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Protein Synthesis in Delayed
Implanting Mouse Ova

Progress Report

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Protein Synthesis in Delayed Implanting Mouse Ova

Technical Progress Report

COO 1801-29

This report covers work completed and in progress under AEC Contract number AT(11-1)-1801 during the period May 1, 1972-May 1, 1973. The specific areas of experimentation include: 1) Effect of ovarian steroids on protein synthesis by blastocysts in vitro; 2) Chemical determination of changes in protein content in normal and delayed implanting blastocysts; 3) Effect of blocking RNA synthesis on the rate of protein synthesis by delayed implanting blastocysts in vitro; 4) Estimation of the rate of aerobic metabolism and the effect of estrogen on the rate of aerobic metabolism by normal and delayed implanting embryos in vivo and in vitro; and 5) The effect of ovarian steroids on changes in the dry weight of normal and delayed implanting embryos in vivo and in vitro. The specific experiments were contained in section B (paragraphs 1b and c; and 2a, b, and c) of the renewal proposal submitted for 1972-1973, and Section B (paragraph 1a) of the proposal submitted for 1971-1972.

I. In vitro uptake and incorporation of amino acids by blastocysts from intact and ovariectomized mice. Section B (paragraph 1a and b) of the proposal for 1971-1972.

This work is completed and has been published (See COO 1801-21).

Blastocysts were recovered from the uteri of intact or ovariectomized mice and incubated with a mixture of ^{14}C amino acids in vitro. The level of uptake and incorporation of the amino acids as estimated by scintillation counting was higher in blastocysts from intact than in those from ovariectomized animals. Furthermore, when the two types of eggs were "pre-incubated" for varying periods in non-radioactive medium and then placed in ^{14}C amino acids, the level of incorporation by blastocysts from intact animals remained constant while that in blastocysts from ovariectomized animals increased with time. The increasing ability to take up and incorporate amino acids in vitro by blastocysts recovered from the uteri of ovariectomized animals suggest that in the absence of ovarian hormones, the uterus exerts a negative influence on the metabolism of the blastocysts. In addition, incubation of the blastocysts with estradiol 17 β and/or progesterone did not affect the uptake or incorporation of amino acids suggesting that the hormones do not influence protein synthesis in the eggs by direct action.

II. Changes in the protein content of blastocysts from normal and delayed implanting mice. Section B (paragraph 1c) of the 1972-1973 proposal.

This work is complete and has been accepted for publication in the Anatomical Record (See COO 1801-22).

Total protein content of preimplantation mouse embryos was measured at 06.00 hr and 16.00 hr on day 5 of pregnancy (day 1 = day of vaginal plug formation). During this ten hour period, just prior to implantation, the amount of protein increased from 23 to 35 ng per blastocyst; a net increase of 52 per cent. In contrast, protein content was unchanged in

delayed implanting embryos recovered from ovariectomized animals during a comparable period on days 5 or 10 (day 5, 24 ng at 06.00 hr and 24 ng at 16.00 hr; day 10, 29 ng at 06.00 hr and 30 ng at 16.00 hr).

These results demonstrate that the different uptake and incorporation of amino acids between normal and delayed implanting embryos (Weitlauf & Greenwald, '65, '68; Weitlauf, '73) reflect a difference in the actual net synthesis of protein by the two types of blastocysts.

III. Effect of actinomycin D on activation of delayed implanting mouse blastocysts in vitro.

This work was outlined in Section B (paragraph 1b) of the renewal proposal submitted for 1972-1973. The work is in progress and a preliminary report was presented at the 1973 meeting of The Anatomists Association in New York (See COO 1801-23).

Mouse embryos remain in the relatively 'dormant' state characteristic of delayed implantation until exogenous estrogen and progesterone are provided. Within a few hours of hormone administration the level of protein synthesis increases and the 'activated' blastocysts go on to implant (Weitlauf and Greenwald, '68). Conversion of 'dormant' delayed implanting blastocysts to 'active' embryos with high levels of protein synthesis also occurs in vitro (Weitlauf, '73). The origin of RNA necessary for the increased protein synthesis in the 'active' blastocysts is unknown, two possibilities are: 1) it is synthesized just prior to the increase in protein synthesis; or 2) it is present and non-functional (masked) in the 'dormant' phase and becomes functional (unmasked) as the embryos are 'activated'. To answer this question blastocysts were recovered on day 5 from intact animals or on day 10 from animals ovariectomized on day 4 (day 1 = day of vaginal plug). The embryos were incubated in medium with ^{14}C labelled amino acids for 30 minutes; they were washed, placed on membrane filters, and treated with TCA. Acid insoluble radioactivity was 88 CPM/embryo in the embryos from normal animals and 12 CPM in those from ovariectomized animals. Additional groups of embryos were incubated for 4 hours in non-radioactive medium with or without actinomycin D (0.1 $\mu\text{g}/\text{ml}$) then transferred to radioactive medium for 30 minutes. Acid insoluble radioactivity in normal embryos incubated without actinomycin was 81 CPM/embryo and 79 CPM in those incubated with actinomycin. Acid insoluble radioactivity in embryos from ovariectomized animals was 62 CPM with, and 69 CPM without actinomycin. The failure of actinomycin to influence the increase in protein synthesis as the embryos are 'activated' suggests that the RNA necessary for the increase is present and masked during the 'dormant' phase of delayed implantation.

Although the concentration of actinomycin used (0.1 $\mu\text{g}/\text{ml}$) is reported to be adequate to block RNA synthesis in normal mouse blastocysts (Tasca & Hillman, '70; Monesi *et al.*, '70) normal and delayed embryos were incubated in non labelled uridine ($1 \times 10^{-6}\text{M}$) with various concentrations of actinomycin (0.00 $\mu\text{g}/\text{ml}$, 0.01 $\mu\text{g}/\text{ml}$, 0.10 $\mu\text{g}/\text{ml}$, 1.00 $\mu\text{g}/\text{ml}$) then incubated in medium with ^3H uridine and actinomycin for 30 minutes to test the effect in delayed implanting embryos. The 0.1 $\mu\text{g}/\text{ml}$ concentration reduced RNA synthesis by only 20%; 1.0 $\mu\text{g}/\text{ml}$ reduced RNA

synthesis by 70%; and 10 $\mu\text{g/ml}$ reduced RNA synthesis by 87%. Thus, the interpretation that new RNA is not essential for activation is untenable.

The amino acid experiments are therefore being repeated with the higher doses of actinomycin. Preliminary results indicate that protein synthesis is not effected even when RNA synthesis is "completely" blocked.

IV. Comparison of $^{14}\text{CO}_2$ evolution by normal and delayed implanting mouse embryos in vitro.

This work was not specifically contained in last year's renewal but is the preliminary study for the work contained in Section B (paragraph 2a, b and c) of the proposal submitted for 1972-1973. The work is completed and is being readied for publication. A preliminary report was made at the 1973 meeting of The Anatomists Association in New York (See COO 1801-25).

The production of CO_2 by delayed implanting blastocysts is low compared to that of normal blastocysts (Menke & McLaren, '71). Studies of amino acid uptake and incorporation by delayed implanting blastocysts suggests that the uterus, devoid of ovarian hormones, induces a depressed state in the embryos. After being removed from the uterus, the embryos apparently escape the uterine inhibition and incorporate amino acids at a higher rate (Weitlauf, '73). The purpose of the present experiments was to determine if the reduced metabolic rate of delayed implanting blastocysts is also mediated by the uterus. Blastocysts were collected from intact mice on day 5 of pregnancy, and on day 10 from animals ovariectomized on day 4 (day 1 = day of vaginal plug). The embryos were pre-incubated in Brinster's medium for: 0, 4, 8, or 24 hours before being transferred to medium containing uniformly labeled ^{14}C glucose. After a 2 hour incubation in the radioactive medium the embryos were killed (1N H_2SO_4) and the ^{14}C CO_2 trapped in Hyamine Hydroxide. The radioactivity was determined in a scintillation counter and converted to picomoles CO_2 produced per embryo per hour. Normal day 5 embryos produced about 6 picomoles of CO_2 regardless of the length of pre-incubation in non-radioactive medium. In contrast, the amount of CO_2 evolved by delayed implanting blastocysts is initially low (0 h, 1.62) and increases with longer pre-incubation, (i.e., 8 h, 4.82; 24 h, 5.29). CO_2 production after 8 or 24 hours pre-incubation is not significantly different from that of normal blastocysts. These data suggest that the uterus has an inhibitory effect on the metabolic rate of delayed implanting blastocysts from which the embryos "escape" when removed from the uterus.

In addition, it has been shown that in vivo both estrogen and progesterone are necessary for the embryos to become active and that activation requires 18-21 hours; neither hormone alone will cause this response. In vitro, the hormones appear to have no effect, suggesting that in vivo they work indirectly via the uterus to activate the embryos.

V. Influence of estrogen and progesterone on the dry weight of delayed implanting embryos.

This work was not included in last year's request but was suggested by the findings reported in COO 1801-19. The work has been done by Mr. H. Hensleigh under my direction and was presented at the 1973 meeting of The American Association of Anatomists (See COO 1801-24) and is being prepared for publication.

The dry weight of mouse embryos increases sharply prior to implantation on day 5 of pregnancy (day 1 = vaginal plug). The increase in weight from 21 ng at 0600 h to 40 ng at 1600 h is coincident with the trophoblastic giant cell transformation (Dickson, '69). The increase in weight and giant cell transformation do not occur in delayed implantation; however, unimplanted blastocysts in uteri of ovariectomized mice given daily injections of progesterone can be induced to undergo deferred giant cell transformation by a single injection of estrogen. The present experiment was undertaken to determine whether an increase in weight occurs with deferred giant cell transformation and if so, what hormonal conditions are necessary. Mice were ovariectomized at 1000 h on day 4 and divided into three groups. Animals in the first group were given progesterone daily (2.0 mg/day, days 6-9); animals in the second group were given progesterone daily and estradiol-17 β on day 9 (0.025 μ g); animals in the third group were given oil vehicle daily (0.1 mg/day, days 6-9) and estradiol-17 β on day 9. Control animals were given oil vehicle daily. Blastocysts were collected from animals in the progesterone treated group and the control group on day 9 and from all groups on day 10, freeze-dried, and weighed individually on a Lowry fishpole balance. The weight of blastocysts from animals treated with both progesterone and estrogen increased markedly between days 9 and 10 (30 to 53 ng). In contrast, there was little or no change in the weight of blastocysts from animals treated with progesterone only, with estrogen only, or from control animals between days 9 and 10. Thus, an increase in weight comparable to that occurring normally on day 5 occurs with deferred giant cell transformation; and this increase is dependent on the presence of both estrogen and progesterone.

VI. Time Spent: The principal investigator (H. Weitlauf) has spent 90% time on these projects and will continue to do so during the remainder of the current term.

References for Progress Report

- Dickson, A. D. 1969. Cytoplasmic changes during the trophoblastic giant cell transformation of blastocysts from normal and ovariectomized mice. *J. Anat.*, 105:371-380.
- Hensleigh, H. C. 1973. Influence of estrogen and progesterone on the dry weight of delayed implanting embryos. American Association of Anatomists, 86th Meeting. Paper from the platform.
- Menke, T. & McLaren, A. 1971. CO₂ output of mouse blastocysts in vitro, in normal pregnancy and in delay. *J. Reprod. Fert.*, Suppl. 14: 23-29.
- Monesi, V., Molinaro, M., Spalletta, E. and Davoli, C. 1970. Effect of metabolic inhibitors on macromolecular synthesis and early development in the mouse embryo. *Exptl. Cell Res.*, 59:197-206.
- Tasca, R. J. & Hillman, N. 1970. Effects of actinomycin D and cyclohexamide on RNA and protein synthesis in cleavage stage mouse embryos. *Nature*, 225:1022-1025.
- Torbit, C. 1973. Comparison of ¹⁴CO₂ evolution by normal and delayed implanting mouse embryos in vitro. American Association of Anatomists, 86th Meeting. Paper from the platform.
- Weitlauf, H. M. 1973. Effect of actinomycin D on activation of delayed implanting mouse blastocysts in vitro. American Association of Anatomists, 86th Meeting. Paper from platform.
- Weitlauf, H. M. 1973. In vitro uptake and incorporation of amino acids by blastocysts from intact and ovariectomized mice. *J. Exp. Zool.* 183:303-308.
- Weitlauf, H. M. & Greenwald, G. S. 1965. A comparison of ³⁵S methionine incorporation by the blastocysts of normal and delayed implanting mice. *J. Reprod. Fert.*, 10:203-208.
- Weitlauf, H. M. & Greenwald, G. S. 1968. Influence of estrogen and progesterone on the incorporation of ³⁵S methionine by blastocysts in ovariectomized mice. *J. Exp. Zool.* 169:463-470.