The appearance and quantitation of cytoplasmic ribonucleic acid in the early chick embryo

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SUMMARY

This paper seeks to extend our knowledge about RNA synthesis in early embryogenesis to the domestic fowl, Gallus domesticus. Using this species for research, apart from increasing our knowledge of higher vertebrate embryology, has certain advantages such as rapid uptake of isotopic precursors and ease of microdissection in culture.

The following results are presented:

1. The cell number in the whole chick embryos is shown to be increasing logarithmically between the time of laying and the early neurula stage; with a doubling time of 7.4 h.

2. The onset of ribosomal RNA synthesis has been shown to be during mid-cleavage of the chick embryo, while development is taking place in the oviduct and uterus of the mother.

3. In a cumulative labelling experiment, embryos were labelled at the unincubated-egg stage, allowed to develop to various morphological stages up to neurulation, and their cytoplasmic RNA prepared and analysed by gel electrophoresis.

4. The specific activity of the precursor pool for RNA synthesis was measured at several stages, using the same labelling conditions, and the results were used to quantitate the RNA synthesis from the incorporated radioactivity.

5. Using these techniques, it was found that newly synthesized cytoplasmic RNA accumulates steadily in the whole chick embryo, reaching a level of 104 µg by the early neurula stage. On a per cell basis, however, the amount of newly synthesized cytoplasmic RNA seems to decrease slightly.

These findings are discussed in the light of present knowledge about embryos of other vertebrates and certain invertebrates.

INTRODUCTION

This paper seeks to extend recent work on ribonucleic acid (RNA) metabolism in early embryos (see Davidson, 1969, for review) to that of the domestic fowl Gallus domesticus.

There seems to be no obvious phylogenetic pattern to the easily measurable parameters concerning RNA metabolism in early embryos, e.g. time of onset of ribosomal RNA (rRNA) synthesis. This first becomes visible in profiles of embryonic, radioactively labelled RNA at a variety of times in different species (see Davidson, 1969, for table). However, there must be an evolutionary basis

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for these molecular events. Things likely to determine the start of rRNA syn-
thesis, e.g. amount of ribosome stockpiling during oogenesis, or quantity of
available nutrient, are presumably themselves the results of natural selection.

A preliminary study of RNA synthesis in early avian embryos (Lerner, Bell &
Darnell, 1963) has demonstrated that stable RNA is already being synthesized at
the onset of mesoderm formation. This paper demonstrates that the start of
rRNA synthesis is sometime during mid-cleavage of the embryo. RNA synthesis
during the first 24 h of incubation is studied by labelling with radioactive uridine.
Newly synthesized cytoplasmic RNA is then prepared and analysed quantita-
tively and qualitatively at morphological stages up to and including the begin-
ning of neurulation. The absolute amounts of RNA synthesis are calculated by
measuring the specific activity of the nucleotide precursor pool. The number of
cells in the chick embryo, at each of the stages used, has been measured in order
to quantitate the RNA synthesis per cell.

MATERIALS AND METHODS

(1) Labelling of embryos. Newly laid, fertilized, White Leghorn eggs were
inoculated 'in ovo' with high specific activity (29 Ci/μM) [5-3H]uridine (Radio-
chemical Centre, Amersham) in isotonic buffered saline (Pannett & Compton,
1924). The radioactive solution, 10–20 μCi in 0.1 ml, was injected into the yolk
beneath the blastoderm. The embryos were allowed to develop for the required
time in a humidity-controlled incubator at 37.5 °C. They were then explanted
into ice-cold saline, staged carefully under a dissecting microscope, pooled, and
stored at −70 °C.

Embryos at pre-laying stages were removed from the oviducts and uteri of
first-year laying White Leghorns. Some of these were at such an early stage of
development that neither shell nor shell membrane had been applied to the egg
surface. In these cases the yolk, together with adherent albumen, was incubated
in sterile saline at 37.5 °C and the isotope injected beneath the cleaving embryo
as above.

(2) RNA Preparation. The frozen batches of pooled embryos (usually from
3–12 embryos/batch) were thawed into a Tris buffer (TEP₂ = 0.01 M Tris,
10⁻³M EDTA, 20 μg/ml polyvinyl sulphate, buffered to pH 7.2 with HCl) con-
taining 1 % (w/v) of the non-ionic detergent Brij 58. The lysate thus obtained
was spun at 10000 rev/min for 10 min on a Misco centrifuge to remove nuclei,
mitochondria, cytoplasmic membranous debris, and as much yolk as possible.
To the supernatant, sodium dodecyl sulphate (SDS) was added to a final con-
centration of 1 % and the preparation warmed to 37 °C for 3 min. Cold phenol
extractions were carried out until a clear interphase was obtained, followed by
further extractions with 1 % iso-amyl alcohol in chloroform. The aqueous phase
was purified from low-molecular-weight impurities by gel filtration through
a 5 ml G 50 Sephadex column. The low-molecular-weight fraction was kept for
further analysis and the RNA fraction was analysed by gel electrophoresis on either 1.8 % or 4 % agarose gels (Evans, 1969).

(3) Chick-embryo cell counts. The method used was that of Solomon (1957). Batches of 2–3 embryos were explanted at various times after the start of incubation, and homogenized in 0.5 ml of ice-cold 1 % citric acid; 0.5 ml of 1 % aqueous methyl green was then added and the staining mixture left on ice for 15 min. The cell nuclei were centrifuged off in a bench centrifuge and the pellet resuspended in a known volume of tap-water. The nuclei were counted in a Neubauer haemocytometer.

(4) The specific activity of the precursor pool. This was measured in batches of 20–50 embryos labelled at stage 1 (Hamburger & Hamilton, 1951) with 5 μCi [3H]uridine each, and incubated to various stages up to stage 6. The frozen batches of embryos were thawed into 0.32 M sucrose, 0.003 M magnesium chloride, buffered to pH 7.2 with bicarbonate, and containing 1 % (w/v) Brij 58. The nuclei were spun from the lysate and the supernatant was made to 5 % trichloracetic acid. 5'-Nucleoside monophosphates were prepared by the method of Brown & Littna (1966) by hydrolysing the di- and tri-phosphates with n-HCl, while adsorbed on to active Norit A (Hopkin & William).

Two methods, using column chromatography, were used to separate the four 5'-mononucleotides.

(i) Anion exchange using QAE Sephadex, was found to give good separation of 5' UMP. The following parameters were found to give the best results:

<table>
<thead>
<tr>
<th>Sephadex</th>
<th>QAE A25 anion exchange</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column</td>
<td>20 × 0.9 cm</td>
</tr>
<tr>
<td>Starting buffer</td>
<td>0.025 M CO₃/HCO₃ buffer, pH 9</td>
</tr>
<tr>
<td>Salt gradient</td>
<td>300 ml 0–0.4 M sodium chloride in starting buffer, convex gradient</td>
</tr>
<tr>
<td>Pump speed</td>
<td>36 ml/h</td>
</tr>
</tbody>
</table>

(ii) Separation of 5'-CMP from the other mononucleotides was achieved by the method of Blattner & Erickson (1967), using a Dowex formate column and elution at 0.5 ml/min with 0.1 M ammonium formate pH 3.2. The column length was 45 cm.

RESULTS

Cell counts in early chick embryos

The results of this experiment are presented in Fig. 2. The number of cells in the embryos is seen to be increasing logarithmically over the period studied, with a mean doubling time of 7.5 h.
Using the methods described, the specific activities of 5'-UMP and 5'-CMP were measured at various times over a period of 24 h after a single injection of [3H]uridine at stage 1 (Hamburger & Hamilton, 1951). Fig. 1 shows two sample separations of 5'-mononucleotide mixtures using both QAE Sephadex and Dowex. As well as the four 5' monophosphates normally found (arrowed in Fig. 1) there are several unlabelled peaks whose nature is unknown. Table 1 gives the specific activities calculated from separations of this type. Fig. 1 shows that 5'-UMP and 5'-CMP are labelled whereas 5'-AMP and 5'-GMP are not. This suggests the conversion of the injected uridine into cytidine.

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**Table 1**

<table>
<thead>
<tr>
<th>Morphological stage</th>
<th>No. of embryos</th>
<th>Incubation (h)</th>
<th>5'-CMP Specific activity</th>
<th>5'-UMP Specific activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>47</td>
<td>5</td>
<td>6.78 x 10^3</td>
<td>1.65 x 10^3</td>
</tr>
<tr>
<td>2</td>
<td>46</td>
<td>9.7</td>
<td>3.92 x 10^3</td>
<td>0.85 x 10^3</td>
</tr>
<tr>
<td>3</td>
<td>29</td>
<td>15</td>
<td>5.55 x 10^3</td>
<td>0.79 x 10^3</td>
</tr>
<tr>
<td>4</td>
<td>30</td>
<td>22</td>
<td>7.12 x 10^3</td>
<td>0.67 x 10^3</td>
</tr>
<tr>
<td>5</td>
<td>28</td>
<td>23</td>
<td>1.67 x 10^3</td>
<td>—</td>
</tr>
<tr>
<td>5</td>
<td>12</td>
<td>25</td>
<td>—</td>
<td>0.303 x 10^3</td>
</tr>
<tr>
<td>6</td>
<td>22</td>
<td>25</td>
<td>3.01 x 10^3</td>
<td>0.32 x 10^3</td>
</tr>
</tbody>
</table>
RNA metabolism in the early chick embryo

Table 2

<table>
<thead>
<tr>
<th>Stage</th>
<th>Incubation (h)</th>
<th>Ratio 5'-CMP:5'-UMP</th>
<th>Specific activity of 5'-mononucleotide pool = (UMP+CMP)/4</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5</td>
<td>0.143</td>
<td>2108.8</td>
</tr>
<tr>
<td>2</td>
<td>9.7</td>
<td>0.165</td>
<td>1092.5</td>
</tr>
<tr>
<td>3</td>
<td>15</td>
<td>0.192</td>
<td>1580.3</td>
</tr>
<tr>
<td>4</td>
<td>22</td>
<td>0.171</td>
<td>1944.2</td>
</tr>
<tr>
<td>5</td>
<td>23</td>
<td>0.085</td>
<td>467.1</td>
</tr>
<tr>
<td>6</td>
<td>25</td>
<td>0.109</td>
<td>835.1</td>
</tr>
</tbody>
</table>

Log\(_{10}\) number of nuclei x 10\(^3\) per embryo

Specific activity (cpm/\(\mu g\)) x 10\(^3\)

![Graph Fig. 2](image)

**Fig. 2.** The number of cells per embryo. Each point represents the number of isolated, stained nuclei per embryo in batches of two or three embryos.

**Fig. 3.** The change in specific activity (cpm/\(\mu g\)) of the 5'-monophosphate pool, following a single dose of [\(^3\)H]uridine at the unincubated egg stage.

These results demonstrate therefore the conversion of uridine into cytidine nucleotides in the early chick embryo.

5'-GMP was always found to be in minute quantities or not demonstrable at all in these experiments (see Fig. 1a), suggesting that it may be a rate-limiting step in the biosynthesis of RNA in these early embryos. In calculating the specific activity of the overall 5'-nucleotide pool and therefore that of the RNA made from it, it was assumed that a negligible amount of radioactivity enters the

nucleotides. This is further suggested by the fact that there is always less 5'-CMP found in these preparations than 5'-UMP, and its specific activity is always higher. There is also a fairly constant ratio of 5'-CMP:5'-UMP in these preparations (see Table 2).
RNA via adenine or guanine nucleotides, since these were both found to be unlabelled in these experiments. It is also assumed that cytidine and uridine nucleotides contribute approximately 25% each to the RNA synthesized. The specific activity of the 5' nucleotide pool will therefore roughly equal half of the average specific activity of CMP and UMP (i.e. $\frac{1}{2}(\text{CMP} + \text{UMP})$) (see Table 3).

The change in this overall figure is plotted in Fig. 3. The specific activity of the 5'-mononucleotide pool was found to change very little during the first 24 h of embryogenesis.

**The onset of stable RNA synthesis in chick embryos**

In this experiment, two batches of embryos were taken from the oviducts and uteri of adult hens. The first batch were taken from the upper oviducts; no shell or shell membrane had been applied to the albumen-covered embryo. They were therefore considered to be 6 h old or less (Patterson, 1910). The second batch was taken from the uteri and had both shell and shell membrane attached. The embryos were incubated *in ovo* with 50 $\mu$Ci each of [5-3H]uridine for 3 h, and their cytoplasmic RNA prepared. Morphological controls from both batches were fixed and micrographs of these are shown in Fig. 4. This shows that the embryos of the first batch are in early cleavage, with about ten and 100 cells respectively. The second batch have finished cleavage and are at the stage of epiblast and hypoblast formation. Note the prominent nucleoli in these embryos (Fig. 4).

Gel-electrophoresis profiles of the cytoplasmic RNA from these two batches of embryos are presented in Fig. 5. There is very little RNA synthesis in the early cleavage embryos (Fig. 5a) and this slight incorporation is spread hetero-

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**Table 3**

<table>
<thead>
<tr>
<th>Developmental stage</th>
<th>No. of cells</th>
<th>Doubling time</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>At laying</td>
<td>$0.74 \times 10^4$</td>
<td>7.4 h</td>
<td>Emanuelsson, 1965</td>
</tr>
<tr>
<td>18–20 h</td>
<td>$2.0 \pm 1.0 \times 10^4$</td>
<td>—</td>
<td>Richenbacher, 1956</td>
</tr>
<tr>
<td>0–18 h</td>
<td>—</td>
<td>8.0 h</td>
<td>Emanuelsson, 1962</td>
</tr>
<tr>
<td>18–20 h</td>
<td>$1.93 \times 10^4$</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>33 h</td>
<td>$4.06 \times 10^4$</td>
<td>10.7 h</td>
<td>Woodard, 1948</td>
</tr>
<tr>
<td>48 h</td>
<td>$9.07 \times 10^4$</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>72 h</td>
<td>$3.5 \times 10^4$</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>
RNA metabolism in the early chick embryo
geneously along the gel. There is no increased incorporation into the rRNA regions of the gel, which were shown up by the use of high optical density, unlabelled cytoplasmic RNA from 15-day chick-embryo liver.

The level of radioactivity in Fig. 5(a) was demonstrated statistically to be over the background level by use of the Student's $t$ test. For this purpose the gel was considered to be divisible into two parts: that between the origin and the 4S region containing RNA, and that beyond the RNA containing only natural radioactivity of the agarose. A series of bottles containing only scintillation fluid was first counted for 40 min/bottle. Gel slices were then added and the bottles recounted for the same time. The groups of results were then compared as populations using the Student's $t$ test as follows: (1) RNA-containing gel v, (2) empty bottles, (3) beyond the 4S region v, (4) empty bottles. If there is RNA present in these gels, the probability ($P_a$) that populations (1) and (2) are identical will be much lower than the probability ($P_b$) that populations (3) and (4) are identical. This was in fact the result obtained: For Fig. 5(a) $P_a = 0.0005$, $P_b = 0.01$.

Fig. 5(b) demonstrates that by late cleavage or delamination a large proportion of the incorporated radioactivity is found in the stable RNA types: about 62% of the counts per minute (cpm) found in electrophoresis gel.

It can be concluded therefore that synthesis of the stable RNA types starts sometime during cleavage in the chick, while the embryo is still in the hen, and at least 7–10 h before mesoderm formation starts.

**Synthesis of cytoplasmic RNA during the period of embryogenesis between laying of the fertilized egg and neurulation**

In this experiment embryos were labelled with [5-3H]uridine at the laid, unincubated egg stage; and incubated for various lengths of time before explanting and preparing their cytoplasmic RNA.
RNA metabolism in the early chick embryo

Electrophoresis profiles of two of these preparations are shown in Fig. 6(a, b). It can be seen that in one electrophoresis period (about 2.5 h) on 1.8% gels the 28 S and 18 S rRNA species are well separated from each other but the 5 S and 4 S species are not. Electrophoresis of another sample of the same RNA preparation on 4% agarose gels, however, allows a complete separation of 5 and 4 S RNA (Fig. 6c).

The stable RNA species are being synthesized and transported to the cytoplasm at all the stages studied, up to 26 h after the start of incubation, i.e. the
neurula stage. Some kinetics of this process were studied by measuring such parameters as the radioactivity incorporated into the RNA and into different components of RNA at different times after labelling.

(i) Quantity of RNA synthesized and transported to the cytoplasm

This is shown as the radioactivity incorporated into the cytoplasmic RNA per embryo and per cell after different labelling periods (Fig. 7a, b). On a per embryo basis the incorporation continues to rise throughout the labelling period,
Fig. 8. Quantities of various cytoplasmic RNA components synthesized (a) per embryo and (b) per cell; after a single dose of [³H]uridine at the unincubated-egg stage.

whereas on a per cell basis there is an initial rise up to the 10 h stage, i.e. the appearance of the primitive streak, followed by a gradual decline over the next 16 h.

These progress curves reflect the quantity of RNA appearing in the embryonic cell cytoplasm during the first 26 h of incubation. However, the specific activity of the precursor pool does not remain constant over this period, but falls off slowly (Fig. 3). Therefore the radioactivity incorporated at later stages represents proportionally more RNA synthesis than that at early stages. The absolute amounts of RNA synthesis were calculated from the equation:

$$\mu g \text{ RNA synthesized during time } 0- t = \frac{\text{RNA cpm at time } t}{\text{average specific activity over time } 0- t \text{ in cpm/µg}}.$$  

The progress curve of the quantity of RNA (in µg) synthesized per embryo (Fig. 7c) shows that this rises steadily to reach a level of about 100 µg by the onset of neurulation. Expressing the results on a per cell basis (Fig. 7d) shows that the level of newly synthesized cytoplasmic RNA rises steeply from the 5 h stage to about 0·2 ng RNA/cell at the 10 h stage and falls off slowly for the rest of the labelling period.

The separation of the embryonic RNA by gel electrophoresis allows quantitation by the same process, to be applied to each component of the RNA. Since the heterogeneous RNA is obviously not fractionated by this procedure, this is
all lumped together as one class. Fig 8 shows the appearance of newly synthesized RNA components in the embryonic cytoplasm and their accumulation during the 26 h labelling period.

Considerable variation is seen in these progress curves, which may reflect the *in vivo* situation or the range of biological or experimental scatter. This scatter is probably located primarily at the level of uptake of uridine into the cellular low molecular weight pool. When this source of scatter is eliminated by expressing the RNA radioactivity as a percentage of the low molecular weight pool radioactivity, most of the variation is eliminated (Fig. 9). The straight-line graph suggests that there is a cumulative increase in the amount of labelled precursor taken up into RNA from a precursor pool, which is kept fairly constant (Fig. 7).

(ii) *The change in relation to each other of the stable components*

This is demonstrated by calculating the cpm under each stable RNA peak, over the level of heterogeneous labelling in the gel profiles; and plotting these as percentages of the total RNA cpm (Fig. 10). Three points from this graph bear further discussion.

(a) Initially the percentage of the total cytoplasmic RNA in 18 S is greater than that in 28 S. This situation then reverses until the ratio of 28S:18S is about 2:1. This suggests, especially in the light of previous work (see Discussion) that the 18S species of rRNA enters the cytoplasm before 28S, during the biosynthesis of early chick embryo ribosomes.

(b) The proportion of the total cytoplasmic RNA occupied by each stable RNA species reaches a plateau level by about 10 h of incubation. If the percentage of the total cytoplasmic RNA occupied by all the stable RNA species is
RNA metabolism in the early chick embryo

Fig. 10. The percentage, plotted against time, of the total RNA radioactivity occupied by the stable RNA components, following a single dose of [3H]uridine at the un-incubated egg stage.

Fig. 11. The percentage, plotted against time, of the total RNA radioactivity occupied by the sum of the stable RNA components, during the cumulative labelling period.

Fig. 12. Stable RNA radioactivity minus heterogeneous RNA radioactivity in the embryonic cytoplasm during the cumulative labelling period.

Fig. 13. Ratio of 5S:28S RNA radioactivity in the embryonic cytoplasm during the cumulative labelling period.
plotted against incubation time (Fig. 11) this too rises to a plateau level at about 10 h. This plateau is not horizontal however – a fact which is demonstrated by plotting the difference between stable and heterogeneous RNA radioactivity. The resulting graph (Fig. 12) shows a continual increase of the stable RNA over the heterogeneous RNA level.

(c) The proportion of the total cytoplasmic RNA occupied by both 5S and 4S RNA are more or less constant throughout the labelling period. The 28S RNA, however, rises to a plateau level. This means that the 5S:28S ratio in the cytoplasm drops over the labelling period (Fig. 13). Presumably therefore the 28S and 5S species become associated in the cytoplasm during the formation of the large ribosomal subunit.

**DISCUSSION**

Despite certain obvious disadvantages, e.g. large pool size and manipulative difficulties, the avian embryo is probably the most amenable of the higher vertebrate embryos to a molecular study of this kind, e.g. the embryo grows initially as a flat disc, which, combined with its successful growth *in vitro* (New, 1955) makes micro-dissection relatively simple.

**Cell counts**

Surprisingly few studies of cell number and cell generation time have been carried out on chick embryos at this early age. Results taken from the literature can be seen in Table 3.

The chick embryo develops for about 22 h in the oviduct of the hen (Olsen, 1942; Patterson, 1910). In order to achieve the number of cells seen at laying (see above), the average doubling time must be about 1-4 h. It does not maintain this average during the pre-laying stages; the generation time falls off to 7-4 h just before laying (Emanuelsson, 1965). The results presented here suggest that a doubling time of 7-5 h is maintained for the first 24 h, when the number of cells reaches about $6 \times 10^5$ and then falls off to about 10-7 h (Woodard, 1948).

**The onset of stable RNA synthesis**

This has already been demonstrated to be under way at the onset of mesoderm formation in the chick embryo (Lerner, *et al.* 1963), i.e. about 7 h after incubation of the laid egg. This last-mentioned paper has been misinterpreted in the literature (Davidson, 1969) to mean that rRNA is being synthesized 4–18 h after fertilization. In fact, there is no previously published study which demonstrates synthesis of rRNA before the early primitive streak stage, i.e. gastrulation, an event which takes place about 27 h after fertilization. This paper demonstrates that rRNA and 4S RNA synthesis and transport to the cytoplasm are occurring many hours before this, while the embryo is still undergoing cleavage in the oviduct or uterus of the hen. This approximately coincides with the time of nucleolar appearance in the chick embryo (Bechtina, 1960).
Among the vertebrate species studied, this time of onset of stable RNA synthesis is morphogenetically earlier than that seen in amphibians (Brown & Littna, 1964; Waddington & Perkowska, 1965) and fishes (Aitkhizin, Belitsina & Spirin 1964); but later than that in mammals (Mintz, 1964; Ellem & Gwatkin, 1968; Woodland & Graham, 1969; Piko, 1970). It has to be remembered, however, that the times of onset cited above are from a very small number of species, which tend to be assumed to represent typically the class of vertebrates to which they belong. The comparison between chick and mouse embryos is by no means accurate, since it rests on evidence from only six chick embryos. It is also open to the criticism that the synthesis of small quantities of rRNA may be obscured by heterogeneous RNA synthesis (Emerson & Humphreys, 1970). Repeated gel electrophoresis of the 28 S area, using a non-radioactive marker 28 S rRNA, would probably elucidate this point.

It would be interesting to look for the start of rRNA synthesis in other vertebrates to see if any phylogenetic basis for this phenomenon could be established. For example, the earlier time of onset of rRNA synthesis in chick embryos may reflect the fact that the store of maternal ribosomes in the fertilized egg is not as great as in the amphibian species cited above, or in the echinoderm species studied (Gross, 1967).

It would be interesting for this reason to see if rRNA synthesis in the chick oocyte proceeds on amplified rDNA, and to measure the store of maternal ribosomes in the mature egg. This work is already progressing in this laboratory.

**Precursor pool size in the early chick embryo**

Although these were measured primarily to quantitate the RNA synthesis from the incorporation of [3H]uridine, there are two interesting points to be made from them: (1) the rapid conversion of uridine to cytidine in the chick embryonic cells; (2) the vanishingly small pool of guanosine phosphates in early chick embryo cells. This fact has also been reported in *X. laevis* embryos (Brown & Gurdon, 1966) and may indicate that the passage of guanosine across the cell membrane, or its de novo synthesis within the cell, represents a quantitative control mechanism for RNA synthesis. Its very small pool size suggests that guanosine may be a more efficient isotopically labelled precursor than uridine in RNA studied. In fact, further experiments are already demonstrating this (C. C. Wylie, in preparation).

**The appearance of newly synthesized cytoplasmic RNA from laying to neurulation**

The newly synthesized cytoplasmic RNA per embryo, after a lag period of about 5 h, rises linearly to reach a level of about 104 μg by the onset of neurulation (Fig. 7 c). On a per cell basis, however (Fig. 7 d), the initial dramatic rise is followed by a slow decline. This presumably reflects the fact that after about 6 h of incubation the rate of cell production becomes greater than the rate of accumulation of newly synthesized RNA. This fact has been reported recently
in certain echinoderms (Kijima & Wilt, 1969). Whether this change represents an embryo-wide phenomenon or reflects differentiation within the embryo is an interesting problem which can be tackled in the chick embryo due to the ease of micro-dissection (C. C. Wylie, in preparation). The lag period in appearance of cytoplasmic RNA from 0 to 6 h probably represents the time taken for the egg to warm up in the incubator to a temperature at which development can proceed.

The variation seen in these progress curves has been shown to be largely a variation in the passage of labelled precursor across the membranes into the embryonic cells. Whether this is a rate-limiting step (i.e. a quantitative control mechanism) in the synthesis of nucleic acids is a subject requiring further investigation. The case of guanosine is particularly interesting. The alternative is simply that it is a process subject to considerable experimental variation, especially in view of the fact that small batches of embryos (4–12 embryos in each) have been used in this study.

There are large variations between different species of RNA appearing in the embryonic cytoplasm during this labelling period. The total cytoplasmic RNA graph is made up of numerous species of RNA whose proportions are seen to be shifting in relation to one another (Figs. 8, 10) as the labelling period increases.

(1) The relationship of the 28S and 18S rRNA is as expected in the light of their known biosynthetic pathway (Vaughan, Warner & Darnell, 1967). the 18S being the first to appear in the cytoplasm, followed by the 28S rRNA, which then rises to a value double that of the 18S (Fig. 10).

(2) The 5S rRNA component, known to be associated with the 28S molecule in the structure of the larger ribosomal subunit (Knight & Darnell, 1967), maintains a more or less constant percentage of the total cytoplasmic RNA (Fig. 10). It therefore does not maintain a constant relationship with the 28S rRNA, which would be expected if these two molecules were either (a) transcribed molecule for molecule from their respective genes and/or (b) associated in the nucleus and transported together to the cytoplasm. The 5S and 28S molecules become associated in the cytoplasm rather than the nucleus during the formation of the large ribosomal subunit, therefore, in the early chick embryo. These results do not demonstrate whether the genes for 28S rRNA and 5S rRNA are functionally linked in chick embryos, i.e. whether there is co-ordinate synthesis of these two RNA types. This is thought to be so in prokaryotes (Roschenthaler et al. 1969) but not in HeLa cells (Knight & Darnell, 1967), L-cells (Perry & Kelley, 1968), Drosophila melanogaster (Tartof & Perry, 1970), Xenopus laevis oocytes (Ford, 1971) and X. laevis embryos (Abe & Yamana, 1971).

(3) The heterogeneous RNA shows wide fluctuations in quantity over the labelling period (Fig. 8), which seem to be fairly independent of the stable RNA components. It shows a relative excess at some times compared with the 28S rRNA and a relative deficiency at others. Work is in progress to demonstrate whether these represent large-scale changes in populations of messenger RNAs
or are extremes of practical scatter. This will only be established after careful repetition, together with pulse-chase experiments.

I wish to thank Professor J. Z. Young, F.R.S., Dr Ruth Bellairs and Dr Martin Evans for stimulating and helpful discussion during the course of this work. Expert technical help was provided by Miss M. McCrossan and Miss M. McNeill. Grateful thanks are also due to the Medical Research Council, who supported me financially during the course of this work.

REFERENCES


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