The Viral Nucleic Acids formed in Chick-Embryo Cells Infected with Semliki Forest Virus

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When chick-embryo cells, previously infected with Semliki Forest virus, were treated with actinomycin and then pulsed with [³H]uridine before extraction of the nucleic acids with phenol plus sodium dodecyl sulphate, three species of RNA were found. These were single-stranded viral RNA, sedimenting at 45s in sucrose density gradients, and a 26s single-stranded species and a 20s double-stranded species, which were only partially resolvable on the gradients (Friedman, Levy & Carter, 1966; Son nabend, Martin & Mecs, 1967).

The three species were separable by electrophoresis in 2.5% polyacrylamide gels by using the system of Peacock & Dingman (1967), or by sequential extraction of the infected cells with phenol and then with phenol plus sodium dodecyl sulphate. Phenol extracted only the 20s double-stranded viral RNA, which was further purified by treatment with 4M-lithium chloride, to give a product that was about 75% resistant to ribonuclease (1 µg./ml. for 10 min. in 2 × SSC buffer), had a density in caesium sulphate of 1.635 g./cm.³ and a thermal transition temperature of 107°, and gave a single band on polyacrylamide-gel electrophoresis with a mobility intermediate between that of the 28s and the 16s ribosomal markers. After the phenol extraction, the 26s and 45s species were extracted with phenol plus sodium dodecyl sulphate. Both species had a density of 1.680 g./cm.³, were completely degraded by ribonuclease, and were readily separable by polyacrylamide-gel electrophoresis, the 45s species having a very low mobility and the 26s species a mobility slightly less than that of the 28s ribosomal marker. Attempts to demonstrate transfer of radioactivity from 20s RNA to 26s or 45s RNA by pulse-chase techniques were unsuccessful, but both ³H-labelled 26s and 45s RNA displaced radioactivity from ¹⁴C-labelled 20s RNA on heating to 110° and cooling. Short pulses showed that 26s RNA was the first single-stranded species formed. Comparison of the sedimentation velocities and electrophoretic mobilities of the 26s and 45s species, together with the sharp thermal transition of 45s to 26s at 60°, suggest that 45s RNA comprises two 26s RNA species, rather than these being different conformational states of the same molecule as suggested by Sreevalsan, Lockart, Dodson & Hartman (1968).

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The Use of Polyethylenesulphonate in the Fractionation of Proteins and some Aspects of its Application to the Separation of Chromatin Proteins

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Recent interest in this laboratory has centred on the biological importance of the non-histone proteins of chromatin (Paul & Gilmour, 1968; Gilmour & Paul, 1969) and a number of methods for their isolation are currently being developed (MacGillivray, Ingles & Krauze, 1969).

Chromatin proteins can be obtained in high yield in the supernatant after ultracentrifugation of chromatin dissociated in 2M-NaCl-5M-urea (Bekhor, Kung & Bonner, 1969). Since removal of the salt and urea from this supernatant causes precipitation of the proteins, we considered that the presence of the soluble polyanion polyethylenesulphonate (PES) during this process should lead to the selective precipitation of the histones, leaving the non-histone proteins in solution.

In model systems PES was found to be an effective precipitant of histones, maximum precipitation being dependent on the pH, urea concentration and the PES/histone ratio. However, at ionic strengths up to 0.1, maximum precipitation was independent of these parameters. Under somewhat different conditions of pH etc., serum albumin and globulin could also be precipitated with PES. Surprisingly, at low ionic strengths both albumin and globulin were maximally precipitated below pH 4, but PES could distinguish between these proteins, since on increasing the ionic strength globulin was precipitated at a more acidic pH. The ability of PES to fractionate a group of proteins was confirmed by using a mixture of histones, albumin and globulin. Histones were precipitated at pH 7.5 and 1.0-0.5, and then the ionic strength was increased to 0.4. Albumin was precipitated at pH 3.8-3.45 and globulin subsequently precipitated at pH 3.0.

Ultracentrifuge supernatants from calf thymus chromatin were concentrated tenfold by ultrafiltration. For the precipitation of histones the salt and urea concentrations were lowered to 0.1M and 0.25M respectively in the presence of PES at

pH 7.5. The non-histone proteins were then recovered from the supernatant with ammonium sulphate or alternatively were themselves precipitated with PES at pH 4. Electrophoresis at pH 2.4 showed the presence of a number of slow-migrating components together with a series of fractions with mobilities higher than those of histones. It is possible that the latter represent degradation products of chromatin proteins.

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The Effect of Feeding With a Tryptophan-Free Amino Acid Mixture on Albumin Synthesis by Rat Liver Slices

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The number of albumin-containing rat hepatocytes has been shown to decrease with decreasing protein content of the diet (Chandrasakharam, Fleck & Munro, 1967). Fleck, Shepherd & Munro (1965) observed an immediate loss of heavy polyosomes and a concomitant accumulation of oligosomes in rat liver after the animals were given an amino acid mixture lacking tryptophan (T- diet), compared with the polysome pattern of animals fed on the complete amino acid mixture (T+ diet). They also showed that the animal fed on the T- diet had a decreased uptake of DL-[1-¹⁴C]leucine by microsomes *in vitro* 1 hr. after feeding.

In view of these effects of the diets on liver protein synthesis it was of interest to investigate the short-term effects of the T+ and T- diets on albumin production by rat liver slices. Animals were starved for 18 hr. overnight, and then fed by stomach tube with the T+ or T- amino acid mixture (Wunner, Bell & Munro, 1966) and killed 1 or 2 hr. after feeding. Tryptophan alone was fed to animals in the 1 hr. group only. The tryptophan content of the diets corresponded to the original egg-albumin value and was thus a quarter of the amount used by Wunner *et al.* (1966). Liver slice preparation and albumin assays were performed by the methods of Campbell & Stone (1957).

Albumin production in the 1 hr. groups was linear for the first 1 hr. of incubation. There was a significantly greater ($0.05 > P > 0.025$) albumin production by the animals fed on the T+ diet at all

incubation times (0, 30 and 120 min.) except 60 min., although the rate of production was similar ($4 \mu\text{g.}/\text{min.}/\text{g. wet wt. of liver.}$). There were no differences between the 2 hr. groups and the production of albumin was linear for the first 30 min. of incubation. The group given tryptophan alone was not significantly different from the other 1 hr. groups. It thus seems likely that tryptophan is a limiting amino acid only when all the other essential amino acids are available for protein synthesis. Our results suggest that the amino acid supply acutely affects the synthesis of serum albumin by the liver.

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The Effect of Feeding with a Tryptophan-Free Amino Acid Mixture on Rat Liver Nuclear Ribonucleic Acid Nucleotidyltransferase

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The size of the rat liver nucleolus has been shown to be responsive to starvation and high-protein diets and changes in its size have been detected as early as 3 hr. after the animal had been given a high-protein diet (Lagerstedt, 1949). The nucleolus is the site of ribosomal RNA synthesis in rat liver (Steele, Okamura & Busch, 1965), and Siebert (1966) has shown that the nuclear Mg^{2+} -activated RNA nucleotidyltransferase (EC 2.7.7.6) is probably situated entirely in this organelle. Widnell & Tata (1966) have suggested that this enzyme synthesizes ribosomal RNA.

As the morphological evidence indicates that the nucleolus is effected by diet, the activity of the nuclear Mg^{2+} -activated RNA nucleotidyltransferase was examined after the feeding of a complete amino acid mixture (T+ diet) and the feeding of the same mixture with tryptophan omitted (T- diet) (Wunner, Bell & Munro, 1966). The tryptophan content of the diets corresponded to the original egg-albumin value and was thus a quarter of the amount used by Wunner *et al.* (1966). The enzyme assay was based on that given by Widnell & Tata (1966), and enzyme activity was determined by the initial velocity during the first 3 min. of the incubation. It was expressed as pmoles of [³H]-GTP incorporated/min./mg. of DNA.

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