negligible over $R(x)$). No matter where $I(x)$ is located, alternate integers in it must be even, four out of every six (regularly arranged) divisible by 2 or 3, etc. This regularity of deletion by the sieve of Eratosthenes extends to all the smallest primes whose product $2.3.5 \ldots p = N \leq t \log x$. About $te^{-\gamma}\log x/\log_3 x = tg(x)$ integers in $I(x)$ will survive. Any p not a factor of N need not be the smallest prime factor of a surviving integer in $I(x)$ and a prime larger than $t \log x$ need not even have a multiple in $I(x)$, so that one of the "survivors" being deleted by any such prime is now a matter of chance with probability $1/p$. By the prime number theorem, the expectation of primes in $I(x)$ is exactly t (in the limit), hence the compound probability for primality of a "survivor" is asymptotic to $1/g(x)$. Moreover, if some k of these survivors be tested and found composite or prime (without revealing their numerical values), the knowledge does not modify the probability for primality for the rest. In all this, x is merely a background parameter, whose principal use is to furnish relative magnitudes of the various functions involved, as $x \to \infty$.

It follows that if P_r be the probability for precisely r primes in $I(x)$, then in the limit $P_0 = \lim(1 - 1/g)^{tg} = e^{-t}$. Using textbook definitions and procedures, the limit $P_1 = \lim(1 - 1/g)^{tg-1}(tg)(1/g) = te^{-t}$, and so on, with limit $P_r = e^{-t}t^r/r!$ But any limiting distribution over $R(x)$ as $x \to \infty$ will obviously be the distribution over the entire x-line, here the Poisson distribution with parameter t, as before.

[1] Prachar, K., *Primzahlverteilung* (Berlin, 1957), ch. 3.

[2] Hardy, G. H., and E. M. Wright, *An Introduction to the Theory of Numbers* (Oxford University Press, 1945), Theorem 430, pp. 349–354.

[3] Prachar, K., *op. cit.*, ch. 2, Theorem 4.4.

[4] *Ibid.*, Theorem 2.4.7.

[5] Feller, W., *An Introduction to Probability Theory and Its Applications* (New York: 1950), vol. 1, p. 366 *et passim.*

[6] For general known results on gaps in the sequence of primes, see Prachar, *op. cit.*, p. 154 ff.

[7] Ricci, G., "Sul pennello di quasi-asintoticità delle differenze di interi primi consecutivi," *Rend. Atti. Accad. Naz. Lincei*, **8**, 192–196 and 347–351 (1954–5).

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*DEPRESSION OF HOST-CONTROLLED RNA SYNTHESIS IN HUMAN CELLS DURING POLIOVIRUS INFECTION**,†

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It was shown in a preliminary communication[1] that poliovirus infection of HeLa cells leads to synthesis of RNA with greatly altered base ratios. The rate of p-RNA (phenol-extractable RNA) synthesis was not greatly altered during this shift in base ratios during the first 6 hr of infection, although the rate of r-RNA (residual phenol-nonextractable RNA) synthesis showed a slight decline during infection.[2] Thus, it was suggested[1] that normal host RNA synthesis must be suppressed in order for the synthesis of predominantly virus-type RNA to proceed at about the normal rate, and the present study demonstrates that this does in fact occur. Salz-

man *et al.*[3] noted a continued incorporation of cytidine-2-C^{14} into RNA of poliovirus-infected HeLa cells despite the absence of net synthesis of RNA, and a gradual loss of cellular components into the medium beginning at about 6 hr post-infection. They suggested that an early effect of poliovirus infection is activation of a ribonuclease since the acid-soluble pool of infected cells increased starting 3 hr after infection. The work of other investigators[4] indicates that guanine from degraded host RNA may be used for virus synthesis.

The present study demonstrates that poliovirus infection depresses the synthesis of normal host-controlled RNA. This is shown by employing guanidine to inhibit virus-directed RNA synthesis. Guanidine·HCl at low concentrations has been shown[5] to inhibit poliovirus synthesis at concentrations which failed to alter normal cell growth detectably.[6] Guanidine acts to inhibit poliovirus synthesis at any stage of the infectious cycle and prevents virus-mediated abnormal accumulations of cytoplasmic RNA as detected with acridine orange.[6] It is shown here that guanidine strongly inhibits the synthesis of infectious RNA in poliovirus-infected HeLa cells without suppressing the rate of normal cell RNA synthesis. If guanidine was added to virus-infected cells 3.5 hr after infection to suppress virus-induced RNA synthesis, it was found that about 90 per cent of total p-RNA and r-RNA synthesis was subsequently arrested. It appears that virus infection strongly depresses host-controlled RNA synthesis while directing the synthesis of viral type RNA at near-normal rates. If, in turn, virus-directed RNA synthesis is suppressed with guanidine, the total synthesis of RNA drops to as little as 10 per cent of normal. This virus-induced inhibition of RNA synthesis seems analogous to phage DNA inhibition of host cell nucleic acid synthesis.

Materials and Methods.—Methods have been described in the preceding paper.[2] Guanidine·HCl was employed at a concentration of 10^{-3} *M* in cell culture medium with or without P^{32} as indicated. Infectious RNA was determined using only p-RNA from infected HeLa cells and plating on HeLa cell monolayers using 2 *M* $MgSO_4$ during exposure.[7] All base ratios presented in this paper are based on P^{32} in 2′3′ nucleotides of newly synthesized RNA.[2]

Results.—Preliminary experiments showed that guanidine at 10^{-3} *M* inhibits poliovirus production and the development of cytopathic changes in HeLa cells as it has been reported to do in monkey kidney cells.[5, 6] At this level of guanidine, uninfected HeLa cells continued to multiply in a normal manner over a period of 7 days or more. If HeLa cells were infected with a high multiplicity of Type 1 poliovirus and immediately placed into guanidine medium and incubated at 37°, no cytopathic effects were visible within the first 8 hr (by which time untreated cells have started to degenerate), but extensive cellular degeneration was evident 1 to 2 days later. Addition of Type 1 antiserum to the medium together with guanidine did not prevent this late degeneration; so it is not due to spread of guanidine-resistant mutants of virus. It appears then, that guanidine greatly delays but does not completely prevent virus-induced cell death.

Next, it was shown that 10^{-3} *M* guanidine arrests (or greatly retards) replication of infectious RNA just as it retards virus development.[5, 6] Table 1 shows that 10^{-3} *M* guanidine completely prevented infectious RNA accumulation up to 6 hr post-infection if added immediately after virus adsorption. Even when added 2 hr post-infection, 10^{-3} *M* guanidine arrested 97 per cent of infectious RNA synthesis.

TABLE 1

GUANIDINE INHIBITION OF INFECTIOUS RNA SYNTHESIS IN HeLa CELLS INFECTED WITH TYPE 1 POLIOVIRUS

Treatment of cells	Total PFU infectious RNA 6 hours after infection*	Per cent infectious RNA formed‡
Control cells—RNA extracted after 6 hr incubation at 37° following virus absorption.	9×10^5	100
Control cells—RNA extracted 20 min after virus adsorption	2×10^3	0.2
Cells placed in guanidine medium† immediately following virus adsorption and incubated 6 hr at 37°	1×10^3	0.1
Cells incubated in normal medium for 2 hours following virus adsorption, then incubated in guanidine medium for 4 additional hours.	2×10^4	2.2

* 6×10^6 HeLa cells per bottle were infected with Type 1 poliovirus at an adsorption multiplicity >10. At the indicated intervals after infection, p-RNA was extracted with phenol and its infectivity determined on HeLa cell monolayers.
† Where indicated, guanidine·HCl was added to the medium at a final concentration of 10^{-3} *M*.
‡ Per cent plaque-forming units formed as compared to control cells.

The fact that 2.2 per cent of infectious RNA was produced, however, indicates that 10^{-3} *M* guanidine greatly retards, but does not completely prevent, virus RNA synthesis.

Table 2 shows the effect of 10^{-3} *M* guanidine on the synthesis of RNA in uninfected HeLa cells and in cells infected with Type 1 poliovirus. It can be seen that guanidine had little or no effect on the rate of RNA synthesis in uninfected cells. Nor did it greatly affect the rate of RNA synthesis when added to infected cells immediately after virus adsorption, although a small decrease in rate of synthesis was always observed.

It can also be seen that 10^{-3} *M* guanidine caused alteration in base composition of p-RNA but no significant changes in r-RNA. It is pointed out in reference 2 that other treatments of normal HeLa cells often lead to abnormal incorporation of pyrimidines into p-RNA. These changes vary in magnitude from experiment to experiment and were not usually found if the cells were allowed to adapt to 10^{-3} *M* guanidine for several days before testing. It can be seen that poliovirus infection

TABLE 2

EFFECT OF 10^{-3} *M* GUANIDINE ON p-RNA AND r-RNA SYNTHESIS IN NORMAL AND IN POLIOVIRUS INFECTED HeLa CELLS

Treatment of cells before RNA extraction*	Moles per 100 moles P^{32} in each nucleotide of RNA				Per cent P^{32} incorporated into RNA
	U	G	A	C	
	p-RNA				
Untreated, uninfected cells	24.7	31.5	16.5	27.3	100 (control)
Uninfected cells incubated 6 hr in guanidine medium	29.3	29.3	15.6	25.8	87
Cells infected with Type 1 poliovirus and incubated for 6 hr in guanidine medium	35.0	25.0	17.0	23.0	67
	r-RNA				
Untreated, uninfected cells	29.5	24.1	24.2	22.2	100 (control)
Uninfected cells incubated 6 hr in guanidine medium	28.6	23.0	25.0	23.4	109
Cells infected with Type 1 poliovirus and incubated for 6 hr in guanidine medium	27.0	23.0	25.2	24.8	65

* P^{32} was added 3.5 hr prior to RNA extraction in each case. Where indicated, guanidine was added to Eagles medium (without and with P^{32}) to a final concentration of 10^{-3} *M*.

TABLE 3

REPRESSION OF HELA CELL RNA SYNTHESIS WHEN GUANIDINE IS ADDED SEVERAL HOURS FOLLOWING INITIATION OF TYPE 1 POLIOVIRUS INFECTION

Hours after infection before guanidine addition*	Hours incubation after addition of guanidine until RNA extraction	Moles per 100 moles P^{32} in each nucleotide of RNA				Per cent P^{32} incorporation into RNA
		U	G	A	C	
		p-RNA				
0 (control)†	0 (control)†	24.2	30.8	16.5	28.5	100
1.5	4	35.3	24.7	16.0	24.0	80
2.0	4.5	28.6	28.5	16.7	26.2	35
2.3	2.5	42.8	24.1	14.1	19.0	43
3.5	3.5	45.7	21.2	14.6	18.5	23
3.5	5	48.5	22.2	12.3	17.0	14
3.5	8.5	41.6	25.7	13.9	18.8	9
		r-RNA				
0 (control)†	0 (control)†	29.7	23.8	22.9	23.6	100
1.5	4	28.0	23.2	23.8	25.0	70
2.0	4.5	30.3	23.1	22.3	24.3	25
2.3	2.5	29.0	23.0	24.0	24.0	45
3.5	3.5	28.9	22.0	25.1	24.0	13
3.5	5	27.5	23.3	24.9	24.3	14
3.5	8.5	28.5	23.8	24.2	23.5	11

* In each case P^{32} was added 3.5 hr prior to extraction of RNA. Guanidine was added to a final concentration of 10^{-3} *M* at indicated times post-infection.
† Controls are uninfected, untreated, normal HeLa cell.

for 6 hr in the presence of guanidine caused little alteration in base ratios of r-RNA and slight alteration in p-RNA as compared with uninfected cells in guanidine.

Next, guanidine was added to cells in which poliovirus infection had been allowed to proceed normally for various intervals after virus adsorption. It can be seen in Table 3 that when poliovirus infection had been allowed to proceed for 3.5 hours before introduction of guanidine, the subsequent addition of the drug led to the suppression of up to 90 per cent of p-RNA and r-RNA synthesis. After shorter periods of normal virus infection, less inhibition of RNA synthesis was obtained upon guanidine treatment. Addition of the drug after 1.5 hr of infection effected no greater suppression of RNA synthesis than did addition immediately after infection (see Table 2). Table 3 shows (as did Table 2) that treatment of either infected or uninfected HeLa cells with guanidine usually caused distorted base ratios (heavy uridylate label) in p-RNA but not in r-RNA. Guanidine addition after virus infection prevented the usual virus-induced base ratio shifts in r-RNA. Even when guanidine was added at 3.5 hr post-infection (within 30 min of the time at which large shifts in base ratio are normally apparent during poliovirus infection[1]), there were no changes in base ratio, and the r-RNA, produced at a greatly reduced rate, resembled normal cell r-RNA in base composition. Thus, it appears that poliovirus infection causes a progressive repression of host-controlled RNA synthesis. Under usual conditions of infection, virus-controlled RNA synthesis replaces host-controlled RNA synthesis, but when a specific inhibitor of viral RNA synthesis is added, all RNA synthesis is depressed.

Cells that were treated with guanidine beginning 3.5 hr post-infection exhibited cytopathic effects and degenerated at about the same time as did untreated, poliovirus-infected HeLa cells, despite the fact that poliovirus-induced RNA synthesis was strongly inhibited. It is possible that the major cause of the rapid cytopathic effects of poliovirus infection is inhibition of vital host-directed RNA synthesis. It has been observed (unpublished observations) that 90 per cent depression of

HeLa cell RNA synthesis with actinomycin D leads to cell degeneration within 5 to 10 hr.

Discussion.—It might be argued that the effect described here is not actually poliovirus-induced repression of host RNA synthesis but some synergistic effect between guanidine action and virus action. This seems unlikely, however, since the two together did not significantly inhibit RNA synthesis unless the virus infection had proceeded in a normal manner for several hours.

The mechanism of repression can only be speculated upon at present. The apparent inhibition of synthesis could possibly be due to rapid breakdown of cell RNA synthesized at the normal rate. Such an explanation seems unlikely for several reasons. First, if there were a nuclease capable of selectively degrading host RNA so rapidly that only 10 per cent remained within an hour of synthesis, there should be very little host RNA present 5 or 6 hr after infection. This is not the case, since so much original host RNA remains during infection that P^{32} labeling must be employed to detect virus-induced RNA synthesis.[1] Second, rapid degradation of newly formed RNA would probably (depending on the nuclease) leave resistant acid-insoluble oligonucleotides that might greatly alter the base ratios of newly formed RNA. This was not observed (Table 3). Although the virus repression of RNA synthesis described here probably does not depend upon nuclease activity, there are good indications that poliovirus may activate nucleases capable of slow degradation of host RNA.[3, 4]

Other mechanisms which might be suggested are: (1) attachment of poliovirus RNA to critical DNA template sites, (2) activity of an interferon-like material. Poliovirus RNA base ratios are not incompatible with its being complementary to sequences along one or both strands of host DNA. Studies are in progress in an attempt to relate this phenomenon to interference, which might be viewed as another case of repression of heterologous nucleic acid synthesis. Preliminary results suggest that certain very concentrated interferon preparations from chick allantoic fluid may slightly suppress RNA synthesis in chick fibroblast cultures, but it is questionable whether this is a direct effect or merely a nonspecific result of cell damage. Work to be presented elsewhere will show that repression of RNA synthesis is not due to loss of capacity of DNA in infected cells to act as a template for RNA synthesis, although DNA-primed RNA polymerase activity is depressed in extracts from infected cells.[8]

The specific ability of guanidine to prevent poliovirus-infectious RNA synthesis and to prevent virus-induced synthesis of RNA with altered base ratios without inhibiting normal host-controlled RNA synthesis suggests that it may have considerable potential for elucidation of mechanisms involved in viral RNA replication.

Summary.—Guanidine at 10^{-3} *M* inhibits poliovirus-infectious RNA synthesis and virus-induced RNA base ratio alterations but does not greatly inhibit normal cell RNA synthesis. When guanidine was added to HeLa cells several hours after poliovirus infection in order to inhibit virus-directed RNA synthesis, host cell-directed RNA synthesis was found to be drastically inhibited. It is suggested that this inhibition of host RNA synthesis may be the chief reason for the rapid cell destruction caused by poliovirus infection.

* This investigation was supported by a grant from the National Science Foundation.

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

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

[2] Holland, J. J., these PROCEEDINGS, **48**, 2044 (1962).

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

[4] Diamond, L., and M. E. Balis, *Virology*, **15**, 210 (1961).

[5] Loddo, B., *Boll. Soc. It. Biol. Sper.*, **37**, 395 (1961); Rightsel, W. A., J. R. Dice, R. J. McAlpine, E. A. Timm, J. W. McLean, Jr., G. J. Dixon, and F. M. Schable, Jr., *Science*, **134**, 558 (1961).

[6] Crowther, D., and J. L. Melnick, *Virology*, **15**, 65 (1961).

[7] Holland, J. J., B. H. Hoyer, L. C. McLaren, and J. T. Syverton, *J. Exp. Med.*, **112**, 831 (1960).

[8] Holland, J. J., *Biochem. Biophys. Res. Comm.* (in press).

Note added in proof: Baltimore, D., and Franklin, R. M., these PROCEEDINGS, **48**, 1383 (1962) have recently reported inhibition of host-controlled RNA synthesis in cells infected with mengovirus, another small RNA animal virus.

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*TRANSFORMATION INDUCED BY SIMIAN VIRUS 40 IN NEWBORN SYRIAN HAMSTER RENAL CELL CULTURES**

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In recent reports,[1-3] we have described transformation of human renal cells *in vitro* by simian virus 40 (SV40). Similar results were communicated shortly thereafter by Koprowski *et al.* using a different cell type.[4] This agent also has been found to induce sarcomas on subcutaneous inoculation into newborn (NB) Syrian hamsters.[5, 6] It became of interest, therefore, to determine whether SV40 would induce transformation in newborn hamster cells *in vitro*. Results of an experiment of this sort will be described.

Materials and Methods.—Virus: SV40 strain, VA 45–54 GMK 6/9/61, was originally obtained from M. R. Hilleman and passaged twice in human fetal renal cell cultures. Infectivity titer in grivet monkey kidney cultures was $10^{6.5}$ TID^{50}/0.1 ml.

Cultures: Newborn Syrian hamster renal cell cultures were obtained as confluent monolayers in screw-capped tubes from Microbiological Associates, Inc. Screw caps were replaced with rubber stoppers and cultures were thereafter treated as previously described[2] for maintenance of human renal cell cultures employed in experiments on transformation.

Viral inoculation and subcultivation of cells: Procedures were the same as previously described.[2]

Immunofluorescence techniques: Monolayers in Leighton tubes were fixed and stained by the indirect Coon's technique as previously described.[3]

Experimental.—Morphology and reproducibility of transformation: Ninety-four