THE SYNTHESIS OF SOLUBLE-RNA IN DEVELOPING PRE-IMPLANTATION MOUSE EMBRYOS CULTURED *IN VITRO* IN THE PRESENCE OF ^{14}C -LABELLED PYRUVATE, GLUCOSE AND CARBON DIOXIDE

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The extent to which the early mouse embryo is able to synthesize the components required for the formation of RNA from naturally occurring exogenous energy sources which support development *in vitro* remains to be determined. Recent reports have described the synthesis of RNA and DNA in pre-implantation mouse embryos when cultured or incubated *in vitro* with labelled nucleosides (Ellem & Gwatkin, 1968; Pikó, 1970), but an exogenous supply of these nucleic acid precursors is not necessary for early development (Thomson TenBroeck, 1968). The present study was, therefore, undertaken to discover whether pyruvate and glucose, as exogenous energy sources, act as precursors for the synthesis of soluble-RNA (s-RNA) during this time. Moreover, since mouse embryos require the presence of a bicarbonate buffer system for their development *in vitro*, and since the embryos gain a substantial amount of carbon through the fixation of CO_2 (Wales, Quinn & Murdoch, 1969; Graves & Biggers, 1970) incorporation of ^{14}C from bicarbonate into the s-RNA of the embryos has also been examined.

Two-cell and eight-cell embryos were recovered from the reproductive tracts of superovulated female albino mice at specific times after ovulation (Wales & Biggers, 1968). The basic medium used in the study and the method of washing the embryos free of substrate before cultivation have been described previously (Wales & Biggers, 1968). After washing, embryos were collected in a minimum of substrate-free medium and two-cell embryos were cultivated in 20- μl droplets of medium (twenty embryos/drop) containing $[2\text{-}^{14}\text{C}]\text{pyruvate}$ (0.5 mM; specific activity 5.3 $\mu\text{Ci}/\mu\text{mol}$) with and without the addition of unlabelled lactate (5.0 mM). For the study of the incorporation of glucose into s-RNA, eight-cell embryos were cultured in 25- μl droplets of medium (twenty-five embryos/drop) containing $[\text{U-}^{14}\text{C}]\text{glucose}$ (5.56 mM; specific activity 2.9 $\mu\text{Ci}/\mu\text{mol}$). Carbon dioxide fixation was studied by culturing embryos in the presence of $\text{NaH}^{14}\text{CO}_3$ (specific activity 10 $\mu\text{Ci}/\mu\text{mol}$) as described by Wales *et al.* (1969). All embryos were cultivated in plastic culture dishes (Falcon Plastics) containing light paraffin oil (Brinster, 1963), and were allowed to develop to the blastocyst stage at 37° C under a gas phase of air: CO_2 (95:5).

At the end of the culture period, total incorporation of radioactivity in the embryos was assessed by separating a small number of blastocysts from the culture medium as described by Wales & Biggers (1968). The remaining embryos which had undergone normal development were collected, washed twice in 2.0 ml of substrate-free medium, and stored in sealed culture tubes at -20°C for 2 or 3 days until assayed. Carrier cells, in the form of 13-day-old pronase-dispersed mouse embryos (Gwatkin & Thomson, 1964), were added to these blastocysts and nucleic acids extracted after the method described by Ellem (1966). Soluble-RNA was then isolated from the nucleic acid extract by chromatography on DEAE-cellulose as described by Holley, Apgar, Doctor, Farrow, Marini & Merrill (1961). The s-RNA in the eluate from the DEAE-cellulose column was precipitated, after the addition of 3 mg of yeast s-RNA, by mixing with 3 vol of cold 95% ethanol and storing overnight at -20°C . The RNA was collected on membrane filters of $0.45\text{-}\mu$ pore size, washed with 80% and 95% ethanol and dissolved in 2 ml of 0.05 M-tris-HCl solution, pH 7.3.

TABLE 1
INCORPORATION OF RADIOACTIVE CARBON FROM $[2\text{-}^{14}\text{C}]\text{PYRUVATE}$,
 $[\text{U}\text{-}^{14}\text{C}]\text{GLUCOSE}$ AND $\text{NaH}^{14}\text{CO}_3$ INTO THE s-RNA OF DEVELOPING TWO-
AND EIGHT-CELL MOUSE EMBRYOS CULTURED *IN VITRO*

<i>Labelled compound</i>	<i>Replicate No.</i>	<i>No. of embryos cultured</i>	<i>% Developing into blastocysts</i>	<i>Carbon accumulated from position labelled (pg atoms/blastocyst)</i>	<i>Carbon incorporated from position labelled into s-RNA (pg atoms/blastocyst)</i>
$[2\text{-}^{14}\text{C}]\text{Pyruvate without lactate}$	1	900	32	16.9	0.35
	2	400	22	12.3	0.22
$[2\text{-}^{14}\text{C}]\text{Pyruvate with lactate}$	1	600	57	10.1	0.18
	2	376	50	11.2	0.11
$[\text{U}\text{-}^{14}\text{C}]\text{Glucose}$	1	350	69	269	5.60
	2	210	75	501	4.35
	3	300	87	327	7.13
$\text{NaH}^{14}\text{CO}_3$	1	400	29	—	0.15
	2	475	63	—	0.45

Aliquots of this solution were taken for radioassay. A preliminary examination revealed that protein and DNA contamination was negligible when the s-RNA was isolated by this method. Furthermore, no ribosomal-RNA (r-RNA) could be detected in the preparation after separation on Sephadex G-75 columns by the method of McCoy & Carter (1968).

The results from Table 1 show that a significant amount of labelled carbon from pyruvate accumulated in two-cell mouse embryos during their development into blastocysts. Between 1 and 2% of the pyruvate-carbon was incorporated into s-RNA. The addition of unlabelled lactate to the medium increased the number of embryos developing into blastocysts but decreased the intracellular accumulation of labelled carbon from pyruvate. The percentage of pyruvate-carbon incorporated into s-RNA under these conditions, however, was similar to that in the absence of lactate.

Of the glucose-carbon accumulated intracellularly by eight-cell embryos

cultured to blastocysts, approximately 1 to 2% was incorporated into s-RNA (Table 1). Table 1 also shows that developing eight-cell mouse embryos possess the ability to fix CO_2 and incorporate the carbon into s-RNA.

The results demonstrate that early mouse embryos are able to incorporate carbon into their s-RNA from exogenous energy sources which support development during cultivation *in vitro*, and from the fixation of CO_2 . The results also confirm the observations of Brinster (1965a) that the combination of pyruvate and lactate in the culture medium facilitates a better developmental response than pyruvate alone. However, the presence of lactate in the culture medium reduced the amount of pyruvate-carbon incorporated into the s-RNA of developing two-cell embryos suggesting that the unlabelled pyruvate arising from the conversion of lactate catalysed by lactate dehydrogenase (Brinster, 1965b) may compete, as an alternative s-RNA precursor, with the radioactive pyruvate.

Graves & Biggers (1970) recently showed that CO_2 fixation occurs at all stages of pre-implantation development in the mouse embryo and demonstrated the incorporation of radioactivity from $\text{NaH}^{14}\text{CO}_3$ into nucleic acids. The results from the present investigation indicate that, of the CO_2 fixed into the nucleic acids of these embryos, a significant amount is incorporated into s-RNA.

Thomson & Biggers (1966) have shown that some actinomycin-sensitive RNA synthesis in early mouse embryos is necessary in order for cleavage divisions to occur and have indicated that the RNA is probably of the messenger or transfer (soluble) type. Monesi, Molinaro, Spelletta & Davoli (1970) have also suggested that development of the early mouse embryo is regulated by continual synthesis of RNA. Despite this apparent necessity for RNA synthesis during early development, the extent of overall RNA synthesis in the mouse embryo is small between the two-cell and eight-cell stage but steadily increases up to the end of the blastocyst stage and is further increased after outgrowth (Ellem & Gwatkin, 1968).

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