ALTERED BASE RATIOS IN RNA SYNTHESIZED DURING ENTEROVIRUS INFECTION OF HUMAN CELLS, †*

By John J. Holland

DEPARTMENT OF MICROBIOLOGY, SCHOOL OF MEDCINE, UNIVERSITY OF WASHINGTON, SEATTLE

Communicated by Colin MacLeod, October 4, 1962

Ackermann *et al.*[1] reported increased synthesis of RNA and protein in resting (nondividing) HeLa cells after infection with poliovirus. The RNA produced showed normal base ratios. Salzman *et al.*,[2] however, found no significant increase in synthesis of RNA after poliovirus infection of actively multiplying HeLa cells, and we have confirmed this. In a preliminary report,[3] it has been shown that in actively multiplying HeLa cells poliovirus infection led to altered base ratios in newly synthesized (P^{32}-labeled) RNA without causing a significant shift in total cell RNA base ratios or rate of RNA synthesis. The base ratios in newly synthesized RNA shifted in the direction of the base composition of RNA reported for purified poliovirus by Schaffer *et al.*[4] This suggests that RNA virus can cause infected cells to produce mainly viral type RNA, just as the DNA of bacterial viruses induces production of viral DNA. Whether this RNA is mainly virus RNA or nonviral RNA whose synthesis is directed by viral RNA (as phage DNA was shown to direct synthesis of new RNA by Volkin and Astrachan[5]) or simply host cell-directed RNA of different composition is not certain.

The present report provides evidence that the altered RNA is virus RNA and/or virus-directed RNA. It is also shown that poliovirus RNA metabolism may involve a high specific activity fraction of cell RNA (r-RNA or residual RNA)[6–9] which is not extractable with aqueous phenol in addition to the phenol-extractable RNA (p-RNA), which is most studied.

No shift in base composition in HeLa cell DNA could be detected during poliovirus infection, which is further evidence that DNA does not participate in RNA virus infection.[10, 11]

Materials and Methods.—HeLa cells were employed as monolayer cultures in glass bottles. Cell stocks were grown in a medium containing 10% calf serum and 0.1% yeast extract in Hanks' balanced salt solution. Only rapidly growing, recently fed cells were used. Primary human amnion cell monolayers were prepared by trypsinization as described previously.[12]

Type 1 poliovirus (Mahoney), Type 2 poliovirus (MEF1), and Coxsackie B_1, B_3, and B_5 were employed as high-titered pools from HeLa cells after at least seven passages in HeLa cells. Cell monolayers were infected by 10-min adsorption of virus to give an adsorption multiplicity greater than 10.

P^{32}-labeling of cells was accomplished by incubation of about 10^7 cells in 1 ml of Eagle's medium containing 10% dialyzed calf serum with 50–200 microcuries/ml of P^{32} orthophosphate as the sole added source of phosphorus. To attain the desired labeling of RNA, incubation for 3.5 hr was employed. After 1.5 hr of incubation, 2 ml of fresh P^{32} medium was added to the cells. Under these conditions, most of the P^{32} entering RNA and DNA was incorporated during the final hour of incubation. After thorough washing to remove excess P^{32} following the 3.5 hr incubation period, RNA was extracted from the cells by the phenol method of Gierer and Schramm.[13] The cells were shaken at room temperature and then at 0° with an equal volume (5 ml) of phenol and 0.02 *M* phosphate buffer pH 7.2. Most of the cellular RNA (p-RNA), consisting mainly of ribosomal RNA plus soluble RNA, is recovered in the aqueous supernatant phase after centrifugation. This p-RNA was repeatedly precipitated with ethanol until only traces of inorganic P^{32} were present in the supernatant. After removal of p-RNA in the aqueous phase by the above procedure, the nucleoprotein coagulum at the interphase between phenol and aqueous phases was

freed of remaining buffer and phenol by careful pipetting and decantation and was re-extracted twice more with 5 ml each of phenol and 0.02 *M* phosphate buffer. The nucleoprotein residue was then washed and precipitated 5 times with 10 ml ethanol. The final precipitate consisted mainly of DNA, protein, and r-RNA. The r-RNA was released and degraded to 2'3' nucleotides by 18 hr incubation in 0.3 *N* KOH at 37°.[14] The pH was then adjusted to about 2.0 with perchloric acid, and the DNA-protein precipitate which formed was removed by centrifugation. The 2'3' nucleotides in the supernatant were separated by paper ionophoresis at pH 3.5.[14,15] The 2'3' nucleotides from the p-RNA were likewise obtained by KOH digestion and separated by ionophoresis. Nucleotides were eluted into 0.01 *N* HCl, and molar concentrations were determined by UV spectrophotometry.[16] Radioactivity was determined with a Nuclear-Chicago D-47 gas flow counter with micromil window. Where only radioactivity of each nucleotide, rather than molar concentration or specific activity, was to be determined, liver RNA was added as carrier before the ethanol precipitation steps. Under the above conditions of labeling, nucleotide specific activities exceeding several hundred cpm/mμM were obtained.

DNA was also labeled with P^{32} by incubation for 3.5–4.0 hr in P^{32} medium. DNA was isolated after cell disruption and deproteinization with 5% sodium dodecyl sulfate containing 0.01 *M* sodium citrate.[17] DNA was repeatedly precipitated with ethanol, the fibers collected on glass rods, and resuspended in 0.15 *M* NaCl containing 10 μg/ml pancreatic ribonuclease. After incubation for 5 min at 37°, DNA was precipitated and treated 4 or 5 times with ribonuclease to reduce to a minimum contamination with the more heavily labeled RNA. Finally, the DNA preparation was shaken once with chloroform-octanol,[18] denatured by heating at 100° for 3 min in 0.15 *M* NaCl, reprecipitated with ethanol, and treated alternately with micrococcal diesterase and spleen phosphodiesterase to recover the 3' nucleotides as described by Josse *et al.*[19] DNA nucleotides were separated by electrophoresis and eluted as with the RNA nucleotides. Purified spleen phosphodiesterase and crystallized pancreatic ribonuclease were purchased from Worthington Co. Micrococcal diesterase was prepared from *Micrococcus aureus*.[20]

Actinomycin D was generously supplied by Merck, Sharp & Dohme Co.

Results.—Base ratio alterations in p-RNA of HeLa cells and primary human amnion cells during poliovirus or Coxsackie virus infection: Base composition changes in p-RNA during poliovirus infection have been reported in a preliminary communication.[3] Table 1 shows base ratio shifts in newly formed p-RNA after poliovirus infection. There have been some discrepancies in the base ratios of RNA from

TABLE 1

MOLAR BASE RATIOS BASED ON P^{32} OF 2'3' NUCLEOTIDES IN HELA CELL p-RNA BEFORE AND AT INTERVALS AFTER TYPE 1 POLIOVIRUS INFECTION

Time after infection at which RNA was extracted* (hr)	Moles per 100 moles of P^{32} in each nucleotide of HeLa Cell p-RNA††			
	U	G	A	C
Uninfected 0 (Control cells)†	22.0 ± 0.6	30.2 ± 1.3	17.2 ± 0.7	30.6 ± 0.9
Uninfected 0 (Control cells)‡	25.1 ± 1.2	30.6 ± 0.09	16.5 ± 0.7	27.5 ± 1.0
1, 2, and 3§	21.5 ± 0.6	30.6 ± 0.8	17.1 ± 1.0	30.8 ± 0.9
4.3	25.2 ± 1.2	23.3 ± 1.3	26.0 ± 0.6	25.5 ± 0.8
5	24.9 ± 1.2	23.9 ± 1.2	26.4 ± 1.5	24.8 ± 0.5
6	24.3 ± 0.4	24.1 ± 0.7	25.4 ± 0.7	26.2 ± 0.6
7‡‡	23.0	24.5	25.5	27.0
8	37.2	20.6	21.1	21.1
9	Variable	—	—	—
Poliovirus RNA base ratios**	25.2 ± 0.7	24.0 ± 1.0	28.5 ± 0.5	22.0 ± 1.0

* P^{32} was added 3.5 hr prior to RNA extraction in each case.
† Data from 14 experiments performed between July and October 1961.
‡ Data from 12 experiments performed between February and April 1962.
§ Averages from RNA extracted at 1, 2, or 3 hr post-infection.
** Poliovirus RNA data from Schaffer *et al.*[4]
†† Data expressed as mean of five or more determinations plus or minus one standard deviation.
‡‡ Data for 7 and 8 hr are averaged results from single experiments.

cultured human cells as determined in different laboratories.[1, 3, 4] It has been observed that the strain of HeLa cells maintained in this laboratory has undergone a significant shift in its normal base ratios over a period of nine months. For this reason, two sets of values for P^{32}-labeling ratios are shown in Table 1 for normal HeLa cells. Despite these differences, it is clear the poliovirus infection induced synthesis of an RNA having different base ratios and that the shift was in the direction of the base composition of RNA from purified poliovirus.[4] This change in RNA synthesis was not evident until about four hr after infection.

Differences in base composition have also been observed at different times in the total p-RNA of normal HeLa cells as determined spectrophotometrically. In some experiments, there was excellent agreement between RNA base ratios measured spectrophotometrically and by P^{32} incorporation in normal cells. The ratios of specific activities then approximate 1:1:1:1 in normal cells and greatly deviate from these equal ratios during infection.[3] Normal cell RNA did not always have approximately equal ratios of specific activities, but in every case poliovirus infection led to significant shifts in specific activity and ratios of specific activities. This is the result of a large shift in base composition in newly synthesized (P^{32}-labeled) RNA without any significant change taking place in base composition of total RNA.[3] No major alterations in total p-RNA base composition have been observed at any stage of poliovirus infection prior to onset of viral cytopathic changes. Nor was there a major change in rate of p-RNA synthesis as measured by P^{32} incorporation before six hr after infection, when virus RNA synthesis is essentially complete.

It was found that other enteroviruses cause similar shifts in RNA synthesis in HeLa cells (Table 2) and that poliovirus induces base ratio changes in newly synthesized RNA of primary human amnion cells (Table 3).

It is of obvious importance to determine whether the observed shifts in base ratio in newly synthesized RNA actually represent a shift in the direction of base

TABLE 2

MOLAR BASE RATIOS BASED ON P^{32} OF 2′3′ NUCLEOTIDES IN HELA CELL p-RNA 5.5 HR AFTER INFECTION WITH TYPE 2 POLIOVIRUS OR COXSACKIE B_1, B_3, B_5

Infecting virus	Moles per 100 moles of P^{32} in each nucleotide of HeLa Cell p-RNA* U	G	A	C
None (control cells)	21.1	31.7	16.5	30.7
Type 2 poliovirus	24.5	26.5	23.2	25.8
Coxsackie B_1	24.0	26.3	23.2	26.5
Coxsackie B_3	24.5	25.2	25.0	25.3
Coxsackie B_5	23.8	26.5	23.8	25.9

* P^{32} was added 3.5 hr prior to RNA extraction in each case.

TABLE 3

MOLAR BASE RATIOS BASED ON P^{32} OF 2′3′ NUCLEOTIDES IN PRIMARY HUMAN AMNION CELL p-RNA BEFORE AND DURING TYPE 1 POLIOVIRUS INFECTION

Time after infection at which RNA was extracted* (hr)	Moles per 100 moles of P^{32} in each nucleotide of amnion cell p-RNA U	G	A	C
0 (uninfected control)	20.0	31.9	18.5	29.6
4.5	25.0	23.8	25.0	26.2
6.5	26.2	25.0	24.5	24.3
7	23.7	24.3	27.0	25.0

* In each case, P^{32} was added 3.5 hr prior to RNA extraction.

TABLE 4

MOLAR BASE RATIOS BASED ON P^{32} IN 2'3' NUCLEOTIDES OF RNA OBTAINED FROM PURIFIED TYPE 1 POLIOVIRUS AND COXSACKIE B_1

Source of labeled RNA*	Moles per 100 moles of P^{32} in each nucleotide‡			
	U	G	A	C
Type 1 poliovirus	24.0 ± 0.4	23.4 ± 0.5	30.0 ± 0.5	22.6 ± 0.5
Coxsackie B_1	24.0 ± 0.4	24.2 ± 0.4	29.0 ± 0.7	22.8 ± 1.0
Poliovirus RNA base ratios†	25.0 ± 0.7	24.0 ± 1.0	28.5 ± 0.5	22.0 ± 1.0

* Virus purified by ultracentrifugation and chromatography on DEAE[21, 22] after labeling infected HeLa cells with P^{32} between 1 and 5 hr post-infection.
† Poliovirus RNA base ratios determined by Schaffer *et al.*,[4] using spectrophotometric quantitation of bases and nucleotides obtained by acid hydrolysis of purified virus.
‡ Data are means from six determinations plus or minus one standard deviation.

ratios to be found in viral RNA. Although degradation of RNA by KOH to 2'3' nucleotides tends to randomize P^{32} distribution when RNA is synthesized from unequally labeled 5' nucleotides, large differences in specific activity among the nucleotides of the acid-soluble pool might (depending on nearest-neighbor relationships) cause a large variance from the actual base ratios. Therefore, poliovirus was isolated and purified from HeLa cells labeled with P^{32} in the usual way, and viral RNA was extracted with phenol and degraded with KOH. Table 4 shows the molar base ratios calculated from the radioactivity of 2'3' nucleotides of purified P^{32}-labeled poliovirus RNA. There is good agreement between these results and the established values for the base composition of poliovirus RNA. Coxsackie B_1 RNA shows nearly the same base ratios.

Base ratios of r-RNA in HeLa cells: The base ratios in P^{32}-labeled r-RNA were examined in normal and in poliovirus-infected cells. It can be seen in Table 5 that the r-RNA from normal HeLa cells and from cells early after infection had a base composition which was almost complementary to poliovirus RNA. Whether this near correlation is of any significance is open to doubt, especially since the base ratios in r-RNA of normal cells are a composite value from many hundreds or thousands of molecular species rather than from a homogeneous population of molecules. Table 5 also shows that r-RNA synthesized later in infection changed in base ratio, until at 8 hr post-infection it exhibited the high adenylate values charac-

TABLE 5

MOLAR BASE RATIOS BASED ON P^{32} OF 2'3' NUCLEOTIDES IN HELA CELL r-RNA, BEFORE AND AT INTERVALS AFTER TYPE 1 POLIOVIRUS INFECTION

Time after infection at which RNA was extracted* (hr)	Moles per 100 moles of P^{32} in each nucleotide of HeLa Cell r-RNA				Per cent P^{32} incorporated into r-RNA†
	U	G	A	C	
0 (uninfected control cells)	29.0 ± 1.0	24.0 ± 0.5	24.3 ± 0.7	22.7 ± 0.8	100
2	28.6	23.1	24.4	23.9	88
3.5	28.4	24.2	24.0	23.4	58
4.0	28.0	24.4	24.0	23.6	53
5.0	28.3	23.2	25.0	23.5	27
6.0	26.8	22.2	25.8	25.2	26
7.0	25.2	20.8	27.3	26.7	27
8.0	23.7	21.7	28.6	26.0	24
Predicted poliovirus RNA base-pair partner	28.5 ± 0.5	22.0 ± 1.0	25.2 ± 0.7	24.0 ± 1.0	—
Poliovirus RNA	25.2 ± 0.7	24.0 ± 1.0	28.5 ± 0.5	22.0 ± 1.0	—

* P^{32} was added 3.5 hr prior to extraction of RNA.
† Per cent of P^{32} incorporated into nucleotides of r-RNA at each interval after infection as compared with uninfected control cells.

teristic of poliovirus RNA. However, the guanylate and cytidylate values were not in good agreement with those of poliovirus RNA. The time after infection at which r-RNA base ratios shifted was found to vary in different experiments.

In agreement with the Russian[7, 8] and Japanese[6] investigators who have studied phenol fractionation of RNA from mammalian tissues and organs, it has been found that r-RNA of HeLa cells represents about 15–20 per cent of the total RNA. Also in HeLa cells as in mammalian tissues, much higher specific activities are found in r-RNA than in p-RNA (approximately 5 to 10 times as great) after several hours of P^{32} labeling.

It can be seen in Table 5 that the rate of r-RNA synthesis slowly declined during poliovirus infection, whereas the rate of p-RNA synthesis was fairly stable until virus RNA synthesis was nearly complete.[3]

Infectivity of viral suspensions, p-RNA, and r-RNA during poliovirus infection of HeLa cells: Figure 1 shows the time course of production of infectious molecules of r-RNA, p-RNA, and mature virus in HeLa cells infected with Type 1 poliovirus and incubated at 37°. It can be seen that infectivity is present in r-RNA of HeLa cells after infection with poliovirus. All infectivity was lost after treatment of r-RNA with 1 μg/ml pancreatic ribonuclease. From these data, it is not possible to determine whether the infectivity of r-RNA represents a precursor molecule of p-RNA or an RNA complementary to p-RNA or simply contamination of the nucleoprotein precipitate with p-RNA.

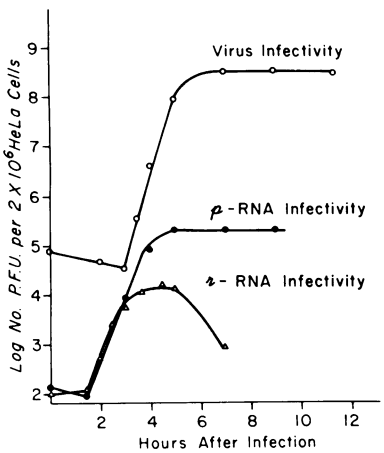

FIG. 1.—Time course of development of infectivity in r-RNA, p-RNA, and whole virus after type 1 poliovirus infection of 2×10^6 HeLa cells. After extraction of p-RNA from infected cells, the interphase nucleoprotein precipitate was washed several times with phenol-0.02 *M* phosphate buffer, and r-RNA was released from the precipitate by treatment with 5 per cent duponol at 60–65°.

Base ratios in newly synthesized DNA of normal and poliovirus-infected HeLa cells: There is ample evidence that replication of the RNA animal viruses does not require participation of pre-existing cell DNA or synthesis of new DNA.[10, 11] But these findings do not rule out the possibility that DNA synthesis, although unnecessary for RNA virus replication, may nevertheless become involved in, or altered by, RNA virus infection. However, when the base ratios of newly synthesized DNA in normal and poliovirus-infected cells were compared by measuring the P^{32} content of DNA 3′ nucleotides, not significant differences were observed. Table 6 shows the results of a typical experiment. Some variation was obtained in the DNA base ratios based on 3′ nucleotide P^{32} in different experiments, but in no case did the infected cells differ significantly from the controls. Thus, it can be concluded that the replication of RNA virus does not lead to synthesis of DNA with grossly altered base ratios.

Maintenance of RNA synthesis in poliovirus-infected HeLa cells after treatment with actinomycin D: Reich *et al.*[23] have shown that actinomycin D inhibits normal cell RNA synthesis and DNA virus synthesis at levels which failed to arrest mul-

TABLE 6

MOLAR BASE RATI BASED ON P^{32} IN 3′ NUCLEOTIDES OF DNA FROM NORMAL AND FROM POLIOVIRUS-INFECTED HELA CELLS

Time after infection at which DNA was extracted* (hr)	Moles per 100 moles of P^{32} in each nucleotide of Hela Cell DNA†				Per cent P^{32} incorporated into DNA‡
	T	G	A	C	
0 (uninfected control cells)	28.6	22.2	27.2	22.0	100
2	28.0	22.6	26.9	22.5	97
3	28.3	22.1	27.5	22.1	91
5	27.8	22.0	28.0	22.2	29

* In each case cells were labeled for four hours with P^{32} before DNA extraction.
† 3′ nucleotides were obtained by successive digestion with micrococcal diesterase and spleen phosphodiesterase.[19]
‡ Per cent P^{32} incorporated into DNA nucleotides as compared to uninfected control cells.

tiplication of an RNA virus (Mengo virus). They suggested that actinomycin inhibits DNA-directed RNA synthesis without affecting viral RNA-directed RNA synthesis. Actinomycin was therefore employed in the present study to suppress host DNA-controlled RNA synthesis in order to examine virus-induced RNA base ratio alterations against a low background of host cell RNA synthesis. Preliminary experiments established that poliovirus multiplication is unaffected by the presence of 1–5 μg/ml actinomycin D in the medium during infection. By adjustment of actinomycin concentration and time of exposure, it was found that the rate of p-RNA synthesis in normal cells could be greatly reduced without equal reductions in poliovirus-infected cells. Table 7 shows the results of one experiment in which about 90 per cent of normal cell p-RNA synthesis was arrested after 3.5-hr treatment with 5 μg/ml actinomycin, whereas similarly treated infected cells showed nearly normal p-RNA synthesis 5 or 6.5 hr post-infection, but not at 2 hr post-infection. The base ratios of p-RNA in these actinomycin-treated infected cells closely resembled the base ratios of poliovirus RNA within 5 hr post-infection. These results suggest strongly that the base ratio shifts in virus-infected cells are indeed virus-induced and that the altered RNA is viral RNA and/or RNA synthesized under the

TABLE 7

SYNTHESIS OF RNA DURING 3.5-HR TREATMENT WITH ACTINOMYCIN D IN POLIOVIRUS-INFECTED HELA CELLS AS COMPARED TO UNINFECTED CELLS

Time after infection at which RNA was extracted* (hr)	Moles per 100 moles of P^{32} in each nucleotide of HeLa Cell RNA				Per cent P^{32} incorporation into RNA
	U	G	A	C	
		p-RNA			
0 (uninfected, untreated with actinomycin)	22.0	31.5	17.2	29.3	100 (control)
0 (uninfected, actinomycin-treated cells)	72.0	13.0	5.8	9.2	11
2‡	61.5	20.3	7.2	11.0	11
5‡	26.3	21.6	28.2	23.9	95
6.5‡	25.2	22.5	28.5	23.8	78
		r-RNA			
0 (uninfected, untreated cells)	28.5	23.8	24.3	23.4	100 (control)
0 (uninfected, actinomycin treated cells)	20.3	11.0	25.3	43.4	2
2‡	23.1	13.7	27.1	36.1	2
5‡	22.0	22.5	29.0	26.5	17
6.5‡	22.0	21.0	31.0	26.0	8
Poliovirus RNA†	25.2	24.0	28.5	22.0	—

* P^{32} was added 3.5 hr prior to RNA extraction. Actinomycin D was added to a final concentration of 5 μg/ml 3.5 hr prior to RNA extraction (except for the control cells).
† Poliovirus RNA base ratios of Schaffer *et al.*[4]
‡ Infected, treated.

direction of virus RNA. It appears the virus-induced p-RNA is too asymmetrical to be composed of base-paired, double-stranded RNA or separated strands of equal numbers of complementary molecules. It has the high adenine levels characteristic of single-stranded poliovirus RNA. Similar results are also seen in Table 7 for r-RNA, except that actinomycin arrested 98 per cent of normal cell r-RNA synthesis, and poliovirus infection did not restore synthesis fully. This is not surprising, since poliovirus infection itself depresses r-RNA synthesis (Table 5). It should be emphasized that actinomycin concentration and period of exposure were found to be rather critical for demonstration of virus-induced RNA synthesis.

Apparently, the abnormal base ratios induced in normal cell RNA by actinomycin were reflected in virus-infected cells to a slight extent (Table 7). Base ratios found in actinomycin-treated uninfected cells varied widely in different experiments. Generally, very high proportions of uracil were found in the newly synthesized RNA, although higher levels of actinomycin and longer exposure times led to synthesis of RNA with very high cytidylate content. The nature of this abnormally labeled polynucleotide has not yet been investigated. Similar pyrimidine-dominant base ratios were often found after treatment of HeLa cells with mitomycin (unpublished results) or guanidine.[24] This might possibly represent radioactive contamination of these 2′3′ nucleotides.

Poliovirus-like base ratios in P^{32}-labeled RNA of infected HeLa cell ribosomes: Table 8 shows that newly synthesized RNA recovered from microsomes and ribo-

TABLE 8

BASE RATIOS RESEMBLING POLIOVIRUS RNA IN P^{32}-LABELED p-RNA OF HELA CELL MICROSOMES AND RIBOSOMES 4.5 HR AFTER TYPE 1 POLIOVIRUS INFECTION

Source of RNA	Moles per 100 moles of P^{32} in each nucleotide of p-RNA			
	U	G	A	C
Control microsomes from uninfected HeLa cells*	20.2	32.5	19.3	28.0
Microsomes from HeLa cells infected 4.5 hr*†	25.6	24.0	28.8	21.6
Ribosomes from HeLa cells infected 4.5 hr*†‡	25.8	23.1	29.0	22.1
Poliovirus RNA base ratios[4]	25.2	24.0	28.5	22.0

* Cells were homogenized at 0° in 0.01 *M* tris buffer pH 7.5 containing 10^{-2} *M* $MgCl_2$. Homogenates were brought to 0.35 *M* sucrose by addition of 2 *M* sucrose, and the microsomal fraction was recovered by centrifugation for 90 min at 105,000 × *g* after sedimentation of nuclear and mitochondrial fractions.

† Over 80 per cent of P^{32}-labeled RNA of infected cell microsomes and ribosomes was rendered acid-soluble by ribonuclease, showing that this RNA was not contained in intact virus.

‡ Ribosomes were recovered from microsomal preparations by sodium desoxycholate treatment followed by centrifugation at 105,000 × *g*. for 2 hr.

somes of poliovirus-infected cells had base ratios nearly identical to poliovirus RNA base ratios. At 4.5 hr after infection, only a small part of this RNA was in mature virus particles associated with the microsomes and ribosomes, as shown by the fact that most of the label was sensitive to ribonuclease. Later in infection, much of the labeled microsomal RNA is within virus particles. It was not always possible to show virus-like base ratios in RNA associated with microsomes of infected HeLa cells, possibly due to RNA release or breakdown during cell fractionation. However, the data seen in Table 8 are typical of results obtained in three out of six experiments and they suggest strongly that virus-like RNA does become associated with microsomes during poliovirus replication, possibly in fulfilling a messenger function and possibly even replicating there.

The data presented here suggest that poliovirus RNA can enter a human cell containing over a million times as much RNA, and eventually direct nearly all

RNA synthesis. It will be shown in another paper that host-directed RNA synthesis is greatly depressed during the course of virus-directed RNA synthesis.[24]

* This investigation was supported by the National Science Foundation.

† Presented at the Atlantic City meeting of the Federation of American Societies for Experimental Biology, April, 1962.

[1] Ackermann, W. W., P. C. Loh, and F. E. Payne, *Virology*, **7**, 170 (1959).

[2] Salzman, N. P., R. Z. Lockart, Jr., and E. D. Sebring, *Virology*, **9**, 244 (1959).

[3] Holland, J. J., *Biochem. Biophys. Res. Comm.*, **6**, 196 (1961).

[4] Schaffer, F. L., H. V. Moore, and C. E. Schwerdt, *Virology*, **10**, 530 (1960).

[5] Volkin, E., and L. Astrachan, *Virology*, **2**, 149 (1956).

[6] Sibatani, A., K. Yamana, K. Kimura, and H. Okagaki, *Biochem. Biophys. Acta*, **33**, 590 (1959).

[7] Georgiev, G. P., and V. L. Mant'eva, *Biokhimiya*, **25**, 143 (1960).

[8] Georgiev, G. P., V. L. Mant'eva, and I. B. Zbarsky, *Biochem. Biophys. Acta*, **37**, 373 (1960).

[9] Sibatani, A., S. R. deKloet, V. G. Allfrey, and A. E. Mirsky, these PROCEEDINGS, **48**, 471 (1962).

[10] Simon, E. H., *Virology*, **13**, 105 (1961).

[11] Reich, E., and R. M. Franklin, these PROCEEDINGS, **47**, 1212 (1961).

[12] Holland, J. J., *Virology*, **15**, 312 (1961).

[13] Gierer, A., and G. Schramm, *Nature*, **177**, 702 (1956).

[14] Davidson, J. N., and R. M. S. Smellie, *Biochem. J.*, **52**, 594 (1952).

[15] Markham, R., and J. D. Smith, *Biochem. J.*, **52**, 552 (1952).

[16] Beaven, G. H., E. R. Holiday, and E. A. Johnson, in *The Nucleic Acids*, ed. E. Chargaff and J. N. Davidson (New York: Academic Press, 1955), vol. 1, p. 493.

[17] Kay, E. R. M., N. S. Simmons, and A. L. Dounce, *J. Am. Chem. Soc.*, **74**, 1724 (1952).

[18] Sevag, M. G., D. B. Lackman, and J. Smolens, *J. Biol. Chem.*, **124**, 425 (1938).

[19] Josse, J., A. D. Kaiser, and A. Kornberg, *J. Biol. Chem.*, **236**, 864 (1961).

[20] Cunningham, L., B. W. Catlin, and M. Private DeGarilhe, *J. Am. Chem. Soc.*, **78**, 4642 (1956).

[21] Hoyer, B. H., E. T. Bolton, R. A. Ormsbee, G. LeBouvier, D. B. Ritter, and C. L. Larson, *Science*, **127**, 859 (1958).

[22] Hoyer, B. H., personal communication.

[23] Reich, E., R. M. Franklin, A. J. Shatkin, and E. L. Tatum, *Science*, **134**, 556 (1961).

[24] Holland, these PROCEEDINGS, in press.

ONCOGENIC EFFECTS IN HAMSTERS OF HUMAN ADENOVIRUS TYPES 12 AND 18

BY ROBERT J. HUEBNER, WALLACE P. ROWE, AND WILLIAM T. LANE

LABORATORY OF INFECTIOUS DISEASES, NATIONAL INSTITUTES OF HEALTH, BETHESDA, MARYLAND

Communicated October 29, 1962

The importance of demonstrable oncogenic activity on the part of prevalent human viruses is obvious. Recently Trentin *et al.*[1] reported the induction of cancer in NIH Syrian hamsters (*Mesocricetus auratus*) by the prototype strain of adenovirus type 12 supplied by us through the Viral and Rickettsial Registry of the American Type Culture Collection (ATCC).[2] We have confirmed the observations of Trentin's group, and in addition found that type 12 strains other than the prototype and the prototype strain of adenovirus 18 also produce cancers in hamsters.

Adenovirus Type 12.—Using a virus preparation (infectivity titer in human embryo kidney cultures 10^6 $TCID_{50}$ per 0.1 ml) which was three KB cell passages[3]