotides of RNA from Normal and Poliovirus Infected HeLa Cells at Intervals Following Infection

Time Interval after Infection at Which RNA Was Extracted*		Moles per 100 Moles of P^{32} in Each Nucleotide of Cell RNA**			
		U	G	A	C
None (uninfected control)		22.0±0.6	30.2±1.3	17.2±0.7	30.6±0.9
1, 2, and 3 hrs. (averages of all 3)		21.5±0.6	30.6±0.8	17.1±1.0	30.8±0.9
4.3 hours		25.2±1.2	23.3±1.3	26.0±0.6	25.5±0.8
5 hours		24.9±1.2	23.9±1.2	26.4±1.5	24.8±0.5
6 hours		24.3±0.4	24.1±0.7	25.4±0.7	26.2±0.6
7 hours		23.0	24.5	25.5	27.0
9 hours	Expt. 1	22.0	26.0	22.0	30.0
	Expt. 2	24.8	35.0	17.2	23.0
Poliovirus RNA base ratios†		25.2±0.7	24.0±1.0	28.5±0.5	22.0±1.0

*In each case P^{32} was added 3.5 hrs. prior to extraction time.
**Data expressed as mean of 5 or more determinations plus or minus standard deviation, except for 7 and 9 hr. times where data from individual experiments are given.
†Schaffer et al., 1960.

Despite the changed base ratios in newly formed (P^{32}-labelled) RNA observed above, the molar base ratios in total RNA (determined spectrophotometrically) are not changed significantly during infection. This agrees with the report of Ackermann et al. (1959) who found no alteration of base composition of HeLa cell RNA during infection. Table 2 shows a typical experiment in which RNA nucleotide ratios were unaltered, but in which specific activity of the nucleotides underwent drastic alteration during infection. After infection, the ratios of specific activities of the 2', 3' nucleotides shifted from approximately 1:1:1:1 typical of uninfected cells to lower levels of guanine and cytosine and higher levels of adenine and uracil. The equal specific activities of nucleotides from control cell RNA shows that the labelling of 2', 3' nucleotides under these conditions reflects the base ratios of the bulk of cell RNA.

These results with RNA from infected cells suggest that most of the RNA synthesized by HeLa cells during the interval between 4 and 7 hours after

Table 2

Specific Activities and Ratios of Specific Activities of P^{32}-Labelled 2', 3' Nucleotides from RNA of Normal and Poliovirus-Infected HeLa Cells*

RNA Obtained from Cells at Indicated Time after Infection***	U	G	A	C
Uninfected cells (control: specific activity (CPM/μM)	1650	1610	1660	1580
Ratios of specific activities**	1.01	0.99	1.02	0.98
Cells infected 4 hrs: specific activity (CPM/μM)	1810	1340	2320	1240
Ratios of specific activities	1.08	0.79	1.39	0.74
Cells infected 6 hrs. specific activity (CPM/μM)	1920	1470	2480	1290
Ratios of specific activities	1.07	0.82	1.39	0.72

* Data obtained from a single typical experiment
** Ratios calculated to an arbitrary total of 4.
*** In each case P^{32} was added 3.5 hours prior to extraction time.

poliovirus infection is either virus RNA or RNA synthesized under the direction of virus RNA. However, the pre-existing cellular RNA is present in such quantities as to prevent detection of this virus-induced RNA by spectrophotometric methods.

These results with an RNA animal virus seem analogous to studies of a number of phage systems in which DNA made after infection exhibits base ratios resembling phage DNA rather than cell DNA. In the present study it is possible that much if not all of the newly synthesized RNA is viral RNA since it is produced at about the same time that infectious RNA accumulates (Darnell et al., 1961) (Holland et al., 1960). Whether non-viral RNA is produced during poliovirus infection is not tested here. It is possible that much of the newly-formed RNA is non-viral, but virus-directed similarly to DNA-directed synthesis of RNA during phage infection (Astrachan and Volkin, 1958).

Since predominantly virus-type RNA appears to be produced without any large increase in total RNA synthesis between 4 and 7 hours post-infection, it seems probable that net synthesis of normal cell RNA is

interfered with. Salzman et al. (1959) suggested virus activation of a ribonuclease.

Further work is in progress on poliovirus control of RNA synthesis, and the effect of other enteroviruses is being investigated. Type 2 poliovirus and Coxsackie B1 have shown similar effects in preliminary experiments.

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