Determination of the Nucleoside Triphosphate Contents of Eggs and Oocytes of *Xenopus laevis*

By H. R. WOODLAND and R. Q. W. PESTELL*

Department of Zoology, University of Oxford, South Parks Road, Oxford OX1 3PS, U.K.

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The ribonucleotide and deoxyribonucleotide contents of eggs and oocytes of *Xenopus laevis* were measured. Eggs contained most deoxyribonucleotide in the form of triphosphates. dCTP, dTTP, dATP and dGTP were present in similar amounts. The egg contained sufficient deoxynucleotide triphosphate to make approximately 2500 nuclei. Oocytes contained less pyrimidine deoxyribonucleoside triphosphates than did eggs, and purine deoxyribonucleoside triphosphates were not detected. These differences may be correlated with the ability of eggs to induce nuclear DNA synthesis, a property not shown by oocytes. Both oocytes and eggs seem to contain non-phosphorylated, α -unsubstituted aldehydes, which may be deoxyribose derivatives. Eggs and oocytes contain similar amounts of ribonucleoside triphosphates. The low rate of RNA synthesis found in eggs, but not in oocytes, is therefore not caused by simple precursor control.

In Amphibia the conversion of an ovarian oocyte into an egg involves a total change in nucleic acid metabolism, for the oocyte makes only RNA and the zygote only DNA. Since in these cells the activity of the nucleus has been shown to be under cytoplasmic control [see review articles by Gurdon (1968) and Gurdon & Woodland (1968)] the comparison of oocytes and eggs is likely to be especially helpful in analysing the control of DNA and RNA synthesis. One possible way in which a gross change in nucleic acid synthesis might be mediated is through the cellular content of DNA and RNA precursors. The amount of DNA and its precursors in the eggs and embryos of frogs has been a matter of controversy for many years (see review by Grant, 1965). The confusion about DNA content has been clarified by Dawid (1965, 1966) and by Baltus *et al.* (1968), who showed that most of the high-molecular-weight DNA of the egg was mitochondrial, or else associated with the yolk platelets. The nature and content of low-molecular-weight deoxyribose derivatives is still uncertain. In the present paper we report the determination of the amounts of nucleoside triphosphates in the eggs and oocytes of the toad *Xenopus laevis*. We have determined these molecules because they are generally believed to be the precursors of DNA and RNA.

Experimental

Materials

Chemicals. These were all obtained from the sources indicated by Pestell & Woodland (1972).

Biological material. Female *X. laevis* were kept and induced to lay eggs as described by Gurdon (1967a).

* Present address: Department of Zoology, University of California, Berkeley, Calif. 94720, U.S.A.

Oocytes and eggs were handled in standard saline modified from that of Barth & Barth (1959), and containing 88 mM-NaCl, 1 mM-KCl, 2.4 mM-NaHCO₃, 15 mM-tris-HCl buffer, pH 7.6, and 0.01 g each of sodium benzylpenicillin and streptomycin sulphate/l. Unfertilized eggs were de-jellied immediately after laying by using a solution of 1% papain and 2% cysteine hydrochloride in the standard saline, which was adjusted to pH 8.0 with NaOH (Brown, 1967). They were frozen and stored at -70°C.

Ovaries were removed from females killed by decapitation. The lobes of the ovary were dissected open and the oocytes released by agitation in the cysteine-papain solution. As the oocytes were released they were transferred to the standard saline solution. The large oocytes were separated from the other sizes by passing the saline solution upward between them in a 2.5 cm-diam. chromatography column. As the flow rate is increased, progressively larger oocytes are removed. The oocytes were stored at -70°C.

The number of oocytes or eggs accumulated by these procedures was estimated in one of two ways. In the first, the volume of packed cells in the sample was measured and compared with the volume of samples of a known number of cells. In the second, lots of 100 oocytes or eggs were stored and homogenized, as for the main batch. They were dried down at 100°C overnight, as were portions from the main sample. The number of oocytes or eggs in the sample was calculated from the dry weights.

Methods

Extraction. The frozen cells were thawed out in 0.5 M-HClO₄ containing a known quantity of [³H]-dTTP (13 Ci/mol) or [³H]dATP (4 Ci/mmol). They

Fig. 1. Fractionation of deoxyribonucleotides from eggs

This was done by Dowex-1 chromatography of the periodate-methylamine-treated acid-soluble extract of 24000 unfertilized eggs by using a 2-litre linear gradient from 0 to 1.0M-HCO₂NH₄. The wash fractions are not included. (a) U.v. extinction at 260nm (○) and 280nm (●); the positions of dCTP, dTTP, dATP and dGTP are indicated by C, T, A and G respectively. The other peaks have not been fully identified, but the substances that eluted between dTTP and dATP have spectra characteristic of adenine derivatives. (b) Fluorescence in arbitrary units after the 3,5-diaminobenzoic acid reaction. The fractions between about 150 and 300 ml contained a substance, originating from the periodate-methylamine treatment, which interfered with fluorescence measurements. (c) Radioactivity (c.p.m.) of 0.5 ml samples from each fraction. [³H]dTTP was added to the original homogenate to act as a marker and to enable the recovery of nucleotide to be estimated.

were then homogenized in a Teflon-glass homogenizer and centrifuged at 3000g for 15min at 0°C. The supernatant was removed and neutralized with KOH at 0°C. The precipitate of KClO₄ was removed by centrifugation at 3000g for 10min. The supernatant was then extracted successively with equal volumes of ether, chloroform and ether, with centrifugation to separate the two phases. Ether was removed from the final aqueous phase by aeration.

Destruction of ribonucleotides. A portion (one-tenth or one-fifth) of the sample was removed at this stage for analysis of total nucleotides. The 2'- and 3'-unsubstituted ribonucleotides of the remainder were destroyed by the method developed in the work described in the preceding paper (Pestell & Woodland, 1972). The resulting solution was used for column chromatography immediately, or stored at -70°C overnight.

Chromatography and assay of deoxyribonucleotides. The extract was run on to a column (1cm x 50cm) of Dowex-1 (formate form) prepared as described by Hurlbert *et al.* (1954). It was washed with deionized water until no further u.v.-absorbing material was eluted, then in all experiments except one it was eluted with a 2-litre linear gradient from 0 to 1M-NH₄HCO₃. One total nucleotide fraction was separated by the method of Hurlbert *et al.* (1954). A column (30cm x 2cm) of Dowex-1 (formate form) was eluted by the gradient system described by these authors. Batches (500ml) of the following solutions were used in the reservoir: 4M-HCO₂H; 4M-HCO₂H-0.3M-HCO₂NH₄; 4M-HCO₂H-0.6M-HCO₂NH₄; 4M-HCO₂H-1.2M-HCO₂NH₄. In both sorts of separation fractions of about 9ml were collected by counting drops. Samples (0.5ml) were taken from each tube, 0.05ml of 100% formic acid was added to neutralize the bicarbonate and the liquid was dissolved in 14ml

of scintillation fluor, containing 625ml of toluene, 375ml of 2-ethoxyethanol, 4g of 2,5-diphenyloxazole and 0.1g of 1,4-bis-(5-phenyloxazol-2-yl)benzene/litre. The volume of the remaining fraction was measured to enable the total radioactivity per tube to be calculated.

The fractions from chromatography were freeze-dried and the NH₄HCO₃ was removed by sublimation *invacuo* at 60°C. U.v.-absorption spectra of peak tubes were measured after dissolving the contents in 1ml of water. The derivatives of deoxyribose were then determined by the procedure described in the preceding paper (Pestell & Woodland, 1972). This involved bromination and reaction with diaminobenzoic acid.

Injection of oocytes and analysis of thymidine phosphorylation. This was performed by the procedures described by Woodland (1969).

Results

Determination of deoxyribonucleotide pools in eggs

Our procedure destroys all nucleotides with unsubstituted 2'- and 3'-hydroxyl groups and causes the purine or pyrimidine moiety of the molecule to be washed straight through the column (Pestell & Woodland, 1972). [³H]dTTP was added to the homogenate and was eluted predominantly in the dTTP position. Therefore the procedure resulted in very little breakdown of pyrimidine triphosphate, as shown by Fig. 1(c). The small amounts of [³H]-thymidine, [³H]dTMP, [³H]dTDP and other unidentified radioactive substances were shown to be present in the original radiochemical stock in similar proportions. A similar experiment with oocytes showed that [³H]dATP was also stable in this procedure. All of the radioactivity added to the egg homogenate was recovered from the column.

Table 1. Deoxyribonucleotide content of eggs and oocytes

Results with eggs are from the chromatography of an extract of 24000 unfertilized eggs, shown in Fig. 1. Results with oocytes (Expt. A) are from the chromatography of an extract of 19000 oocytes taken from two female toads 3 days after they had laid eggs; the chromatography is represented in Fig. 2. Results with oocytes (Expt. B) are from the chromatography of 12000 oocytes taken from one toad a week after it had laid eggs; the chromatographic separation was similar to that in Fig. 2 in all respects, except that [³H]dATP was added to the homogenate, rather than [³H]dTTP. In the experiment with eggs there was sufficient u.v. absorption to calculate the content of the deoxynucleotide peaks by u.v. absorption as well as fluorescence. This was not possible in the experiments with oocytes.

Type of cell	Method of measurement	Content (pmol/cell)			
		dATP	dCTP	dGTP	dTTP
Eggs	<i>E</i> ₂₆₀	13	19	12	12
	Fluorescence	13	16	11	9
Oocytes					
Expt. A	Fluorescence	<2	8	<1	7
Expt. B	Fluorescence	<1	2	<1	7

Table 2. *Phosphorylation of [³H]thymidine by oocytes*

Females 1 and 2 are the two toads which provided oocytes for experiment A in Table 1, and the chromatography shown in Fig. 2. From each toad 30 oocytes were injected with [³H]thymidine at 1 mCi/ μ l (specific radioactivity 22.4 Ci/mmol) and the cells incubated for 10 h at 21°C. The HClO₄-soluble extract was fractionated on a column (10 cm $\times$ 1 cm) of Dowex-1 (formate form) with a 200 ml linear gradient from water to 4 M-formic acid–1.2 M-HCO₂NH₄, as described by Woodland (1969). Insufficient [³H]dTTP was formed for it to be measured accurately.

Toad no.	% of total radioactivity as		
	[³ H]Thymidine	[³ H]dTDP	[³ H]dTTP
1	56	10	33
2	63	7	30

About nine main peaks of u.v.-absorbing material occurred in the eluate (Fig. 1a). The first of these contained substances which interfered with the 3,5-diaminobenzoic acid reaction, and probably were produced in the periodate–methylamine reactions. It is therefore not known whether or not deoxyribose was present in this region. Substances which react to give very much of the appropriate fluorescence are found only in four regions. These were at the salt concentrations expected of dCTP, dTTP, dATP and dGTP, and each possessed the expected u.v.-absorption spectrum. The amounts of deoxyribonucleoside triphosphate recovered from each egg are shown in Table 1. There is good agreement between the amounts calculated by fluorescence and u.v. extinction at 260 nm. It may be concluded that there are similar amounts of each triphosphate, and that this class of nucleotide seems to constitute the major part of the deoxyribonucleotide pool.

Determination of deoxyribonucleotide pools in oocytes

The oocytes used for nucleotide determinations were taken from females that had laid eggs either 3 days or 1 week previously. Oocytes of this sort were used because it is known that the thymidine $\rightarrow$ dTTP phosphorylation pathway is very active in them (Woodland, 1969), even though they do not induce injected nuclei to synthesize DNA (Gurdon, 1967b). We suspected that they were more likely to contain deoxyribonucleoside triphosphates than were oocytes from females that had not laid recently, for in these oocytes the thymidine phosphorylation pathway is not active. We justify this choice on the grounds that our interest in the nucleotides of oocytes mainly concerned whether deoxynucleotides are ever present in cells that do not make DNA.

The particular oocytes we used were tested for their ability to convert [³H]thymidine into [³H]dTTP by the method of Woodland (1969), which involves injecting [³H]thymidine into the cells, extracting the

HClO₄-soluble products and chromatographing them on Dowex-1 (formate form) by using a formic acid–ammonium formate elution system. The results of such an experiment (Table 2) shows that about one-third of the injected [³H]thymidine is converted into [³H]dTTP. Such a high proportion is not found if oocytes are used from a female that has not laid recently. In oocytes of this sort less than 5% of the injected [³H]thymidine is converted into [³H]dTTP. These oocytes were judged less likely to contain deoxyribonucleoside triphosphates than those from females that had just laid.

Fig. 2(a) shows that oocytes from females that had laid recently contained various substances, which after periodate treatment were eluted as peaks from a Dowex column. They corresponded in general to those extracted from unfertilized eggs, but there were no discrete extinction peaks in the regions expected for deoxyribonucleoside triphosphates. Even so, the fluorimetric assay (Fig. 2b) showed two small peaks in the positions expected for dCTP and dTTP, the latter being eluted with [³H]dTTP. The amounts of these pyrimidine triphosphates in each oocyte are shown in Table 1. A maximum possible value for the purine triphosphates is given, although we have no evidence that there is any at all. The apparent lower content of pyrimidine triphosphates that we have estimated to be present in oocytes, as compared with eggs, might mean that there is indeed less in every cell; alternatively it might mean that some cells contain the same amount as eggs and others much less.

There are two possible reasons why the low deoxyribonucleoside triphosphate contents estimated for oocytes might be artifacts. One is that the triphosphates were destroyed during processing and storage. To check that this did not occur the experiments were performed with [³H]dTTP and with [³H]dATP added to the initial homogenate, as described in the section on eggs. In both cases the recovery of radioactivity was high, and there was a similar proportion of radioactive nucleoside mono-, di- and tri-phosphate as in the original radiochemical stock. It therefore seems

that both deoxypyrimidine and deoxypurine triphosphates in the original homogenate are recovered with high efficiency. In addition, oocytes and egg extracts were processed in the same way, and thus should be subject to the same artifacts. However,

although the chemical procedure used in experiments on oocytes was identical with that for eggs, the method used to isolate the cells was rather different. A limited amount of enzyme digestion and considerable agitation in saline solution was necessary to

Fig. 2. Fractionation of deoxyribonucleotides from oocytes

This was done by Dowex-1 chromatography of the periodate-methylamine-treated extract of 19000 oocytes, chromatographed in the same way as the egg extract (Fig. 1). (a) U.v. extinction at 260 nm (○) and 280 nm (●). (b) Fluorescence in arbitrary units after the 3,5-diaminobenzoic acid reaction. (c) Radioactivity (c.p.m.) of 0.5 ml samples from each fraction. [^3H]dTTP was added to the original homogenate. The radiochemical sample used contained a lower proportion of [^3H]dTTP than that used for eggs.

Table 3. *Ribonucleoside triphosphate content of eggs and oocytes*

The content of ribonucleotides is calculated from the u.v. absorption at 260nm. The u.v.-extinction profile of the eggs (Expt. A) is shown in Fig. 3, and of the oocytes in Fig. 4.

Type of cell	No. of cells	Gradient system used for column elution	Content (pmol/cell)			
			ATP	CTP	GTP	UTP
Egg						
Expt. A	390	NH ₄ HCO ₃	900	200	200	610
Expt. B	1570	NH ₄ HCO ₃	1030	270	470	360
Expt. C	14000	Formate	740	230	200	500
Oocyte	2090	NH ₄ HCO ₃	610	180	150	290

Fig. 3. *Fractionation of total nucleotides from eggs*

This was done by Dowex-1 chromatography of an acid-soluble extract of 1570 unfertilized eggs. The sample was washed on to the column with 450ml of water and then eluted with a 2-litre linear gradient from 0 to 1.0M-HCO₂NH₄. The second peak of the wash contains hypoxanthine and/or inosine, the third peak contains guanine. The positions of CTP, UTP, ATP and GTP are indicated by C, U, A and G respectively. The extinction was measured at 260nm (o) and 280nm (●).

separate the oocytes from ovarian stroma, and then further agitation occurred when the largest oocytes were separated from other size classes. These procedures might have produced considerable leaching or destruction of deoxynucleotides. Such phenomena seem unlikely to have had a significant effect, for similar amounts of ribonucleotides (on a per-cell basis) were extracted from oocytes as from unfertilized eggs (Table 3, and the next section). It seems unlikely that the deoxyribonucleotides should be much more susceptible to degradation or leakage than the ribonucleotides.

Measurement of ribonucleoside triphosphate pools in eggs

Samples taken from the extracts intended primarily for deoxyribonucleotide analysis were chromatographed without periodate-methylamine destruction of the ribonucleotides. Typical results are shown in Figs. 3 and 4. A considerable amount of u.v.-absorbing material passes straight through the column. A large proportion of this material is free guanine, and a little less is free hypoxanthine or inosine. These purines are present throughout development (H. R. Woodland, unpublished work). The most abundant

Fig. 4. Fractionation of an acid-soluble extract of 2090 oocytes

Details are as given for Fig. 3.

nucleotide derivative is eluted in the position expected for ATP. There are also peaks of u.v.-absorbing material in the positions expected for CTP, UTP and GTP; the substances present have the expected spectra and the putative UTP is co-eluted with [^3H]UTP. The results obtained by column chromatography were confirmed by H. R. Woodland and S. E. Ayers (unpublished work). Nucleotides extracted from eggs were chromatographed on polyethyleneimine t.l.c. plates by the first procedure of Neuhaard *et al.* (1965). ATP, GTP and UTP were found to be the polyphosphates present in largest amounts. These compounds were identified by their mobilities on the plates, fluorescence under alkaline and neutral conditions, phosphorescence at 77°K and by their spectra when eluted. There was insufficient dCTP for it to be identified with certainty.

Figs. 3 and 4 show that triphosphates are the most abundant ribonucleoside derivatives. We have also fractionated total nucleotides on Dowex-1 (formate form) by using the formic acid elution system of Hurlbert *et al.* (1954). This method produces a different order of elution of nucleotides from that produced by the NH_4HCO_3 system, but as in the NH_4HCO_3 system most of the u.v.-absorbing materials are eluted in the nucleoside triphosphate position.

The amounts of ribonucleoside triphosphates per cell are shown in Table 3. Roughly similar amounts are present in eggs and in oocytes, and in eggs similar amounts are shown to be present by using the two types of chromatography. In contrast to deoxyribo-

nucleotides, there are rather different amounts of the four ribonucleoside triphosphates in a given extract of eggs or oocytes. It is also evident that the various preparations of eggs give rather different amounts of the ribonucleoside triphosphates. The reason for this is not known, but eggs or oocytes from different females of *X. laevis*, or at different seasons, have previously been observed to show differences in the activity of their nucleotide phosphorylation pathway (Woodland, 1969). Variable nucleotide-pool sizes in eggs of *Rana pipiens* have also been reported by Bieber *et al.* (1959). Bearing in mind these problems, it is reasonable to conclude that the ribonucleoside triphosphate pools of oocytes and eggs are similar in size and composition.

Determination of non-phosphorylated deoxyribose derivatives

It is not possible directly to measure the deoxyribose content of non-phosphorylated substances eluted from the Dowex column after periodate-methylamine treatment, because there is far too much material present. Much of this material is produced by the periodate-methylamine treatment, and is not present in the wash fractions of the ribonucleotide separation. Therefore the wash fractions of experiments intended for the analysis of ribonucleotides, such as those shown in Figs. 3 and 4, were each divided into two and treated with 3,5-diaminobenzoic acid with and without prior bromination. The fluorescence produced in the absence of

Table 4. *Non-phosphorylated compounds in eggs and oocytes reacting with 3,5-diaminobenzoic acid*

These values were obtained by assaying the substances washed through the column before the ribonucleotide separations shown in Figs. 3 and 4.

Type of cell	Content (pmol/cell)	
	Without bromination	With bromination
Egg	52	51
Oocyte	37	53

bromination should indicate the deoxyribose and purine deoxyriboside content; that measured after bromination should include the pyrimidine deoxyribonucleosides. Several peaks of fluorescence were obtained from the columns and the total contents of deoxyribose derivatives are shown in Table 4. Great caution should be exercised in interpreting these results. The samples assayed contain a very heterogeneous collection of molecules, which may interfere with the assay in such a way as to increase or to decrease the fluorescence. The fluorescence assay is specific for α -unsubstituted aldehydes, so our results suggest that eggs and oocytes contain roughly similar amounts of α -unsubstituted aldehydes, substances which are likely to be deoxyribose derivatives. About one-third of them are apparently linked to pyrimidines in oocytes. In eggs there are apparently similar amounts of non-phosphorylated unsubstituted aldehyde derivatives as in oocytes, though all of it seems to react without bromination. The total amount of such non-phosphorylated substances in eggs seems to be similar to the content of deoxyribonucleoside triphosphate.

Discussion

Deoxyribose and ribose derivatives in eggs

Our results indicate that eggs contain similar amounts of the four common deoxyribonucleoside triphosphates. This is as one expects if the only function of these substances is in DNA synthesis. Eggs contain sufficient deoxyribonucleoside triphosphate to support DNA synthesis to the 2500-cell stage, which is an early stage-8 blastula according to the Nieuwkoop & Faber (1956) table of development. Our finding may be correlated with the observation that the eggs of *R. pipiens* cleave normally in the presence of amethopterin, but gastrulation is inhibited, an effect that seems to depend mainly on inhibition of the synthesis of thymidine derivatives (Grant, 1960). These results seem to indicate that early embryos of *R. pipiens* contain enough DNA precursors for cleavage to occur. It would clearly be interesting to know at what stage *Xenopus* embryos become sensitive to inhibitors of folate metabolism.

Our results also correlate fairly well with those of Kuriki & Okazaki (1959), who worked with a later stage of a different species and used a microbiological assay for deoxyribose. They found that blastulae of *Bufo vulgaris formosus* contained 38 pmol of dCTP, 76 pmol of dATP, 32 pmol of dGTP and 66 pmol of dTTP. These values are 3–7 times larger than ours, a difference that may result from the different stage analysed or the different species used.

Like us, Kuriki & Okazaki (1959) found few deoxynucleoside mono- and di-phosphates, but they found only 41 pmol of deoxyribose plus deoxynucleosides, i.e. much less than the total deoxyribonucleoside triphosphate. We estimate that there are similar amounts of these compounds and total deoxyribonucleoside triphosphates in eggs of *Xenopus*, although we are rather sceptical about the accuracy with which deoxyribose derivatives can be measured in column wash-through fractions by using the fluorimetric assay. Nevertheless other workers have reported the presence of quite large amounts of deoxynucleosides in animal tissues. For example, Rotherham & Schneider (1958) found that a range of mammalian organs contained deoxyribonucleosides in roughly similar amounts to deoxyribonucleotides, and Travaglini *et al.* (1958) found that deoxyribonucleosides were abundant in the eggs of *Drosophila melanogaster*. Although pathways of deoxyribonucleotide synthesis are thought not to pass through the deoxyribonucleoside level, the occurrence of deoxyribonucleosides in tissues correlates well with the firmly established correlation between increased thymidine kinase activity and DNA synthesis observed in organisms of all sorts.

We may therefore conclude that the egg is well primed with deoxyribonucleoside triphosphates to act as precursors for the rapid and frequent DNA synthesis that occurs during cleavage. There may in addition be a store of oligonucleotides or 'yolk DNA' from which deoxyribonucleoside triphosphates are synthesized. This matter has been subject to long controversy, fully reviewed by Grant (1965). Dawid (1965) has shown that eggs of *X. laevis* contain 300–500 diploid DNA equivalents of alcohol-soluble deoxyribose derivatives (possibly oligodeoxyribonucleotides), and there appears to be about ten times

this amount of high-molecular-weight DNA firmly bound to the yolk (Baltus *et al.*, 1968). However, it has not yet been established that these compounds are sources of precursors for nuclear DNA synthesis.

Eggs are clearly well supplied with ribonucleotides, even though they do not make a significant amount of RNA in their nuclei (Gurdon & Woodland, 1969). The ribonucleoside triphosphates are 20–100 times more abundant than the corresponding deoxyribonucleotides. In contrast to the deoxyribonucleoside triphosphates, ribonucleoside triphosphates are present in rather different amounts, the explanation presumably lying in their various roles in cell metabolism. The most abundant ribonucleotide is ATP. If we assume that an egg represents 1 μ l of solution (this actually is its total volume), then the concentration of ATP is 0.6–1 mM and those of the other ribonucleotides are 0.2–0.6 mM. This is roughly the concentration range found in other cells.

Deoxyribose derivatives in oocytes

The main interest in the results relating to oocytes lies in their comparison with those relating to eggs. This is because the two types of cells differ so much in the types of nucleic acid that they make. Eggs are characterized by their ability to induce DNA synthesis in nuclei which come into contact with their cytoplasm, and such nuclei (which include the normal male and female haploid nuclei) do not synthesize RNA. In contrast, nuclei in oocyte cytoplasm synthesize RNA, but not DNA (see review articles by Gurdon, 1968; Gurdon & Woodland, 1968). Two main points relevant to these differences emerge from our results. The first is that eggs and oocytes contain similar amounts of the four common ribonucleoside triphosphates. The inhibition of RNA synthesis that occurs when an oocyte has matured to form an egg does not therefore result from a simple change in ribonucleoside triphosphate content. The second notable point is that there are less deoxyribonucleoside triphosphates in oocytes than in eggs. dTTP and dCTP are present, but we could not detect any deoxypurine triphosphates. Taken at face value this result indicates that deoxypurine triphosphates might control DNA synthesis in eggs and oocytes. Other workers have failed to find purine deoxyribonucleotides in adult organs (Potter *et al.*, 1957; Schneider, 1955), so this deficiency could be characteristic of cells not engaged in very frequent DNA synthesis. However, although the oocyte contains less deoxyribonucleoside triphosphates than the egg, which has to form about 30000 nuclei in the first 10h of development, it might contain a considerable amount relative to that needed to replicate a single nucleus.

An indication that some deoxypurine as well as deoxypyrimidine triphosphates are indeed present in these eggs has been obtained by C. C. Ford & H. R. Woodland (unpublished work), for they found that oocytes can replicate single-stranded DNA injected into them. It therefore seems unlikely that the cytoplasmic state associated with the induction of DNA synthesis in nuclei is total presence or absence of precursors.

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