INCORPORATION OF [^{14}C]GLUCOSE AND [^{3}H]URIDINE INTO THE MAJOR CLASSES OF RNA IN MOUSE EMBRYOS DURING PREIMPLANTATION DEVELOPMENT

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Abstract

The incorporation of [U-^{14}C]glucose into the major classes of RNA in mouse embryos cultivated *in vitro* was studied at stages from eight-cell to the blastocyst. Comparative studies using [5-^{3}H]uridine were also included. Chromatography on columns of methylated bovine serum albumin-coated kieselguhr (MAK) was employed to separate soluble RNA (sRNA), ribosomal RNA (rRNA), and RNA having a high affinity for MAK (TD-RNA).

The uptake and rate of incorporation of [^{14}C]glucose into the RNA of each cell of the embryo was low at the eight-cell stage but increased abruptly at the morula or early blastocyst stage and then fell at the late blastocyst stage of development. TD-RNA incorporated the bulk of the glucose carbon entering into the RNA at all stages of development and gained more radioactivity in relation to the other RNA classes during transition from the eight-cell to the morula. Embryos cultured with [^{3}H]uridine, however, incorporated the bulk of the nucleoside into rRNA, rather than into TD-RNA, suggesting the possibility of either different precursor pools or the differential entry of glucose and uridine into the pools. Actinomycin D at concentrations of 10^{-7} and 10^{-6}M greatly depressed the incorporation of glucose into sRNA and rRNA but only slightly depressed the incorporation of glucose into TD-RNA.

The inability of actinomycin D to suppress completely the utilization of labelled precursor pools to form TD-RNA prevented a valid assessment of the relative stability of the various RNA classes labelled with radioactive glucose.

Morulae developing into blastocysts over a 24-hr period *in vitro* in the presence of either [1-^{14}C]glucose or [6-^{14}C]glucose incorporated carbon-1 and carbon-6 of the hexose into RNA at an equal rate, confirming that most of the sugar is metabolized via the Embden–Meyerhof pathway and tricarboxylic acid cycle rather than via the hexose monophosphate shunt.

I. INTRODUCTION

In recent years increasing attention has been paid to the synthesis of RNA during early development with the aim of assessing the times of intervention and utilization of the genome and the nature of its expression. Although autoradiographic and biochemical analyses have shown that the synthesis of both transfer and ribosomal RNA (tRNA and rRNA) begin at the four-cell stage of cleavage in the mouse embryo (Mintz 1964; Woodland and Graham 1969), some earlier genetic

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intervention may be required for embryogenesis in this species since embryos at the two-cell stage display a marked sensitivity to actinomycin D (Silagi 1963; Mintz 1964; Thomson and Biggers 1966; Monesi *et al.* 1970). RNA synthesis intensifies from the morula stage to the blastocyst (Monesi and Salfi 1967) and, from the eight-cell stage on, rRNA is a major product of RNA synthesis (Ellem and Gwatkin 1968; Pikó 1970). The rabbit embryo also shares the property of very early synthesis of tRNA with the mouse embryo but differs in the time of onset of rRNA synthesis (Manes 1969, 1971).

In contrast to the early dependence of embryonic development on gene activity in the mouse, the externally developing eggs of echinoderms and amphibians appear to be completely independent of genetic intervention until gastrulation. Although some messenger RNA (mRNA) synthesis does occur in these embryos during cleavage, their main support is probably derived from stable mRNA and ribosomes synthesized during oogenesis (Gross and Cousineau 1964; Gross *et al.* 1964; Tyler 1967; Gross 1968).

Using chromatography on methylated bovine serum albumin-coated kieselguhr (MAK) for the separation and analysis of [^{3}H]uridine incorporation into the various classes of RNA, Ellem and Gwatkin (1968) detected synthesis of low molecular weight soluble RNA (sRNA), of rRNA, and of RNA having a high affinity for MAK (TD-RNA) in the mouse embryo at stages from the eight-cell to late blastocyst. No information, however, is available on the extent to which the early mouse embryo is able to synthesize the components required for the formation of the three major classes of RNA from exogenous energy sources which alone support development *in vitro*. This paper presents a study of the synthesis of sRNA, rRNA, and TD-RNA in preimplantation mouse embryos during cultivation *in vitro* in the presence of [^{14}C]glucose, an energy substrate which supports cleavage from the eight-cell stage to the blastocyst (Brinster and Thomson 1966). Comparative studies using [^{3}H]uridine are also presented.

II. Materials and Methods

(*a*) *General*

Embryos were obtained at various developmental stages by flushing the reproductive tracts of superovulated random-bred albino mice at specific times after ovulation (Brinster 1967*a*). Modified Krebs–Ringer bicarbonate solution containing 25 mM sodium DL-lactate, 0·25 mM sodium pyruvate, 1 mg/ml bovine serum albumin, 60 μg/ml penicillin, and 50 μg/ml streptomycin sulphate (Brinster 1965) was used as the medium for collection of embryos. After recovery the embryos were transferred through two changes of a similar medium devoid of energy substrates (2 ml per wash) before incubation in medium containing either [^{14}C]glucose or [^{3}H]uridine (Amersham Laboratories, England). Eight-cell embryos failed to develop satisfactorily in the presence of [U-^{14}C]glucose unless the sugar was purified by paper chromatography using n-butanol–acetic acid–water (4 : 1 : 5 v/v) solvent. Labelled glucose was adjusted with substrate-free medium to give a final concentration of 5·56 mM and specific activities of either 3 or 28 μCi/μmole depending on the requirements of the experiment. [5-^{3}H]Uridine was adjusted with medium containing lactate and pyruvate or with medium containing unlabelled glucose to give a final concentration of 0·01 mM and specific activity of 5 mCi/μmole.

(*b*) *Number of Cells in Embryos*

The number of cells in mouse morulae and blastocysts was determined by counting free nuclei prepared by the method of Tarkowski (1966). The nuclei were stained with Giemsa (Ellem and Gwatkin 1968) and the cells were counted in 10 embryos.

(c) *Culture of Embryos*

The required number of 25-μl drops of radioactive medium were prepared in a plastic culture dish (Falcon Plastics) containing 10 ml of paraffin oil (Brinster 1963) and 25–100 embryos were incubated in each drop for the required periods at 37°C using air–carbon dioxide (95 : 5 v/v) as the gas phase.

At the completion of the incubation period 10 apparently normal embryos were separated from the incubation medium by centrifugation through non-radioactive medium and assayed for radioactivity (Wales and Whittingham 1970). The remaining embryos which were normal morphologically at the completion of culture were washed twice in 2 ml of substrate-free medium and stored at −20°C in 1 ml of 0·1M acetate buffer (pH 5·0) containing 4 μg of polyvinyl sulphate for 2–3 days until assayed.

(d) *Extraction of RNA*

Approximately 100 mg of mouse liver was homogenized in 9 ml of 0·1M acetate buffer (pH 5·0) containing 4 μg/ml of polyvinyl sulphate and added to the embryos just prior to extraction in order to provide carrier RNA. RNA was then extracted from the embryos by the cold phenol method essentially as described by Brown and Littna (1964). Sodium lauryl sulphate was added to give a final concentration of 2% (w/v) and the suspension was shaken with an equal volume of redistilled water-saturated phenol at 5°C for 15 min. After centrifuging, the aqueous phase and interphase were removed and again extracted with water-saturated phenol for a further 15 min. Nucleic acids were precipitated from the aqueous phase after the second extraction by the addition of 0·1 vol. of 1M NaCl and 2·5 vol. of ethanol followed by storage for approximately 6 hr at −20°C. The precipitated nucleic acids were collected by centrifugation, washed in 75% (v/v) ethanol and ether, and then dissolved in 5 ml of 0·01M acetate buffer (pH 5·0) containing 1 μg/ml of polyvinyl sulphate. Ribonuclease-free deoxyribonuclease (15 μg/ml) and 1 mM $MgCl_2$ were added and the solution was held for 15 min at room temperature. At the end of this period, RNA was again precipitated as described above and allowed to stand overnight at −20°C. The RNA collected by centrifugation was washed in 75% (v/v) ethanol and then ether, and finally dissolved in 2 ml of 0·05M NaCl in 0·05M Tris–HCl buffer (pH 6·7). An aliquot of this solution was taken for radioassay and the remainder applied to a column of MAK.

(e) *Chromatography on MAK*

The preparation of the methylated albumin has been described by Mandell and Hershey (1960); the construction of the column followed the simplified method of Monier *et al.* (1962). Similar chromatographic profiles were obtained when the column was constructed by the method of Mandell and Hershey (1960) or of Monier *et al.* (1962). Kieselguhr (16 g) was suspended in 80 ml of 0·05M Tris–HCl buffer (pH 6·7) containing 0·05M NaCl and coated with methylated serum albumin by adding 4 ml of a 1% (w/v) solution of the protein in water. MAK columns (1 by 12 cm) were poured by sections on top of a cellulose powder pad under an air pressure of 0·3 kg/cm^2. The RNA extract was applied to the column and then washed with approximately 60 ml of the buffer solution until the radioactivity in the eluate had fallen to low levels.

Nucleic acids were eluted with a linear 0·1–2·0M NaCl gradient in 0·05M Tris–HCl buffer (pH 6·7) at a pressure of 0·1 kg/cm^2 at room temperature and 2-ml fractions were collected and retained for optical density measurements at 260 nm and for radioassay. At the completion of the gradient elution, TD-RNA, having a high affinity for MAK, was eluted from the column with 1M ammonia and each fraction was neutralized immediately on collection (Ellem 1966; Kunz *et al.* 1970). Washing with ammonia was usually initiated after 30 fractions had been collected from the NaCl gradient.

(f) *Determination of Radioactivity*

A 1-ml sample of each fraction was added to 10 ml of a scintillator consisting of toluene–Triton X100 (2 : 1 v/v) (Patterson and Greene 1965) containing 0·4% (w/v) 2,5-diphenyloxazole and 0·01% (w/v) 1,4-bis(4-methyl-5-phenyloxazol-2-yl)benzene and counted in a Nuclear-Chicago liquid scintillation spectrometer. Where necessary counts were corrected for quenching by internal standardization.

(g) *Statistical Analyses*

Where necessary the primary data were converted to logarithms and the significance of the results was assessed by statistical analyses. Values given in the text are means ± standard errors of the means.

III. Results

(a) *Incorporation of [U-^{14}C]Glucose into the RNA of Mouse Embryos Cultured for 24 hr* in vitro

MAK chromatographic elution profiles of labelled RNA extracted from mouse embryos following culture for 24 hr *in vitro* in medium containing [U-^{14}C]glucose (3 μCi/μmole) as the sole energy source are shown in Figure 1. A similar qualitative elution pattern was obtained consistently and the results were highly reproducible. Optical density peaks of RNA from the unlabelled carrier liver cells, although not shown in the figure, always corresponded to peaks of radioactivity. Major fractions of RNA were eluted from the MAK column in turn as sRNA, rRNA, and TD-RNA (see Ellem and Gwatkin 1968; Kunz *et al.* 1970).

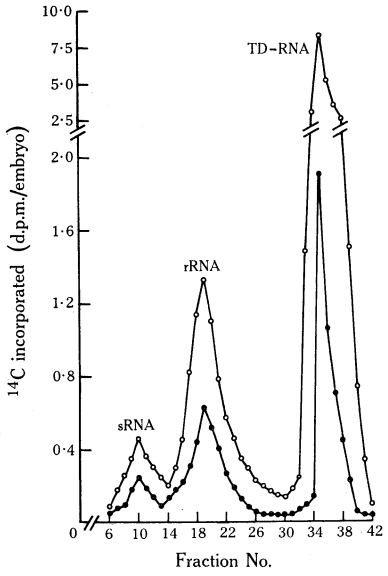

Fig. 1.—MAK column chromatography of RNA from mouse embryos following culture for 24 hr with [U-^{14}C]glucose. Each point represents the mean of three replicates. The average number of embryos used per replicate was 255. ● Eight-cell embryos developing into morulae. ○ Morulae developing into blastocysts.

Eight-cell embryos which developed into morulae over the 24-hr culture period *in vitro* accumulated 58·7 ± 0·9 pg-atoms of glucose carbon per embryo intracellularly and incorporated radioactive carbon into all major RNA classes. About 11% of the total uptake of radioactivity was accounted for in the RNA of which 47% was incorporated into TD-RNA, 40% incorporated into rRNA, and the remaining 13% occurred in sRNA. The amount of radioactivity accumulated intracellularly increased to 252·3 ± 19·2 pg-atoms of glucose carbon per embryo when morulae were permitted to develop for 24 hr into blastocysts in the presence of the labelled glucose. In this case about 12·5% of the radioactivity occurred in RNA. The increased uptake of glucose at this stage was accompanied by a twofold increase in

the amount of glucose incorporated into sRNA and rRNA, and a sevenfold increase in the amount incorporated into TD-RNA. The total amount of radioactivity incorporated into TD-RNA was very much greater than that into rRNA ($P < 0{\cdot}01$) which, in turn, was greater than that into sRNA ($P < 0{\cdot}05$).

(*b*) *Incorporation of [U-^{14}C]Glucose into the RNA of Mouse Embryos Cultured for 5 hr* in vitro

In order to assess more precisely the time at which glucose incorporation into embryonic RNA is accelerated during preimplantation development mouse embryos at the eight-cell, morula, and late-blastocyst stages were cultured for 5 hr with [U-^{14}C]glucose of high specific activity (28 μCi/μmole) and their RNA was again subjected to MAK chromatographic analysis. The results are presented in Figure 2.

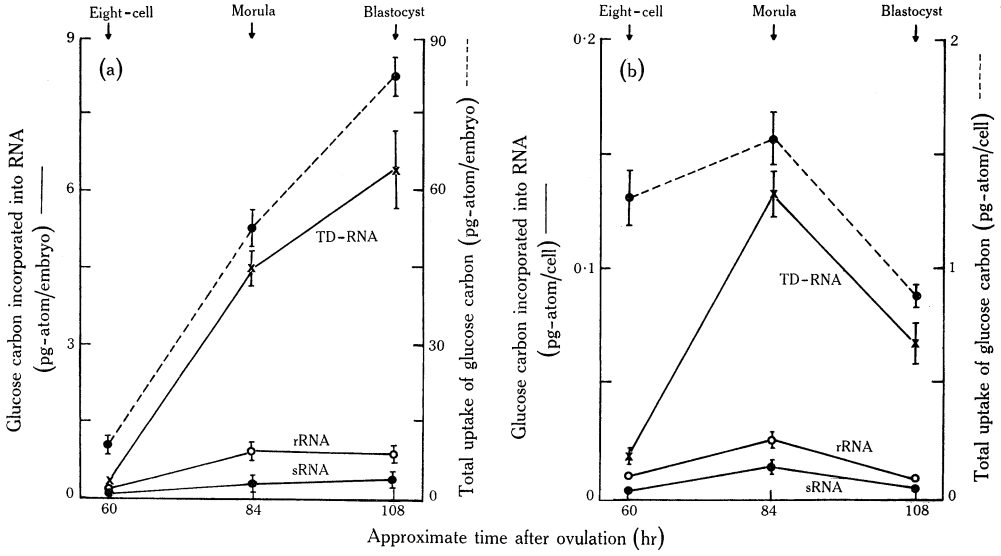

Fig. 2.—Total uptake and incorporation of [U-^{14}C]glucose into the various RNA classes of mouse embryos at the eight-cell, morula, and blastocyst stages of development during 5 hr culture. Values are expressed either in terms of whole embryos (*a*) or in relation to the number of cells present at each stage (*b*). Each point represents the mean ± standard error for three replicates. The average number of embryos used per replicate was 200.

In addition to assessing RNA synthesis on a per embryo basis, the data were also related to the number of cells present at each stage of development. The embryos contained $34{\cdot}1 \pm 3{\cdot}5$ cells at the morula and $94{\cdot}0 \pm 9{\cdot}0$ cells at the blastocyst stage.

Eight-cell embryos accumulated $10{\cdot}41 \pm 0{\cdot}99$ pg-atoms of glucose carbon per embryo intracellularly over the 5-hr culture period and incorporated only about 2% of this radioactive carbon into RNA. About 59% of the radioactivity in the RNA occurred in the TD-RNA at this stage. The uptake of glucose increased to $53{\cdot}0 \pm 4{\cdot}2$ pg-atom/embryo at the morula stage and was accompanied by an abrupt acceleration of incorporation of radioactivity into all RNA classes, and particularly into TD-RNA.

This increase in uptake and incorporation was not due merely to an increase in the number of cells present in the embryo since the increase was still evident when the data were expressed on a per cell basis. The radioactivity in the RNA at the morula stage represented about 11% of that incorporated intracellularly. Although the accumulation of glucose by whole embryos further increased to 82·6±4·0 pg-atoms of glucose carbon per embryo at the late-blastocyst stage, the rate of uptake and incorporation of the sugar into the RNA of each cell of the embryo decreased.

TD-RNA gained more radioactivity in relation to the other RNA classes during transition from the eight-cell to the morula stage and at the morula and blastocyst stages it contained 80 and 84% respectively of the radioactivity in the total RNA. The amount of radioactivity incorporated into rRNA at all developmental stages was significantly greater ($P < 0{\cdot}05$) than that into sRNA.

(c) *Comparison of the Incorporation of [U-^{14}C]Glucose and [5-^{3}H]Uridine into the RNA of Morulae Cultured for 5 hr* in vitro

An experiment was designed to compare the relative rates of incorporation of labelled glucose and uridine into the various RNA classes of mouse morulae following culture *in vitro* for 5 hr. Aliquots of the labelled nucleoside as obtained from the supplier were dried under a stream of nitrogen and reconstituted to give the required concentration in either lactate–pyruvate medium as described by Ellem and Gwatkin (1968) or in unlabelled glucose medium. To gauge the effect of uridine on glucose incorporation, [U-^{14}C]glucose was reconstituted in substrate-free medium with and without the addition of 0·01M uridine. The results are shown in Table 1.

TABLE 1

INCORPORATION OF [^{14}C]GLUCOSE AND [^{3}H]URIDINE INTO THE MAJOR RNA CLASSES OF MOUSE MORULAE

The isotopic incorporation values represent the means ± standard error for three replicates. The average number of embryos used per replicate was 189. Embryos were cultivated *in vitro* with the labelled compounds for 5 hr. G = 5·56 mM glucose; U = 0·01 mM uridine; LP = 25 mM lactate and 0·25 mM pyruvate

Parameter measured	Culture medium	10^2 × Glucose carbon (pg-atom) or uridine (pmole) incorporated per embryo into:			Percentage distribution of radioactivity in:		
		sRNA	rRNA	TD-RNA	sRNA	rRNA	TD-RNA
Glucose incorporation	G	21±3	65±7	413±77	4	13	83
	G+U	15±4	52±10	330±98	4	13	83
Uridine incorporation	LP+U	1·3±0·3	5·9±0·8	3·1±0·7	13	57	30
	G+U	0·9±0·1	5·2±1·2	2·2±0·4	11	62	27

Following culture in the presence of [U-^{14}C]glucose about 83% of the radioactive carbon incorporated into RNA occurred in the TD-RNA fraction while about 13% occurred in rRNA. No significant effect of uridine on the rate of glucose incorporation was detected and the results are in good agreement with those of the previous experiment. Culture of morulae in the presence of [5-^{3}H]uridine, however, produced a different MAK chromatographic profile of isotopic incorporation into RNA. In

this case, only about 29% of the radioactivity incorporated into RNA occurred in TD-RNA while about 60 and 11% occurred in the rRNA and sRNA fractions respectively.

(*d*) *Incorporation of Specifically Labelled Glucoses into the RNA of Morulae Cultured for 24 hr* in vitro

Morulae developing into blastocysts over a 24-hr period *in vitro* in the presence of either [1-^{14}C]- or [6-^{14}C]glucose (3 μCi/μmole) incorporated carbon-1 and carbon-6 of the hexose into RNA at about an equal rate (Table 2). The MAK chromatographic elution profile of labelled RNA obtained following culture with [1-^{14}C]glucose was almost identical to that obtained with [6-^{14}C]glucose. The intracellular accumulation of carbon-1 and carbon-6 over the 24-hr period was calculated as $55{\cdot}3 \pm 3{\cdot}8$ and $54{\cdot}5 \pm 2{\cdot}6$ pg-atom/embryo respectively. As in the previous experiments, the bulk of the radioactive carbon occurring in RNA was found in TD-RNA while more was incorporated into rRNA than into sRNA.

TABLE 2

INCORPORATION OF [1-^{14}C]- AND [6-^{14}C]GLUCOSE INTO THE MAJOR RNA CLASSES OF MOUSE MORULAE

The isotopic incorporation values represent the means ± standard error for three replicates. The average number of embryos used per replicate was 293. Embryos were cultivated *in vitro* with the labelled sugars for 24 hr

Labelled sugar	$10^2 \times$ Glucose carbon incorporated from position labelled (pg-atom/embryo):			Percentage distribution of radioactivity in:		
	sRNA	rRNA	TD-RNA	sRNA	rRNA	TD-RNA
[1-^{14}C]Glucose	47 ± 8	138 ± 21	754 ± 182	5	15	80
[6-^{14}C]Glucose	41 ± 8	120 ± 22	653 ± 175	5	15	80

(*e*) *Incorporation of* [*U*-^{14}*C*]*Glucose and* [5-^{3}*H*]*Uridine into the RNA of Morulae following a Pulse-labelling Period of 2 hr and a Subsequent Chase Period of 24 hr*

Morulae were incubated for 2 hr in glucose medium containing either [U-^{14}C]-glucose (28 μCi/μmole) or [5-^{3}H]uridine (5 mCi/μmole) and then washed thoroughly in two changes of 2 ml of non-radioactive medium. Half of the embryos were deep-frozen immediately and stored until assayed while the remaining half were cultured for a further 24 hr in non-radioactive medium. The results are shown in Figure 3.

Morulae accumulated $22{\cdot}36 \pm 0{\cdot}42$ pg-atoms of glucose carbon per embryo intracellularly over the 2-hr pulse-labelling period. After chasing for 24 hr in non-radioactive medium, $7{\cdot}40 \pm 1{\cdot}66$ pg-atoms of glucose carbon per embryo remained. TD-RNA incorporated the bulk of the radioactive glucose carbon entering into RNA during the 2-hr incubation and only a relatively small amount of ^{14}C was isolated in the rRNA fraction. The content of labelled carbon in sRNA was low over this period and a well-defined peak of radioactivity was not obtained. However, a small peak of radioactivity, which eluted from the MAK column at an earlier stage than sRNA,

was consistently detected. This peak was not observed in the previous experiments where longer periods of labelling were employed, and disappeared after the embryos were cultured in non-radioactive medium. The amount of radioactive glucose carbon contained in the TD-RNA decreased after culture in non-radioactive medium while substantial increases occurred in the amount of radioactivity contained in the sRNA and rRNA fractions.

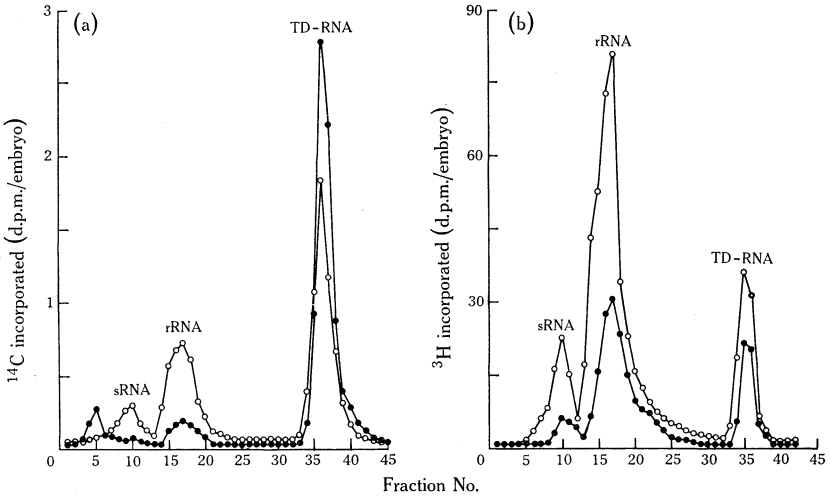

Fig. 3.—MAK column chromatography of RNA from mouse morulae pulse-labelled for 2 hr with [U-^{14}C]glucose (*a*) or [5-^{3}H]uridine (*b*). Each point represents the mean of three replicates. The average number of embryos used per replicate was 275. ● Embryos pulse-labelled for 2 hr with no further treatment. ○ Embryos pulse-labelled for 2 hr and cultured for a further 24 hr in non-radioactive medium.

Uridine was rapidly incorporated into the RNA of morulae and after 2 hr appeared in all the classes separated by MAK chromatography. The rRNA fraction incorporated the bulk of the radioactivity and there was no evidence of a peak of radioactivity appearing before the elution of sRNA. Although the amount of radioactivity contained in each RNA class increased during culture in non-radioactive medium, the increase in the TD-RNA fraction was comparatively smaller than the increases that occurred in the sRNA and rRNA fractions.

(*f*) *Effect of Actinomycin D on the Incorporation of* [*U-*^{14}*C*]*Glucose into the RNA of Morulae*

The effects of 10^{-7} and 10^{-6}M actinomycin D on the rate of incorporation of [U-^{14}C]glucose (28 μCi/μmole) into the various RNA classes of mouse morulae are shown in Table 3. Embryos were incubated for 10 min in substrate-free medium containing the desired dose of actinomycin D before the 5-hr labelling period in the presence of actinomycin D.

Actinomycin D inhibited markedly the incorporation of glucose carbon into sRNA and rRNA but only slightly depressed incorporation into TD-RNA. The

antibiotic was particularly effective in inhibiting the incorporation of glucose carbon into rRNA while the incorporation of radioactivity by sRNA was more resistant to the inhibitor. The total uptake of glucose by morulae was depressed slightly in the presence of 10^{-6}M actinomycin D.

TABLE 3

EFFECT OF ACTINOMYCIN D ON THE INCORPORATION OF [U-^{14}C]GLUCOSE INTO THE MAJOR RNA CLASSES OF MOUSE MORULAE

Values represent the means ± standard error for three replicates. The average number of embryos used per replicate was 204. Embryos were cultivated *in vitro* with the labelled glucose and the various doses of actinomycin D for 5 hr

Concn. of actinomycin D	Total uptake of glucose carbon (pg-atom/embryo)	$10^2 \times$ Glucose carbon incorporated (pg-atom/embryo) into:		
		sRNA	rRNA	TD-RNA
0	69·5±1·6	28±6	99±14	310±17
10^{-7}M	69·3±4·5	13±2	30±8	295±15
10^{-6}M	59·9±3·6	4±1	11±3	238±33

(*g*) *MAK Chromatography of RNA following Treatment with Ribonuclease, Pronase, and 0·3*N *Alkali*

This experiment was conducted to assess whether any of the RNA classes isolated by MAK chromatography were contaminated by other radioactive moieties produced during culture with [^{14}C]glucose.

A total of 1200 morulae were cultured for 5 hr in the presence of [U-^{14}C]glucose (28 μCi/μmole) and then extracted for RNA. The RNA was dissolved in 4·0 ml of 0·05M NaCl in 0·05M Tris–HCl (pH 6·7), divided into four equal aliquots of 1·0 ml, and treated as follows. One aliquot was subjected to MAK chromatography without further treatment and served as a control. Another aliquot was treated with 100 μg of boiled bovine pancreas ribonuclease in the presence of 0·015M trisodium citrate for 1 hr at 37°C. The third aliquot was treated with 0·25% (w/v) pre-digested Pronase for 1 hr at room temperature. The RNA was precipitated from the fourth aliquot by adjusting the NaCl concentration to 0·1M, adding 3 vol. of ethanol, and storing overnight at −20°C. The precipitate collected by centrifugation was dissolved in 2 ml of 0·3N NaOH and incubated for 18 hr at 37°C. The sample was then neutralized with HCl. The RNA in all treated samples was reextracted after adding carrier liver cells as described in Section II and subjected to MAK chromatography.

The sRNA and rRNA fractions of the extract were extremely susceptible to ribonuclease and contained amounts of radioactivity following treatment with the enzyme which were not significantly different from background. TD-RNA, however, was more resistant to the effects of the enzyme and approximately 48% of the radioactivity remained in the fraction after treatment. Treatment of the extract with Pronase did not affect the radioactive content of the individual RNA classes. Of the radioactivity originally contained in the sRNA, rRNA, and TD-RNA fractions, only 10, 4, and 18% respectively remained following digestion in 0·3N alkali.

IV. Discussion

The development of the early mouse embryo appears to be regulated by continual synthesis of RNA (Monesi *et al.* 1970). However, an exogenous supply of nucleic acid precursors is not necessary for early development and it is likely that the embryo either contains adequate endogenous amounts or is able to synthesize the precursors required for the formation of RNA from exogenous energy sources (Thomson Ten Broeck 1968). From the eight-cell stage to the blastocyst exogenous glucose supports cleavage (Brinster and Thomson 1966) and the results of the present investigation indicate that the embryo incorporates carbon from glucose into all the major classes of RNA separated by MAK chromatography. Murdoch and Wales (1971) have also shown that early mouse embryos are able to incorporate carbon into their sRNA from other naturally occurring exogenous energy sources which support development during cultivation *in vitro*, and from the fixation of carbon dioxide.

Care must be exercised in interpreting the present isotopic incorporation values as valid measures of RNA synthesis since little knowledge exists of precursor pool sizes and their effects in the embryos (Epstein and Daentl 1971). The entry of glucose into a wide variety of carbon pools within the embryo (Pike and Wales 1972) increases the problem of determining precursor pool effects in isotopic incorporation studies where a sugar, rather than a nucleoside precursor such as uridine, is used to label the nucleic acids. Until further work resolves the size and nature of the internal pool, it cannot be claimed that the increased incorporation of glucose into RNA during transition from the eight-cell to the morula reflects increased RNA synthesis resulting from an increase in the rate of transcription of the genome. Such a speculation would also require that the percentage recovery and stability of RNA and the retarding effect of culture on development be the same, or at least known, at each of the stages studied. Although the course of incorporation of glucose into total RNA between the eight-cell and morula stages is similar to the course of incorporation of [^{3}H]uridine as reported by Ellem and Gwatkin (1968), the patterns of incorporation of glucose and uridine appear to be different during transition from the morula to the late-blastocyst stage. Measured on a per cell basis, the rate of glucose incorporation into RNA decreases during this time while Ellem and Gwatkin (1968) suggest no such decrease in the total RNA incorporation of uridine. This difference may be related to changes in the metabolism of the cultured embryo affecting the rate of incorporation of glucose into precursor pools.

TD-RNA appears to be the predominant class of RNA synthesized from exogenous glucose. Similar observations have been made by other investigators using various tissues and [^{32}P]orthophosphoric acid and [^{3}H]orotic acid as RNA precursors (Ellem 1966; Emerson and Humphreys 1970; Kunz *et al.* 1970). In contrast, the pattern of isotopic incorporation obtained with [^{3}H]uridine indicates that the bulk of this nucleoside enters into the rRNA rather than the TD-RNA of developing mouse embryos and agrees with the findings of Ellem and Gwatkin (1968) and Pikó (1970). These differences in the relative rates of incorporation of glucose and uridine into the various RNA classes make it difficult to assess which type of RNA is in fact being preferentially synthesized during early development. The differences could indicate the possibility of either different precursor pools for each

RNA or the differential entry of glucose and uridine into these pools. The high level of incorporation of [^{14}C]glucose into TD-RNA is unlikely to be due to residual labelled protein or polysaccharide moieties which could possibly be extracted together with the RNA, absorbed onto the MAK column, and eluted by the 1M ammonia. Treatment of the RNA extracts with the non-specific proteolytic enzyme, Pronase, did not reduce the radioactive content of the TD-RNA. Furthermore, incubation of the RNA extract in 0·3N alkali degraded about 82% of the labelled material originally contained in the ammonia fraction, suggesting that the TD-RNA is composed predominantly of RNA. Even if it is assumed that the 18% of labelled material which is not susceptible to hydrolysis by alkali is not RNA and is subtracted from the total radioactivity contained in the ammonia fraction, the results still indicate that TD-RNA is the major class of RNA synthesized from exogenous glucose. The sRNA and rRNA classes labelled with glucose carbon and isolated by MAK chromatography in the present study also appear to be relatively free of radioactive contaminants. Only low levels of radioactivity were recovered in these fractions when the embryos were incubated with actinomycin D or when the RNA extracts were treated with alkali or ribonuclease. Treatment with Pronase did not affect the radioactive content of these fractions.

The large proportion of ribonuclease-resistant material in the TD-RNA fraction may be due to the presence of RNA with a nucleotide structure which is not susceptible to the hydrolysing effects of the ribonuclease enzyme. West and Todd (1964) have cited evidence to indicate that pancreatic ribonuclease will not hydrolyse phosphate bonds linking pyrimidine nucleotides at the 5′-position with purine nucleotides at the 3′-position, linkages between adjacent purine nucleotides, or 2′,5′-linkages between nucleotides. Gorski and Nicolette (1963) have noted that much newly synthesized nuclear RNA in rat uteri is resistant to hydrolysis by ribonuclease.

A number of authors have attested to the presence of a high-molecular-weight, heterodisperse, labile nuclear RNA in a variety of eukaryotic cells (Harris 1963; Darnell 1968; Penman *et al.* 1968) and Pikó (1970) suggested that this RNA may be identical to the TD-RNA recovered in studies utilizing MAK chromatography. About 90% of the isotope present in the TD-RNA of Hela cells has a DNA-like composition and characteristics which resemble polysomal mRNA (Ellem 1966). The TD-RNA of mouse embryos in the present study may also be composed mainly of DNA-like RNA in view of its relative resistance to the effects of actinomycin D. Actinomycin D at a concentration of 10^{-7}M abolishes nucleolar RNA synthesis (rRNA) but does not appreciably inhibit the extranucleolar nuclear synthesis of DNA-like RNA (Mintz 1964; Ellem and Gwatkin 1968; Pikó 1970). Higher concentrations (10^{-6}M) of the antibiotic depress the extranucleolar nuclear incorporation of [^{3}H]uridine (Mintz 1964) and the rate of incorporation of [^{14}C]glucose into TD-RNA (present study).

The data presented in Figure 3 suggest that a period of greater than 24 hr is required by mouse embryos to deplete the radioactivity contained in the precursor pools after pulsing for 2 hr with either [^{14}C]glucose or [^{3}H]uridine. Since radioactivity continues to be incorporated into all RNA classes of the embryo for extensive periods after pulsing, and since actinomycin D does not completely suppress incorporation

of [^{14}C]glucose into TD-RNA unless levels are used at which its total physiological effects are uncertain, the relative stabilities of the various RNA classes labelled with radioactive glucose could not be demonstrated in the present investigation. However, the smaller changes in the radioactive content of the TD-RNA fraction as compared with those in the sRNA and rRNA fractions following pulsing and culturing in non-radioactive medium in the absence of actinomycin D suggest that TD-RNA is more labile than the other classes of RNA. The negligible amount of radioactivity in the sRNA of mouse embryos pulse-labelled for 2 hr with [^{14}C]glucose may imply the need for a period greater than 2 hr to synthesize the necessary nucleoside triphosphate precursors from exogenous energy sources for this type of RNA. Uridine is incorporated more rapidly than glucose into the embryonic RNA presumably because it does not have to proceed through the complex network of metabolic pathways that glucose carbon must enter in order to be incorporated into RNA.

The similar rates of incorporation of carbon-1 and carbon-6 of glucose into the RNA of mouse embryos supports the findings of Brinster (1967*b*) that shunt activity is negligible in the early mouse embryo and indicates that the sugar is not incorporated into RNA through conversion to ribose in the hexose monophosphate pathway. The absence of extensive shunt activity does not necessarily preclude the entry of glucose carbon into the sugar moiety of the nucleic acids since labelling of the ribose could still occur through the non-oxidative interconversion of hexose and pentose phosphates.

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