Preliminary study on the kinetics of ^{32}P incorporation into protein and ribonucleic acid of Hela cells infected with poliovirus

Preliminary studies on the kinetics of ^{32}P incorporation into proteins and nucleic acids have been made in the early phases of infection of Hela cells with poliovirus type 1. There are reports in the literature [1,2] on the later phases of virus infection of labelled cell cultures.

The Hela cells (obtained in 1953 through the kindness of Dr. J. T. SYVERTON) were maintained as usual[3]. The infection of the cells was carried out in monolayers or in a spinner culture[4], in the presence of a maintenance medium containing 2.3 mg P/l. The cells were removed from the containers by use of disodium ethylenediaminetetraacetate. The cultures were divided in equal amounts for inoculation with a placebo prepared from normal cells, and with Brunilda poliovirus type 1 in a multiplicity varying between 2 and 10. About 1 μC ^{32}P (obtained from Oak Ridge) was used per million cells.

The aliquot samples were spun at 1000 $\times$ g for 3 min at 0°, the supernatant medium discarded and the cell pellet frozen at —25°. Simultaneous samples for cell counts and virus determinations were also taken.

The samples were fractionated following the technique by TYNER[5]. After extraction of the nucleic acids, the protein sediment was exhaustively washed with water and acetone[6]. The absorbance of the dialyzed nucleic acids was measured in a Model DU Beckman Spectrophotometer, between 230 and 290 mμ. The measured ε(P) of RNA at 260 mμ was close to 9.650[7]. Complete DNA precipitation at acid pH was checked by the diphenylamine test[8], performed on the original mixtures and on the two resulting fractions. The protein fraction was free from nucleic acid as tested by the absorbance at 260 mμ or by the diphenylamine test. Paper chromatography showed the absence of labelled inorganic phosphate. Isotope counts were done in planchets and in a Geiger-Müller liquid counter.

At zero time the weights of proteins and RNA were respectively the same in both series, but the specific activity of the proteins in the infected cells was twice that of the control cells, a statistically significant difference (see Table I). However, the specific activity of RNA was about the same in the two series.

At 30 min the specific activity of the proteins had decreased and that of RNA increased in the infected cells.

At 60 min the specific activity of the proteins in the infected cells was slightly above the non-infected cell level, whereas the specific activity of RNA was significantly over that level. In such a system the RNA pool was found not to be homogeneous, as indicated by the different shape of the absorption spectrum of the RNA fraction from the infected cell (data to be published elsewhere).

At 120 min the specific activity of both substances had reached the values of the non-infected cell. At this time there seemed to be an increase in the amount of RNA per infected cell.

In these experiments, there was some increase of DNA specific activity in the infected cells beginning at 30 min, with reference to the level in non-infected cells.

Determinations of the number of virus plaque-forming units[9], showed that

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

TABLE I

PERCENTAGE RATIO OF SPECIFIC ACTIVITY IN PROTEINS AND RNA PER 10^7 CELLS OF INFECTED OVER NON-INFECTED CELL CULTURES

Number of expts.	*Chemical species*	*Time* (min)*	*Percentage ratio***		*Probability (t test)*
5	Protein	0	206	± 41	$P < 0.02$
2		30	166		
5		60	117	± 9	
5		120	106	± 10	
7	RNA	0	110	± 18	
3		30	232		
7		60	140	± 17	$P < 0.02$
6		120	109	± 7	

* At zero time, 8 min had elapsed after isotope and virus inoculation.
** The percentage ratio has been calculated as follows:

$$\frac{\text{Specific activity of infected cells} \times 100.}{\text{Specific activity of non-infected cells}}$$

Specific activity of the proteins = counts/min/mg protein.
Specific activity of RNA = counts/min/μg RNA.
The values given are mean ± standard error of the mean.

there was at 7 h an increase of about 100-fold over the value at the beginning of the experiments.

From the succession of events observed, there appears to be a precursor-like relation between the protein and RNA which shows an inversely related variation of their specific activities. From the protein sediment, we have separated two fractions[6] —the phosphatidopeptide, soluble in acid chloroform–methanol, and the insoluble phosphoprotein fraction. The weight of the former was about 10 times greater in the infected series at zero time, with lower specific activity than in the control series. The specific activity of the phosphoprotein fraction at zero time was 2–3 times greater in the infected cells. At 30 min the findings in these two fractions were reversed.

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