THE EFFECT OF OESTROGEN AND PROGESTERONE ON CO₂ PRODUCTION BY 'DELAYED IMPLANTING' MOUSE EMBRYOS

C. A. TORBIT AND H. M. WEITLAUF*

*Departments of Anatomy and Obstetrics and Gynecology,
University of Kansas Medical Center,
Kansas City, Kansas 66103, U.S.A.*

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Menke & McLaren (1970) reported that CO₂ production by 'delayed implanting' mouse embryos is significantly lower than that of blastocysts implanting at the normal time. They suggested that the metabolic quiescence associated with delayed implantation could result either from an inhibitory effect of progesterone, or from a lack of stimulation by oestrogen. The present experiments were undertaken to distinguish between these possibilities; maternal levels of oestradiol and progesterone were manipulated and the effect on CO₂ production by preimplantation embryos was determined.

Sexually mature, virgin, white Swiss mice at random stages of the oestrous cycle were induced to ovulate with injections of gonadotrophins (Fowler & Edwards, 1957). The females were placed with fertile males and mating was confirmed by the presence of a vaginal plug on the next morning (Day 1 of pregnancy). Pregnant females were allocated to two groups; those in the first group were left intact, and those in the second group were ovariectomized on Day 4 (09.00 to 10.00 hours) to prevent implantation (Weitlauf & Greenwald, 1968). Blastocysts were collected from intact animals on Day 5 and from ovariectomized animals on Day 10 (09.00 to 10.00 hours). Blastocysts were flushed from excised uteri with a stream of culture medium (Brinster & Thomson, 1966) from a blunt needle on a hypodermic syringe. Embryos from each group were pooled in 100 μ l medium in a Maximov slide.

The level of CO₂ production was estimated by the method of Brinster (1967) modified as follows. Twenty to twenty-five blastocysts were washed (three times) in glucose-free medium and transferred to a glass tube (10 $\times$ 45 mm) containing 50 μ l medium with uniformly labelled [¹⁴C]glucose (1 mg/ml; Amersham/Searle, sp. act. 3.0 mCi/mmol); this was covered with 0.07 ml paraffin oil and sealed in a scintillation vial with a second empty tube (10 $\times$ 45 mm). The embryos were incubated for 2 hr at 37°C in 5% CO₂ and air. The embryos were then killed and CO₂ was released by injecting 0.1 ml of 1 N-H₂SO₄ through the stopper. Soluene sample solubilizer (1 ml—Packard) was injected into the empty tube to absorb CO₂. The vials were left sealed for 24 hr to allow for absorption of ¹⁴CO₂; they were then opened and the tubes containing

* Present address: Department of Anatomy, University of Oregon, School of Medicine, Portland, Oregon 97201, U.S.A.

embryos and [^{14}C]glucose medium were removed; 10 ml of the scintillation mixture (4.0 g PPO, 40 mg POPOP—Amersham-Searle/litre toluene—Mallinckrodt) was added and the amount of radioactive CO_2 was estimated in a Packard Tricarb Scintillation counter (Model 314 EX). Using samples of [^{14}C]NaHCO₃, it was determined that the efficiency of the total system was 77%. Two vials without embryos were included with each determination to provide background counts. After adjusting the raw counts for background and overall efficiency, total CO_2 production was calculated and expressed as pmol CO_2 /embryo/hr.

Table 1. Effect of oestradiol and progesterone on the production of CO_2 by blastocysts in ovariectomized mice*

Group	Hormone treatment		Hr after first injection of oestradiol-17 β	Mean $\pm$ S.E. CO_2 production (pmol/embryo/hr)
	Progesterone (2.0 mg/day)	Oestradiol-17 β (0.025 $\mu\text{g/day}$)		
1	None†	None†	—	1.59 $\pm$ 0.10 (10)
2	Days 7 to 10	None†	—	1.36 $\pm$ 0.12 (10)
3	None†	Days 9 to 10	—	1.59 $\pm$ 0.06 (10)
4a	Days 7 to 10	Days 9 to 10	0	1.59 $\pm$ 0.11 (10)
b	Days 7 to 10	Days 9 to 10	12	2.14 $\pm$ 0.10 (10)
c	Days 7 to 10	Days 9 to 10	18	3.05 $\pm$ 0.10 (10)
d	Days 7 to 10	Days 9 to 10	21	4.71 $\pm$ 0.14 (10)
e	Days 7 to 10	Days 9 to 10	24	5.13 $\pm$ 0.25 (11)
5	Normal Day-5 embryos‡		—	7.65 $\pm$ 0.30 (12)

Figures in parentheses represent the number of samples.

* Blastocysts recovered between 09.00 and 10.00 hours on Day 10.

† Sesame seed oil vehicle (0.1 ml subcutaneously).

‡ Blastocysts recovered from intact mice between 09.00 and 10.00 hours on Day 5 (i.e. under the influence of endogenous oestrogen and progesterone); compare the rate of CO_2 production with that of the mice in the experimental groups.

To determine whether progesterone and oestradiol influence the production of CO_2 by delayed implanting blastocysts, ovariectomized females were divided into four groups and given hormone injections according to the schedule shown in Table 1 (all injected material was given subcutaneously at 08.00 hours in 0.1 ml sesame seed oil). The females in Group 4 were killed and the blastocysts were recovered at different times (from 0 to 24 hr) after the first injection of oestradiol.

The experiments were carried out randomly over several weeks to avoid systematic errors. Before being pooled into groups, data from each experimental trial were tested with Bartlett's test for groups of unequal sizes and the variances were found to be homogeneous; the data were also subjected to a one-way analysis of variance to confirm a single population. Group means were then compared by Student's *t* test (Snedecor & Cochran, 1967).

The rate of CO_2 production by embryos from ovariectomized mice treated with oil, progesterone or oestradiol was low compared with that by embryos from intact mothers (compare Groups 1, 2 and 3 with Group 5; $P < 0.001$). By contrast, treatment of animals with oestradiol plus progesterone increased

the rate of CO₂ production; within 12 hr, it was significantly above controls (Group 4a versus Group 4b, $P < 0.005$) and groups treated with either progesterone or oestradiol (Group 4b versus Groups 1, 2 and 3), and by 24 hr (Group 4e), it approached that of normal embryos (Group 5).

The present experiments confirm the observation of Menke & McLaren (1970) that the rate of CO₂ production by 'delayed implanting' mouse embryos is low compared to that of blastocysts implanting at the normal time. Furthermore, the results suggest that the difference in embryonic metabolism is related to maternal oestradiol and progesterone levels.

Two hypotheses have been proposed to explain the influence of maternal hormones on carbohydrate metabolism of the blastocyst: (1) that progesterone, unopposed by oestradiol, has an inhibitory effect, and (2) that oestradiol provides a stimulatory effect on the embryos but they remain dormant in its absence (Menke & McLaren, 1970). An inhibitory rôle for progesterone was not observed in the present experiments since there was no significant difference in CO₂ production by 'delayed implanting' embryos from oil- or progesterone-treated mice ($P > 0.2$). Other work (C. A. Torbit and H. M. Weitlauf, unpublished data) has demonstrated, however, that the CO₂ output by 'delayed implanting' mouse embryos increases with time *in vitro* and that this increase is more rapid in blastocysts from oil-treated animals than in those from progesterone-treated females. With preincubation of 8 hr before pulsing with labelled glucose, the CO₂ produced by embryos from oil-treated animals approaches that of normal embryos while CO₂ production by blastocysts from progesterone-treated mice was significantly lower; CO₂ output by normal embryos does not change with preincubation. Therefore, the discrepancy between the present results and those of Menke & McLaren (1970) appears to be related to the shorter incubation in labelled glucose used in this study (i.e. 2 hr versus 4 hr). 'Delayed implanting' blastocysts from animals treated with progesterone show a significant increase in CO₂ production after the injection of oestradiol, suggesting that activation of the blastocysts is the consequence of a stimulatory effect of oestrogen. Whether the hormones act directly on the embryos or indirectly by way of the uterus cannot be determined from the present results. The observation that carbohydrate metabolism of the blastocyst is only accelerated by oestrogen in the presence of progesterone and that this activation requires 12 to 18 hr is strikingly similar to the results of experiments with protein synthesis in 'delayed implanting' mouse blastocysts (Weitlauf & Greenwald, 1968). Although it seems clear that activation of both aspects of embryonic metabolism must be related, it cannot be determined from the present experiments whether the increase in protein synthesis depends on an increase in carbohydrate metabolism or *vice versa*. Experiments are in progress to answer this question.

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