Alteration in ribonucleic acid metabolism resulting from poliomyelitis virus infection of HeLa cells

Profound changes are observed in the nucleic acid metabolism of a clonal strain of HeLa cells (strain S3)[1] subsequent to infection with poliomyelitis virus and prior to the release into the medium of a significant fraction of the intracellular virus.

Suspension cultures of S3 cells were grown in 2-fold concentrated EAGLE's medium[2] supplemented with 5 % horse serum. A culture containing [2-^{14}C]cytidine (207,000 counts/min/μmole, 0.01 m*M* final concentration) was divided into 2 portions, one of which was infected with the Mahoney strain of poliomyelitis virus by the addition of 20–40 plaque-forming units per cell. Each cell could be shown to be infected at 1.5 h after the addition of virus by (1) the demonstration of each cell as an infective center, and (2) the inability of any cell in the infected culture to give rise to a clone[3]. Aliquots of cells from both cultures were removed at 0, 3, 6, 9, and 12 h for analysis.

The amount of RNA in the control cells increased progressively, showing a net change of 37 % in 12 h. On the other hand, infected cells showed no significant increase in RNA content during the first 6 h, and during the next 6 h showed a pronounced loss, amounting to 70 % of that initially present (Table I). During this same period, 6 to 12 h after infection, 20 % of the cell protein was also lost, but no DNA.

TABLE I

RNA NUCLEOTIDES IN CELL SUSPENSION

The cellular material was collected by centrifugation for 10 min at 1800 rev./min. The cold 5 % $HClO_4$-insoluble material obtained after extraction with alcohol and ether was treated with 1 *N* KOH for 18 h and the nucleotides were separated by paper electrophoresis[4]. The figures represent the total amount of the 4 nucleotides in μmoles/l suspension. Similar results have been obtained when RNA was estimated colorimetrically[5].

	Time (h)				
	0	*3*	*6*	*9*	*12*
Growing culture	20.7	21.7	25.5	27.6	28.3
Polio-infected culture	20.5	22.0	21.1	13.9	7.07

Though no significant net increase in the amount of RNA in infected cultures could be measured, there was a rapid and continued uptake of [2-^{14}C]cytidine into RNA as determined by the specific activities of cytosine and uracil. The pattern of uptake differed from that of normal cultures. Although the specific activities of RNA uracil were similar in normal and infected cultures, the specific activity of RNA cytosine was significantly lower in the infected cells at every time period studied. This pattern of incorporation is not peculiar to polio-infected cells, and may instead be characteristic of non-growing cells, as illustrated in Expt. 2 of Table II.

Infection produced an enhanced nucleotide pool which was first observed at 3 h, and which continued to increase until 9 h. Between 9 and 12 h there was a sharp loss of nucleotides from the pool. The increase of nucleotides in the acid-soluble pool is

Abbreviations: RNA, ribonucleic acid; DNA, deoxyribonucleic acid.

TABLE II

INCORPORATION OF [2-^{14}C]CYTIDINE* AFTER 6 h INTO THE RNA PYRIMIDINE BASES OF INFECTED, GROWING, AND NON-GROWING HeLa CELLS

The RNA bases were isolated by alkaline digestion of the defatted tissue followed by $HClO_4$ hydrolysis. Purification of the bases was as previous described[6]. When an amino acid essential for growth[2] (phenylalanine) was omitted from the medium, there was a complete inhibition of net synthesis of RNA, DNA, and protein.

	counts/min/μmole			
	Expt. 1		*Expt. 2*	
	Polio	*Growing*	*Non-growing*	*Growing*
Cytosine	12,880	17,920	9,533	15,550
Uracil	28,760	28,690	21,390	23,270

* Cytidine added to HeLa cell suspensions is rapidly converted to uridine. Incorporation studies with [2-^{14}C]uridine, and determination of the rate of cytidine deamination indicate that uridine is the true precursor in studies with [2-^{14}C]cytidine.

consistent with either a breakdown of RNA, an increased rate of synthesis of nucleotides, or a decreased rate of utilization.

Other workers have reported a large increase in RNA resulting from polio infection[7]. This has not been found in the present experiments despite the fact that we have consistently obtained high yields of infectious material by 12 h (350 plaque-forming units/cell). An increase in cellular RNA therefore is not necessary for the formation of virus, nor is it a necessary result of virus infection. We have also failed to observe a more rapid rate of turnover of either RNA or DNA in an infected culture as compared with a non-growing uninfected culture, as reported by MASSAB *et al.*[7]. Some of these discrepancies may reflect differences in the metabolic states of the cultures used.

Some of the effects observed in infected cultures parallel those seen in cultures where growth has been prevented by the omission of an essential amino acid (phenylalanine) from the medium. Under both conditions we have observed an inhibition of net synthesis of RNA (and DNA and protein) and a similar pattern of uptake of cytidine into the nucleic acid pyrimidines. Polio infection does however result in an enhanced nucleotide pool early in the infective cycle, and a rapid loss of RNA observed between 6 and 12 h. Neither of these changes is observed in non-growing cultures.

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