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Impaired Estrogen-Mediated Production of Type C Viral DNA Polymerase in Aged NIH Swiss Mouse Uteri (39481)

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For several years our laboratory has been studying the phenomenon of estrogen-mediated enhancement of murine leukemia virus protein synthesis in the uteri of ovariectomized mice (1-5). Viral proteins examined are RNA-directed DNA polymerase ("reverse transcriptase") and p30, the major viral core protein which bears the group-specific antigenic determinants. We have shown that expression of viral markers in the mouse uterus is strain-dependent (3) and that the level of viral proteins is dependent upon the relative biological potency of the estrogen (5). Maximum concentration of viral proteins occurred 48 hr after a single injection of estrogen (5). We now report a comparative study of the ability of young and aged ovariectomized NIH Swiss mice to respond to estrogen by production of viral protein markers in the uterus.

Materials and methods. Animals. All mice were NIH Swiss females bilaterally ovariectomized at age 14–22 days. Animals in the "young" group were 2–5 months old and in the "aged" group 2.0–2.5 years old. Estrogen-treated mice were injected intramuscularly with 1.0 μg of 1,3,5(10)-estratrien-3,17 β-diol in peanut oil at 0 and 24 hr and were killed at 48 hr by cervical dislocation.

Tissue preparation and assays. Uterine homogenates were prepared in 0.5 *M* KCl and 0.5% Triton X-100 as described (4) and high speed supernatants were analyzed by sedimentation velocity gradients in 10 → 30% glycerol for RNA tumor virus-type RNA-directed DNA polymerase using poly(A)·oligo (dT) as template-primer (4). Murine leukemia virus p30 antigen was measured by radioimmune precipitation assay using a double antibody technique (6). The first antibody was prepared in goat against disrupted Rauscher murine leukemia virus and was supplied by Dr. Roger Wilsnack of Huntington Laboratories. The second antibody was rabbit anti-goat IgG from Meloy Laboratories. Highly purified p30 was obtained from Dr. Wade Parks, Meloy Laboratories. DNA was determined by the method of Hatcher and Goldstein (7), and protein was measured according to Bramhall *et al.* (8).

Histology. Uteri and adrenal glands were dissected from old and young animals, fixed in 10% formalin and 70% ethanol, and paraffin sections were stained with hematoxylin and eosin, Masson's trichrome, or Sudan black.

Results. Aged NIH Swiss mice, even though bilaterally ovariectomized before sexual maturity and untreated with estrogen, had much larger uteri than younger animals (46 vs 6 mg, respectively, Table I). In fact, unstimulated aged mice had uterine weights approximately equal to those of the estrogen-stimulated young mice. The larger uteri in aged animals may have resulted from estrogen produced by the adrenals or elsewhere. Young mice responded to estrogen treatment with a six- to sevenfold increase in uterine weight while older animals had slightly less than a twofold increase (Table I). The amount of weight gained, however, was about the same for both groups (35 and 43 mg, respectively).

There was a fivefold increase in total protein per uterus in young mice in response to stimulation while in aged animals the increase was only twofold (Table I). The actual amount of increased protein, however, was nearly the same in both groups (1.7 and 2.2 mg). DNA per uterus increased threefold upon stimulation in young animals but did not increase in aged mice.

Estrogen treatment of young, ovariectomized mice increased uterine p30 protein from 0.020 ng p30 per μg protein in unstimulated to 0.051 ng p30 per μg protein in stimulated mice (Table I). On the basis of

TABLE 1. EFFECT OF ESTROGEN TREATMENT ON VARIOUS UTERINE PARAMETERS IN YOUNG AND AGED NIH SWISS OVARIECTOMIZED MICE.[a]

	Unstimulated	Stimulated
p30 (ng p30/μg protein)		
young (3)	0.020	0.051[b]
old (4)	0.033	0.044[b]
p30 (total ng per uterus)		
young (3)	6.6	103.0[b]
old (4)	89.0	215.0[b]
Uterine wt. (mg)		
young (3)	5.9	41.3[b]
old (4)	46.5	89.4[b]
Total uterine protein (mg)		
young (3)	0.4	2.1[b]
old (4)	2.4	4.6[b]
Total uterine DNA (μg)		
young (4)	367	808[b]
old (4)	308	350

[a] Mice in the stimulated group were injected intramuscularly with 1.0 μg of estradiol-17β at 0 and 24 hr and killed at 48 hr. Determinations were made on uterine homogenates as described in the text. The data were analyzed as paired samples (unstimulated and stimulated pools assayed simultaneously). The number of sample pairs is in parentheses.

[b] Significantly greater than value for unstimulated at 95% confidence level.

total p30 per uterus the difference between unstimulated and stimulated was greater (7 and 103 ng, respectively). The aged animals also showed increased p30 specific activity in response to estrogen treatment (0.033 ng/μg protein in unstimulated vs 0.044 ng/μg protein in stimulated) and an approximately 2.5-fold increase (89 ng to 215 ng) in total p30. As with uterine weight and total protein values, the total amount of p30 increase in response to estrogen was about the same for both groups (96 ng for young and 126 ng for old).

Figure 1 compares glycerol sedimentation velocity gradient profiles of viral-type polymerase from supernatants of uteri from untreated and estrogen-treated, ovariectomized NIH Swiss mice of the two age groups. In the young animals marked increase in DNA polymerase upon estrogen administration is evident, while the aged group shows no change. A mixing experiment was conducted in which equal volumes of supernatants from aged and young mouse uteri were mixed and run on glycerol gradients. The resulting profile of DNA polymerase activity was essentially superimposable on the profile obtained by plotting the sum of the corresponding fractions of gradients run separately with each supernatant (data not shown). This experiment suggests that the low enzyme activity in the old mice is not due to a soluble inhibitor present in excess in the supernatant.

In a previous study (5), we determined that concentrations of viral marker proteins in the uteri of ovariectomized NIH Swiss mice were greatest 48 hr after a single intramuscular injection of estrogen. The time sequence may be different in the aged mice. To test this possibility, a group of animals was given estrogen on Days 0, 4, 6, 8, 11, and 12 and sacrificed on Day 13. Glycerol gradient profiles of polymerase from uteri of these animals were not different from those of mice given estrogen only at 0 and 24 hr and killed at 48 hr.

When preparing homogenates we noticed

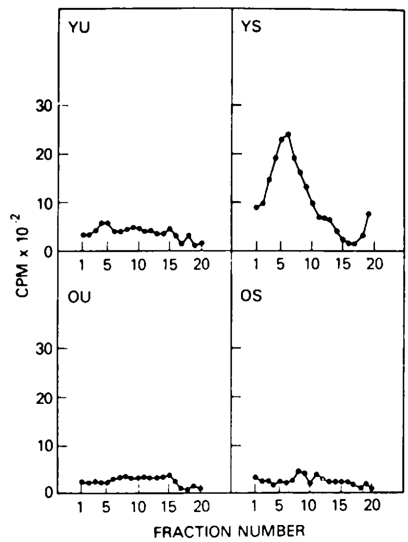

FIG. 1. Glycerol sedimentation velocity gradient profiles of viral-type DNA polymerase from old and young mouse uterine tissue. Portions of high speed supernatants (100 μl) from 25% homogenates of uteri from young unstimulated (YU), young estrogen-stimulated (YS), old unstimulated (OU), and old estrogen-stimulated (OS) NIH Swiss overiectomized mice were layered onto 4 ml 10 → 30% glycerol gradients containing 500 mM KCl, 2 mM dithiothreitol, 10 mM Tris-HCl (pH 8), 2 mM magnesium acetate, and 0.05% Triton X-100. Gradients were centrifuged 15 hr in an SW56Ti rotor, the bottom of each tube was punctured and drops collected. Fractions were assayed for DNA polymerase activity using poly(A)·oligo (dT) as template-primer as previously described (4).

that the uteri from aged mice were larger and firmer than uteri from young, estrogen-stimulated mice. Histological examination of the uteri of young, intact (nonovariectomized) mice (Fig. 2B) showed the expected hyperplasia of all the tissue layers. The uteri of the aged mice (Fig. 2C), whether estrogen-treated or not, did not show the atrophic changes seen in the estrogen-withdrawn organ (Fig. 2A). All tissue layers in the uteri of aged animals were hyperplastic as in the intact organ. However, in contrast to the uterus of an intact animal, numerous cysts were present in the endometrial stroma, and there was an abundance of connective tissue in both the stroma and the myometrium (Masson's trichrome stain, not shown). Using fluorescent antibody techniques and electron microscopy, we have previously shown localization of viral proteins in the glandular and lumenal epithelial cells (9).

In many of the aged mice we observed grossly enlarged adrenals, and some appeared malignant, with destructive invasion of the kidney. Histological comparison of adrenals from aged and young, ovariectomized mice indicated the presence of adrenal carcinoma in the aged animals. Characterization of these tumors will be reported elsewhere.

Discussion. We have previously shown (4) that uteri from ovariectomized NIH Swiss mice respond to estrogen by increased levels of a DNA polymerase which used poly(A)·oligo(dT) as template-primer. The enzyme will also copy the poly C strand of poly(C)·oligo(dG). Although of higher molecular weight than the polymerase from Rauscher murine leukemia virus, the uterine enzyme cross-reacts in enzyme inhibition assay with specific antisera made against purified Rauscher virus polymerase (4). We suggested that the enzyme of mouse uterus might be a precursor of the DNA polymerase associated with mature viral particles.

Since the function of the viral-type RNA-directed DNA polymerase we have described in the mouse uterus is unknown, the physiological significance, if any, of the failure of aged mice to produce the enzyme in response to estrogen is not clear. However, the lack of increased levels of DNA in the uteri of older animals after estrogen treatment may implicate this enzyme in DNA replication. The presence of elevated p30 levels in the absence of increased viral-type DNA polymerase suggests that these proteins are under independent regulatory mechanisms. In previous studies we have found the behavior of these two viral marker proteins to be coordinated, i.e., estrogen administration elicits both or neither, depending upon the mouse strain used.

It is reasonably clear that a lack of estrogen receptors in the uterus cannot explain our results. There is histological evidence of endometrial hyperplasia in the uteri of the "unstimulated" old mice, which implies both an endogenous estrogen source and the presence of receptors. Studies in postmenopausal women suggest that in the absence of functional ovaries, estrogens can arise by peripheral (*e.g.*, adipose tissue) conversion of androgens (10). Others have implicated the adrenals as an estrogen source in women (11) and in mice (12). It has been suggested (13) that the estrogen receptor population degenerates after long-term hormone absence. Eisenfeld and Axelrod (14) demonstrated that pretreatment of ovariectomized rats with estrogen increased the capacity of the uterus to bind estradiol. We found no change in viral-type DNA polymerase in the uterus if the older mice were pretreated for 2 weeks with estrogen. As we have pointed out, the actual hormone-mediated increases in uterine weight, total protein, and p30 were nearly the same in both groups. Expressed on a percentage basis, however, the increases are greater because of the very low baseline values for the young, unstimulated ovariectomized animals. In spite of these indications of uterine growth, the DNA data imply that cellular proliferation in response to estrogen has not occurred in the uteri of the aged mice, whereas there was a threefold increase in DNA per uterus in the young animals upon stimulation.

Gonadectomy has been known for some time to produce adrenal cortical tumors (for review, see Ref. 15). Adrenal cortical tumors might well product hormones which could block an estrogen effect at the genetic level in derepressing or activating genes and

A

FIG. 2. Longitudinal sections of NIH Swiss mouse uteri stained with hematoxylin and eosin. A. Uterus from young, ovariectomized animal. B. Uterus from young, nonovariectomized mouse. C. Uterus from aged, ovariectomized animal. Arrow points to the wall of an endometrial cyst frequently seen in uteri of aged, ovariectomized mice. All magnifications are ×31.

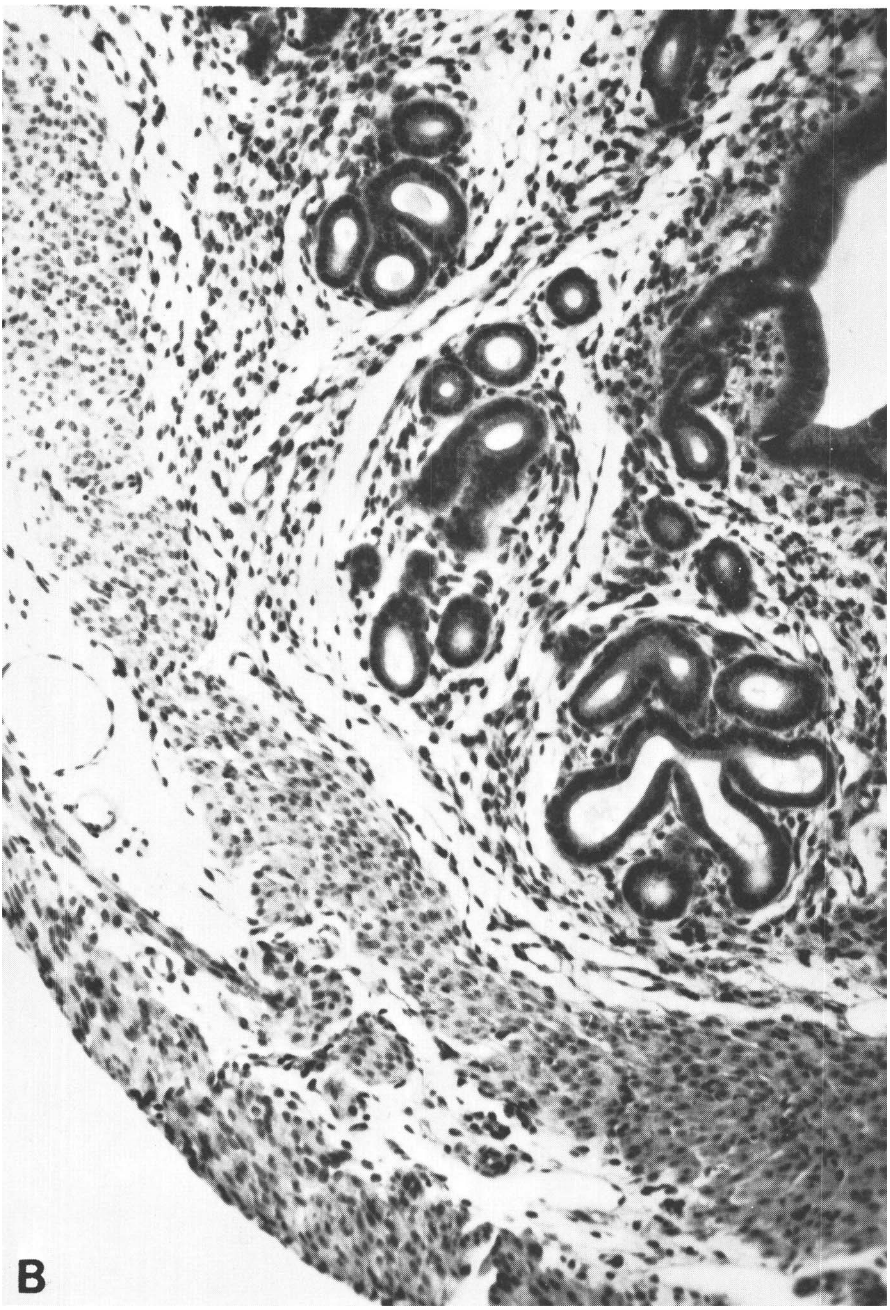

FIG. 2. *Continued.*

DNA replication while not affecting as much cytoplasmic effects such as membrane permeability and translational steps reflected by increases in uterine weight and total protein. Indeed, we have found in preliminary experiments that cortisol can partially block estrogen-mediated increases in uterine p30 levels in young ovariectomized NIH Swiss mice (data not shown).

Summary. Effects of estrogen administration were compared in young (2–5 months) and aged (2.0–2.5 years), ovariectomized

C

FIG. 2. *Continued.*

NIH Swiss mice. Two murine leukemia virus proteins, p30 and RNA-directed DNA polymerase, were markedly elevated by estrogen in the uteri of young, ovariectomized mice, but behaved differently in aged animals. The polymerase was low or absent in the uteri of aged mice and showed no increase in response to estrogen. In contrast, p30 responded to estrogen in the aged animals much the same as in the younger mice. This implies that the production of these viral proteins is under separate control mechanisms.

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