The Incorporation of Labelled Nucleosides by Mouse Morulae

The rate of early cleavage of the mouse is not yet proved to be genetically controlled[1], but slightly later a lethal gene (t^{12}) is known to stop the development of the morulae[2]. Several other genes act prior to the implantation of the mouse embryo[3] and, in other mammals, cytological research also suggests precocious differentiation[4]. Experiments with Echinoderm and Amphibian eggs instead have offered no evidence of genetic control prior to the blastula stage[5]. Therefore their nucleic acid metabolism during cleavage might be expected to differ from that of the mouse cleaving egg. Since the latter is barely known in this respect, here we present information that permits us to draw a comparison.

Method. The morulae (8–10 blastomeres) from superovulated random-bred mice were cultured at 37°C in a basic medium consisting of Ringer-Krebs bicarbonate at pH 7.2, supplemented with 0.1% dextrose and 0.1% crystalline bovine albumin[6]. The precursors used were either H^3-thymidine, H^3-cytidine or H^3-uridine (New England Nuclear Corp.), at 10 μc/ml of basic medium and in pulse-chase experiments, the morulae were transferred to 0.2% cytidine or uridine (California Found.).

The ova were fixed in formalin-ethanol-acetic acid, embedded in paraffin and sectioned 6 μ thick. In some slides, RNA was digested with 0.2% ribonuclease (Worthington) at pH 6 for 1 h at 40°C; controls were treated in the same way, without the enzyme. All slides were covered with Kodak AR.10 stripping film and exposed during 6 to 10 days. Approximately 400 ova were used in these experiments.

Results. A brief incubation (30 min) with H^3-thymidine is enough to label every cell that is not in mitosis.

A short pulse of H^3-cytidine causes the labelling of the nuclei but not of the nucleolus (Figure 1). After 1 to 2 h of incubation, the nucleolus and the cytoplasm become labelled; the latter showing comparatively more label the longer the incubation (Figures 2, 3). It is not possible to decide whether the labelling of the nucleolus is previous to or simultaneous with that of the cytoplasm, because the resolution of the method does not permit differentiation of the rim of the nucleolus from the associated chromatin[4].

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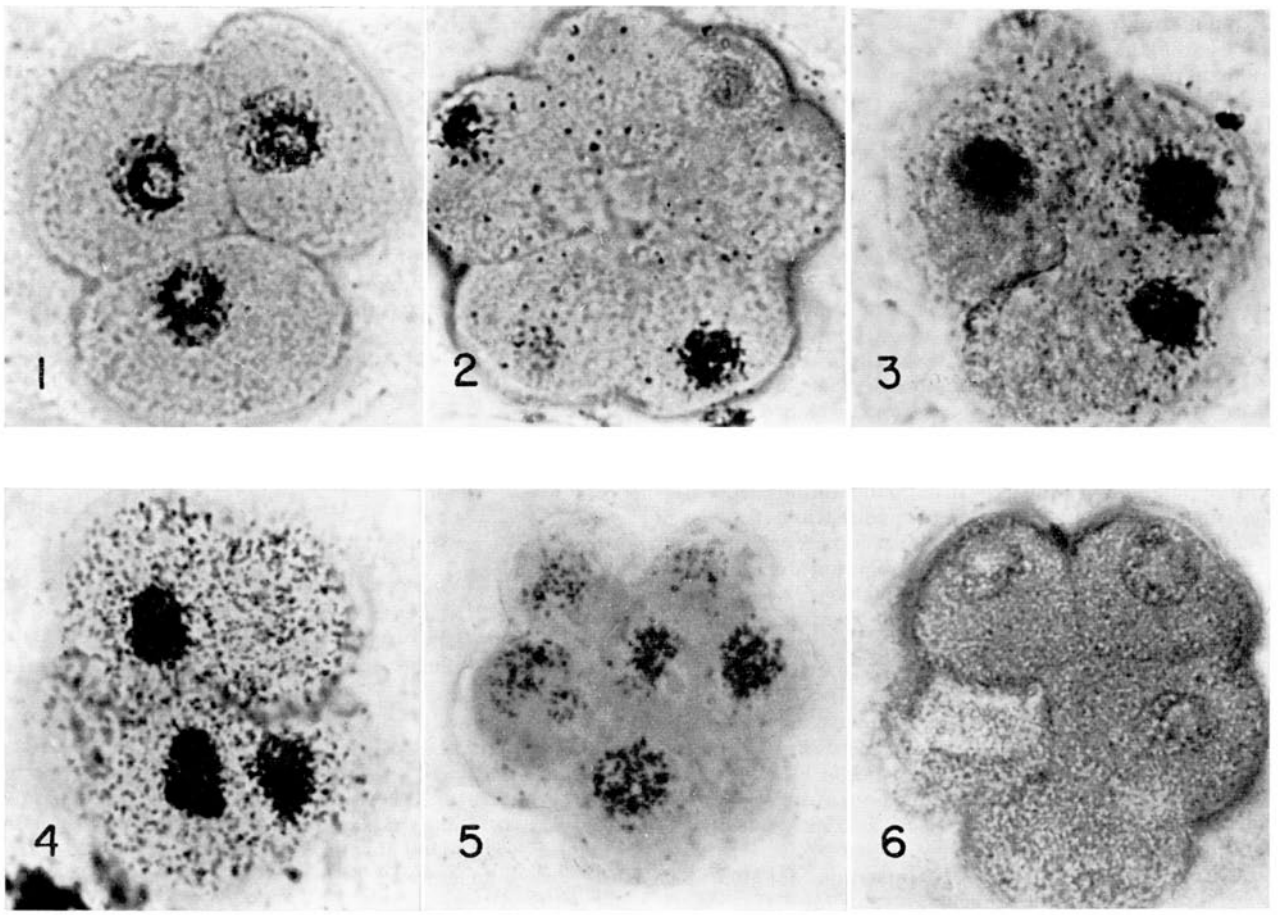

Fig. 1. 8-cell ovum: 1 h incubation with H^3-cytidine. – Fig. 2. 10-cell ovum: 2 h incubation with H^3-cytidine. – Fig. 3. 8-cell ovum: 3 h incubation with H^3-cytidine. – Fig. 4. 8-cell ovum: 1 h incubation with H^3-cytidine and $1^1/_2$ h incubation with cytidine. – Fig. 5. 12-cell ovum: 30 min incubation with H^3-uridine. – Fig. 6. 8-cell ovum: digestion with ribonuclease.

The cytoplasm is also labelled after a short pulse, when this is followed by a chase with cold precursor (Figure 4). We have not noticed a decrease of nuclear label in this case; however, the chase periods tried were not longer than $1\,^1/_2$ h.

The incorporation of H^3-uridine follows the same pattern described for H^3-cytidine, and the extraction with ribonuclease yields negative radioautographs (Figures 5, 6).

Discussion. Our experiments with H^3-thymidine prove that, even if a cytoplasmic store of DNA exists, an exogenous precursor can also be utilized.

The results obtained with H^3-cytidine and H^3-uridine give evidence of uptake into RNA and show a pattern of incorporation similar to that seen in tissue cultures[7]. The cytoplasmic label probably corresponds to ribosomic RNA, as is suggested by its time of appearance in relation to the nucleolar label[7], and by the higher probability of retaining heavy ribosomic RNA after the fixation and the embedding procedure. In Echinoderms and Amphibia, the incorporation of labelled uridine during cleavage is low and it is not recovered from the heavy ribosomal fraction[8–11].

Furthermore, labelled uridine in our preparations is extracted by ribonuclease, while it is partially incorporated into the DNA of cleaving Amphibia eggs[12]; a finding also reported for the egg of the marine snail *Ilyanassa*[13].

The effect of actinomycin D also points to a difference between mouse ova, their development being arrested[14], and Amphibia or Echinoderm eggs, which are not affected prior to the blastula stage[15,16].

In conclusion the pattern of nucleic acid metabolism of mouse morulae differs from that known for cleaving Echinoderm and Amphibia eggs; this coincides with their difference in genetic control[17].

Résumé. L'incorporation de précurseurs marqués des acides nucléiques dans les morulae de souris, suit un cours différent de celui qui est connu pour les œufs en segmentation d'Echinodermes et d'Amphibiens; ceci pourrait se rapporter à leur contrôle génétique différent.

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Muscle Spindles in the Rat Diaphragm

Antidromic activity (ADA), which under certain conditions occurs in motor nerves and ventral roots, was first demonstrated by Masland and Wigton[1]. Their finding has been confirmed and subjected to further study by other authors (Feng and Li[2]; Eccles et al.[3]; Riker et al.[4]; Werner[5]; Blaber and Bowman[6]). Under appropriate conditions, ADA has been demonstrated also in the phrenic nerve-diaphragm preparation (Van der Meer and Meeter[7]; Barstad[8]; Randić and Straughan[9]). As the recordings in the latter experiments have been made from the phrenic nerve trunk, activity in afferent fibres within this nerve presents a potential source of error. Recording from the cervical ventral roots of the rat being technically difficult, Randić and Straughan[9] circumvented the intricacy by sensory denervation of the phrenic nerve three weeks in advance of their experiments. For a similar purpose, van der Meer and Meeter[7] employed longitudinal splitting of the nerve.

Studying ADA in the rat phrenic nerve-diaphragm preparation, the present authors occasionally recorded series of spike potentials which had the main characteristics of the afferent firing from muscle spindles. Records from these experiments are shown in Figure 1.

Afferent firing in the phrenic nerve has been recorded in the rabbit by Cardin[10,11] and Cuénod[12] and in the cat by Yasargil[13,14]. According to Hinsey et al.[15] approximately 10% of myelinated fibres in the cat phrenic nerve are sensory. Landau et al.[16] found 35–45% afferents in the phrenic nerve of the dog.

Histologically several authors have demonstrated the occurrence of muscle spindles in the diaphragms of different mammalian species, including man (Dogiel[17];

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