Uptake and incorporation of [^3H]uridine by normal or diapausing rat blastocysts after various periods of culture

V. Bitton-Casimiri, J. L. Brun and A. Psychoyos

*Laboratoire de Physiologie de la Reproduction, CNRS, E.R. 122,
Hôpital de Bicêtre (INSERM), 94270 Bicêtre, France*

The transition from morula to blastocyst is accompanied by an increase in metabolic activity (see Brinster, 1973). In lactating rats and mice or under certain experimental conditions, i.e. after ovariectomy in early pregnancy, blastocysts do not implant and exhibit a state of dormancy during which mitotic activity is absent (Baevsky, 1963), the ability to synthesize DNA, RNA and protein is almost non-existent (Weitlauf & Greenwald, 1965; Prasad, Dass & Mohla, 1968; Gulyas & Daniel, 1969) and energy metabolism is markedly lower (Menke & McLaren, 1970). For activation and implantation of such 'dormant' blastocysts to occur *in utero*, it is obligatory for the uterus to be under the influence of both ovarian hormones (Psychoyos, 1973). However, 'dormant' blastocysts transferred to extrauterine sites recover their mitotic activity, whereas those left in the uterus remain depressed (Kirby, 1967; Psychoyos, 1967), and after culture for some hours uridine uptake (Psychoyos & Bitton-Casimiri, 1969) and amino acid incorporation (Weitlauf, 1974), initially low, increase.

In order to study the synthetic processes of blastocysts removed from the uterine environment, we compared the pattern of [^3H]uridine uptake and incorporation by normal and 'dormant' blastocysts during 24 hr of culture.

Normal blastocysts were recovered from rats killed at 13.00 hours on the 5th day of pregnancy. 'Dormant' blastocysts were recovered on the 9th day of pregnancy from rats which had been ovariectomized on the 2nd day *post coitum* and then treated daily with 5 mg progesterone. Blastocysts were washed twice with culture medium and groups of 10 were placed in Petri dishes under paraffin oil in 20 μl Eagle L medium supplemented with 1% dialysed fetal calf serum, and incubated at 37°C in an atmosphere of 5% CO_2 in air (Bitton-Casimiri & Psychoyos, 1968). After incubation for 0, 3, 6, 12, 18 or 24 hr a 20 μl drop of medium containing 2 μCi [^3H]uridine (sp. act. 20 Ci/mm: Commissariat à l'Energie Atomique) was added for 45 min. At the end of the incubations, an ice-cold solution of Tyrode containing non-radioactive uridine was added to each dish. The blastocysts were then washed three times with 1 ml Tyrode solution containing unlabelled uridine, recovered and deposited between two discs of Whatman fibre-glass filters. Background radioactivity was determined in an equal amount of the wash solution. The filters were placed in 1 ml cold 5% trichloroacetic acid (TCA) for 20 min, followed by two successive treatments with 1 ml cold 95% ethanol for 10 min each, and then with 1 ml ether for 5 min. The filters were finally air dried. The dried filters and 0.5 ml TCA were counted with 10 ml Bray's scintillation fluid in a Beckman LS 100 counter. The radioactivity in the filters gives an estimate of uridine incorporated into TCA-insoluble material containing the RNAs, whereas the estimation of free uridine is given by the radioactivity of the TCA-soluble fraction.

The results are shown in Table 1. The uptake of uridine (TCA-soluble fraction) by 'dormant' blastocysts was lower than normal at the time of recovery; during the first hours *in vitro* it doubled, and a sharp rise occurred around the 18th hour while the level of uptake of normal blastocysts showed a distinct rise only by 24 hr of culture. However, there was an apparent drop of the uptake values after 6 hr for normal and after 12 hr for 'dormant' blastocysts; such variations may be related to the series of rhythmic contractions and dilatations of blastocysts *in vitro* which have been observed through microcinematographic studies, the first strong contraction appearing after 5-6 hr for normal (Bitton-Casimiri, Brun & Psychoyos, 1970), and about the 10th hr for 'dormant' (unpublished data) blastocysts. The similarity of these times suggests that the decrease of radioactivity is related to changes in the volume of the blastocoel.

Table 1. Mean ($\pm$ S.E.M.) uptake and incorporation (ct/min/blastocyst) of [3 H]uridine by normal and 'dormant' rat blastocysts (50–70/group) after various periods of incubation

Incubation period (hr)	No. of determinations	TCA-soluble fraction		TCA-insoluble fraction	
		Normal	Dormant	Normal	Dormant
0	3	343 $\pm$ 31	100 $\pm$ 18	42 $\pm$ 5	20 $\pm$ 2
3	3	326 $\pm$ 11	189 $\pm$ 19	49 $\pm$ 8	25 $\pm$ 4
6	3	140 $\pm$ 9	207 $\pm$ 40	57 $\pm$ 6	34 $\pm$ 3
12	3	266 $\pm$ 48	157 $\pm$ 43	81 $\pm$ 13	32 $\pm$ 10
18	4	432 $\pm$ 22	569 $\pm$ 89	136 $\pm$ 3	105 $\pm$ 12
24	4	901 $\pm$ 14	592 $\pm$ 56	154 $\pm$ 5	139 $\pm$ 19

Incorporation of uridine into RNA (TCA-insoluble fraction) of normal blastocysts showed a progressive increase throughout the entire period of culture. The level of incorporation by 'dormant' blastocysts, lower than normal at the beginning, exhibited a small but significant increase during the first 6 hr and a sharp increase after 18 to 24 hr to reach values comparable to those for normal blastocysts. Studies of 'dormant' mouse blastocysts have indicated that there is an augmentation of protein synthesis after 4–8 hr of incubation which is inhibited by addition of actinomycin-D to the medium (Weitlauf, 1974). One of the first events after removal of the 'dormant' blastocysts from the uterine environment may therefore be the synthesis of DNA-dependent RNA which eventually codes the synthesis of some new proteins essential for further activation. The augmentation of RNA synthesis recorded around the 18th hr of culture may depend on the formation of such proteins.

Rapid activation of the synthetic processes in the 'dormant' blastocyst *in vitro*, as shown by the present study, further supports the hypothesis that the uterus may exert an inhibitory influence on blastocysts during delayed implantation, inducing a state of embryonic diapause (Psychoyos & Bitton-Casimiri, 1969; Psychoyos, 1973).

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