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Synthesis of Ribonuclease-Resistant Ribonucleic Acid by Fowl-Plague-Virus-Induced Ribonucleic Acid Polymerase

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An RNA-dependent RNA polymerase is induced in chick-embryo fibroblast cells infected with fowl-plague virus (Skehel & Burke, 1968; Scholtissek & Rott, 1969). The RNA synthesized by this enzyme *in vitro* has now been investigated and compared with RNA formed in infected cells *in vivo*.

Chick-embryo fibroblast cells grown in roller bottles were infected at an input multiplicity of 5–10 plaque-forming units/cell (Borland & Mahy, 1968). The microsomal fraction prepared from cells at 6 hr. after infection was incubated at 28° with a reaction mixture containing (μ moles/ml.): tris-HCl, pH 7.8, 250; 2-mercaptoethanol, 12; $MgCl_2$, 2.5; ATP, CTP and GTP, 0.15 each; [3H]-UTP, 0.005 (5 μ C). Incorporation into acid-insoluble radioactivity increased linearly with time of incubation up to 60 min. When samples of the reaction mixture taken at any time were treated with ribonuclease (1 μ g./ml. for 10 min. in 0.15M-NaCl–0.015M-sodium citrate buffer), only about 50% of the radioactivity was rendered acid-soluble. Labelled RNA extracted from the reaction mixture after 30 min. incubation with phenol–sodium dodecyl sulphate or phenol–cresol (Kirby, 1965) was 37% resistant to ribonuclease treatment. The ribonuclease-resistant fraction showed a sharp thermal transition at about 90° in 0.15M-NaCl–0.015M-sodium citrate buffer.

The polymerase product RNA sedimented in the 7–18s region on sucrose density gradients, and ribonuclease-resistant fractions were found at 12–14s. If the product RNA was treated with ribonuclease before sedimentation, all the acid-insoluble radioactivity banded sharply at 12s. To compare the RNA product obtained *in vitro* with RNA formed *in vivo*, cells were treated with actinomycin D (2 μ g./ml.) at 5.5 hr. after infection then pulse-labelled with [3H]uridine (10 μ C/ml.)

for 15 min. before extraction of RNA from the microsomal fraction. The bulk of the RNA labelled *in vivo* sedimented in the 18s region, i.e. in a similar position to RNA from purified fowl-plague virus (Barry & Davies, 1968), but 5–10% was resistant to ribonuclease and sedimented at 12s.

Hybridization experiments were carried out between 3H -labelled polymerase product RNA and ^{32}P -labelled fowl-plague-virus RNA. The virus RNA was completely susceptible to ribonuclease treatment, but became 37% resistant after mixing with polymerase product RNA under annealing conditions. The results of further annealing studies will be presented.

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The Use of Gradient Centrifugation to Study Events in the Life Cycle of Cultured Cells

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Exponentially growing P815Y neoplastic mast cells (Schindler, Day & Fischer, 1959) were briefly exposed to radioactive precursors, collected by centrifugation, washed and layered on to 40 ml. of a gradient (5–10%, w/v) of Ficoll in growth medium (Morris, Cramer & Reno, 1967). After centrifugation at 80g_{av} for 7 min. the tube was punctured and samples were collected. The number of cells in each sample, and their mean volume, was measured with a Coulter counter. Precursor incorporated into cells was determined by filtering a portion of each sample, washing with trichloroacetic acid and measuring the radioactivity.

The distribution of cells through the gradient showed a broad peak near the centre. The mean volume of cells near the top was about half that at the bottom. When thymidine was used as a precursor, the incorporated radioactivity/cell showed a broad maximum just below the cell peak, in agreement with the results of Morris *et al.* (1967). When proline, uridine or choline was used, the incorporated radioactivity/cell increased from the top to the bottom of the gradient.

When cells that had been separated by gradient centrifugation were resuspended in medium, they grew with a certain degree of synchrony; those from the top of the gradient divided after 10–15 hr. (mean generation time 15 hr.) whereas those from the bottom began division within 5 hr. Hence cells near the top of the gradient appear to be