PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 158, 260–262 (1978)

Influence of Pregnancy and Postpartum Period on *in Vitro* incorporation of dl-Valine-1-^{14}C into Oviducal and Uterine Protein (40174)

SAMIR K. ROY JR.[1,2] SUDHIR R. MUKHERJI, AND SARIT K. ROY

Division of Endocrinology, Central Drug Research Institute, Lucknow, India

It has been established (1) that estrogen stimulates synthesis and storage of protein in the oviducts; progesterone causes a release of such stored protein into the lumen in rabbits. An increase in oviduct protein synthesis during early pregnancy has also been reported in this species (2). In rats, estrogens are known to accelerate the incorporation of labelled valine into uterine protein but progesterone antagonizes this effect (3).

A rise in the uterine fluid protein and concurrent decrease in oviducal fluid protein during implantation has also been demonstrated in hamsters (4). However similar studies pertaining to stages after implantation are sparse.

The present study is focussed on the influence of endogenous hormonal changes on oviducal and uterine protein synthesis during pregnancy and the postpartum period.

Materials and methods. Adult cycling female albino rats (150–180 g) of the Institute colony were mated with coeval males of proven fertility. Females with sperm positive vaginal smears were separated and that time designated as day 1 PC (postcoitum). Six animals were collected for each day of postcoitum (day 1–12 and 20 PC) and postpartum (day 1–5 PP = postpartum) period. Six animals were also sacrificed during estrus and diestrus stages of the estrus cycle. Oviducts and uterii (both sides) from each animal were removed and cleaned of the adhering fat. Fetuses were stripped away from the uterus prior to slicing and incubation. Tubal tissues from both sides were pooled and so were the uterine slices from each rat. The pool of tissues from both sides from each rat constituted one estimation. The tissue slices were incubated with dl-valine-^{14}C (Bhabha Atomic Research Center, Trombay, India) in Krebs Ringer buffer solution (ph 7.4) at 37° using a shaking incubator in an atmosphere of 95% O_2 and 5% CO_2. The incubation was terminated after 2 hr by transferring the incubation flasks to ice. The adhering radioactivity was removed by washing the tissue slices in fresh buffer solution. The incorporation of radioactive valine into TCA precipitable protein was measured by the method of Little and Lincoln (3) using a Packard Tricarb Scintillation Spectrometer (Model 3320) with 90% counting efficiency for ^{14}C. Protein concentration was estimated using Lowry's method (6).

Statistical analysis. Significance of all comparisons has been determined by *t* test after analysis of variance.

Results. Oviduct. Radioactive valine incorporation increased from diestrus to day 1 PC but declines between days 1 and 6 PC followed by a sharp fall during day 7 and 8 PC (vs. day 6, $P < 0.01$). From day 8 PC onwards up to day 12 there is a steady increase in the rate of amino acid incorporation in the oviduct reaching a peak on day 20 PC. At the time of parturition (day 1 PP) a very significant drop in valine incorporation is observed (vs. day 20 PC, $P < 0.01$), followed by a rise on day 5 PP (vs. day 1 PP, $P < 0.01$).

Uterus. Incorporation of dl-valine-1-[^{14}C] into uterine protein is significantly higher during estrus than at diestrus ($P < 0.01$: Fig. 1). The rise in valine incorporation between day 1 and 4 PC is gradual but a significant increase is seen on day 5 PC (vs. day 1 to 4 PC, $P < 0.01$). This is followed by a continuous decline during days 6–8 PC. A sharp rise in uterine amino acid incorporation is observed between day 12 and 20 PC (vs. day 8, $P < 0.01$). On day 1 PP a significant fall in valine incorporation is observed (vs. day 20 PC, $P < 0.01$) followed by a rise on day 5 PP (vs. day 1 PP, $P < 0.01$).

A close similarity exists between the patterns of oviducal and uterine valine incorporations during various stages following day 8

[1] Reprint request: Dr. Samir K. Roy Jr., Nichols Institute, 1300 So. Beacon Street, San Pedro, CA.

0037-9727/78/1582-0260$01.00/0

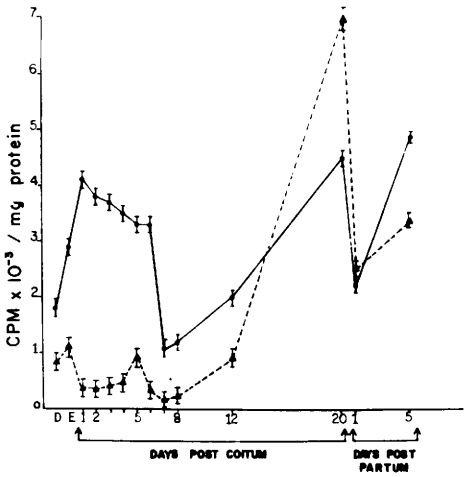

FIG. 1. *In vitro* incorporation of dl-valine-1-[^{14}C] into protein of rat oviduct and uterus during pregnancy and postpartum period. One determination consists of pool of tissues (fallopian tube/uterus) from both sides per animal.

Solid lines indicate valine incorporation into oviducts with the mean of six animal values represented by each solid circle (●—●—●—●). Broken lines indicate incorporation of valine into uterii with each solid triangle as the mean of six animal values (▲---▲---▲---▲). The standard error of mean values are indicated by vertical bars. D = diestrus. E = estrus.

PC i.e., late pregnancy and postpartum. However, the level of radioactive valine uptake in the oviduct seems to be higher than that of the uterus except on day 20 PC.

Discussion. The similarity between hormonal responses of the oviduct and the uterus has been reported (7, 8). Our observation on increase in oviducal and uterine protein synthesis during estrus agrees with previous findings (2, 4, 9). The pattern of amino acid incorporation into oviduct and uterine protein during implantation reflects the following: (a) Oviducal valine incorporation tends to decline with the transport of ova toward the uterus between days 1 and 4 PC, (b) uterine valine uptake is stimulated as a preparatory phenomenon prior to ova reception, followed by implantation. The work of Noske and Daniel (4) showed a decline in oviducal but an increase in uterine fluid protein of rodents during implantation. A similar rise in rabbit uterine fluid protein (Blastokinin) has been reported by Arthur and Daniel (5).

During postimplantation stages (days 6 to 20 PC) a close parallelism between oviducal and uterine valine incorporation exists. A significant fall in valine incorporation and estrogen uptake (8) in both organs on day 7 and 8 PC deserves further exploration.

The close similarity in the radioactive valine incorporation into the oviduct and uterus during late pregnancy and postpartum indicates a parallelism in their biochemical response to gestation and parturition. The present finding is in accordance with our previous observations of similarity between oviducal and uterine estrogen uptake patterns during pregnancy and parturition in rats (8). The rising endogenous estrogenic titer during pregnancy (11) seems to directly influence the uterine amino acid corporation (2, 12). However, a simultaneous increase in the level of valine incorporation in the oviduct protein could also be envisaged on similar grounds. A puerperal fall in plasma estrogen levels causing a decrease in mean total content of uterine nucleic acid has been documented in the rat (15). In view of this, a regression in the oviducal valine incorporation during the postpartum period deserves attention.

After parturition, uterine involution begins in order to regain a prepregnant status (16) involving changes in the biochemical composition and hormonal sensitivity (8, 13, 15). A rise in oviducal and uterine valine incorporation on day 5 PP, after an initial fall on day 1 PP, seems to be correlated with the critical changes in the endogenous level of estrogen and progesterone during puerparium. Our previous work (8, 13) on *in vitro* oviducal and uterine uptake of estrogen during postpartum period is in agreement with the above findings.

In conclusion, the present experiment demonstrates a close similarity in oviducal and uterine valine uptake patterns during pregnancy and postpartum; however, it is not the case during preimplantation stages.

Summary. In vitro incorporation of dl-valine-1-[^{14}C] was measured in oviducts and uterine TCA precipitable protein during diestrus, estrus, early and late pregnancy (PC) and postpartum period (PP) in albino rats. Incorporation of amino acid (valine) into oviduct protein declined between day 1 and 6 PC and a steep fall was observed between day 6 and 7 PC. In uterus, the valine incorporation into protein showed a moderate rise

during implantation (day 5 PC). During post implantation stages (days 7–20 PC) a simultaneous stimulation of oviducal and uterine amino acid uptake occurs. However, on day 1 PP a sharp fall in valine incorporation is observed both in oviduct and uterus followed by a rise on day 5 PP.

Authors wish to thank Mr. P. A. George for statistical analysis and Ms. Kathleen Hatley, CPS for typing the manuscript.

1. Greenwald, G. S., *in* "The Mammalian Oviduct" (E. S. E. Hafez and R. J. Blandau, eds.), 183 pp, University of Chicago Press, (1969).
2. Roy, S. K., Jr., Gupta, D. N., Roy, S. K., Karkun, J. N., and Kar, A. B., Endocrinologie **59,** 221 (1972).
3. Little, B., and Lincoln, E., Endocrinology **74,** 1 (1964).
4. Noske, I. G. and Daniel, J. C., Jr., 9. Reprod. Fertil. **38,** 173 (1974).
5. Arthur, A. T., and Daniel, J. C., Jr., Fertil. Steril. **23,** 115 (1972).
6. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J., J. Biol. Chem. **193,** 265 (1951).
7. Kim, Y. J., Coates, J. B., and Flickinger, G. L., Proc. Soc. Biol. Med. **126,** 198 (1967).
8. Roy, S. K., Jr., and Roy, S. K., Fertil. Steril **28,** 587 (1977).
9. Gupta, D. N., Karkun, J. N., and Kar, A. B. Amer. J. Obstet. Gyncol. **106,** 833 (1970).
10. Holmdahl, T. H., Johnson, E. D. B., and Wide, L., Acta Endocrinol. (kbh) **67,** 353 (1971).
11. Shaikh, A. A., Biol. Reprod. **5,** 297 (1971).
12. Rado, A., Crystle, C. D., and Townsley, J. D., J. Clin. Endocrinol. **30,** 497 (1970).
13. Roy, S. K., Jr., Roy, S. K., Dasgupta, P. R., Engineer, A. D., and Kar, A. B., Amer. J. Obstet. Gynecol., **112,** 229 (1972).
14. Munson, A. K., Mueller, J. R., and Yanone, M. E., Amer. J. Obstet. Gynecol. **108,** 340 (1970).
15. Grant, R. A., J. Reprod. Fertil. **9,** 285 (1965).
16. Vorherr, H., *in* "Biology of Gestation" (N. S. Assali, ed.), 426 pp, Academic Press, New York, (1968).
17. Challis, J. R. G., Davis, I. J., and Ryan, K. J., Endocrinology **93,** 971 (1973).

Received September 30, 1977. P.S.E.B.M. 1978, Vol. 158.