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The oviduct as a barrier to exogenous thymidine in the early development of the mouse embryo

By A. P. DYBAN[1]
NATALIA A. SAMOSHKINA[1]
AND EWA B. MYSTKOWSKA[2]

From the Department of Embryology, USSR Academy of Medical Science and the Department of Embryology, University of Warsaw

SUMMARY

Mouse embryos are able to use exogenous thymidine during cleavage stages *in vivo* if the thymidine is injected directly into the oviduct.

DNA synthesis during cleavage occurs asynchronously and the intensity of DNA synthesis in the blastomeres of the same embryo varies. At all stages, the label in some nuclei is distributed unevenly, suggesting that some chromosomes may be heterochromatinized very early in development.

INTRODUCTION

[^{3}H]thymidine injected subcutaneously into a pregnant female mouse is incorporated by the embryos only when they have entered the uterus, i.e. in the late morula or early blastocyst stage (Samoshkina, 1965). However, if cleaving mouse embryos are cultured in medium containing [^{3}H]thymidine, nuclear labelling is observed from the beginning of cleavage (Mintz, 1962, 1964; Izquierdo & Roblero, 1965; Oprescu & Thibault, 1965; Szollosi, 1966; Samoshkina, 1968; Fraccaro *et al.* 1969). The data presented in this paper show that the reason for this difference between *in vivo* and *in vitro* experiments is that [^{3}H]thymidine, when injected subcutaneously into pregnant female mice, does not reach the cleaving embryos.

MATERIAL AND METHODS

On each of the first 4 days after mating, 0·05–0·1 ml of Brinster's medium (Brinster, 1963), containing 25 or 50 μCi/ml [^{3}H]thymidine, was injected directly into the isthmic part of the left oviduct of female mice of strain *C3HA*. Oviducts and uterine horns on the right side were untreated. The animals were killed 2 or 24 h after operation. Right and left oviducts and uterine horns were fixed in

[1] *Authors' address:* Department of Embryology, Institute of Experimental Medicine, USSR Acad. Med. Sci., Kirovski prospekt 69/71, Leningrad P-22, U.S.S.R.

[2] *Author's address:* Department of Embryology, University of Warsaw, Warsaw 64, Poland.

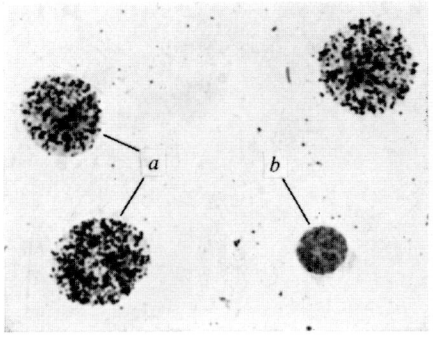

Fig. 1

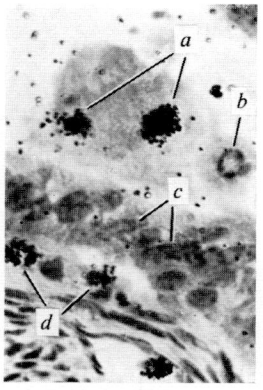

Fig. 2A

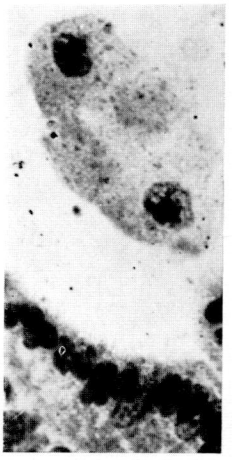

Fig. 2B

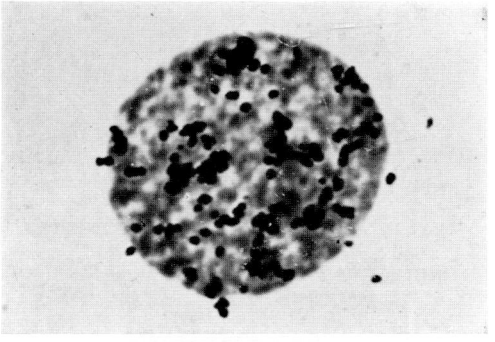

Fig. 3

Autoradiographs of cleaving mouse embryos after [^{3}H]thymidine injection into the oviduct.

Fig. 1. Three-blastomere embryo from [^{3}H]thymidine-injected oviduct (2 h after [^{3}H]thymidine injection). × 400. *a*, Labelled blastomere nuclei; *b*, unlabelled nucleus of polar body.

Fig. 2. Cross-sections of the mouse oviduct on the 3rd day of pregnancy. A. Embryos in thymidine-injected oviduct. *a*, Labelled blastomere nuclei; *b*, unlabelled nucleus of polar body; *c*, oviduct epithelium (none of the epithelial nuclei in this field were labelled); *d*, labelled nuclei of oviduct connective tissue cells. B. Embryo in intact oviduct of the same mouse. No radioactive label in blastomere nuclei. × 900.

Fig. 3. Uneven label distribution (heterochromatization) in blastomere nuclei at six-blastomere stage.

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alcohol–acetic, embedded in paraffin and serially sectioned for histological analysis. In some animals the oviducts and uterine horns were flushed with physiological saline and the embryos air-dried (Tarkowski, 1966). Liquid photoemulsion (type NIKFI-'M' or 'R') was used to prepare autoradiographs. 150 embryos were examined by autoradiography.

RESULTS

The results obtained by sectioning and by air-drying were identical. The embryos from the oviducts injected with [^{3}H]thymidine readily incorporated radioactive label into their nuclei, during the whole of cleavage from the pronuclear stage onwards (Figs. 1, 2A). On the other hand, no radioactive labelling was detected in the embryos of the control oviducts at any stage of cleavage (Fig. 2B). The DNA-labelling pattern in embryos from [^{3}H]thymidine-injected oviducts was identical to that in cleaving ova exposed to [^{3}H]thymidine *in vitro* (Samoshkina, 1968). In both cases DNA synthesis during cleavage occurs asynchronously. For instance, at the 8-blastomere stage, 4 nuclei may incorporate thymidine while 4 others remained unlabelled, or all the blastomeres may be labelled, or none.

The intensity of DNA synthesis in the blastomeres of the same embryo varied, with some nuclei containing a large number of silver grains, and others lightly labelled or unlabelled. Asynchrony of DNA synthesis was found both in blastomeres of the same embryo and in different embryos of the same female.

At all stages the label in some nuclei was distributed unevenly (Fig. 3), which might indicate that some chromosomes are heterochromatinized very early in development (Fraccaro *et al.* 1969). In embryos examined 24 h after [^{3}H]-thymidine injection there was 3- to 4-fold dilution of the radioactive nuclear label when the embryos reached the blastocyst stage, corresponding to the increase in blastomere number. In such embryos nearly 75% of all nuclei were labelled and labelled mitoses were found.

Nuclei of epithelial and connective tissue cells from uninjected oviducts did not contain any label, while some of those from thymidine-injected oviducts were labelled. In contrast, uterine cells both in the left (operated) and in the right (control) side were labelled. This shows that radioactive thymidine injected into the left oviduct, although not incorporated into the cells of the right oviduct, was utilized by the cells in the right uterine horn.

DISCUSSION

Thus our results show that mouse embryos are able to use exogenous thymidine during cleavage *in vivo*. However, this utilization occurs only if thymidine is injected directly into the oviduct. Unsuccessful attempts to find radioactive label in cleaving mouse embryos after direct injection of [^{3}H]thymidine into the oviduct have been reported (Glass & McClure, 1965; Woodland & Graham, 1969; A. McLaren, personal communication). Our positive results may be explained by the higher [^{3}H]thymidine doses used and especially by the very high sensitivity of nuclear photoemulsion (NIKFI) with high-resolution ability which was used in our experiments (Epifanova & Terskich, 1969). Samoshkina's (1965) studies in which subcutaneously injected [^{3}H]thymidine failed to be

incorporated into embryos in the oviduct also used very high doses of [^{3}H]thymidine, and the same highly sensitive nuclear photoemulsion.

We may conclude that during the early stages of pregnancy in mice, subcutaneously injected thymidine penetrates little if at all into the oviduct lumen, i.e. the oviduct tissues serve as a biological barrier for exogenous thymidine, blocking its way to the cleaving embryos. The mouse uterus has no such barrier function, so that when the embryo enters the uterus its cells can utilize exogenous thymidine injected subcutaneously into the mother.

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