Short Communications

The Pattern of Synthesis of Ribonucleic Acid Species under the Action of Spermine in the Chick Embryo

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Several workers have reported numerous effects of polyamines in a large variety of biological systems ranging from bacteria (Cohen & Raina, 1967; Abraham, 1968) to the chick embryo (Caldarera, Moruzzi, Rossoni & Barbiroli, 1969) and the rat ventral prostate (Caldarera, Moruzzi, Barbiroli & Moruzzi, 1968; Pegg, Lockwood & Williams-Ashman, 1970).

As far as nucleic acid biosynthesis in the chick embryo is concerned, it has been shown that it is possible to modify their concentrations (Caldarera, Barbiroli & Moruzzi, 1965) and the rate of incorporation of labelled precursors into the nucleic acids (Moruzzi, Barbiroli & Caldareira, 1968) by causing corresponding modifications of the concentrations of the polyamines. Nevertheless the nature of the changes in the pattern of RNA synthesis has not been investigated in detail.

In the present studies RNA was isolated from spermine-treated and -untreated whole chick embryos at the eighth day of incubation, after the injection of a labelled precursor of RNA. Embryos were obtained from White Leghorn $\times$ New Hampshire fertilized eggs from a commercial source. The isolated RNA was fractionated on columns of kieselguhr coated with methylated serum albumin and the distribution of radioactivity in the various RNA fractions was determined.

Embryos were divided into two groups and 1 h before they were killed $50 \mu\text{Ci}$ (in 0.2 ml of 0.9% NaCl) of [^3H]uridine (specific radioactivity 27.7 Ci/mmol) was injected into the air space. At 45 min before they were killed the test group received in the same way 0.1 ml of a 2.5 mM solution of spermine chloride in 0.9% NaCl, and the control group received the same volume of 0.9% NaCl.

Four embryos from the control and from the test groups were freed from the yolk sac and the extra-embryonic membranes and pooled separately for the isolation of RNA.

RNA was extracted by the hot-phenol method of Warner, Soeiro, Birnboim, Girard & Darnell (1966) with the modification used by Billing, Barbiroli & Smellie (1969). Methylated-albumin-kieselguhr

chromatography was performed as described by Billing & Barbiroli (1970).

Fractions (2 ml) were collected and their extinctions at 260 nm were measured. The RNA in each fraction was precipitated by the addition of 0.5 ml of 50% (w/v) trichloroacetic acid and collected on 25 mm Millipore filter membranes of 0.22 μm pore size. After a washing with 5% (w/v) trichloroacetic acid the membranes were dried at 50°C and placed in scintillation vials with 10 ml of toluene-based scintillator [0.5% (w/v) 2,5-diphenyloxazole].

Radioactivity was measured with a Nuclear-Chicago model 725 liquid-scintillation spectrometer and corrections for quenching were made by using the channels-ratio method. The average recovery of radioactivity from methylated-albumin-kieselguhr columns was 98%.

Fig. 1(a) shows the elution pattern for RNA from the control group and Fig. 1(b) shows the pattern obtained from embryos treated with spermine.

No significant differences occur in the distribution of u.v.-absorbing material from the two groups. However, there is an increase in total radioactivity of RNA recovered from the test group of 290% above that in the control. The greatest change is found in the sodium dodecyl sulphate-eluted RNA, which shows an increase in radioactivity of 330% over the controls, and in the Q_2 -RNA peak, which shows an increase in radioactivity of 320%. Smaller increases of about 200% occur in the radioactivity of Q_1 -RNA and ribosomal RNA peaks.

The present results show that the injection of spermine into the air space of fertilized eggs at the eighth day of incubation results 45 min later in an increased incorporation rate of the labelled precursor into all RNA species. The magnitude of the increase is much higher for the DNA-like species (Q_2 -RNA and sodium dodecyl sulphate-eluted RNA peaks), showing a preferential influence of spermine on this type of RNA. It is noteworthy that these species of RNA have been included in the mRNA fraction by Yoshikawa-Fukada, Fukada & Kawade (1965).

Fig. 1. Chromatography of whole chick-embryo RNA on columns of kieselguhr coated with methylated serum albumin. (a) Elution pattern of E_{260} and ^3H radioactivity for the RNA from four pooled control whole chick embryos at the eighth day of incubation; 43 E_{260} units were applied to the column. Controls received 50 μCi of [^3H]uridine 60 min before they were killed and 0.1 ml of 0.9% NaCl 45 min before they were killed. (b) Elution pattern of E_{260} and of ^3H radioactivity for the RNA from four pooled spermine-treated whole chick embryos at the eighth day of incubation; 43 E_{260} units were applied to the column. Test embryos received 50 μCi of [^3H]uridine 60 min before they were killed and 0.1 ml of a 2.5 mM-spermine solution in 0.9% NaCl 45 min before they were killed. —, E_{260} ; ----, ^3H radioactivity. Abbreviation: SDS, sodium dodecyl sulphate. The various RNA species are as defined by Billing & Barbiroli (1970).

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