Ribonucleic Acid Synthesis in Rabbit Erythroid Cells

ANALYSIS OF RATES OF SYNTHESIS OF NUCLEAR AND CYTOPLASMIC RIBONUCLEIC ACID

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(Received 4 June 1976)

Kinetic studies on the synthesis of RNA in mature bone-marrow erythroid cells from rabbits were made by measuring the incorporation of [2-³H]adenosine into the ATP pool and RNA over periods up to 8 h. By use of equations to fit the pool specific radioactivity and an equation using the same type of pool to generate the rate of linear DNA synthesis, good agreement between the pool parameters is found, provided that the ATP pool is measured in whole cell extracts, and assuming that the dATP and ATP pools equilibrate rapidly. RNA-synthesis rates were measured by using curve fits to equations developed by using the pool specific-radioactivity curves. The rate of synthesis of poly(A)-containing RNA varied in three experiments from 90 to 220 mol/min per cell, with a half-life of nuclear processing of 12–22 min with a mean of 16 min. Ribosomal RNA is synthesized at a rate of 70–200 mol/min per cell with an average half-life of nuclear processing of 37 min for the 18S RNA and 214 min for the 28S RNA. When the stable rRNA components are subtracted from the nRNA synthesis, the rate of nRNA synthesis is between 2 and 6 fg/min per cell with an average half-life of degradation of 27 min. The rate of synthesis of poly(A)-containing RNA is 1.5–3.5% of the RNA-synthesis rates. These rates are compared with the RNA-synthesis rates found in L cells and concentrations of globin mRNA found in various erythroid-cell preparations.

The analysis of rates of synthesis of RNA in developing sea-urchins by using labelled adenosine and measurement of ATP-pool sizes was pioneered by Humphreys and his colleagues (Emerson & Humphreys, 1971). In these cells (Brandhorst & Humphreys, 1971) they showed that 85% of the total RNA synthesized in blastulae nuclei turned over in the nucleus and the remaining 15% was transported to the cytoplasm (Brandhorst & Humphreys, 1972). These cells were not active in rRNA synthesis. In the pluteus the rate of rRNA synthesis could also be measured. In cultured cells, Emerson (1971) has measured rates of synthesis of rRNA and its precursors under conditions of exponential growth and contact inhibition in the chick fibroblast using [³H]adenosine and ATP-pool measurements. Brandhorst & McConkey (1974) have measured the rate of stable cytoplasmic RNA and nRNA accumulation in mouse L cells by similar methods. Work by Penman *et al.* (1968) used [³H]uridine labelling under continuous labelling conditions to determine relative nuclear and cytoplasmic RNA-labelling rates, but they did not measure the UTP-pool-labelling characteristics, so that no absolute rates of synthesis could be obtained. The same criticism is true for the work on duck

erythroblasts by Spohr *et al.* (1974). Despite the paucity of quantitative data, many statements have been made about the rates of RNA synthesis in differentiating cells with regard to rate of synthesis and turnover of nRNA, rates of synthesis and turnover of mRNA, and rRNA-synthesis rates. The lack of such data, and the current speculation as to whether the excess of nRNA is part of a transcript which contains mRNA or is independent of mRNA production, means that an accurate account of the synthesis and degradation of such RNA is required. In this paper, the rates of synthesis of cytoplasmic rRNA, 4S and 5S RNA and poly(A)-containing RNA, as well as the rate of synthesis and degradation of nRNA, are examined in late erythroblasts from rabbit bone marrow and contrasted with the rate of synthesis and degradation of RNA from other cells.

Materials and Methods

Chemicals and biochemicals

All chemicals were reagent grade unless otherwise stated. Sucrose (ribonuclease-free) was from Schwartz/Mann (Orangeburg, NY, U.S.A.). Phenol

was redistilled under N_2 before use. Sodium dodecyl sulphate was recrystallized from ethanol. Bovine serum albumin (fraction V) was from Armour Pharmaceutical Co. (Phoenix, AZ, U.S.A.). Eagle's minimal essential medium (MEM), with glutamine but without $NaHCO_3$ and foetal calf serum, was from Grand Island Biological Co. (Grand Island, NY, U.S.A.). Poly(U) and digitonin were from Sigma Biochemical Co. (St. Louis, MO, U.S.A.). Acrylamide and *NN'*-methylenebisacrylamide were electrophoresis purity grade from Bio-Rad Laboratories (Richmond, CA, U.S.A.). PPO (2,5-diphenyloxazole) and dimethyl-POPOP [1,4-bis-(4-methyl-5-phenyloxazol-2-yl)benzene] were from Isolab (Akron, OH, U.S.A.). BBS-3 solubilizer and Triton X-100 were from Beckman Instruments (Fullerton, CA, U.S.A.). Glass-fibre filters (GF/C) were from Reeve Angel (Clifton, NJ, U.S.A.). All glassware used for extraction of RNA was sterilized before use.

Radiochemicals. [3H]Poly(U) (12 mCi/mmol) and [^{14}C]poly(A) (2.15 mCi/mmol) were from Schwartz/Mann. [2-^3H]Adenosine (16 mCi/mmol) was from Nuclear Dynamics (El Monte, CA, U.S.A.).

Enzymes. Firefly lantern extract was from Sigma Biochemical Co.

Methods

Induction of anaemia. Adult New Zealand White rabbits were given 0.3 ml of neutralized 2.5% (w/v) phenylhydrazine/kg (aqueous) intraperitoneally for 5 days, and blood and bone marrow were taken from the animals on the seventh day.

Fractionation of bone marrow. Bone marrow was blown from the long bones of exsanguinated rabbits into 75% heat-treated anaemic rabbit plasma/25% 0.115 M- NaH_2PO_4 / Na_2HPO_4 , pH 7.5, as described by Borsook *et al.* (1969). The suspended cells were fractionated on 40 ml bovine serum albumin gradients (density 1.06–1.09 g/cm³) by the method of Borsook *et al.* (1969). Three main fractions are collected corresponding to the upper two-thirds of the gradient, with fraction 3, which is used in these experiments, representing the denser portion of the major band of cells. The cells from one rabbit (approx. 3×10^9) were fractionated on one gradient.

Cell incubations. Bone-marrow cells were incubated in spinner flasks (Bellco, Vineland, NJ, U.S.A.) at 37°C in an atmosphere of CO_2 +air (5:95) for 30 min at a concentration of 2.5×10^7 cells/ml of 20% foetal calf serum/20% dialysed anaemic rabbit plasma in Eagle's minimal essential medium with 30 μ g of ferrous ammonium sulphate/ml, 100 i.u. of penicillin/ml and 50 μ g of streptomycin/ml. [2-^3H]Adenosine (18–25 μ Ci/ml) was added, and duplicate 2.5 ml portions were taken for each time-point. In Expt. 3, duplicate 0.5 ml portions were also taken to measure total cell ATP specific radioactivity.

Total incubation time was from 3 to 8 h, with 11–14 time-points.

Determination of rates of protein synthesis by using [^{14}C]valine was as described by Lingrel & Borsook (1963).

Determination of ATP specific radioactivity. The specific radioactivity of [3H]ATP was determined on 0.5 M-HClO₄ supernatants of cell lysates in Expts. 1 and 2 and on 0.5 M-HClO₄ supernatants of washed cells in Expt. 3 by the firefly luciferase assay described by Emerson & Humphreys (1971). In the second experiment, some difficulty in obtaining accurate results for the ATP-pool specific radioactivity was encountered, owing to presumed breakdown of the [3H]ATP in the cell lysate, so that in Expt. 3 total cell ATP specific radioactivity was measured.

Cellular fractionation. The cells from each time-point were diluted with 2 vol. of ice-cold incubation medium without adenosine and washed three times in the same medium. Osmotic-shock lysis was performed by using 1 ml of rat liver supernatant from rat livers homogenized in 0.01 M-Tris/HCl (pH 7.6)/0.01 M-KCl/0.005 M-MgCl₂/0.001 M-ethanethiol (Grau & Favelukes, 1968), and the nuclei and unlysed cells were separated by centrifugation at 2000g for 10 min. The remaining cells were lysed with 1 ml of medium containing 1 mg of digitonin/ml of 0.25 M-sucrose/0.05 M-Tris/HCl (pH 7.6)/0.025 M-KCl/0.001 M-MgCl₂ (Medium A) followed by centrifugation as above. The nuclear pellet was washed once with 1 ml of Medium A. Samples (0.1 ml) of each lysate or wash were spotted on to filter paper for radioactivity determination, and the remainder was combined and made 6% in sodium *p*-aminosalicylate for extraction of RNA, except for in Expts. 1 and 2 where additional 0.2 ml portions were taken from each of the first lysates for ATP determination before extraction of the remainder.

The nuclei after the wash were washed three times with 0.5 M-HClO₄/0.9% sodium pyrophosphate, taken up in 0.5 ml of 0.3 M-KOH and incubated at 37°C for 90 min to dissolve the DNA and RNA. Samples (0.1 ml) were taken for radioactivity determination. Another portion (0.1 ml) was taken for DNA determination by precipitation by addition of 0.1 ml of ice-cold 15% (w/v) trichloroacetic acid, and the DNA collected on glass-fibre filters and washed with cold 5% (w/v) trichloroacetic acid before radioactivity determination. Nuclear RNA was determined in a further 0.1 ml sample by precipitation of the DNA with 0.2 ml of 0.5 M-perchloroacetic acid and assay of the supernatant.

The remainder of the nuclear extract in 0.3 M-KOH was hydrolysed for a further 16 h at 37°C for base analysis by paper electrophoresis after neutralization with perchloroacetic acid. In all cases, more than 95% of the label was found in AMP

as was expected, since conversion of [2-³H]adenosine into guanosine results in loss of the ³H label.

No attempt was made to measure the amount of end adenosine labelling in the cytoplasm, since the rRNA and poly(A)-containing components were separated from the 4S and 5S RNA, which contain the tRNA subject to terminal adenosine addition. In previous experiments, as much as 50% of the label in 3M-NaCl-soluble RNA from the cytoplasm was found in terminal adenosine, indicating a high turnover in the tRNA.

Extraction of cytoplasmic RNA. Lysate supernatants from the first and second lysis and wash were made 6% in sodium *p*-aminosalicylate and extracted with an equal volume of water-saturated phenol/*m*-cresol/8-hydroxyquinoline (500g:70ml:0.5g); the phenol phase was re-extracted with 0.1M-sodium acetate, pH5.0, and the combined aqueous phases, made 3% in NaCl, were re-extracted with 0.5 vol. of phenol/*m*-cresol/8-hydroxyquinoline (Kirby, 1968). The aqueous phase was precipitated with 2 vol. of ethanol, and the RNA precipitate washed with 3 × 1 vol. of 75% ethanol/1% NaCl in water, before being dissolved in water. All operations were at 4° or 0°C.

Determination of poly(A)-containing RNA. Poly(A)-containing RNA in the cytoplasmic extracts was assayed on photopolymerized poly(U) glass-fibre filters as described by Sheldon *et al.* (1972). All batches of poly(U) filters were checked for efficiency of binding by use of [¹⁴C]poly(A).

Determination of rRNA. The components of the cytoplasmic RNA were separated by polyacrylamide-gel electrophoresis in 2.5% or 3% (w/v) polyacrylamide gels at 2.5mA/tube for 2.5h at room temperature (24°C) in 0.04M-Tris/HCl/0.02M-sodium acetate/0.1% sodium dodecyl sulphate, pH7.8. Before application, the RNA samples were heated at 65°C for 10min in the same buffer to destroy aggregates. After electrophoresis, the gel was fractionated by extrusion from a syringe directly into vials for radioactivity determination.

Determination of haemoglobin. The amount of [¹⁴C]valine in haemoglobin in lysates of cell fractions was determined by electrophoresis in 10% (w/v) polyacrylamide gels by using a buffer containing 0.2M-sodium phosphate and 0.1% sodium dodecyl sulphate, pH7.2, at 9 mA/tube for 2h at 24°C. Slices of the gel were cut for radioactivity determination after staining with Wool Fast Blue to determine the position of the haemoglobin.

Radioactivity determination. Lysate and wash samples which had been spotted on to Whatman 3MM paper strips (1 cm × 5 cm) were placed in 0.5M-HClO₄ (5 ml/strip) for at least 5 min and washed with 6 × 5 ml of 0.25M-HClO₄/0.9% sodium pyrophosphate/strip for 10 min and with 2 × 5 ml of ethanol/strip for 10 min. All the above washes were at 0°C. The

papers were dried by washing twice with diethyl ether/ethanol (1:1, v/v) and twice with diethyl ether both at room temperature for 10 min and air-drying before being placed in scintillation vials for radioactivity counting in 0.5% PPO/0.05% dimethyl-POPOP in toluene scintillator solution in a Packard 3000 series liquid-scintillation spectrometer.

DNA precipitates on glass-fibre filters were washed by the same procedure and counted for radioactivity in the same way. The poly(U)-filter assays were dried and counted for radioactivity in the scintillator solution as described above.

The ATP samples, which has been assayed for luciferase in scintillation vials, were counted for radioactivity by direct addition of a mixture of 0.5% PPO/0.05% dimethyl-POPOP in toluene and Beckman BBS-3 solubilizer (5:1, v/v); 10 ml of the scintillation mix was used for 1 ml of the luciferase assay.

Similarly, the nRNA samples, which had been freed of DNA by precipitation with HClO₄, were neutralized with 0.5M-NaOH, and the scintillator/BBS-3 solubilizer mix was added in the ratio 10 ml of scintillator per ml of RNA sample.

Radioactivity in the RNA in fractionated polyacrylamide gels was determined by elution of the gels with 0.2 ml of 0.1M-NaOH for 120 min at 37°C followed by neutralization with 0.5M-HCl and addition of a mixture of 0.5% PPO/0.05% dimethyl-POPOP in toluene and Triton X-100 (6:7, v/v) in the ratio of 13 ml per ml of extract.

The relative efficiency of counting of [³H]ATP from the luciferase assay to [³H]RNA from the nuclear extract to [³H]RNA on filter paper and [³H]DNA and RNA on glass fibre was 1:0.81:0.62. Normal efficiencies were 32, 25 and 20%; although there was some variation, the ratios were reasonably constant. [³H]Poly(U) was used as the standard for RNA-efficiency determination.

The efficiency of counting [³H]RNA radioactivity in polyacrylamide gels was 7% and that for ¹⁴C-labelled haemoglobin 50%, after solubilizing and bleaching the gel in 0.3 ml of 30% (v/v) H₂O₂ at 60°C for 120 min, after which 5 ml of the Triton X-100 scintillator was added for radioactivity counting.

Treatment of the results. The determination of absolute rates of synthesis of RNA in any cell requires knowledge of the specific radioactivity of the precursor pools. ATP was chosen because of the ease of assay by the firefly luciferase assay (Emerson & Humphreys, 1971). Emerson & Humphreys (1971) and other workers have used an averaging of the specific radioactivity between time-points to convert the radioactivity measured in various cell fractions into absolute amounts. Although this seems reasonable for curves of specific radioactivity that are rising, the logic of correcting

for a specific radioactivity that declines with respect to time is difficult to justify, especially when complex systems are being assayed. The approach used here is to fit the specific radioactivity of the pool to a curve and then to use the parameters from this curve to generate other parameters for RNA synthesis.

Although many of the ATP specific-radioactivity curves that have been measured appear to fit an equation:

$$P = \frac{K_1}{K_2} (1 - e^{-K_2 t}) \quad (1)$$

(where P is specific radioactivity in c.p.m./pmol), the addition of a linear component was found empirically to give a better fit in all cases, and especially so when a pronounced decline of specific radioactivity with time was found. In this case the equation becomes:

$$P = \frac{K_1}{K_2} (1 - e^{-K_2 t}) + K_3 t \quad (2)$$

By fitting the curve of the pool specific radioactivity to eqn. (2) three constants can be obtained: $K_1/K_2 = A$, which has dimensions of c.p.m./pmol of adenosine; K_2 , which has the dimension min^{-1} ; $K_3/A = B$, which has the dimension min^{-1} . These constants are used in the following equations during the curve-fitting process, so that the number of independent variables can be kept to less than three. Alternatively, values of K_2 and B can also be obtained from eqn. (10) below.

The rate of synthesis of several types of RNA can be determined as follows. For all types of RNA that are processed in the nucleus (N), the general equation is:

$$\frac{dN}{dt} = K_4 P - K_5 N \quad (3)$$

where N has the dimension c.p.m., K_4 pmol of adenosine/min and $K_5 \text{ min}^{-1}$. On integration eqn. (3) becomes:

$$N = \frac{K_4 A}{K_5} + \frac{K_4 A}{K_5 - K_2} \left(\frac{K_2}{K_5} e^{-K_5 t} - e^{-K_2 t} \right) + \frac{K_4 A B}{K_5^2} (K_5 t + e^{-K_5 t} - 1) \quad (4)$$

The synthesis of stable cytoplasmic RNA (C) from an unstable precursor in the nucleus is described by the following equation:

$$\frac{dC_{\text{stable}}}{dt} = K_6 N \quad (5)$$

$K_6 = K_5 \times F_u$, where F_u is the fraction of stable RNA precursor in the nucleus that reaches the cytoplasm

and the individual species of RNA has a degradation rate in the nucleus of K_5 with dimensions min^{-1} . Therefore:

$$C_{\text{stable}} = F_u K_4 A t + \frac{F_u K_4 A}{K_5 - K_2} \left[\frac{K_2}{K_5} (1 - e^{-K_5 t}) - \frac{K_5}{K_2} (1 - e^{-K_2 t}) \right] + \frac{F_u K_4 A B}{K_5} \left[\frac{K_5 t^2}{2} + \frac{1}{K_5} (1 - e^{-K_5 t}) - t \right] \quad (6)$$

The rate of synthesis of unstable cytoplasmic RNA is described by:

$$\frac{dC_{\text{unstable}}}{dt} = K_7 N - K_8 C \quad (7)$$

$K_7 = K_5 F_u$, where F_u is the fraction of unstable RNA precursor in the nucleus that reaches the cytoplasm, and its degradation rate in the nucleus is K_5 with the dimensions and parameters described for eqn. (5).

In this case, on integration:

$$C_{\text{unstable}} = \frac{F_u K_4 A}{K_8} (1 - e^{-K_8 t}) + \frac{F_u K_4 A K_2}{(K_5 - K_2)(K_8 - K_5)} (e^{-K_5 t} - e^{-K_8 t}) - \frac{F_u K_4 A K_5}{(K_5 - K_2)(K_8 - K_2)} (e^{-K_2 t} - e^{-K_8 t}) + \frac{F_u K_4 A B}{K_8^2} (e^{-K_8 t} - 1) + \frac{F_u K_4 A B t}{K_8} - \frac{F_u K_4 A B}{K_5 K_8} (1 - e^{-K_8 t}) + \frac{F_u K_4 A B}{K_5 (K_8 - K_5)} (e^{-K_5 t} - e^{-K_8 t}) \quad (8)$$

Finally, if it is assumed that the rate of equilibration of the ATP and dATP is rapid, the rate of DNA (D) synthesis can be calculated by using the equation:

$$\frac{dD}{dt} = K_9 P \quad (9)$$

and hence

$$D = K_9 A t + \frac{K_9 A}{K_2} (e^{-K_2 t} - 1) + \frac{K_9 A B t^2}{2} \quad (10)$$

The values of A , B and K_2 may be obtained from the pool analysis. In no equation are more than three independent variables present. Such equations are thus suitable for curve-fitting procedures by a non-linear least-squares program as described by Wolberg (1967).

Results

Characterization of the fractionated cells

Table 1 shows the cellular composition of the fraction of bone-marrow cells used in these experiments. The cell composition is highly reproducible from experiment to experiment. Total protein synthesis in these cells was assayed by incorporation of [^{14}C]valine and compared with other cell fractions both in the amount synthesized and the proportion found in haemoglobin. Table 2 summarizes the results obtained from three different cell fractions. Fraction 3 is used in the present experiments because of the minimal contamination by white cells and the high proportion of protein synthesis found in haemoglobin.

Incubation conditions and accuracy of measurements

In preliminary experiments the difference between incubating cells in a shaking water bath was compared with incubation in a spinner culture with the use of CO_2 + air (5:95) at the same relative concentrations.

From these experiments, the spinner-culture method was chosen because of the greater ease of operation, the greater incorporation of [^3H]adenosine into RNA, and the ease of removal of samples for each time-point. Studies in quintuplicate with individual tubes for each assay allowed measurement of the accuracy of sampling and handling the cells and fractionation procedures. The standard deviation for ^3H c.p.m. in nuclear and cytoplasmic RNA was 7.2% and for DNA measurements 9.5% at each time-point. There was no evidence of a change in the standard deviation as the amount of labelling increased. These values were used for weighting during analysis of the data, except where the 18S and 28S rRNA components were determined as a percentage of the total counts by polyacrylamide-gel electrophoresis. In this case, the standard deviation used was 1% of the total counts, since this was the estimated accuracy of the measurement of RNA fractions from a gel.

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Pool specific-radioactivity determination

The accuracy of determining the pool specific radioactivity and the goodness of fit of the data to the empirical equation are of importance to the determination of rates of RNA synthesis and also important in determining rates of degradation and processing of nuclear and cytoplasmic RNA when these rates are close to the rate of approach to equilibration of the pool specific radioactivity. In preliminary experiments an independent test was made to determine whether this method was a viable one by assuming that the pool of dATP rapidly equilibrates with the ATP pool and that during the relatively short incubation periods the rate of DNA synthesis was linear. Under these conditions the constants K_2 and $K_2K_3/K_1 = B$ (eqn. 3), obtained from the pool as determined on

Table 1. Cell composition of fraction-3 cells used in the RNA-synthesis experiments

Some 1000 cells were counted for each measurement. No cells were counted for Expt. 3.

Percentage composition						
	Pronormoblasts	Basophilic erythroblasts		Polychromatophilic erythroblasts	White cells	Reticulocytes
		Large	Small			
Expt. 1	1.2	12.0	26.1	42.0	9.0	9.9
Expt. 2	0.8	8.6	22.4	50.8	5.3	13.3

Table 2. Protein synthesis in erythroid-cell fractions

The cell fractions were incubated with $2.5\mu\text{Ci}$ ($270\text{mCi}/\text{mmol}$) of [^{14}C]valine for each 10^8 cells in 1 ml of medium for 1 h before and after preincubation at 37°C for 4 h. The cells were lysed by osmotic shock, and total protein synthesis and haemoglobin synthesis determined as described in the Materials and Methods section.

Cell fraction	Percentage composition				Incorporation of [¹⁴ C]valine (c.p.m./10 ⁵ erythroid cells) after preincubation for:		Percentage of [¹⁴ C]valine in haemoglobin
	Basophilic erythroblasts	Poly-chromatophilic erythroblasts	Reticulocytes	White cells			
					0h	4h	
1	56.8	11.9	5.2	26.0	2.4	5.0	30
3	33.8	47.6	14.8	4.9	3.9	6.2	85
4	15.8	50.4	30.3	4.7	3.5	5.0	90

Fig. 1. Curve fits of the ATP-pool specific radioactivity by using eqn. (2)
(a) Expt. 1; (b) Expt. 2; (c) Expt. 3. The vertical bars indicate s.d. in repeat determinations of the ATP extracts.

Table 3. Pool specific-radioactivity curve fitting

Summary of the parameters derived from fitting the pool specific radioactivity to eqn. (2). The dimensions of *A* are c.p.m./pmol of adenosine; *K*₂, min⁻¹; *K*₃, c.p.m./min per pmol of adenosine. The covariances are normalized, and the least-squares values normalized according to the variance of each point (Wolberg, 1967).

Parameter	Expt. ... 1	2	3
$K_1/K_2 = A$	181 ± 8.4	479 ± 88*	266.8 ± 14.1
K_2	0.166 ± 0.018	(2.5)	0.081 ± 0.021
K_3	-0.240	-1.36 ± 0.6*	-0.033 ± 0.05
Covariance A, K_2	-0.95	—	-0.6
Covariance A, K_3	-0.91	0.97	-0.85
Covariance K_2, K_3	-0.84	—	-0.49
Least-squares value	5.1	173	9.9
$B = K_3/A$	-0.00132 ± 0.0005	-0.0029 ± 0.0012	-0.000124 ± 0.00018

* These values in this experiment were derived from the equation $P = A - K_3t$. This gave essentially the same values as eqn. (2) when *K*₂ was 2.5, but with the much smaller variance shown.

Fig. 2. Curve fit of the DNA-labelling kinetics by the best fit to eqn. (10) and the values of K_2 and B derived from the pool (a) Expt. 1; (b) Expt. 2; (c) Expt. 3. —, Computer-generated best fit; ---, best fit by using K_2 and B derived from the respective ATP-pool specific radioactivities.

whole cells, were very similar to the constants K_2 and B obtained from the DNA-labelling experiment (eqn. 10). The fit of the pool specific-radioactivity curves for the three experiments is shown in Fig. 1 and Table 3. The fit of the DNA by best fit and K_2 and B derived from the pool is shown in Fig. 2. In the first two experiments performed in spinner cultures which are reported here, the ATP pool was measured in the first lysate. However, problems were encountered owing to degradation of the ATP, which tended to produce artificially high specific-radioactivity measurements of the ATP because of overlap of the ATP and ADP in chromatography. This is most evident in the second experiment (Table 3 and Fig. 1) where it was difficult to obtain a good fit of a curve

to the data. However, the DNA analysis in this case gave values that indicated that the constants obtained for K_2 and B in this case were reliable (Table 4). In Expt. 3 the pool specific radioactivity was measured in whole cells.

Measurement of rates of nuclear and cytoplasmic RNA synthesis

The incorporation of $[2\text{-}^3\text{H}]\text{adenosine}$ into the pool, mRNA, DNA and cytoplasmic RNA obtained by osmotic-shock lysis, digitonin lysis and in a nuclear wash are shown in Figs. 1–5 for three experiments with different incubation times, performed on three

Table 4. Curve fitting for the DNA incorporation

The dimensions of K_9A are c.p.m./min per 3.8×10^7 cells for Expt. 1 and c.p.m./min per 5×10^7 cells for Expts. 2 and 3.

(a) Summary of the parameters derived from fitting the DNA incorporation to eqn. (10)

Parameters	Expt. ... 1	2	3
K_9A	25700 ± 1100	23700 ± 1020	17100 ± 1300
K_2	∞	1.69 ± 2.8	0.131 ± 0.0065
$k_3/A = B$	-0.0033 ± 0.0007	-0.00143 ± 0.0005	$+0.00024 \pm 0.00032$
Covariance K_9A, K_2	—	-0.85	-0.87
Covariance K_9A, K_3K_9	-0.92	-0.92	-0.95
Covariance K_2, K_3K_9	—	+0.72	+0.77
Least-squares value	1.8	1.4	25.8

(b) Summary of the parameters derived from curve fitting for DNA incorporation when constants K_2 and B are obtained from the pool-curve fit.

Parameters	Expt. ... 1	2	3
K_9A	26400 ± 1800	25700 ± 600	18500 ± 400
Least-squares value	519	6.5	28.7

Fig. 3. Labelling kinetics of nuclear and cytoplasmic RNA and DNA from Expt. 1

(a) Nuclear labelling: ●, DNA; ○, nRNA; ▲, wash RNA. (b) Cytoplasmic RNA: ●, osmotic-shock lysate; ○, digitonin lysate.

different occasions. The fit of the DNA curve to eqn. (10) by three independent parameters is shown in Table 4 and Fig. 2. There was no significant difference in the value of K_9A , which was generated by using the data from the pool curve fit

given in Table 3(b), so that in all subsequent curve-fitting procedures the values for K_2 and B given in Table 3 were used, except where specifically noted.

Poly(A)-containing RNA. The percentage of poly(A)-containing RNA found in the extracts of

Fig. 4. Labelling kinetics of nuclear and cytoplasmic RNA and DNA from Expt. 2

(a) Nuclear labelling: ●, DNA; ○, nRNA; ▲, wash RNA. (b) Cytoplasmic RNA: ●, osmotic-shock lysate; ○, digitonin lysate.

Fig. 5. Labelling kinetics of nuclear and cytoplasmic RNA and DNA from Expt. 3

(a) Nuclear labelling: ●, DNA; ○, nRNA; ▲, wash RNA. (b) Cytoplasmic RNA: ●, osmotic-shock lysate; ○, digitonin lysate.

the osmotic-shock lysates for each experiment are shown in Fig. 6. These percentages were used to calculate the total poly(A)-containing RNA in the total cytoplasmic RNA from the osmotic-shock lysates. These data are shown in Fig. 7, and have been fitted to eqn. (8), a half-life of either 5 or 15 h for the poly(A)-containing RNA being used. The values generated for $F_n K_4 A$ and K_5 are given in Table 5(a). The values of K_5 are equivalent to a half-life of pro-

cessing of poly(A)-containing RNA in the nucleus varying from 12 to 29 min, although within each half-life for the poly(A)-containing RNA the range is smaller, and the average for the 5 h half-life is 20 min and that for the 15 h half-life 15 min. When values for K_2 and B , obtained from the DNA-curve fits, were used, the values shown in parentheses in Table 5(a) were obtained. The largest differences are found in the values for K_5 , which, on the

Fig. 6. Percentage of poly(A)-containing RNA in the RNA extracted from osmotic-shock lysates

(a) Expt. 1; (b) Expt. 2; (c) Expt. 3.

average, now give values for the half-life of processing of 24 min and 17 min for the 5 h and 15 h half-life models respectively.

Ribosomal RNA. The relative amounts of 18S and 28S rRNA in the osmotic-shock lysate were calculated from the polyacrylamide-gel profiles. Examples of the profiles and the way in which the background radioactivity was subtracted from the peak areas are shown in Fig. 8. The curves of rRNA synthesis and their fit to eqn. (6) are shown in Fig. 9. The values of the parameters $F_s K_4 A$ and K_5 derived from eqn. (6) are shown in Table 5(b). In Expts. 1 and 2, although reasonable fits were obtained by using eqn. (6) for the 18S RNA, no single fit to a curve could be obtained for the 28S RNA, and the values given are estimates obtained by fitting a curve to the total rRNA-synthesis rate and finding the value of K_5 , which was best when $F_s K_4 A$ was closest to the expected value obtained

by subtracting the rate of synthesis of 18S RNA from the total rRNA-synthesis rate. However, in Expt. 3, because the experimental measurements were taken for up to 8 h, the fit for the 28S RNA was somewhat better, but was still not obtained with the same degree of certainty as for the 18S RNA. With these reservations in mind, it is possible to assess the rate of processing (K_5) of the rRNA in the nucleus. For the 18S RNA, this rate is equivalent to a half-life of its precursor of between 35 and 58 min with a mean of 37 min. For the 28S RNA, the half-life of its nuclear precursor is a mean of 214 min with a range of 165–287 min. This somewhat large value for the processing time of the 28S RNA precursors in the nucleus indicates that a large proportion of the steady-state concentration of rRNA precursors will be for the 28S RNA (namely 45S, 32S, 30S etc.) and a much smaller proportion for the 18S RNA (45S and 20S).

Fig. 7. Curve fit of the labelling kinetics of poly(A)-containing RNA by using either a 5 h or 15 h half-life of RNA degradation in eqn. (8)

(a) Expt. 1; (b) Expt. 2; (c) Expt. 3. ----, 5 h half-life; —, 15 h half-life.

Other cytoplasmic RNA. No extensive study of the 4S and 5S fraction obtained from the polyacrylamide-gel electrophoresis has been made. General curve fitting to eqn. (6) indicates that the processing of the RNA in the nucleus is extremely rapid or instantaneous. The value of $F_s K_d A$ for these fits is given in Table 5(c). However, this interpretation could be affected by the cytoplasmic addition of terminal adenosine to tRNA, which was not measured in these experiments, since the 5S and tRNA were not separated, but which could

represent approx. 50% of the labelling of the tRNA. The values for the rates of synthesis of 4S and 5S RNA in Table 5(a) are thus maximum values.

The RNA extracted from the second (digitonin) lysis was less easy to characterize, since it appeared to contain a certain amount of degraded RNA, which prevented an accurate estimation of the rRNA content. In addition, the amount of poly(A)-containing RNA was never higher than 4–5% of the total. Since this RNA was presumably derived in part from non-erythroid cells and represented less

Table 5. *Curve fitting for cytoplasmic RNA*

The dimensions of $F_u K_4 A$ are c.p.m./min per 3.8×10^7 cells for Expt. 1 and c.p.m./min per 5×10^7 cells for Expts. 2 and 3; the dimensions of K_5 are min^{-1} .

(a) Poly(A)-containing RNA

Summary of the parameters derived from fitting the incorporation into poly(A)-containing RNA to eqn. (8) by using either a 5h or 15h half-life for the RNA ($K_8 = 0.00231$ or 0.00077 respectively) and the values of K_2 and B derived from the pool- or from the DNA-curve fit.

Parameters	Expt. ... 1		2		3	
Half-life (h)	... 5	15	5	15	5	15
$F_u K_4 A$	278±17 (382±42)*	245±16 (327±35)	671±96 (524±60)	550±67 (440±43)	953±69 (893±68)	747±58 (699±59)
K_5	0.041±0.007 (0.018±0.0004)	0.049±0.01 (0.022±0.005)	0.024±0.007 (0.036±0.011)	0.032±0.010 (0.051±0.017)	0.037±0.008 (0.032±0.007)	0.058±0.017 (0.049±0.014)
Covariance	-0.86 (-0.94)	-0.84 (-0.93)	-0.91 (-0.88)	-0.89 (-0.85)	-0.78 (-0.80)	-0.75 (-0.76)
Least squares-value	6.7 (9.9)	8.3 (11.1)	75.0 (66.3)	68.3 (59.1)	26.1 (28.0)	34.3 (38.0)

(b) Ribosomal RNA

Summary of the parameters derived from fitting the incorporation into rRNA to eqn. (6). The values obtained from the 28S RNA fit were not absolute for Expts. 1 and 2 and are derived in part from use of total rRNA-incorporation curve fitting (details not shown). The constants for K_2 and B were derived from pool fitting experiments.

Parameters	Expt. ... 1		2		3	
	18S RNA	28S RNA	18S RNA	28S RNA	18S RNA	28S RNA
$F_s K_4 A$	652 ± 177	1500 ± 185	836 ± 283	1974 ± 850	1011 ± 88	2905 ± 343
K_5	0.020 ± 0.006	0.0042 ± 0.00008	0.016 ± 0.012	0.0025 ± 0.00015	0.012 ± 0.002	0.003 ± 0.0002
Covariance	-0.92	-0.86	-0.94	-0.89	-0.88	-0.88
Least-squares value	65	17	104	50	17	58

(c) 4S and 5S RNA

Summary of the parameters derived from fitting the incorporation into 4S and 5S RNA by using eqn. (6).

Parameters	Expt. ... 1	2	3
$K_5 F_4 A$	2020 ± 165	2180 ± 260	2610 ± 431
K_5	∞	∞	∞
Least-squares value	27.1	1419	305

* The values in parentheses are obtained by using the values of K_2 and B calculated from the DNA-curve fit.

than 30% of the total rRNA synthesis of the cells in the first two experiments and less than 15% from Expt. 3, the only use made of these data was in the correction for the determination of the rate of synthesis of non-ribosomal nuclear RNA.

Nuclear RNA. Because the kinetics for the incorporation of label into the wash fraction are very similar to those obtained for incorporation into the nucleus and unlike the kinetics for incorporation into the two lysates, and the amount incorporated represents about 30% of the label in the nucleus, it was decided to pool the counts from the wash and nRNA fractions, allowing for the difference in efficiency of counting, for use in further analysis. Since the nucleus is known to consist of at least two compartments in which RNA

synthesis takes place, i.e. the nucleolus and nucleoplasm, and the rates of labelling and processing of the 18S and 28S RNA are known, these values were subtracted from the total nuclear counts during the process of curve fitting for the residual RNA synthesis by use of eqn. (4). This technique does not take any account of the rate of processing of the 45S, 32S, 30S and other rRNA precursors, so that the final curve-fit data include the processing and synthesis of the non-conserved portion of the rRNA precursors. The curves were fitted by using the values for K_2 and B obtained from both the pool-curve fitting and the DNA-curve fitting by use of eqn. (4), and the results are shown in Fig. 10 and Table 6. The average value for the half-life of processing the RNA for Expts. 2

Fig. 8. Polyacrylamide-gel analysis of RNA extracted from the osmotic-shock lysate of Expt. 3

(a) 30min incubation; (b) 60min incubation; (c) 120min incubation; (d) 240min incubation; (e) 360min incubation; (f) 480min incubation. ----, Background for estimation of the amount of 18S and 28S rRNA labelled.

and 3, obtained by using the pool values for K_2 and B , is 33min, and the average value from all of the experiments, when DNA-derived values for K_2 and B were used, is 27min.

Discussion

When an attempt was made to fit the kinetic data by the method of Brandhorst & McConkey (1974), no reasonable values could be obtained for the

nRNA kinetics, so that this method was abandoned in favour of the fit to the equations.

Accuracy of the pool-fitting criteria

A comparison between the DNA-curve fit by using the best fit to the data for eqn. (10) and that by using the constants for K_2 and B obtained from the pool in eqn. (10) shows some differences in Expts. 1 and 2, but very little difference in Expt. 3 (Fig. 2). As

Fig. 9. Curve fit to cytoplasmic rRNA-labelling kinetics from osmotic-shock lysates by using eqn. (6)

(a) Expt. 1; (b) Expt. 2; (c) Expt. 3. ●, 18S RNA; ○, 28S RNA.

mentioned above, the ATP used to measure the pool specific radioactivity in the first two experiments was isolated from osmotic-shock lysates and was liable to degradation by this technique. In Expt. 3 and in previous experiments, there was a much greater concordance between the values of K_2 and B obtained from the pool specific radioactivity from total cells and those obtained from the DNA incorporation. Hence in all of the curve-fitting procedures the values obtained were checked by both sets of parameters, and where there are significant differences in the data obtained, such as in the poly(A)-containing RNA and the rRNA curve fits (Tables 5a and 6), the values from both sets of constants are given. The assumption that the DNA has a linear synthesis rate over these time-periods and that the nuclear ATP- and dATP-synthetic pools are in very rapid equilibrium are justified by the curve fits obtained, and the

agreement between the values of K_2 and B obtained from the pool- and DNA-labelling kinetics in Expt. 3. Because of this, the values of K_2 and B obtained by fitting the DNA-labelling kinetics are favoured over the values obtained from the ATP pool in evaluating the curve fits for Expts. 1 and 2, especially for the rRNA kinetics, where the values of K_2 have the greatest effect on the estimation of the rate of RNA degradation (K_5).

Comparison of the rates of synthesis obtained in erythroid cells with those from other cells

Rates of rRNA synthesis have been measured in exponentially growing and contact-inhibited chick fibroblasts by Emerson (1971) and found to be 5 and 2 fg/min per cell respectively. The average obtained in the experiments reported here, allowing for the factors used in Table 6, is 0.9 fg/min per cell.

Fig. 10. Curve fit to nuclear and wash RNA-labelling kinetics, with the stable 18S and 28S rRNA components removed, by using eqn. (4)

(a) Expt. 1; (b) Expt. 2; (c) Expt. 3. —, Curve fit by using K_2 and B from the ATP pool. ----, Curve fit by using K_2 and B from the DNA-curve fit.

Table 6. Curve fitting for nRNA

Summary of the parameters derived from curve fitting with nuclear and wash RNA radioactivity to eqn. (4). The components of the 18S and 28S rRNA were removed by using values obtained from Table 5(b) for $F_s K_4 A$ and $K_4 A$ and K_5 . In addition, in Expts. 1 and 2, $F_s K_4 A$ was multiplied by 1.5 to account for rRNA synthesis in the second lysate and by 1.1 in Expt. 3. The values of K_2 and B were derived from the pool-curve fit and the DNA-curve fit. The dimensions for $K_4 A$ and K_5 are the same as in Table 5.

Parameters	Expt. ... 1	2	3
$K_4 A$	75 500 ± 14 900 (24 500 ± 4460)*	47 000 ± 4400 (53 700 ± 4330)	20 800 ± 3880 (16 900 ± 3420)
K_5	0.14 ± 0.031 (0.029 ± 0.007)	0.014 ± 0.0022 (0.021 ± 0.0025)	0.028 ± 0.0078 (0.027 ± 0.0082)
Covariance	0.97 (0.89)	0.89 (0.91)	0.91 (0.91)
Least-squares sum	19 (55)	22 (12)	53 (57)

* The values in parentheses are obtained by using the parameters for K_2 and B derived from the DNA-curve fit.

Table 7. Calculation of absolute rates of DNA and RNA synthesis

The absolute rates of DNA and RNA synthesis are converted into pmol of adenosine/min by finding the values of K_4 and K_9 by using the values of A determined from the pool-curve fits. Correction for the base composition of the RNA is made by assuming a 22.2, 16.6, 36.8 and 25% content for 18S, 28S, poly(A)-containing and nuclear RNA and 25% adenosine content for DNA. The conversion into molecular synthesis is done by assuming molecular weights of 220000, 670000 and 1650000 for poly(A)-containing, 18S and 28S RNA respectively. It is assumed that the rATP and dATP pools have the same specific radioactivity.

(a) Expt. 1

	Rates of synthesis		
	(pmol of adenosine/min per 10^7 cells)	(pmol of nucleotides/min per 10^7 cells)	(mol/min per cell)
DNA	61.0	244	—
Nuclear RNA	136 (44)*	542 (176)	[1234, (343)]†
18S RNA	1.6	6.8	199
28S RNA	3.6	21.2	245
Poly(A)-containing RNA (15h half-life)	0.58	1.59	137
4S+5S RNA	4.8	—	—

(b) Expt. 2

	Rates of synthesis		
	(pmol of adenosine/min per 10^7 cells)	(pmol of nucleotides/min per 10^7 cells)	(mol/min per cell)
DNA	16.9	68	—
Nuclear RNA	26 (28)*	97 (111)	[189, (126)]†
18S RNA	0.56	2.54	73
28S RNA	1.33	8.01	93
Poly(A)-containing RNA (15h half-life)	0.37	1.01	89
4S+5S RNA	1.5	—	—

(c) Expt. 3

	Rates of synthesis		
	(pmol of adenosine/min per 10^7 cells)	(pmol of nucleotides/min per 10^7 cells)	(mol/min per cell)
DNA	21.9	87.5	—
Nuclear RNA	19 (16)*	77 (53)	[150, (122)]†
18S RNA	1.22	5.51	159
28S RNA	3.51	21.2	245
Poly(A)-containing RNA (15h half-life)	0.90	2.46	216
4S+5S RNA	3.2	—	—

* The values in parentheses were determined by using the data from the DNA constants.

† The values in square brackets were calculated assuming a mol.wt. of 10^7 .

In mouse L cells, Brandhorst & McConkey (1974) estimated a rate of 18 fg/min per cell, assuming that 82% of the steady-state cytoplasmic RNA is rRNA. Although the doubling time of mouse L cells of 13 h (Perry *et al.*, 1974) is similar to the doubling time of early erythroblasts in normal rats and anaemic mice (12–16 h; Lord, 1970; Hunt, 1974a), the great difference in rRNA-synthesis rates is not reflected in the DNA-synthesis rates, since the value 3 fg/min per cell and a DNA-synthesis time of 6 h (Lord, 1970) and a DNA content of 6 pg/cell indicate that approx. 20% of the cells are in

S phase and approx. 50% of the cells are capable of DNA synthesis. This is a reasonable rate considering that at least 20% of polychromatophilic erythroblasts are inactive in DNA synthesis (Borsook, 1965). It is not clear whether the lower rate of rRNA synthesis is due to synthesis of rRNA only in early erythroblasts or whether there is a gradual decrease in rRNA synthesis as the cells mature. Labelling of mouse erythroblasts *in vivo* (Evans & Lingrel, 1969; Hunt, 1974a) indicates that there is a progressive decline in rRNA synthesis and a constant rate of mRNA synthesis during cell development.

From these comparisons it appears that the fraction of mature erythroid cells used in these experiments, although capable of a reasonable rate of DNA synthesis, are deficient in rRNA synthesis. It is therefore of interest to compare synthesis of other RNA species with that found in other types of cells.

Brandhorst & McConkey (1974) calculated a rate of synthesis of hnRNA* of 54 fg/min per cell with a decay half-life of 21–23 min. In the present experiments the rate of synthesis of hnRNA is 1.5–4.5 fg/min per cell, with a half-life of 27–33 min when the rRNA precursor that is not conserved is included. It thus appears that the hnRNA-synthesis rate is closely comparable with the rate of rRNA synthesis, as is the case in L cells, and is about 5% of the rate found in these cells. The steady-state concentration of hnRNA, including rRNA precursor which is processed in the cell as compared with the 18S and 28S rRNA in the nucleus, is less than 50% of the total, ranging from 25 to 50% in the three experiments. In terms of amounts, the range is from 0.1 to 0.2 pg of hnRNA/cell, which is less than one-tenth of the amount calculated for L cells, as expected from the kinetics. The steady-state concentrations agree with the analysis of short-term (10–20 min) labelled total cell RNA, where it was found that 30–40% of the label chromatographed on methylated albumin/kieselguhr columns in the rRNA region (Johnson & Hunt, unpublished work).

No direct rates of synthesis of poly(A)-containing RNA have been determined in other systems. However, a steady-state concentration of mRNA of 3% of the polyribosomal RNA is quoted by Brandhorst & McConkey (1974), which allows them to calculate the rate of mRNA synthesis in L cells as 1.2 fg/min per cell, which is 2% of the rate of hnRNA synthesis. This compares with a rate of synthesis of poly(A)-containing RNA of 0.03–0.08 fg/min per cell obtained in experiments reported here. These values are 1.4–3.5% of the hnRNA-synthesis rates. However, the accumulated mRNA in the erythroid cells is at least 85% globin mRNA, as indicated by haemoglobin synthesis in these cells and by analysis of the poly(A)-containing mRNA extracted from these cells in a wheat-embryo cell-free system (Hunt & Oakes, 1976). When this is considered the rate of synthesis of poly(A)-containing RNA of 90–200 mol/min per cell represents a maximal rate of synthesis of a single RNA species from a normal gene complement in the haploid cell (Hunt, 1974b).

In several types of cells, the hybridization of total cytoplasmic poly(A)-containing RNA with its own complementary DNA allows an estimate to be made of the relative amount of various DNA sequences that

* Abbreviation: hnRNA, heterogeneous nuclear ribonucleic acid.

are transcribed. In HeLa cells it is found that a few (15–17) types of sequences are present in as many as 8000 copies per cell, another class of some 330 types are present in 440 copies, and a further class of some 30000 types of sequences are present only as 8 copies/cell (Bishop *et al.*, 1974). In the mouse erythroleukaemic cells induced by Friend virus, the corresponding data are three types at 12000 copies, 1700 types at 120 copies and 8700 types at three copies (Birmie *et al.*, 1974), indicating a distribution similar to that found in HeLa cells in a cell that has the capability of differentiating to an erythroid cell. The number of globin mRNA molecules in an erythroid cell has been measured by several groups: their results are 140000/cell and 66000/cell in mouse reticulocytes and foetal liver (Humphries *et al.*, 1976), 1500–1800/cell in mouse-embryo liver erythroid-cell precursors after 20–40 h growth in the presence of erythropoietin (Ramirez *et al.*, 1975), and 21000 and 6600 in mouse reticulocytes and induced mouse Friend cells (Leder *et al.*, 1973). The great disparity between these values appears to be due to differences in measuring cell RNA content rather than in the actual procedures themselves. In rabbit bone marrow, the number of ribosomes is estimated as 8×10^5 – 17×10^5 /cell (Lingrel & Borsook, 1963), which would indicate a globin mRNA content of 10000–200000 copies/cell, if all ribosomes are in a pentameric structure.

It thus appears that in non-erythroid cells more than 80% of the synthesis of poly(A)-containing RNA is accounted for by mRNA present in less than 400 copies per cell, if it is assumed that all of these molecules have the same decay rate. It also indicates that a large portion of the genome may be activated at one time or another in the cell cycle. Although no direct studies have been made on complementary DNA–mRNA re-annealing in erythroid cells, it appears from the experiments reported here and from others that, although the rate of poly(A)-containing RNA synthesis in late erythroid cells is about 5% of that in L cells, the poly(A)-containing RNA is mostly of one species and that at least 50% of the poly(A)-containing RNA synthesized must be globin mRNA. The data given here indicate that there is not a large amount of poly(A)-containing RNA with a half-life of degradation of less than 2 h, but it cannot differentiate between a mixture of lower degradation rates with a half-life between 5 and 20 h. It is therefore tempting to assume that a large proportion of the mRNA synthesis in erythroid-cell nuclei is for the α - and β -chain mRNA for globin and that the concomitant decrease in hnRNA synthesis is coupled with this change.

If the control of hnRNA synthesis is positive and is by synthesis of RNA, as has been suggested by Britten & Davidson (1969), it would be expected that cells that have a more complex genotypic

expression than is expected in late erythroid cells would have a higher ratio of hnRNA synthesis to mRNA synthesis than that in the erythroid cells. However, this is not the case, and, although the complexity of gene expression in erythroid cells has not been measured, it appears that whatever other genes are being expressed in these cells are catered for by a similar hnRNA/mRNA ratio. This constancy of ratio is what would be expected if the mRNA was, in the main, synthesized as a part of a giant precursor.

In conclusion, although there are many more measurements to be made, the data presented here suggest that globin-mRNA synthesis is accompanied by an excess of hnRNA synthesis in the nucleus similar to that found in other cells, even though the globin mRNA synthesized may account for more than 50% of total mRNA synthesis, and that synthesis of DNA, rRNA and mRNA is not co-ordinately controlled in the developing erythroblast.

I thank Barbara Johnson for her excellent technical assistance. This work was supported by grants GM 19076 and GM 22312 from the National Institutes of Health, a grant from the Kui Lee Fund for Cancer Research, and the General Research Support of the John A. Burns School of Medicine.

References

- Birnie, G. D., Macphail, E., Young, B. D. & Paul, J. (1974) *Cell Differentiation* **3**, 221-232
- Bishop, J. O., Morton, J. G., Rosbash, M. & Richardson, M. (1974) *Nature (London)* **250**, 199-204
- Borsook, H. (1965) *Ann. N.Y. Acad. Sci.* **119**, 523-539
- Borsook, H., Ratner, K. & Tattre, B. (1969) *Blood* **34**, 32-41
- Brandhorst, B. P. & Humphreys, T. (1971) *Biochemistry* **10**, 877-881
- Brandhorst, B. P. & Humphreys, T. (1972) *J. Cell Biol.* **53**, 474-482
- Brandhorst, B. P. & McConkey, E. H. (1974) *J. Mol. Biol.* **85**, 451-463
- Britten, R. J. & Davidson, E. H. (1969) *Science* **165**, 349-357
- Emerson, C. P. (1971) *Nature (London) New Biol.* **232**, 101-106
- Emerson, C. P. & Humphreys, T. (1971) *Anal. Biochem.* **40**, 254-266
- Evans, M. J. & Lingrel, J. B. (1969) *Biochemistry* **8**, 3000-3005
- Grau, O. & Favelukes, G. (1968) *Arch. Biochem. Biophys.* **125**, 647-657
- Humphries, S., Windlass, J. & Williamson, R. (1976) *Cell* **7**, 267-277
- Hunt, J. A. (1974a) *Biochem. J.* **138**, 487-498
- Hunt, J. A. (1974b) *Biochem. J.* **138**, 499-510
- Hunt, J. A. & Oakes, G. N. (1976) *Biochem. J.* **155**, 637-644
- Kirby, K. S. (1968) *Methods Enzymol.* **12B**, 87-99
- Leder, P., Ross, J., Gielen, J., Packman, S., Ikawa, Y., Aviv, H. & Swan, D. (1973) *Cold Spring Harbor Symp. Quant. Biol.* **38**, 753-761
- Lingrel, J. B. & Borsook, H. (1963) *Biochemistry* **2**, 309-314
- Lord, B. I. (1970) *Cell Tissue Kinet.* **3**, 13-19
- Penman, S., Vesco, C. & Penman, M. (1968) *J. Mol. Biol.* **34**, 49-69
- Perry, R. P., Kelly, D. E. & LaTorre, J. (1974) *J. Mol. Biol.* **82**, 315-331
- Ramirez, F., Gambino, R., Maniatis, G. M., Rifkind, R. A., Marks, P. A. & Bank, A. (1975) *J. Biol. Chem.* **250**, 6054-6058
- Sheldon, R., Jurale, C. & Kates, J. (1972) *Proc. Natl. Acad. Sci. U.S.A.* **69**, 417-421
- Spohr, G., Imaizumi, T. & Scherrer, K. (1974) *Proc. Natl. Acad. Sci. U.S.A.* **71**, 5009-5013
- Wolberg, J. R. (1967) *Prediction Analysis*, pp. 27-135, Van Nostrand, Princeton, NJ