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Estrous Cycles, Decidual Cell Response and Uterine Estrogen and Progesterone Receptor in Fischer 344 Virgin Aging Rats (40503)

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The aging female rat shows a variety of abnormalities in reproductive function. These include a gradual decline in the number of ova and frequency of ovulation, a decrease in litter size (1), irregular estrous cycles leading to constant estrus (2), pseudopregnancies of irregular length and finally anestrus in the oldest rats (3). Some or all of these changes are also seen in other laboratory rodents including mice, hamsters and rabbits (4, 5). Previous reports have suggested that the uterus may be the initial site of reproductive failure (6). Uteri of older rodents are less able to maintain transplanted ova (7), show a decreased decidual cell response (DCR) (8), have a lower uptake of estrogen and progesterone (9, 10) and show decreased sensitivity to circulating progesterone (11). Since uterine function and the DCR primarily depend on the gonadal steroids, estrogen and progesterone (12), one explanation for the defects in uterine function in the aged animal may be a decreased sensitivity or abnormal biological response to these steroids. We report herein a comparison of the uterine estrogen and progesterone receptors from virgin, young (8–10 months) and old (>20 months) rats.

Materials and methods. Animals. Virgin female rats of the Fischer 344 strain were obtained from the National Institute on Aging (aged animal colony maintained at the Charles River Breeding Laboratory). Young rats were 6–8 months and old rats were over 18 months when received in our laboratory. They were housed in an air conditioned room illuminated between 6 am and 8 pm.

Materials. 1,2-[^{3}H]progesterone (^{3}H-P, 55.7 Ci/mmol) and 6,7-[^{3}H]estradiol-17β (^{3}H-E, 53 Ci/mmol) were obtained from New England Nuclear, Boston, MA. All other chemicals were reagent grade.

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Tissue preparation and receptor assay. Rats were guillotined and the uteri were rapidly dissected out, stripped of adhering fat and mesometrium, slit lengthwise, blotted and weighed immediately. All further steps were done at 4°. For assay of estrogen receptors (ER), the cytosol was prepared in TE buffer (10 m*M* Tris–HCl, 1.5 m*M* EDTA, pH 7.4) and assayed in triplicate for ^{3}H-E binding as described previously (13).

Progesterone receptors were assayed in triplicate using ^{3}H-P in TTG buffer (50 m*M* Tris–HCl, 25% glycerol (v/v), 2.4 m*M* thioglycerol, 0.02% Na azide (w/v), 1 m*M* phenylmethylsulfonylfluoride, pH 7.4) by the dextran coated charcoal (DCC, 1% activated charcoal, 0.05% dextran T-70 in TTG buffer) adsorption method (14).

Sucrose gradients. ER was analyzed by centrifugation through 5–20% sucrose gradients as described previously (13). Progesterone receptors were analyzed by centrifugation through 5–10% sucrose gradients made in TTG buffer. Included in the gradients were 10 n*M* ^{3}H-P plus 1 μ*M* cortisol. After centrifugation in Beckman SW 50.1 rotor for 22 hr at 48,000 rpm (216,000 *g*), approximately 0.2 ml fractions were collected into tubes containing 0.5 ml DCC (in TTG buffer) plus 2 mg/ml bovine serum albumin and 1 μ*M* of unlabeled progesterone. After 10 min in ice with intermittent shaking, the charcoal was removed by centrifugation for 10 min at 1200 *g*. The resulting supernatant was extracted with 3 ml toluene-isoamyl alcohol (19:1 v/v) and 2 ml of this extract was removed for scintillation counting. Sedimentation values were determined from standards (ovalbumin, 3.5S; bovine serum albumin, 4.4S; yeast alcohol dehydrogenase 7.4S; catalase, 11.3S) run at the same time in parallel gradients.

Protein and DNA determination. Protein was determined by the method of Lowry *et al.* (15), and DNA was quantitated by the

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method of Burton (16). Student's t test was employed to test the significance of the results.

Results. Estrous cycles and decidual cell reaction. Vaginal smears were taken daily from all rats for a period of 4 weeks to determine the length of the estrous cycles. All ten young rats cycled regularly with a mean length of 4.7 ± 0.1 days, while none of the aged rats showed regular cycles. Most (8 out of 11) were pseudopregnant (mean duration 16.1 ± 0.9 days), two were in anestrus, while only one rat exhibited two consecutive cycles (length of 5 and 4 days) before entering pseudopregnancy.

The DCR in response to exogenous estrogen (E_2) and progesterone was tested in ovariectomized animals. One week following ovariectomy, rats were primed with E_2 (0.5 μg/100 g bw) daily for 3 days. Beginning on the fourth day rats were given a combination of E_2 (0.05 μg/100 g bw) and progesterone (2.5 mg/100 g bw) daily until autopsy. On the fourth day after E_2 plus progesterone treatment, five sutures were placed in one uterine horn and the contralateral horn served as control. Five days following uterine trauma, all rats were killed and the uteri were weighed and examined for DCR. A response was considered positive if the traumatized horn weighed at least three times the control (12). None of the aged rats (n = 3) showed a DCR while three out of four young rats reacted positively.

Uterine wet weights, protein and DNA. The uteri from aged rats, after ovariectomy and E_2 stimulation, weighed considerably more than uteri from the younger rats (Table I). The lower DNA/protein and soluble/total protein ratios, however, in the uteri of aged compared to young rats seem to indicate that much of the increased wet weight may be due to fibrous tissue, as has been observed in the aged hamster uterus by Rahima and Soderwall (17).

Uterine estrogen and progesterone receptor. After three daily injections of 5 μg E_2, the ER levels were significantly lower ($P < 0.01$, Table II) in the old rats when compared to young rats. When 1.5 mg of progesterone was administered along with E_2 on the third day, the ER levels were reduced significantly in the young rats, but not in the old. It has been shown previously (14, 18) that one of the actions of progesterone in the uterus is to inhibit the synthesis of ER.

The concentration of progesterone receptor in the old rats, although tending to be lower, was not significantly different from that in the young animals. In the gradient analysis of the progesterone receptor, both young and aged rats showed a binding peak sedimenting at about 6S which could be abolished by the inclusion of excess unlabeled progesterone in the reaction mixture and gradients (Fig. 1). The young rats, however, also contained

TABLE I. UTERINE WET WEIGHT, PROTEIN AND DNA IN ESTROGEN TREATED YOUNG AND OLD RATS.

	Wet wt (mg)[a]	mg total protein per mg wet wt	mg cytosol protein per mg total protein	mg DNA per mg Total Protein
Young	429 ±39[b]	0.15 ±0.01	0.26 ±0.01	0.036 ±0.002
Old	785 ±97	0.15 ±0.01	0.19 ±0.02	0.026 ±0.002

[a] One week following ovariectomy, seven young and five old rats were injected sc with 5 μg estradiol daily for 3 days and were killed on the 4th day.
[b] Average ± SE.

TABLE II. UTERINE CYTOSOL ESTROGEN AND PROGESTERONE RECEPTOR LEVELS IN YOUNG AND OLD RATS[a].

	^{3}H Steroid binding (fmoles) per mg	
	Cytosol protein	DNA
Estradiol receptor:		
Young (6)[b]	276 ± 10[c]	2000 ± 100
Old (3)	127 ± 6	800 ± 120
Estradiol receptor after Progesterone treatment:		
Young (3)	169 ± 6	1240 ± 30
Old (3)	133 ± 5	770 ± 180
Progesterone receptor:		
Young (6)	329 ± 59	2430 ± 510
Old (6)	258 ± 47	1850 ± 480

[a] One week following ovariectomy, rats were injected sc with 5 μg estradiol daily for 3 days. For estradiol and progesterone receptor, rats were killed on the 4th day without further treatment. To study the effect of progesterone treatment on the amount of estradiol receptor, rats were injected on the 3rd day with 1.5 mg progesterone in addition to estradiol and were killed on the 4th day.
[b] Number in ( ) denotes the number of animals.
[c] Average ± SE.

peaks of progesterone binding at higher *S* values, which may represent multimers of the 6S species. More striking was the difference in the sedimentation rates of the ER (Fig. 2). The ER from the uteri of aged rats sedimented at about 4.5S. In contrast, the receptor from young rats showed both a 7.5S and a 4.5S peak.

Discussion. The DCR depends on a defined sequence of the proper amounts of E_2 and progesterone (19). Moreover, it is well known that E_2 promotes the synthesis of its own receptor as well as the receptor for progesterone, whereas progesterone inhibits the synthesis of at least the former (20). Our present study shows that abnormalities in the characteristics of the uterine ER are present in the aged rat, which may partially be responsible for the failure in reproduction. The aged rat has a greatly reduced quantity of ER. This is in agreement with preliminary reports in mice (21) and rats (22), though in the latter abstract it was reported that prolonged administration of E_2 (7–11 days, 12 μg/100 g bw) resulted in levels of ER that were not significantly different in mature and senescent rats.

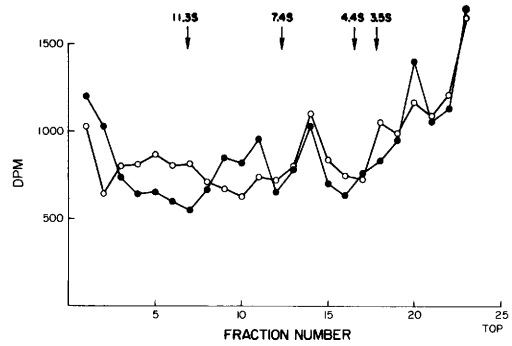

FIG. 1. Sedimentation analysis of the uterine progesterone receptor through 5–10% sucrose gradients made in TTG buffer, with the inclusion of 10 n*M* [^{3}H]progesterone plus 1 μ*M* cortisol. Animals were ovariectomized and 1 week later primed for 3 days with 5 μg estradiol daily. On the 4th day, the rats were killed, cytosol prepared and incubated, and sedimentation performed as described in the methods. Aged rats: ○——○; young rats: ●——●.

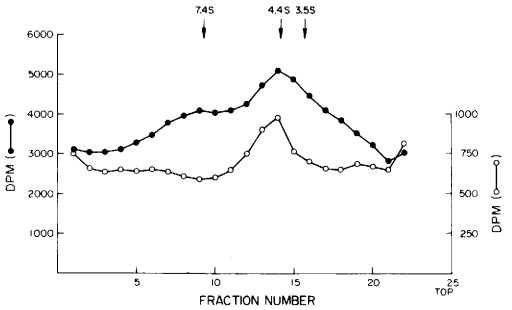

FIG. 2. Sedimentation analysis of the uterine estrogen receptor through 5–20% sucrose gradients made in TE buffer. Hormone treatments were the same as in Fig. 1. Cytosol was prepared, incubated with [^{3}H]estradiol, and centrifuged through the gradients as stated in the methods. Aged rats: ○——○; young rats: ●——●.

The measurements for cytosol ER were all conducted at 4°, at which temperature only available, unbound receptor sites are detected. It is possible that the decreased ER levels in the aged animals were an artifact of the assay in that varying amounts of residual E_2 from the injections might still have been present either in the cells or the interstitial spaces (23). This would have led to binding of the unlabeled E_2 by a fraction of the receptor population during homogenization. That this was not occurring was shown by a preliminary experiment in which the amount of uterine ER was determined 24 hr after the last E_2 injection by both the 4° binding assay and the cytosol exchange assay, which measures both free and filled sites (13). Both methods yielded the same amount.

It has been demonstrated that the level of nuclear bound estrogen correlates with the degree of tissue stimulation by E_2 (24). The decreased quantity of ER in the aged rat uterus, therefore, may render it less sensitive to E_2 and cause the failure in the DCR. Furthermore, we have shown that the aged rat uterus is less sensitive to progesterone as measured by the inhibition of ER synthesis upon progesterone administration. This decline in sensitivity is not due to decreased levels of progesterone receptor since no significant differences between young and old rats were found. Blaha and Leavitt have likewise reported no significant reduction in the content of uterine progesterone receptor in the aged golden hamster (25). Whether these apparent defects in the steroidal response of the tissue are due to alterations in the receptor, as indicated by the altered *S* values, is not known presently. It should be noted, however, that the sedimentation characteristics of receptors are quite sensitive to assay conditions, including ionic strength of the buffers (26).

The aged rat has decreased levels of ER in the brain (27) which was correlated with a reduction in E_2 induced stimulation of acetylcholinesterase activities (28). Also, studies with mice and rats indicate decreased levels of glucocorticoid receptors (29). The infertility of the aged animal may be due to defects in several sites in the hypothalamic–pituitary-gonadal axis. Our results show that end organ (e.g., the uterus) abnormalities in the steroid receptor are present. Indeed, our studies and those cited above may indicate that decreased hormonal sensitivity in the aged animal may be a widespread phenomenon and may be correlated with changes in the receptor for these hormones.

Summary. We have compared the estrous cycles, decidual cell response and the quantities and biochemical characteristics of the estrogen and progesterone receptors in the uterus of aged (>20 months) and young (<10 month), virgin rats. Whereas the young animals showed estrous cycles of normal duration, the aged rats were either pseudopregnant or in anestrus. Old rats failed to show a decidual cell response. Uteri from ovariectomized estrogen primed rats weighed significantly more in the aged compared to the young rats. In the old rat, the absolute amount of estrogen receptor was less than half the amount found in the young animals. The progesterone receptor levels were similar in both age groups. Administration of progesterone failed to decrease estrogen receptor levels in the old rats. The young animals had a greater proportion of progesterone receptor population sedimenting at higher S values than the receptors from aging animals. Similarly, the uterine estrogen receptor of the aged rat sedimented at approximately 4.5S, whereas the young rats showed estrogen receptors sedimenting at both 7.5S and 4.5S. These studies indicate that reproductive failure in the aging rat, as demonstrated by a lack of decidual cell response, is accompanied by changes in the receptors for progesterone and estrogen.

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1. Ingram, D. L., Mandl, A. M., and Zuckerman, T., J. Endocrinol. **17,** 280 (1958).
2. Ingram, D. L., J. Endocrinol. **19,** 182 (1959).
3. Huang, H. H., and Meites, J., Neuroendocrinology **17,** 289 (1975).
4. Finch, C. E., Q. Rev. Biol. **51,** 49 (1976).
5. Soderwall, A. L., Kent, H. A., Turbyfill, C. L., and Britenbaker, A. L., J. Gerontol. **15,** 246 (1960).
6. Connors, T. J., Thorpe, L. W., and Soderwall, A. L., Biol. Reprod. **6,** 131 (1972).
7. Talbert, G., and Krohn, P., J. Reprod. Fertil. **11,** 399 (1966).
8. Holinka, C. F., and Finch, C. E., Biol. Reprod. **16,** 385 (1977).
9. Larson, L. L., Spilman, C. H., and Foote, R. H., Proc. Soc. Exp. Biol. Med. **141,** 463 (1972).
10. Peng, M-T., and Peng, Y-M., Fertil. Steril. **24,** 534 (1973).
11. Blaha, G. C., and Leavitt, W. W., Biol. Reprod. **11,** 153 (1974).
12. DeFeo, V. J., Endocrinology **72,** 305 (1963).
13. Saiduddin, S., and Zassenhaus, H. P., Steroids **29,** 197 (1977a).
14. Saiduddin, S., and Zassenhaus, H. P., Biochemistry **16,** 2829 (1977b).
15. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J., J. Biol. Chem. **193,** 265 (1951).
16. Burton, K., Biochemistry **62,** 315 (1956).
17. Rahima, A., and Soderwall, A. L., J. Reprod. Fertil. **49,** 161 (1977).
18. Hsueh, A. J. W., Peck, E. J., and Clark, J. H., Endocrinology **98,** 438 (1976).
19. Finn, C. A., and Porter, D. G., *in* "The Uterus" (Publishing Sciences Group, Inc.), Acton, Massachusetts (1975).
20. Spelsberg, T. C., and Toft, D. D., *in* "Receptors and Mechanism of Action of Steroid Hormones" (J. R. Pasqualini, ed.), Vol. 1, p. 262, Marcel Dekker, Inc., New York (1977).
21. Nelson, J. F., Holinka, C. F., and Finch, E. E., "The Endocrine Society 58th Annual Meeting," Abstract No. 585 (1976).
22. Holinka, C. F., Nelson, J. F., and Finch, C. E., The Gerontologist **15,** 30 (1975).
23. Michel, G., Jung, I., Baulieu, E. E., Aussel, C., and Uriel, J., Steroids **24,** 437 (1974).
24. Tsai, S. Y., Tsai, M. J., Schwartz, R., Kalimi, M., Clark, J. H., and O'Malley, B. W., Proc. Natl. Acad. Sci. U. S. A. **72,** 4228 (1975).
25. Blaha, G. C., and Leavitt, W. W., J. Gerontology **33,** 810 (1978).
26. Sherman, M. R., Tuazon, Fe B., Diaz, S. C., and Miller, L. K., Biochemistry **15,** 980 (1976).
27. Kanungo, M. S., Patnaik, S. K., and Koul, O., Nature **253,** 366 (1975).
28. Moudgil, V. K., and Kanungo, M. S., Biochem. Biophys. Res. Commun. **52,** 725 (1973).
29. Roth, G. S., and Adelman, R. C., Exp. Gerontol. **10,** 1 (1975).

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