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RNA Biosynthesis during Development of Chick Embryo (38160)

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Recent studies (1) have shown that the incorporation of labeled uridine into RNA is affected by different variables which make it difficult to obtain accurate results about the actual changes in the rate of RNA synthesis. We have measured (1) some of these variables and have found that it is possible to correct the incorporation data by these parameters obtaining more precise indications on the rate of RNA biosynthesis.

On this ground, the present research was undertaken to study the rate of synthesis of the single RNA species as separated by methylated albumin-kieselguhr [1] column chromatography from the third to the twelfth days of incubation. This has been shown to be a very useful technique for fractionating species of RNA (2, 3). Although the individual 18-S and 28-S r-RNA components are not well separated from each other, there is separation between most of the DNA-like RNA (D-RNA) and the r-RNA. Therefore this allows a comparison to be made between the amount of label in r-RNA with that in D-RNA.

If we consider that at least part of D-RNA can be looked at as a regulatory species (4, 5), the detailed study of the synthesis of the single RNA classes enables us to gain some insights into the molecular mechanism responsible for growth and differentiation.

Methods. Embryos. Embryos were obtained from White Leghorn × New Hampshire fertilized eggs obtained from a commercial source and incubated at 39° in a relative humidity of 65%. On every day of incubation studied, 20 μCi of 5-[^{3}H]-uridine (27.7 Ci/mmole, NEN chemicals GmbH, Frankfurt, Germany) were injected in a volume of 0.2 ml of sterile saline into the air space, and the embryos used 60 min later. Embryos were freed from the extraembryonic membranes and yolk sac, washed with cold 0.9% NaCl, blotted dry with absorbent tissue, and weighed.

RNA preparation. The method of RNA extraction was essentially that of Warner *et al.* (6), with hot phenol and SDS, with the modifications previously employed (7). The RNA was precipitated from the pooled aqueous fractions by the addition of sodium acetate to give a final concentration of 2% (w/v), followed by 2 vol of cold ethanol. After at least 30 min at −20° the RNA was collected by centrifugation and extracted with ether three times, and the alcoholic supernatant was stored and the radioactivity measured.

Chromatography on MAK columns. The MAK column used had internal diameter 1.5 cm and consisted of three layers as described by Mandell and Hershey (8). It was surrounded by a jacket so that the temperature could be controlled. The column was eluted with a linear 0.4–1.5 *M* NaCl gradient in 0.02 *M* sodium phosphate buffer, pH 6.7 at 35° at a flow rate of 60 ml/hr. After all the RNA that could be eluted with salt had been removed, the tenaciously bound RNA was removed by eluting first at 35° and then at 80° with 0.2% SDS in 0.4 *M* NaCl in phosphate buffer (2, 3). Fractions (1 ml) were collected and A_{260} of alternate tubes was measured.

Radioactivity. Fractions from the column were pooled in pairs, 50 μg of carrier RNA was added and 50% (w/v) trichloroacetic acid was added to give a final concentration of 10% (w/v). The RNA was collected on Millipore filter membrane (0.22 μm pore

[1] Abbreviations: MAK, methylated albumin—Kieselguhr; D-RNA, DNA-like RNA; TD-RNA, tenaciously bound DNA-like RNA.

size) and washed with 5 ml of 10% trichloroacetic acid. The membranes were placed in scintillation vials and dried at 50°. Then 10 ml of toluene-based scintillation fluid [0.5% (w/v) 2.5-diphenyloxazole] was added and the radioactivity was measured with a Nuclear-Chicago liquid-scintillation spectrometer.

Aliquots from the alcoholic supernatant (0.5–1.0 ml) were mixed with 10 ml of the toluene-based scintillator and the radioactivity measured. The average recovery of radioactivity from MAK columns was 95%.

Results. Figure 1 reports the pattern of the incorporation of labeled uridine into total RNA as extracted by the hot phenol method (continuous line). There is a dramatic fall of RNA specific radioactivity from the third to the twelfth days of incubation, except for the sixth, seventh, and eighth days in which we have found constant values.

The pattern of total uptake of the label (alcohol-soluble plus alcohol-insoluble dpm) is also reported in the same figure by a dashed line. We have found a rapid decrease from the third to the eighth days of incubation which slows down from the eighth to the twelfth.

The dotted line of same figure reports the RNA specific radioactivity referred to the total uptake of label. An almost constant rate of incorporation of labeled uridine into RNA is found at all stages of development studied except for around the eighth day of incubation.

Figure 2 reports a typical elution pattern of RNA extracted by the hot phenol method and fractionated on MAK columns (6 days old embryos). The same amount of O.D. was applied to the column at every day of incubation studied, so that comparisons could be made between the different patterns of radioactivity obtained at different stages of development.

Q_1-RNA has been shown (2) to be the precursor form of ribosomal RNA, and to have a sedimentation coefficient of 45 S. The D-RNA, (DNA-like, A-U-rich RNA) is eluted from the MAK column as two main fractions. One which is eluted toward the end of NaCl concentration gradient is thought to have a sedimentation coefficient of about 50 S. It has been termed Q_2-RNA. The other D-RNA fraction is tenaciously bound to the column after salt elution, but can be removed with a solution of sodium dodecyl sulphate (2). This fraction has been termed TD-RNA and according to Ellem (2) is heterogeneous in nature having a mean sedimentation coefficient of 16–18 S. It has also been shown (7) that Q_2-RNA is a precursor of at least part of TD-RNA.

Figure 3 reports the pattern of incorpora-

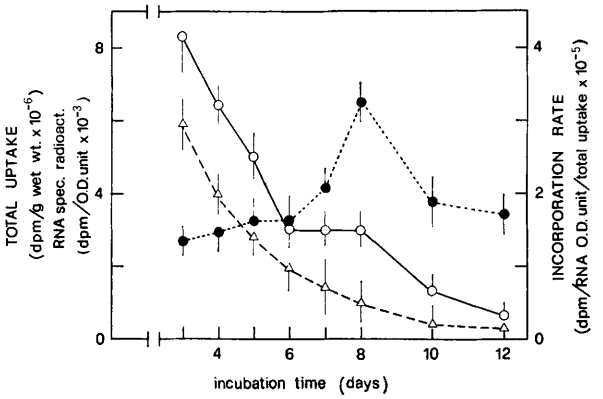

FIG. 1. Incorporation of 5-[^{3}H]-uridine into whole chick embryo total RNA, as extracted by the hot phenol method, as a function of age. Embryos were pulsed with 20 μCi of 5-[^{3}H]-uridine for 60 min. Its incorporation into total RNA was evaluated as described in the Methods section. Left-hand ordinate: O—O, RNA specific radioactivity (dpm/O.D. unit $\times$ 10^{-3}); △---△, total uptake (dpm/g wet wt $\times$ 10^{-6}) defined as alcohol-soluble plus alcohol-insoluble counts; right-hand ordinate: ●····●, incorporation rate (dpm/RNA O.D. unit/total uptake $\times$ 10^{-5}). Data represent the average ± SEM of three determinations performed on pooled embryos (2–8).

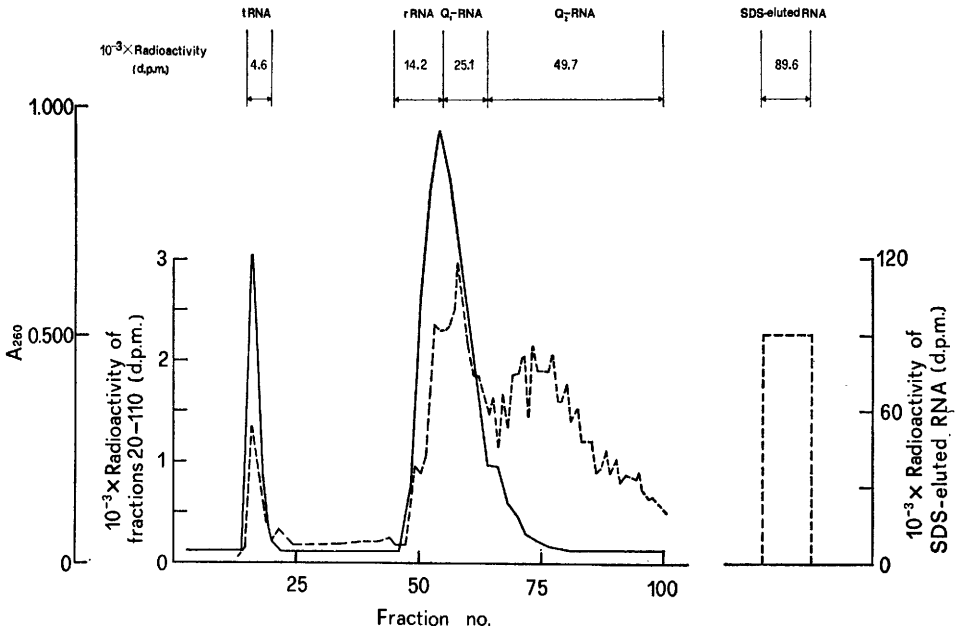

FIG. 2. Chromatography of whole chick embryo RNA on column of Kieselguhr coated with methylated serum albumin (6 days old embryos). 20 μCi of 5-[^{3}H]-uridine were injected into the air space and the embryos pulsed for 60 min. 40 O.D. units of RNA extracted by the hot phenol method (from pooled embryos), as described in Methods, were applied to the column. —, A_{260}; ---, ^{3}H radioactivity. Abbreviations: SDS, codium dodecyl sulphate. The definitions for the various RNA species are reported in Results.

tion of labeled uridine over a period of 60 min into ribosomal, Q_1-RNA, and t-RNA species in relation to the total uptake of the label (defined as incorporation rate). Q_1-RNA does not show large variations during the period of development studied, except for the twelfth day of incubation in which a definite decrease is found.

On the contrary, r-RNA shows a decrease between the third and the sixth days of incubation followed by a large peak of the incorporation rate at the eighth day and a rapid fall thereafter. As far as t-RNA is concerned, it has been found a constant corporation rate of uridine through the first 8 days of incubation, while a definite increase is found at day 10.

In Fig. 4 is reported the pattern of the incorporation of the labeled precursor into Q_2- and TD-RNA species during the same stages of development. TD-RNA shows one peak of incorporation at day 7 of incubation, while Q_2-RNA has two definite separate peaks: one between the fourth and the fifth days of incubation and a second one at the eighth day.

Discussion. The study of the biosynthetic rate of the single RNA species during chick embryo development has been performed after extraction by the hot phenol method. First of all we have studied the incorporation of labeled uridine into total RNA as extracted by the same method, in order to ascertain whether its pattern was following the one previously found (1) for the acid-insoluble material. The results are almost overlapping with those previously reported (1) (see Fig. 1, continuous line).

On the other hand, one of the major problems found in the *in vivo* study of the rate of synthesis of RNA has been the difficulty of distinguishing between the changes that may occur in the sizes or specific activities of the precursor pool, and the changes of the actual rate of synthesis of nucleic acids. A perfect solution to this problem has not been achieved yet. Nevertheless it has been shown (1) that the incorporation of labeled uridine into acid-insoluble material depends on the transport

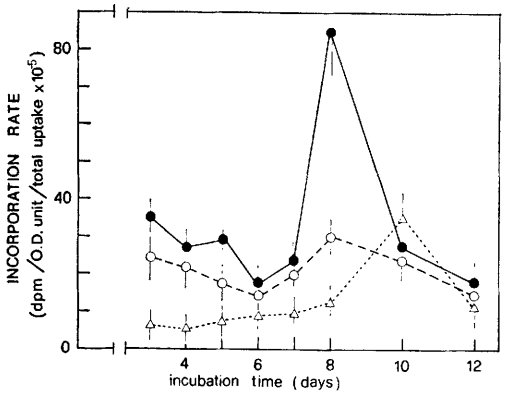

FIG. 3. Incorporation rate of 5-[^{3}H]-uridine into Q_1-RNA, r-RNA, and t-RNA species in the chick embryo as a function of age. The single RNA species have been extracted by the hot phenol method and fractionated on MAK columns as described in the Methods section. The specific radioactivity of the single RNA species has been corrected by the total uptake of labeled precursor into the whole chick embryo in order to obtain what we have defined as the incorporation rate (dpm/O.D. unit/total uptake $\times$ 10^{-5}). Data represent the average of three determinations on pooled embryos (2-8) $\pm$ SEM O---O, Q_1-RNA; ●—●, r-RNA; △···△, t-RNA.

and availability of the label itself. Therefore we have examined the behavior of the total uptake of label defined as the alcohol-soluble plus the alcohol-insoluble counts (Fig. 1, dashed line). This represents the dynamic result of the main parameters that can affect the incorporation of the labeled precursor into RNA (i.e., transport and availability). We have used the total uptake of label to correct the values of RNA specific radioactivity. When the incorporation of labeled uridine into total RNA is expressed in relation to the total uptake of label (Fig. 1, dotted line) we obtain a pattern which is almost identical to that of the incorporation of label into the acid-insoluble material corrected by the UTP specific radioactivity (1).

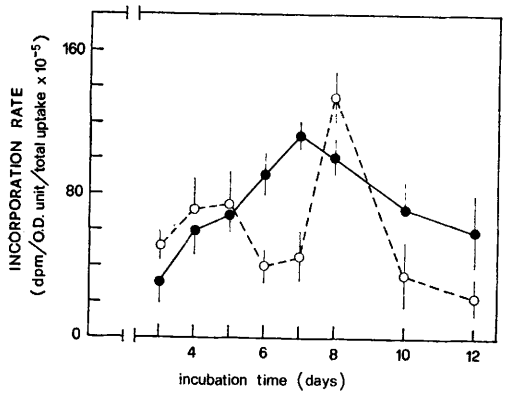

FIG. 4. Incorporation rate of 5-[^{3}H]-uridine into Q_2-RNA and TD-RNA species in the chick embryo as a function of age. For experimental details see legend to Fig. 3 and Methods. Data represent the average of three determinations on pooled embryos (2-8) $\pm$ SEM O---O, Q_2-RNA; ●—●, TD-RNA.

Following these preliminary results, we have studied the synthesis of the single RNA species in the same stages of development as separated by methylated albumin-Kieselguhr column chromatography.

The results obtained for all species of RNA have been corrected for the changes of total uptake. Although we cannot be certain that all species of RNA are synthesized from the same pool of ribonucleotide precursors, it seems reasonable to assume this in absence of evidence to the contrary.

When this assumption is made it is evident that any changes in ribonucleotide pools would affect the labeling of all species of RNA to a similar extent.

The most evident result obtained from the analysis of the rate of synthesis of Q_1-RNA and r-RNA species (Fig. 3) is the quantitatively different behavior found at the eighth day of incubation. In fact both RNA species show a rather similar pattern for all the other stages of development studied.

Q_1-RNA is believed to represent a precursor form of r-RNA (2, 3), and therefore the differential increase of r-RNA over and above the increase of Q_1-RNA represents (1) an increased rate of synthesis of the precursor, and (2) a parallel increased rate of maturation of these species. This becomes evident as an accumulation of radioactivity in the mature form.

This increased rate of synthesis of r-RNA species at the eighth day of incubation seems to be responsible for the increased concentration of total RNA which occurs at the ninth day of incubation (9). At the same time the decreased concentration of total RNA between the third and the sixth days of incubation (9) is more understandable after the present results. In fact the bulk of cellular

RNA is represented by the ribosomal species.

The behavior of t-RNA, peaking at day 10, leads us also to think that its biosynthetic rate follows the increased concentration of maure ribosomes actively engaged in protein synthesis.

As far as the D-RNA species is concerned, it should be pointed out that only a small amount of DNA-like RNA is found in the cytoplasm of the cells in which it has been studied, while a large amount of rapidly turning over D-RNA is found in the nucleus where it seems to be confined (10–12). It has also been shown that at least part of the Q_2-RNA is a precursor form of TD-RNA (7). At the present time the function of nuclear D-RNA is unknown, but it has been postulated that D-RNA is involved in the control of m-RNA synthesis (4, 5). If this is true then its level of activity may be expected to change between different metabolic states or between different differentiation stages. Very little work has been published yet on this aspect of D-RNA synthesis. However it has been observed a higher incorporation of labeled precursors into D-RNA during the resting periods, or non-growing states (13, 14). This would indicate that D-RNA may be required to maintain the resting state of cells and perhaps its differentiated state. If this is the case, the two peaks found in the incorporation rate of label into Q_2-RNA and TD-RNA at the fourth to fifth days and at the eighth day of incubation indicate that the development of chick embryo does not proceed linearly from the first day of incubation to the hatching, but alternates periods of differentiation to periods of growth.

Summary. The incorporation of 5-[^{3}H]-uridine into the single RNA species of chick embryo has been studied between the third and twelfth days of incubation.

Ribosomal RNA precursor, Q_1-RNA, shows an almost constant rate of synthesis during the stages of development studied. r-RNA shows a decreased rate of incorporation of the label from the third to the sixth days, and a large peak at the eighth day of incubation.

DNA-like RNA species show a large peak of the incorporation rate at the seventh day of incubation (TD-RNA) and at the eighth day (Q_2-RNA). Another separate peak is shown by Q_2-RNA at the fourth to fifth days of incubation, while a shoulder is found at the same period in the pattern of TD-RNA.

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